

The Effects of the Storage Conditions of the Juice and the Effects of Nutrient Supplementation
on Wine Fermentation

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Abstract

Storage conditions of Concord grape juice prior to fermentation and types of supplementation during fermentation were studied. Two batches of juice were stored, one at ambient temperature and one at 4.4°C, and were then fermented using four different nitrogen supplementation methods: a control with no supplementation, addition of 100 mg/L nitrogen using diammonium phosphate (DAP), a total target level of 250 mg/L nitrogen using DAP, and complex supplementation using Go-Ferm, Fermaid-K, and DAP. Prise de Mousse yeast was used for fermentation, which is a yeast strain of *Saccharomyces cerevisiae*. Upon completion of fermentation, the wine was analyzed for residual sugar, nitrogen, bound and total SO₂, pH, titratable acidity, volatile acidity, organic acids, and phenols. Sensory evaluation was also performed. There were some significant differences between the different wines, but none of the wines were considered significantly better than the others during sensory analysis.

Introduction

Winemaking: The addition of diammonium phosphate (DAP) to wine to prevent stuck or sluggish fermentations is a common practice performed by many wineries. DAP contains easily accessible nitrogen that is necessary for yeast to make new proteins. Yeasts become stressed when they lack nutrients, and different yeast strains have different nutritional needs (Alexandre & Charpentier, 1998). The type of grapes used plays a role as well, since grape varieties have different nutritional contents. A main source of yeast stress is the amount of nitrogen available to them. Not all nitrogen-containing compounds are easily assimilated by the yeast. Even if there is nitrogen available to the yeast during fermentation, the yeast can still be stressed if it is not easily acceptable to them. Yeast will first consume those nitrogen compounds that are easily accessible and then move on to the less accessible nitrogen compounds (Bauer & Pretorius, 2000).

The ethanol produced by the yeast ultimately builds up to a point of toxicity; the concentration at which ethanol becomes toxic varies by yeast strain. The enzyme H^+ -ATPase controls metabolic activity in the yeast cell as well as regulating the proton gradient. Ethanol inhibits H^+ -ATPase, which decreases metabolic activity within the cells (Bauer & Pretorius, 2000).

Other factors that stress yeast include temperature, high CO_2 and SO_2 levels, and other microorganisms. Ideally, fermentation of red wines should be kept at 18 – 25 °C; higher temperature will cause stress on the yeast. (Bauer & Pretorius, 2000) Ideally, the conditions during fermentation are those that minimize yeast stress. Fermaid-K and Go-Ferm are added during fermentation to further decrease yeast stress.

Sugar is an essential compound for wine fermentation. Yeast use sugar to produce pyruvate, which is then converted to ethanol. Without sugars, fermentation cannot occur. Figure 1 shows the fermentation pathway in yeast. (Moreno-Arribas & Polo, 2008)

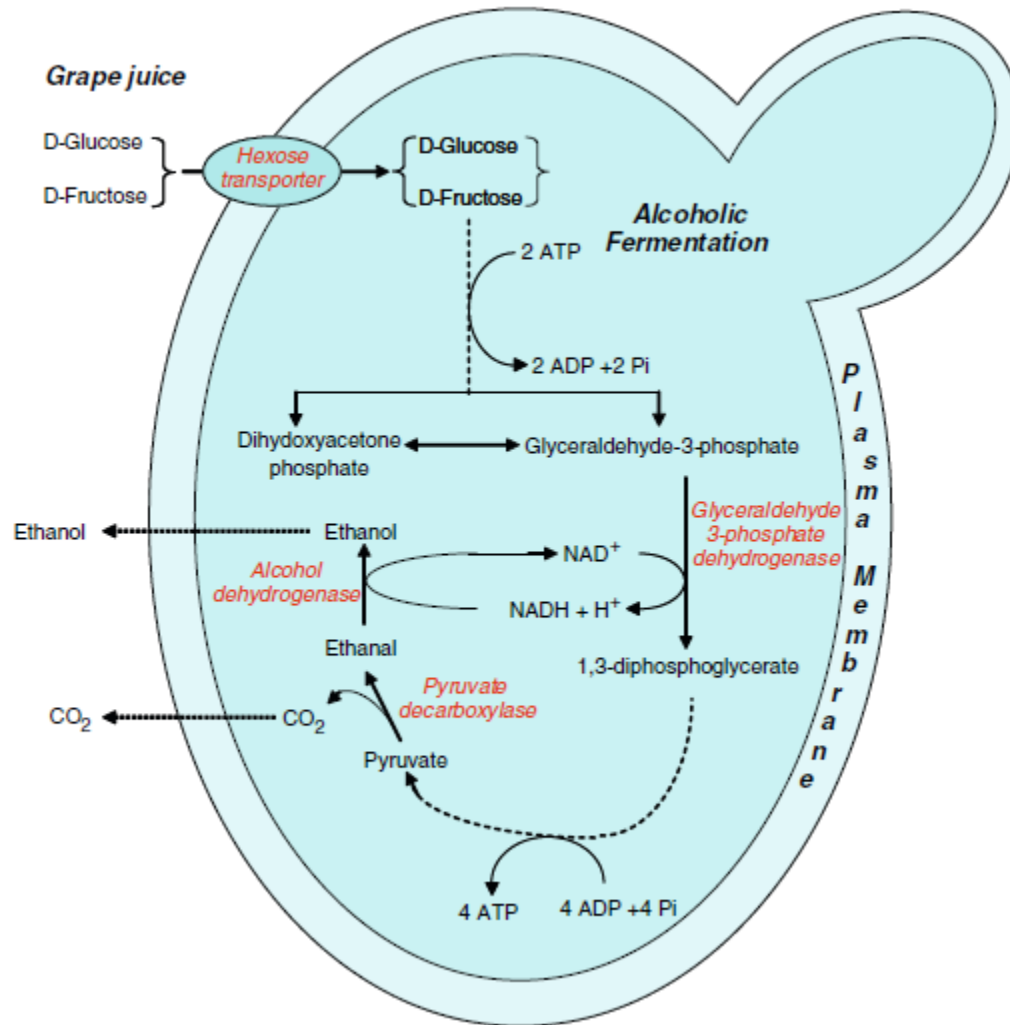


Figure 1. Fermentation process in yeast (Moreno-Arribas & Polo, 2008)

In this cycle, glucose and fructose are converted to ethanol, with a net gain of two ATP. Measuring the decrease in sugar concentration throughout fermentation tracks the rate of

fermentation. It also shows when the fermentation is complete, since almost all of the sugars will be depleted. (Moreno-Arribas & Polo, 2008)

Wine Chemistry: The pH of wines is important for a few reasons. At lower pH levels, harmful microorganisms cannot grow. The pH also plays a role in the color of wines, since at higher pH levels oxidation can cause color loss. The target pH for red wines is 3.3 to 3.6, and for white wines is 3.1 to 3.4. Low values of pH indicate the presence of too many acids, which leads to an unpleasant taste. (Jackson, 2008)

Titrateable acidity (TA) is a measure of the acids present in the wine that can be titrated. In most wines, tartaric acid makes up the greatest concentration of titrateable acids. Malic acid is the second most common. Titrateable acidity gives an estimate of the acids present in wines. (Jackisch, 1985) The TA level is also an indication of taste. Generally, the TA should be kept below 9 g/L. The TA is important in the stability of wines. TA should not drop below 5 g/L. (Jackson, 2008)

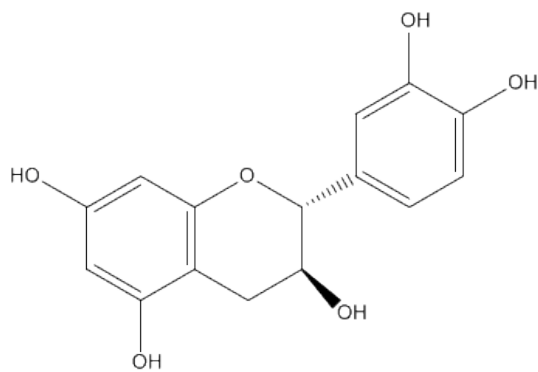
Volatile acidity is a measure of the quantity of those acids in the wines that can be distilled. Volatile acidity is measured in terms of acetic acid. The level of volatile acidity needs to be kept low since acetic acid has a vinegary taste and odor. At the same time, a small amount of acetic acid can add to the complexity of the taste of the wine. Above 0.7 g/L, the taste of acetic acid becomes very unpleasant (Jackson, 2008). Very high levels of acetic acid can cause fermentations to be stuck or sluggish (Alexandre & Charpenter, 1998). Yeast and bacteria produce the majority of acetic acid found in wines (Jackson, 2008).

Both free and total SO₂ in wines must be measured for legal reasons. The amount of total SO₂ needs to be kept below the legal limit of 350 mg/L. The main reason for the legal limit is

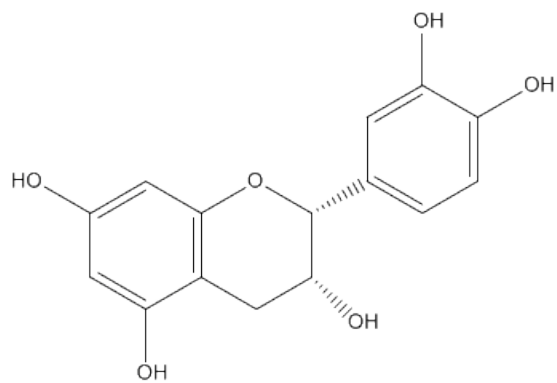
due to health concerns related to consuming too much sulfur dioxide. In addition, some individuals have extra sensitivities or allergies to sulfur dioxide. (Jackson, 2008)

Free SO_2 can exist as dissolved gas, free sulfite ions, and bisulfite ions. SO_2 is often added to wines during fermentation, though this was not done in this experiment due to high SO_2 levels already present in the juice. A range of 15 to 40 mg/L of free SO_2 is the recommended concentration during fermentation. Free SO_2 is important in wines because it prevents microorganism growth. It is actually the most important compound that prevents microorganism growth in wine. Yeast are not as sensitive to sulfur dioxide as other microorganisms are, but high levels of SO_2 can still harm them. (Jackson, 2008) High SO_2 levels are especially harmful in the beginning stages of fermentation (Bauer & Pretorius, 2000).

HPLC Analysis of Phenols and Organic Acids: Phenols affect many properties of wine, including taste and odor. Almost all the phenols in wine come from the grapes themselves. There are two types of phenols: flavonoids and nonflavonoids. Flavonoids have a pyran ring that is connected to two phenolic rings. The common flavonoid groups are flavonols, anthocyanins, and flavan-3-ols. In this experiment, two types of flavan-3-ols, catechin and epicatechin, were analyzed by an HPLC. Their structures are shown below. Both of these compounds are formed in the grapes prior to fermentation (Jackson, 2008). The phenolic rings of flavonoids are reactive. A common reaction with flavonoids is nucleophilic addition. (Moreno-Arribas & Polo, 2008)

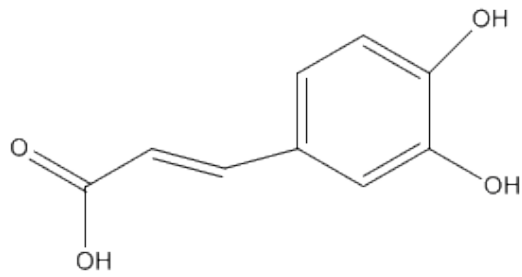


Catechin

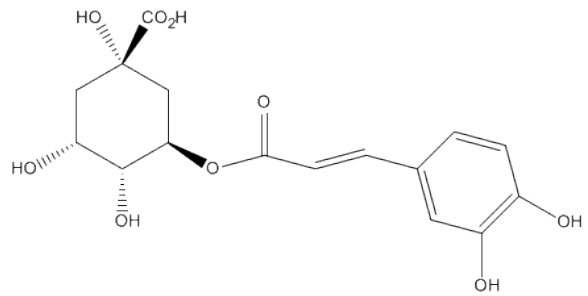


Epicatechin

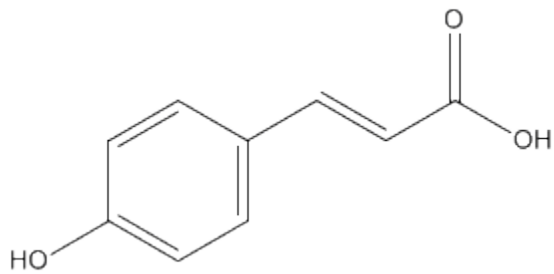
The main classes of nonflavonoids are benzoic acid, benzaldehyde, cinnamic acid, cinnamaldehyde, and tyrosol. In this experiment, one type of benzoic acid, gallic acid, was analyzed by HPLC. Four types of cinnamic acids were also analyzed: coumaric acid, chlorogenic acid, ferulic acid, and caffeic acid. Their structures are shown below.



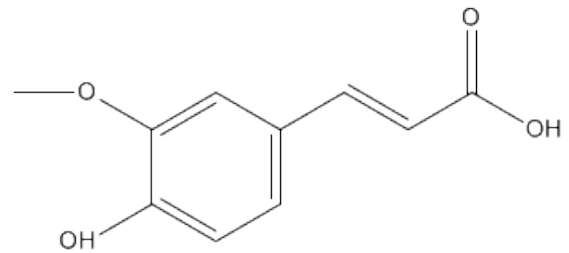
Caffeic Acid



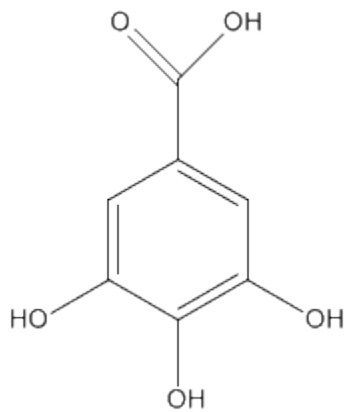
Chlorogenic Acid



Coumaric Acid



Ferulic Acid

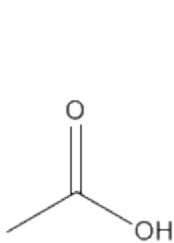


Gallic Acid

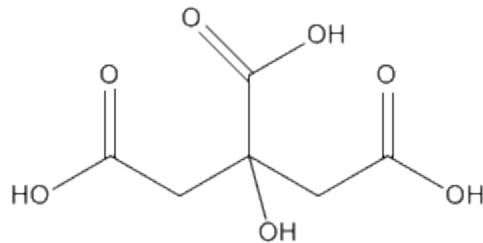
All these acids are formed in the grapes. Gallic acid, coumaric acid, and ferulic acid can also form if wine is stored in oak barrels, but in this experiment, the wines were not aged.

(Jackson, 2008)

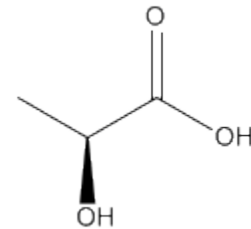
Organic acids play an important role in wines, contributing to the taste and stability of the wine. Five organic acids were analyzed in this experiment: acetic acid, citric acid, lactic acid, malic acid, and tartaric acid. Their structures are shown below.



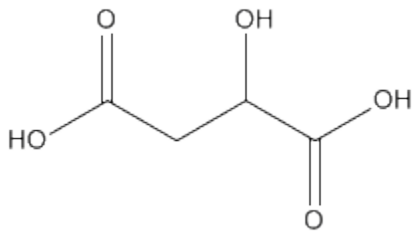
Acetic Acid



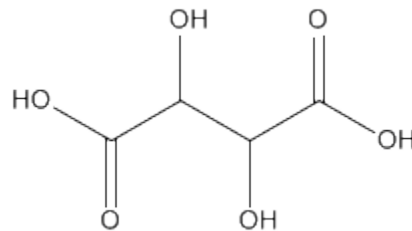
Citric Acid



Lactic Acid



Malic Acid



Tartaric Acid

Most of the organic acids are not volatile acids. The exception is acetic acid. In most wines, tartaric and malic acid usually have the greatest concentration of the organic acids. Not only does tartaric acid have one of the greatest concentrations of organic acids but it is also one of the strongest acids in wines. Due to this, it plays a major role in the pH of wine. The concentration of malic acids in grapes depends on the climate in which they are grown. Cooler climates have a higher concentration of malic acid. A sour taste can develop if the level is too high. During the fermentation process, some of the malic acid can be converted to lactic acid,

through the process of malolactic fermentation. Since lactic acid is found in very small quantities in grapes, malolactic fermentation can be the greatest source of lactic acid in wines. Citric acid is also found in small amounts in wine. It's also a common acid used by winemakers to decrease the pH in finished wines. (Jackisch, 1985)

Some wine companies store their juice prior to fermentation in vats without temperature control. Other companies store their juice indoors in temperature-controlled vats. However, n little research has been done on the effects of juice storage conditions on wines produced from the juice. This study examines the chemical differences between wine produced from juice stored at ambient temperatures (juice stored outside) and cold temperatures (4.4°C). We have also examined chemical differences in wines produced from the two types of juice as a function of the type of nitrogen supplementation used to facilitate the fermentation process.

Experimental

Concord grape juices stored at ambient and cold temperatures were obtained from a large New York winery. Grapes for both lots of juice were harvested from the same vintage on consecutive days. The cold-stored juice was made from grapes crushed between 9/16/09 - 9/17/09 and the temperature of the juice was controlled at 4.4°C. The ambient-stored juice was made from grapes crushed between 9/28/09 – 9/29/09 and was stored in an outside storage facility where the temperature was not controlled. The fermentation process, with the exception of nitrogen supplementation, was standardized.

In this experiment, standard wine tests were performed to determine the levels of residual sugars, pH, titratable acidity (TA), volatile acidity, free and total SO₂, and nitrogen. A small sensory test was performed on the wines as well. The second part of the study involved analyzing the wines by high-pressure liquid chromatography (HPLC). The two types of compounds that were analyzed were phenols and organic acids. The benefit of using HPLC is that you can determine concentrations of individual compounds, something that cannot be achieved as easily or as accurately in other standard analytical practices used in the analysis of wines.

Wine Fermentation: In the first part of the experiment, eight batches of wine were prepared. The fermentation conditions are shown in Table 1.

Table 1: Fermentation Conditions

Juice Storage	Supplementation
Ambient	No Supplementation
Ambient	100 mg/L nitrogen with DAP
Ambient	Total 250 mg/L nitrogen with DAP
Ambient	Complex Nutrition
Cold	No Supplementation
Cold	100 mg/L nitrogen with DAP
Cold	Total 250 mg/L nitrogen with DAP
Cold	Complex Supplementation

Unsupplemented juice was used as the control. One experimental treatment was the straight addition of 100 mg/L of nitrogen using DAP. In this method, the same amount of DAP was added regardless of the concentration of nitrogen already in the juice, reflecting the DAP addition rate standard in many wineries. For the second experimental treatment, the nitrogen level in the juice was measured and then DAP was added to bring the nitrogen level up to 250 mg/L.

In the third experimental treatment, complex supplementation was used. For this method, two additional nutrients, Go-Ferm and Fermaid-K, were added. Go-Ferm is a yeast-rehydrating nutrient that promotes faster and more efficient fermentations. Fermaid-K is a complex blend of nutrients, including DAP, amino acids, unsaturated fatty acids, sterols, and inactive yeast, which helps prevent the death of yeast caused by low acidity levels, high alcohol levels and other toxic materials. It also provides a more efficient supply of nitrogen, which is essential for successful fermentations (Lallemand, 2010). For this supplementation method, the nitrogen level was measured in the juices, and DAP was added to bring the level up to 150 mg/L if necessary, as suggested by the Scott Lab Fermentation Guide.

As shown in Table 1, there were four supplementation methods used on the two types of juices for the fermentation. Replicates were performed for each type of wine, for a total of sixteen batches of wine. The nitrogen levels in the juice were measured by using a ChemWell chemical analyzer. Two reagent kits were used for the analysis. The Primary Amino Nitrogen (PAN) UniTab Reagent Kit contained N-acetyl-L-cysteine (NAC) tablets, ophthaldialdehyde (OPA), and nitrogen standard solutions of 60 mg/L, 120 mg/L, 200 mg/L, 300 mg/L, and 400 mg/L. The second kit was the Ammonia (AMM) UniTab Reagent Kit, containing ammonia reagent tablets, trigger reagent, and ammonia standards of 25 mg/L, 50 mg/L, 100 mg/L, 200 mg/L, and 300 mg/L. The wines were centrifuged before analysis to remove all solids, including yeast.

The procedure used to determine nitrogen was provided in the reagent kits. The “start of day” run was performed to check that the nozzles were dispensing fluids. Next, the channel blank program was run using a performance blanking solution. Two programs were selected: ammonia and primary amino nitrogen. Each wine type was placed into a test tube, which was

then placed into a slot in the ChemWell. The nitrogen and ammonia standards, along with OPA and the trigger reagent, were placed into test tubes and then into the ChemWell. The NAC and ammonia reagent tablets were dissolved and then placed into their appropriate spot in the ChemWell. After the program was run, the “end of day” program was selected, and a 30% chlorine bleach solution was used to rinse the apparatus.

Table 2 shows the amounts of supplements added to each type of fermentation, based on the measured nitrogen levels in the juice.

Table 2. Supplementation Conditions

Juice Storage	Supplementation Type	DAP Added	Fermaid-K Added
Ambient	No Supplementation	-	-
Ambient	100 mg/L nitrogen with DAP	102 mg/L nitrogen	-
Ambient	Total 250 mg/L nitrogen with DAP	204 mg/L nitrogen	-
Ambient	Complex Supplementation	104 mg/L nitrogen	4 g
Cold	No Supplementation	-	-
Cold	100 mg/L nitrogen with DAP	102 mg/L nitrogen	-
Cold	Total 250 mg/L nitrogen with DAP	95 mg/L nitrogen	-
Cold	Complex Supplementation	-	4 g

The Fermaid K was added in two parts. Two grams were added at the beginning of fermentation and another two grams were added at one-third sugar depletion. DAP was added only to the ambient stored juice with complex nutrition. The cold stored juice had a nitrogen level above 150 mg/L, so DAP was not necessary for the complex supplementation fermentation.

The juice, water, high-fructose corn syrup, and appropriate supplements were combined. Prise de Mousse yeast, which is a yeast strain of *Saccharomyces cerevisiae*, was rehydrated in water, and added to each fermentation batch. For the fermentations with complex supplementation, the yeast was rehydrated with Go-Ferm. The fermentation carboys were left at

room temperature for a day and then moved to a 20°C room for the remainder of fermentation. After thirty days, the fermentations were stopped and placed into cold storage (2°C). The wine was kept in cold storage for the remainder of the time.

The sugar levels were measured roughly every other day using the ChemWell. A GF-F150 UniFlex Reagent Kit was used for the analysis, which contained a D-glucose/fructose buffer, hexokinase/G6PDH enzyme suspension, phosphoglucose isomerase (PGI) enzyme suspension, and glucose standards of 1.5, 4.0, and 8.0 g/L. The procedure was taken from the reagent kit. The procedure was very similar to the YAN procedure. The only exception was the reagents used. The D-glucose/fructose program was selected. The glucose standards and PGI enzyme suspension were placed into test tubes and placed into the appropriate slot. The D-glucose/fructose buffer was mixed with water and hexokinase enzyme suspension and then placed into the appropriate spot on ChemWell.

Wine Chemistry: Next, a series of standard wine analysis was performed. The nitrogen was measured in the wines, using the procedure mentioned above. The pH and TA were measured using a Metrohm automatic titrator for all the wine samples and the two juices. It contained three standard buffers for pH 4.0, 7.0, and 9.0.

Volatile acidity was measured by use of a Cash still. In a 250 mL flask, 50 mL of DI water was combined with four drops of a phenolphthalein indicator solution. The solution was titrated with 0.01 N NaOH, used as received from Anachemia, until it reached a steady pink color. DI water was added to the outer portion of the Cash still to cover the coil of the still completely. 10 mL of ambient stored juice was added to the inner portion of the Cash still. The inner portion was then rinsed with a small amount of DI water to ensure that all the juice was in the chamber.



Figure 2. Cash still for volatile acidity analysis. Taken from <http://www.winebusiness.com/wbm/?go=getArticle&dataId=66754>

The water condenser was turned on and the stopcock was closed. The heating mantle was turned on and the juice was brought to a moderate boil. 100 mL of distillate was collected in the 250 mL flask. The distillate was titrated with 0.01 N NaOH. This was repeated for all the wines. The inner chamber was rinsed with DI water between analyses to ensure that there was no juice left in the chamber.

The concentration of SO_2 was measured using the aspiration method, also known as aeration oxidation. First, the free SO_2 was measured. In a pear shaped flask, 10 mL of 0.3% H_2O_2 and six drops of a methylene blue 0.05% and methyl red 0.1% indicator solution were added. The solution turned a light violet color and was titrated with 0.01 N NaOH, used as received from Anachemia, until a dark green endpoint was reached. In a round bottom flask, 20 mL of the

ambient stored juice and 10 mL of 25% phosphoric acid, used as received from Preque Isle Wine Cellar, were combined. The pear shaped flask and round bottom flask were connected to the apparatus and the vacuum was turned on. The juice was aspirated for fifteen minutes, at a flow rate of 1000 mL/min. The vacuum was turned off and the solution in the pear shaped flask was titrated with 0.01 N NaOH until a dark green color was reached.

For the bound SO₂, the pear shaped flask was returned to the apparatus, with the solution still in it. The same round bottom flask was placed over a microburner, the condenser was turned on, and the solution was brought to a boil. The solution was aspirated for fifteen minutes, at a flow rate of 1000 mL/min, while gently boiling. The condensate in the pear shaped flask was again titrated with 0.01 N NaOH until a dark green endpoint was reached. The procedure for measuring free and bound SO₂ was repeated for each of the wines.

A sensory test was performed on the wines. Twenty-one participants, ten males and eleven females over the age of twenty-one, were recruited from Geneva, NY. The participants had a wide range of wine knowledge. There was no screening process. The sensory evaluation was performed over a two-day period with ten participants on the first day and eleven participants on the second day. Each participant was given a tray containing approximately 50 – 75 mL of each type of the eight wines. The wines were in ISO tasting glasses and were covered with petri dishes. Each wine type had a three-digit random number.

The panelists were instructed to taste the wine samples as presented from left to right. They were allowed to retaste the samples as needed. The order of the wine was randomized for each participant. They were instructed to rank the wines in order of preference from one to eight, with one being the most preferred and eight being the least preferred. Each wine was ranked. The tray also included a glass of water and saltine crackers, so that they could clean their palate

between wines. The panelists were told they could have water or crackers at any time during the evaluation. The sensory evaluation was performed at room temperature and under a red light to reduce to effect of color differences.

HPLC Analysis: For the second part of the analysis, HPLC was used to analyze the phenols and organic acids in the wine and the juice. All HPLC instrumental work was performed by Dr. David C. Manns at the NYS Agricultural Experiment Station in Geneva, NY. The juice and wines were microfiltered before injection into the HPLC. For the phenol analysis, a Varian LiChrospher 5 ECAP 250 mm x 4.6 mm column with a RP-18, reverse phase, c18, 5 μm particle size, 100 Å pore size, endcapped fitted with a c18 Microsorb Metaguard column was used. Two mobile phases were used. Mobile phase A contained 99.5% water and 0.5% phosphoric acid by volume and mobile phase B was made up of 50% acetonitrile, 49.5% water, and 0.5% phosphoric acid by volume. All chemicals used were of HPLC grade. An elution gradient was used to vary the percent of mobile phase A and B in the column. Each run time lasted 60 minutes. Table 3 shows the percent of mobile phase B during the analysis.

Table 3. Elution Gradient used for Phenolic Analysis

Percent of Mobile Phase B	Time (min)
0%	2
20%	7
40%	25
80%	35
100%	40
0%	50

The flow rate through the column was 1 mL/min and the column temperature was 30°C. There were four detection wavelengths used: 280, 320, 360, and 520 nm. 50 µL of sample was injected into the HPLC and the needle was rinsed with ethanol between injections.

Standard mixtures containing gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, coumaric acid, and ferulic acid with concentrations of 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm, and 100 ppm were analyzed. Next the wine samples and the juice samples were injected into the HPLC.

For organic acids, a Bio-Rad Aminex HPX-87H column fitted with a Micro-guard cation H refill cartridge was used. The mobile phase consisted of 6% acetonitrile and 0.045 N sulfuric acid. An isocratic elution was used. The injection volume was 20 µL and the needle was rinsed with water between injections. The flow rate through the column was 0.5 mL/min and the column temperature was set at 45°C. The wavelength was set to 210 nm and the run time was set to 30 minutes. All chemicals used were of HPLC grade.

Standard mixtures containing citric acid, tartaric acid, malic acid, lactic acid, and acetic acid with concentrations of 0.1 g/L, 0.5 g/L, 1.0 g/L, 5.0 g/L, and 10.0 g/L were analyzed. Next the wine samples and the juice samples were injected into the HPLC.

Results

The data were analyzed for the sugar levels, nitrogen content, pH, TA, volatile acidity, SO₂, sensory results, and phenolic and organic acid content. Statistically differences are defined as the interval of three standard deviations about the mean value for the first sample not overlapping the interval of three standard deviations about the mean value for the second sample.

Sugars: Figure 3 and Figure 4 show the sugar concentration over the thirty-day period in the wines. All the fermentations were stopped after thirty days.

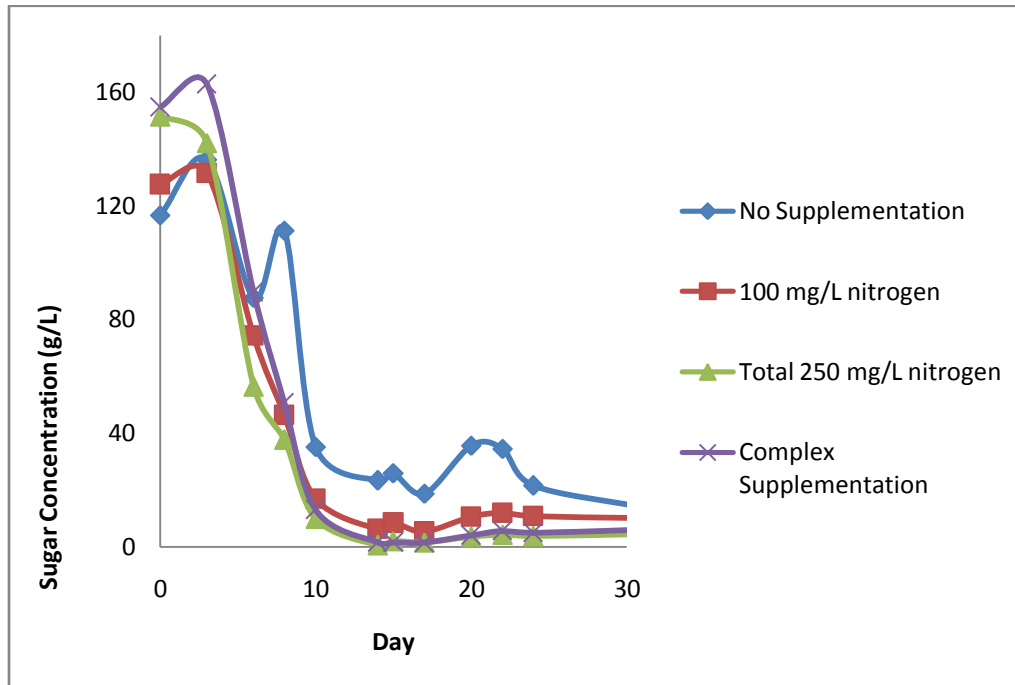


Figure 3. Sugar concentration (g/L) versus day for wines made with ambient stored juice.

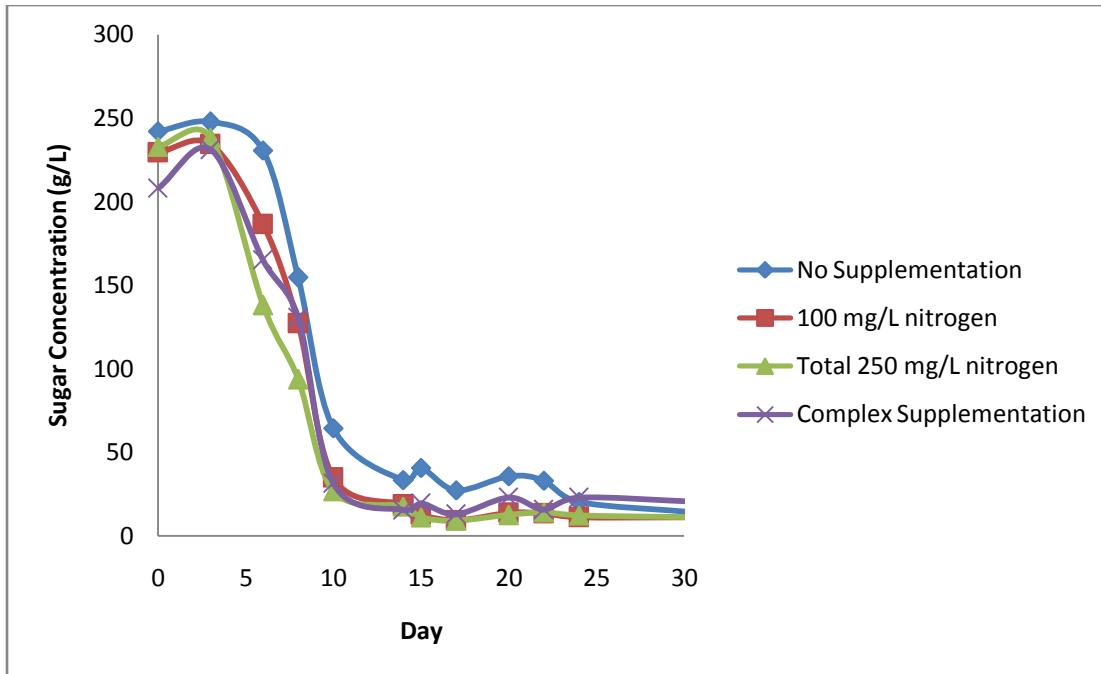


Figure 4. Sugar concentration (g/L) versus day for wines made with cold stored juice.

Nitrogen: Figure 5 and Figure 6 show the nitrogen levels in the juice and the wines. The yeast assimilable nitrogen (YAN) is the sum of the ammonia nitrogen and primary amino nitrogen.

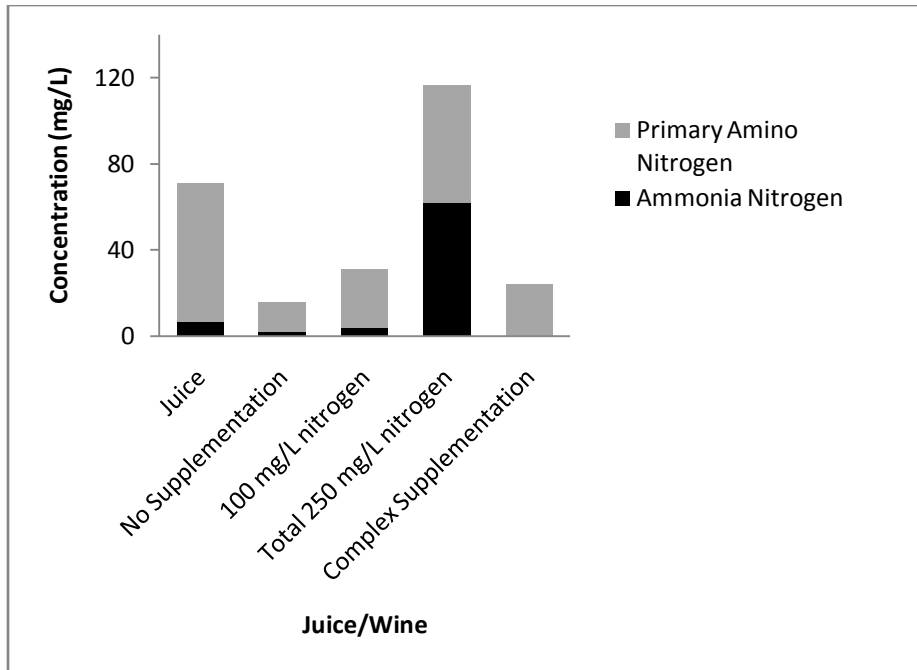


Figure 5. Nitrogen Content in Ambient Stored Juice and Wine

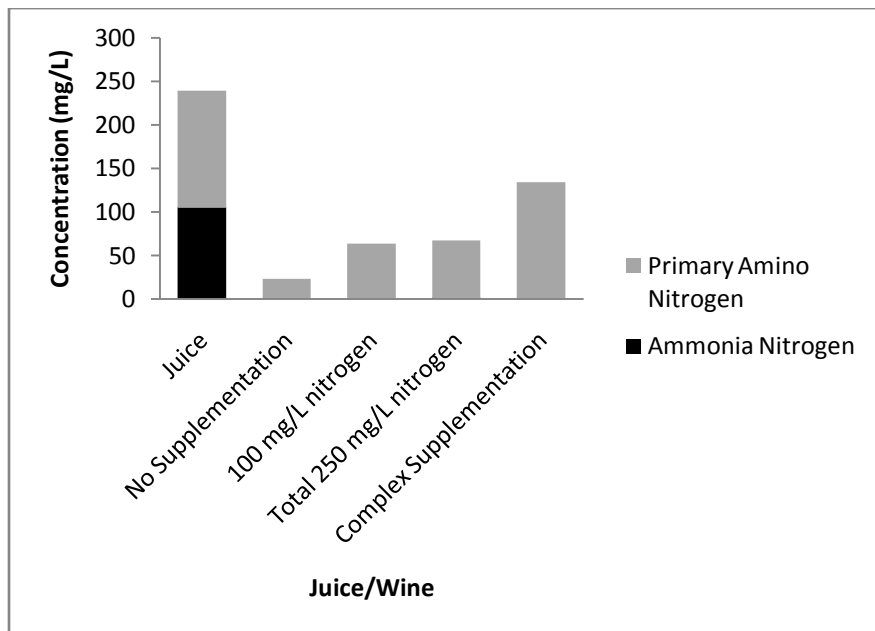


Figure 6. Nitrogen Content in Cold Stored Juice and Wine

Figure 7 shows the percent decrease in total YAN. The starting level is the nitrogen level in the juice after supplementation.

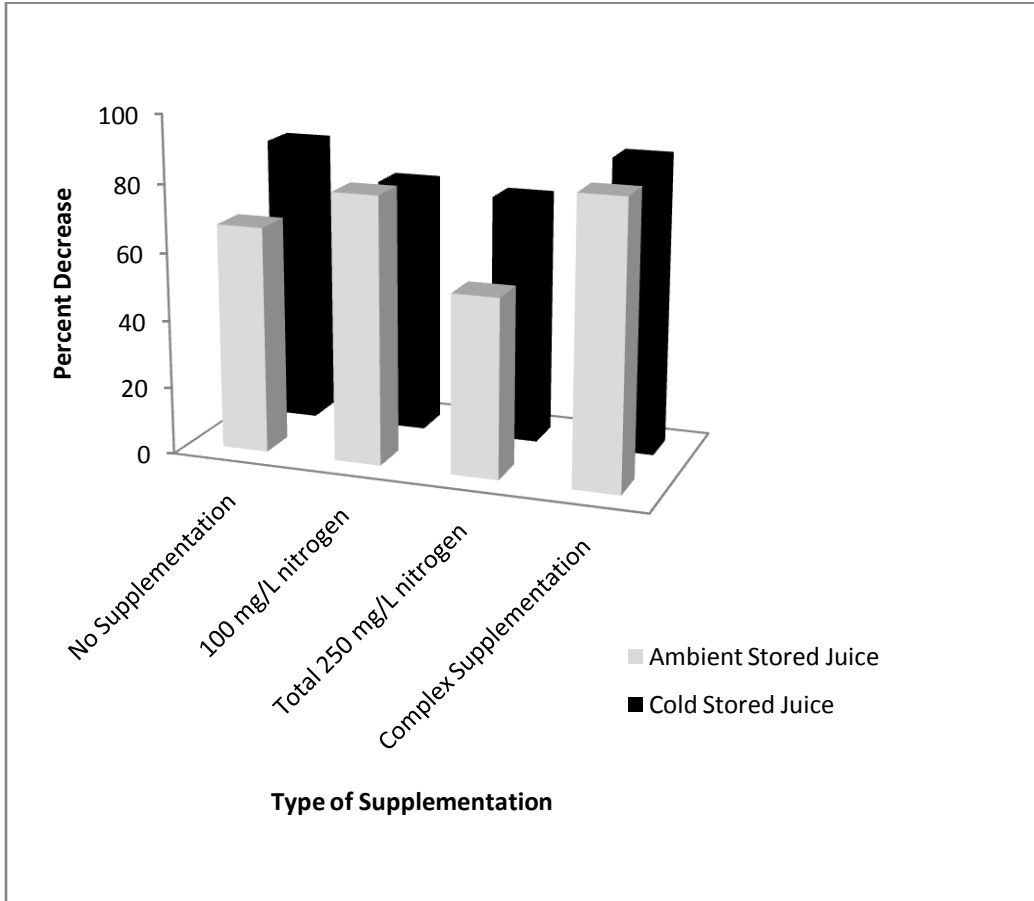


Figure 7. Percent decrease in total YAN in wines

pH: The pH of the juices and wines were measured using an automatic titrator. Table 4 shows the pH levels in the wines and juice.

Table 4. pH in juice and wines

	Ambient Stored Juice	Cold Stored Juice
Juice	3.12	2.98
No Supplementation	3.05 ± 0.02	3.01 ± 0.03
100 mg/L nitrogen	3.04 ± 0.01	2.975 ± 0.007
Total 250 mg/L nitrogen	3.2 ± 0.1	2.99 ± 0.04
Complex Supplementation	3.1 ± 0.1	3.00 ± 0.02

TA: The TA was measured using an automatic titrator and was given in terms of tartaric acid equivalence. Table 5 shows the TA levels in the juice and wines.

Table 5. TA in juice and wines

	Ambient Stored Juice (g/L)	Cold Stored Juice (g/L)
Juice	12.6	16.7
No Supplementation	8.6 ± 0.4	11.07 ± 0.04
100 mg/L nitrogen	9.1 ± 0.1	11.4 ± 0.3
Total 250 mg/L nitrogen	9.1 ± 0.4	11.17 ± 0.07
Complex Supplementation	9.3 ± 0.3	11.18 ± 0.02

Volatile Acidity: Acetic acid makes up the largest amount of volatile acid in the wine, so the volatile acidity was measured in terms of acetic acid. Equation 1 was used to calculate the concentration of acetic acid.

(1)

$$\begin{aligned}
 [\text{Acetic Acid}] = & [\text{NaOH}(M)] \times \frac{\text{mL of NaOH titrated}}{1000} \times \frac{1000}{\text{volume of } \frac{\text{juice}}{\text{wine}} \text{ (mL)}} \\
 & \times \text{Molecular Weight} \left(\frac{\text{g}}{\text{mol}} \right)
 \end{aligned}$$

The molecular weight of acetic acid is $60 \text{ g} \times \text{mol}^{-1}$. The equation can be simplified since 0.01 M NaOH and 10 mL of juice/wine was used for all of the analyses. Equation 2 shows the simplified equation.

(2)

$$[\text{Acetic Acid}] = \text{mL of NaOH titrated} \times 0.06$$

Table 6 shows the volatile acidity levels in the juice and wine.

Table 6. Volatile Acidity in Wines made from Ambient Stored Juice

	Ambient Stored Juice (g/L)	Cold Stored Juice (g/L)
Juice	0.465	0.246
No Supplementation	0.59 +/- 0.07	0.68 +/- 0.01
100 mg/L nitrogen	0.572 +/- 0.006	0.57 +/- 0.03
Total 250 mg/L nitrogen	0.47 +/- 0.02	0.579 +/- 0.008
Complex Supplementation	0.482 +/- 0.002	0.66 +/- 0.01

Sulfur Dioxide: Free SO₂ was calculated using Equation 3.

(3)

$$\text{Free SO}_2 = \frac{[\text{NaOH (M)}]}{2} \times \frac{\text{mL NaOH titrated}}{1000} \times \frac{1000}{\text{volume of juice/wine (mL)}} \\ \times \text{Molecular Weight} \left(\frac{\text{g}}{\text{mol}} \right) \times \frac{1000 \text{ mg}}{1 \text{ g}}$$

For all of the analyses, 0.01 N NaOH was used and the volume of the juice/wine was 20 mL. The molecular weight of SO₂ is 64 g × mol⁻¹. The equation can be simplified for these conditions, as shown below in Equation 4.

(4)

$$\text{Free SO}_2 = 16 \times \text{mL of NaOH titrated}$$

Equation 4 was also used to calculate the amount of bound SO₂. Total SO₂ is the sum of the free and bound SO₂. Figure 8 and Figure 9 show the amounts of SO₂ in the ambient and cold stored juice and wines.

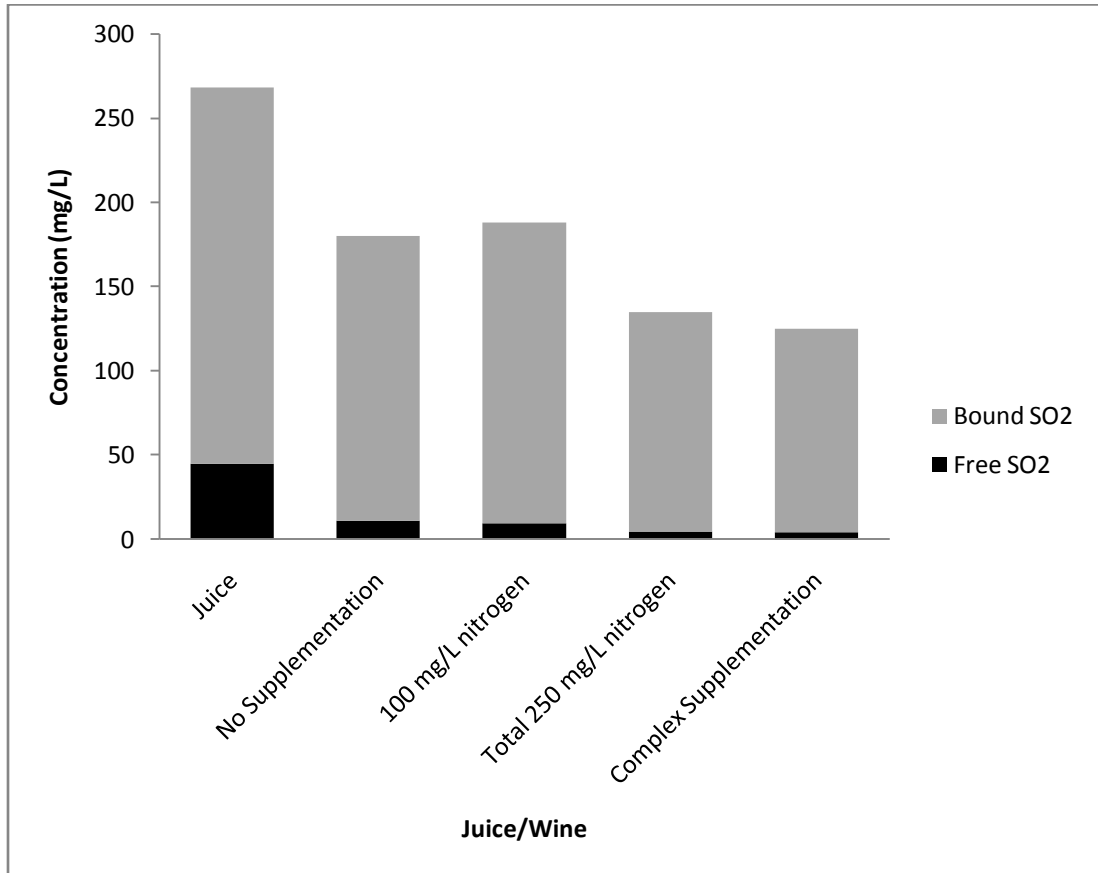


Figure 8. SO₂ Levels in Ambient Stored Juice and Wine

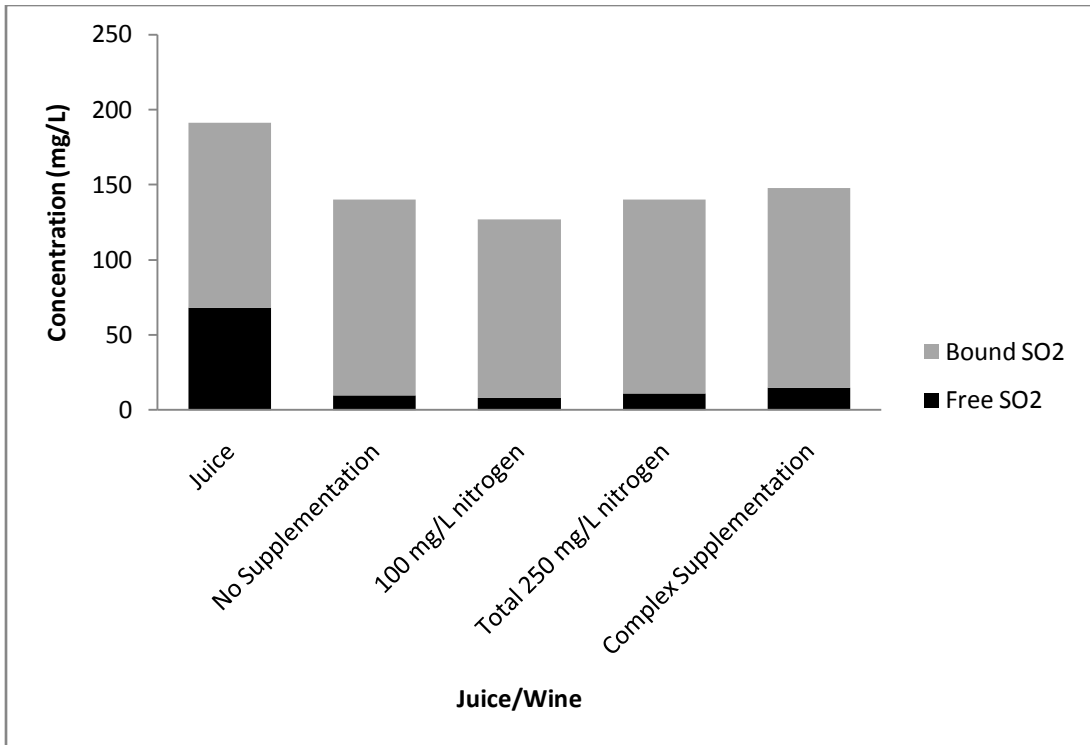


Figure 9. SO₂ Levels in Cold Stored Juice and Wine

Sensory: The participant scores for each wine were summed and the sums were put in order from smallest to largest (highest net preference to lowest net preference). The critical values were obtained at $p = 0.05$ and $p = 0.01$ significance levels (Basker, 1988). The results are shown in the Table 7. The order of preference decreases when moving down the chart.

Table 7. Sensory Test Results

Significance Level		p = 0.05	p = 0.01
Critical Difference		48.1	56
Cold	100 mg/L nitrogen	α	α
Cold	Total 250 mg/L nitrogen	α	α
Ambient	Total 250 mg/L nitrogen	α	α
Ambient	100 mg/L nitrogen	$\alpha\beta$	$\alpha\beta$
Cold	Complex Nutrition	$\alpha\beta$	$\alpha\beta$
Cold	No Supplementation	$\alpha\beta$	$\alpha\beta$
Ambient	No Supplementation	β	β
Ambient	Complex Nutrition	β	β

The Greek letters in the chart show which wines are statistically the same and different. All the wines with the same Greek letter are considered the same. All the wines with an alpha next to them are considered the same and all the wines with a beta next to them are considered the same. The three wines with both an alpha and beta are the same as all the other wines. Note that the sensory evaluation only shows if there are statistical differences in panelist's preferences, not in the actual wines themselves.

Phenolics: Table 8 shows the wavelength at which each compound was detected and the average retention time for the seven phenols analyzed by the HPLC.

Table 8. Phenolics Standards

Phenol	Maximum Wavelength (nm)	Average Retention Time (min)
Gallic Acid	280	10.688
Catechin	280	17.769
Chlorogenic Acid	320	18.131
Caffeic Acid	320	20.730
Epicatechin	280	21.403
Coumaric Acid	320	26.978
Ferulic Acid	320	29.362

Standard curves were made for each phenol. The curves, along with the equations and R^2 values are shown in Appendix B. The smallest R^2 value was 0.99989. The concentrations of each phenol in the different wines were calculated using the standard curves. A representative HPLC chromatogram for the juice and wine are shown in Appendix A for each of the three wavelengths analyzed. Figure 10 shows an example of an HPLC chromatogram for phenolics.

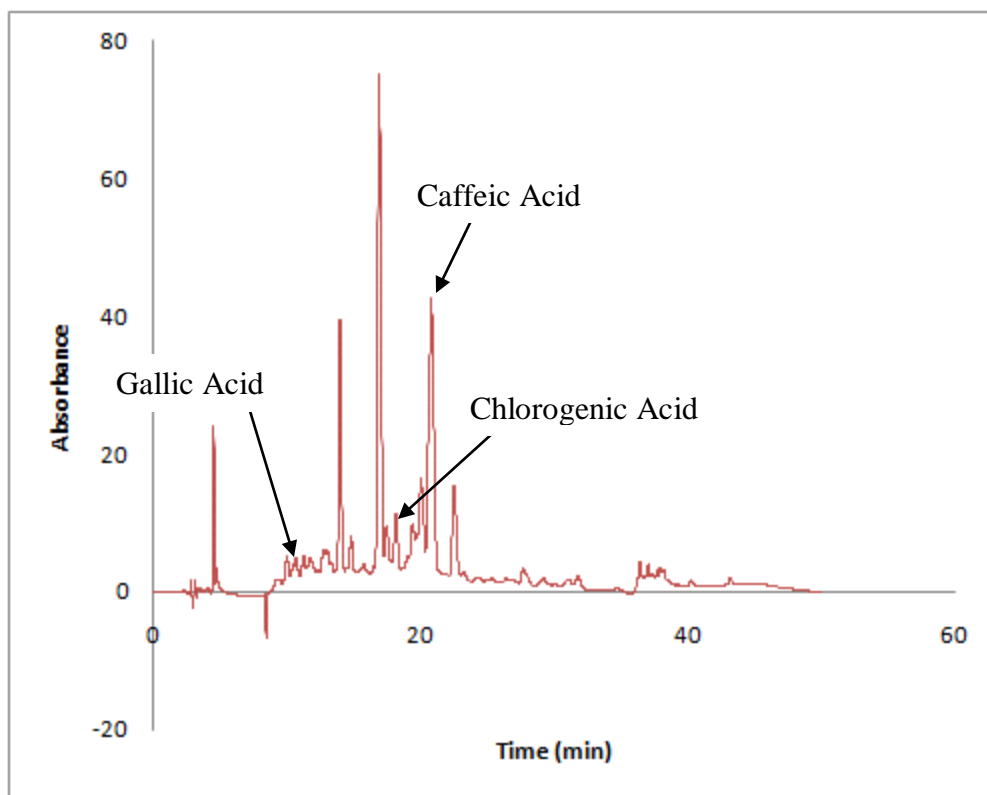


Figure 10. Phenolic HPLC chromatogram for wine with no supplementation made with ambient stored Juice at 320 nm

The concentrations of the phenolics in the juice and the wines are shown in Figure 11 and Figure 12.

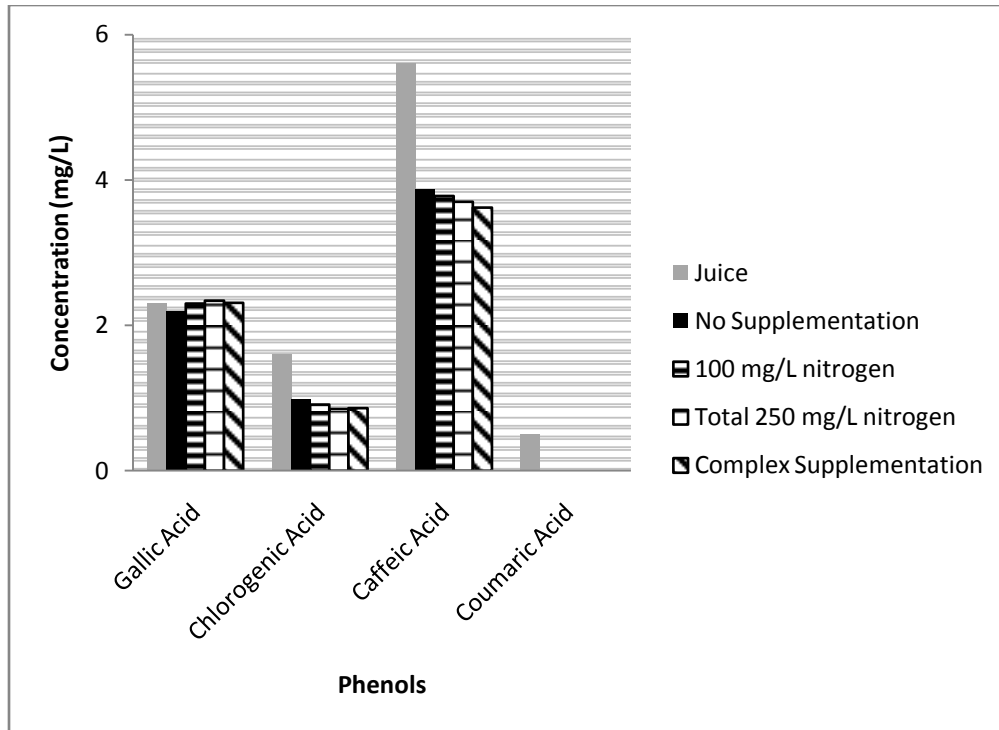


Figure 11. Phenolic content in ambient stored juice and wine

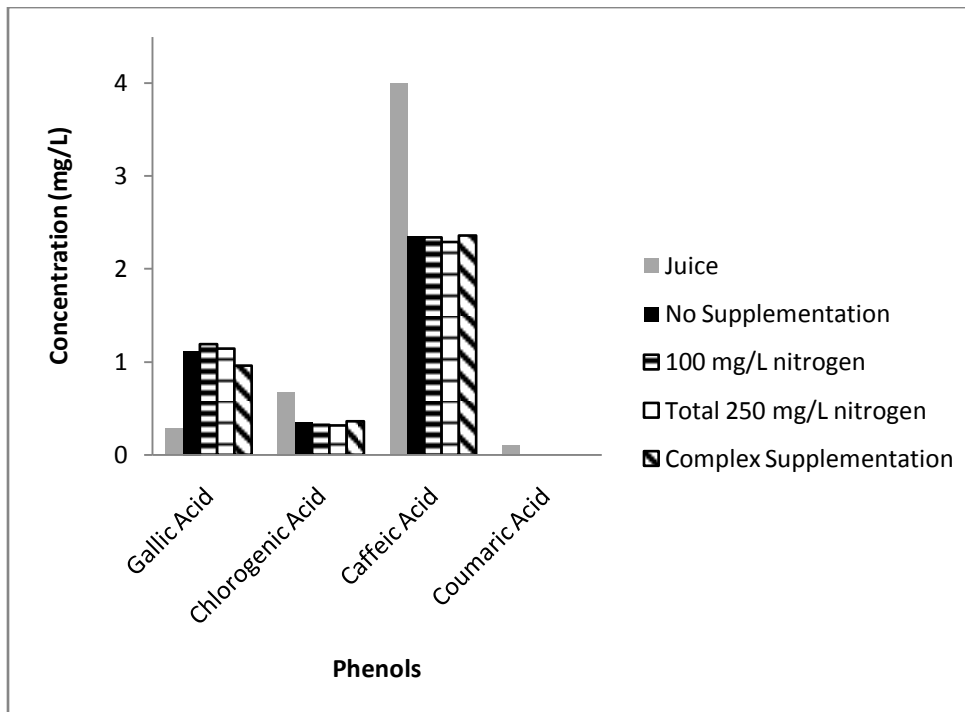


Figure 12. Phenolic content in cold stored juice and wine

No detectable amounts of catechin, epicatechin, and ferulic acid were found in any of the juices or wines. This most likely is due to the type of grapes used to make the juices. Small amounts of coumaric acid were found in the juice, but not in the wines.

Organic Acids: Table 9 shows the average retention time for the five organic acids analyzed. All the organic acids were detected at a wavelength of 210 nm.

Table 9. Organic Acid Retention Times

Organic Acid	Average Retention Time (min)
Citric Acid	9.427
Tartaric Acid	10.238
Malic Acid	11.101
Lactic Acid	14.451
Acetic Acid	17.057

Standard curves were made for each organic acid. The curves and their equations and R^2 values are shown in Appendix D. The smallest R^2 value was 0.99941. The concentration of each organic acid in the different wines was calculated using the standards curves. A representative HPLC chromatogram for the juice and wine are shown in Appendix C. Figure 13 shows an example of an HPLC chromatogram for organic acids

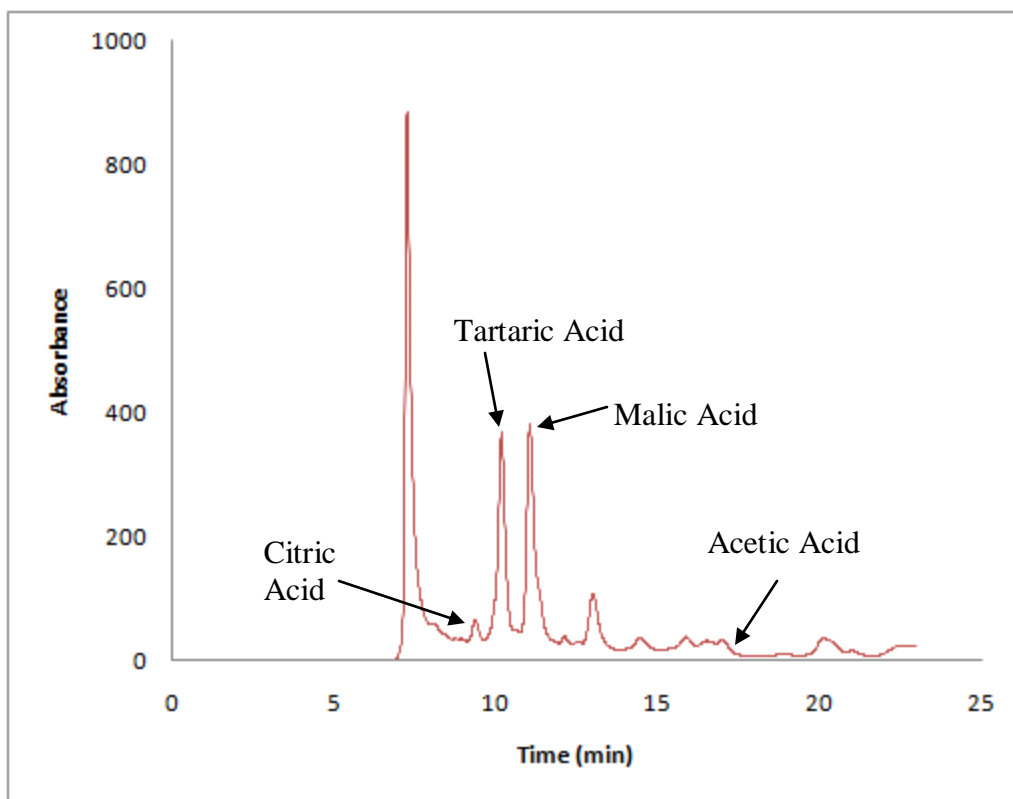


Figure 13. Organic acid HPLC chromatogram for wine with no supplementation made with ambient stored juice

Figure 14 and Figure 15 show the organic acid contents in the ambient and cold stored juice and wine.

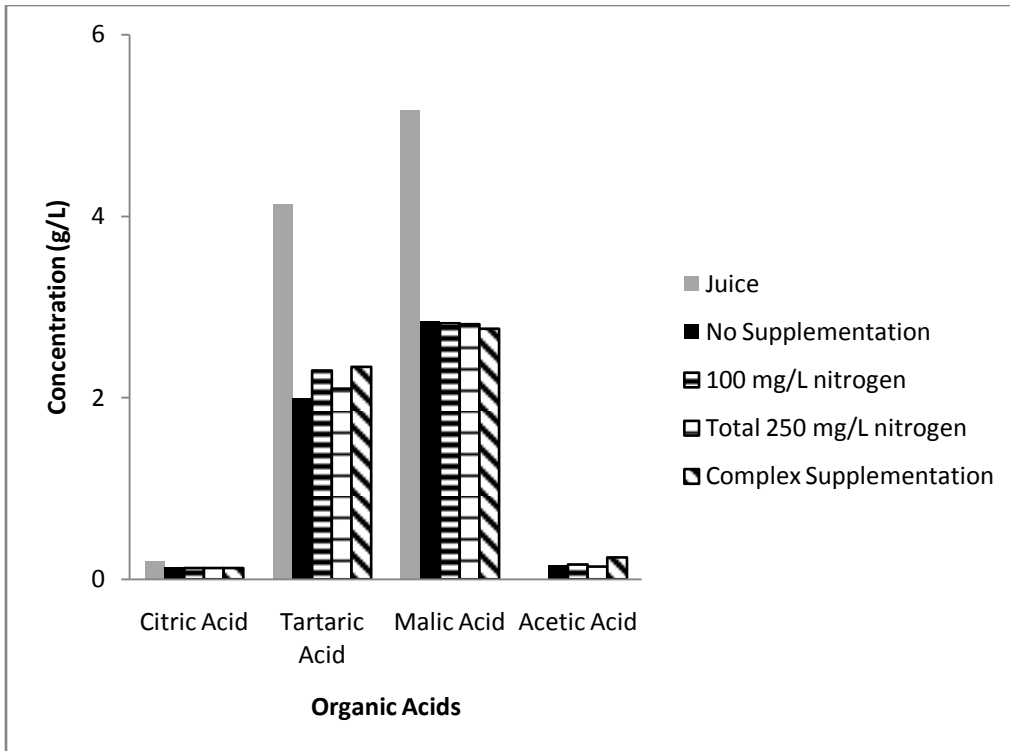


Figure 14. Organic acid content in ambient stored juice and wines

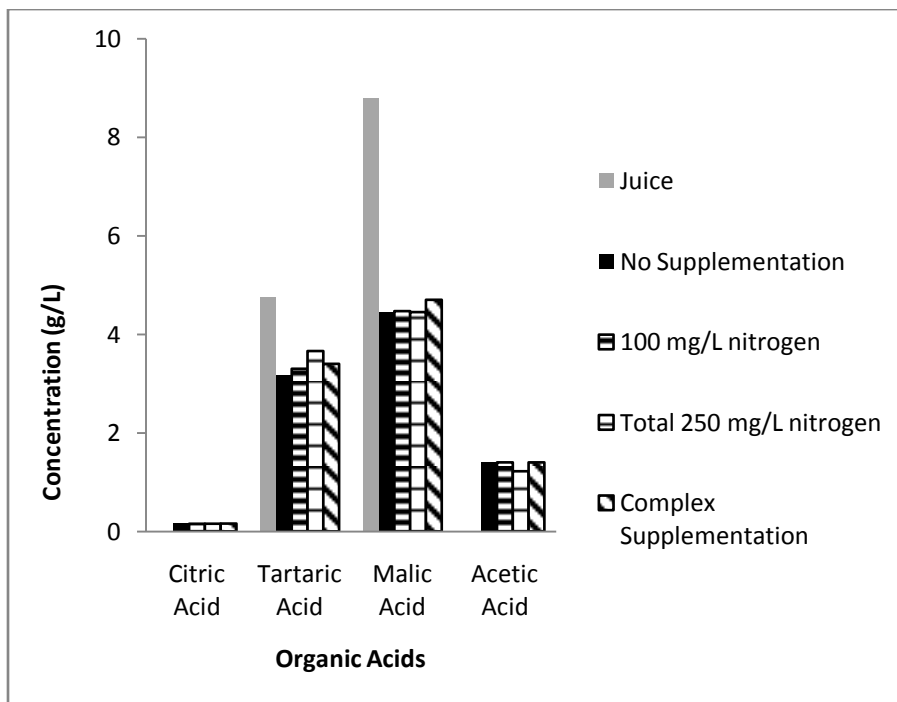


Figure 15. Organic acid content in cold stored juice and wines

Lactic acid was not detected in the juice and the wine. Acetic acid was not detected in the juice but was present in the wines.

Discussion

Nitrogen: The ambient stored juice had a smaller initial nitrogen concentration than the cold stored juice. Both juices had more primary amino nitrogen than ammonia. The wine made with complex supplementation had the largest percent decrease for both types of juice. The wine made with a total of 250 mg/L nitrogen had the lowest percent decrease for both types of juice.

Ammonia nitrogen is consumed first by the yeast during fermentation. The high amount of ammonia nitrogen in the wine made with a total of 250 mg/L in the ambient stored juice indicates that there was extra DAP added. The target level of 250 mg/L nitrogen is therefore too high. On the other hand, the level of ammonia nitrogen in the wines made from complex supplementation for both types of juices is on the low side. The extra supplements added during complex supplementation caused yeast to reproduce at a faster rate. This increase in reproduction consumes more nitrogen than the other types of supplementation methods. It would have been beneficial to increase the amount of DAP added in the complex supplementation method by a small amount to compensate for this.

DAP is a common supplement used by many winemakers. DAP reduces the stress of yeast, which when stressed produce H_2S . H_2S gives wine an unpleasant taste and odor. In addition, DAP affects the expression of the yeast's genes. These genes included orphan genes, as well as genes for amino acid metabolism, small molecule transporters, sulfur assimilation, cell stress, nucleotide metabolism, and protein degradation. Clearly, the effects of DAP on the yeast

are quite complicated, with many of the effects being transcriptional effects on the genes.

Information learned about how exactly DAP causes transcriptional profiling to affect the yeast can help winemakers produce better quality wine. (Marks, van de Merww, & van Vuuren, 2003)

Sugars: The fastest part of fermentation occurred between day three and day ten. After that, the fermentations slowed down. All the fermentations were stopped at the end of thirty days. None of the fermentations had reached completion at this point. The wines made from ambient stored juice and having either a total of 250 mg/L nitrogen supplementation or the complex supplementation were the closest to completion.

The wines made from the cold stored juice had a more consistent rate of fermentation than the wines made from the ambient stored juice. For the wines made with both the ambient and cold stored juice, the control and the complex supplementation method had a slower start to fermentation. Those two supplementation types were more prone to lagging. Interestingly, the control wine was more prone to lagging when made with ambient stored juice and the wine made with complex supplementation was more prone to lagging when made with cold stored juice. This shows that the type of juice and the type of supplementation did affect the sugar levels during fermentation. The type of supplementation played a bigger role in the wines made from ambient stored juice.

Go-Ferm helps the yeast during fermentation. The amount of Go-Ferm added in this experiment followed the manufacturer's recommendation. Research by Threlfall and Morris suggests that adding Go-Ferm in amounts greater than the manufacturer's recommendation for yeast rehydration increases the fermentation rate noticeably. Their research also showed that factors like low pH or low temperatures that can hinder fermentation were overcome by using

Go-Ferm (Threlfall & Morris, 2009). However, the wine made with complex supplementation was still prone to stuck or lagging fermentations. It is possible that part of the reason that complex supplementation did not show better results was that there was not enough Go-Ferm added to the yeast rehydration. The pH and temperature of the fermentations were within the ideal conditions, so that would not be a cause of stuck fermentations. There could also be other factors affecting the fermentations that Go-Ferm could not prevent.

One factor that was never analyzed was whether glucose and fructose were used equally by the yeast, or if the yeast preferred one type over the other. Other research by Berthels et al. has indicated that some yeast strains prefer one type of sugar. They found that *Saccharomyces cerevisiae* yeast strains tend to prefer glucose to fructose, which causes fructose fermentation to be slower. There also will be more fructose than glucose left over at the end of fermentation. Some strains had a greater glucose preference than others. This is a cause of slow or stuck fermentations since fructose has a slower fermentation rate (Berthels, Cordero Orero, Bauer, Thevelein, & Pretorius, 2004).

Ethanol is known to inhibit yeast. In addition, ethanol affects the utilization of glucose and fructose differently. Lower ethanol concentrations inhibited glucose utilization more and higher ethanol concentrations inhibited fructose utilization more. Similarly, nitrogen supplementation affects glucose and fructose utilization differently. Berthels, et al. found that nitrogen supplementation increased the utilization of fructose more than the utilization of glucose (Berthels, Cordero Orero, Bauer, Thevelein, & Pretorius, 2004).

Future research is needed to see if the difference in utilization in fructose and glucose played a role in the fermentations of the wine. This could possibly explain some of the sluggish fermentations in the second half of the fermentation.

pH: There were no statistical differences in pH between both the type of supplementation and the type of juice storage in the wines. There were also no statistical differences between the pH in the juice and the wine. Therefore, the pH was relatively stable throughout fermentation. The wines had an average pH of 3.05 ± 0.09 . The pH levels in the juice and the wine were slightly on the low side for red wines. These lower pH levels could affect the taste of the wines.

Titrateable Acidity: There were no statistical differences in titrateable acidity between the wines made with different supplementation. Only the type of juice influenced the concentration of TA. The wines made from the ambient stored juice had an average TA of 9.0 ± 0.3 . The wines made from the cold stored juice had an average TA of 11.2 ± 0.2 .

The titrateable acidity is on the high side, especially for the wines made from cold stored juice. The cold stored juice had a higher starting concentration than the ambient stored juice. The wines made with cold stored juice had a 33% decrease in TA, while the wines made from ambient stored juice had only a 29% decrease. Since the wine made from both the cold stored and ambient stored juice had the same pH but different TA, the distribution of acid types of the two types of wine must be different. The lower TA of the ambient stored juice wine would mean that there are fewer acids in the wine, but the acids are stronger than the ones in the cold stored juice wines.

Volatile Acidity: Both the type of juice storage and the method of supplementation affected the acetic acid levels in the wine. When the wines were made from ambient stored juice, the wine made using 100 mg/L nitrogen had a volatile acidity level that was statistically different from the

wine made with a total of 250 mg/L nitrogen and the wine made with complex supplementation. When the wines were made from cold stored juice, the wine made with a total of 250 mg/L nitrogen was statistically different from the wine made without supplementation and the wine made with complex supplementation. The levels of acetic acid in all the wines were in the acceptable range.

The ambient juice had a higher starting concentration of acetic acid. However, the wine made from cold stored juice had a higher concentration of acetic acid. The wines made from ambient stored juice had an average percent increase in volatile acidity of 14% and the wines made from cold stored juice had an average percent increase of 152%. Clearly, there was something additional going on in the fermentations in the cold stored juice that was increasing the concentration of acetic acid.

In this experiment, the DAP was added at the beginning of fermentation. Research by Barbosa, Falco, Mendes-Faia, and Mendes-Ferreira showed that adding the DAP during the stationary phase of fermentation had better results than adding it at the beginning. The amounts of ethanol and volatile acidity decreased when nitrogen was added during the stationary phase as compared to when nitrogen was added at the beginning of fermentation in some strains of *Saccharomyces cerevisiae* yeast (Barbosa, Falco, Mendes-Faia, & Mendes-Ferreira, 2009). This raises the question as to why there was not a bigger difference in the wines made with complex supplementation. In complex supplementation, Fermaid-K was added at one-third sugar depletion. Fermaid-K contains nitrogen, but it is a different type of yeast supplement than DAP. It is still interesting to note that in this experiment, adding a source of nitrogen at one-third sugar depletion did not cause significantly different results in fermentation. It did cause a slight

difference in acetic acid concentration when compared to certain wine types, a difference which is discussed later.

Sulfur Dioxide: The concentration of SO₂ in the juice was measured before the juice was diluted for fermentation. Therefore, the starting SO₂ amounts in the juice at the beginning of fermentation were lower. These are still on the high side, especially for free SO₂, which may partially explain the sluggish fermentations. For both wines, the total SO₂ was within the legal limits.

The wines made from ambient stored juice differed in the amount of free SO₂ and total SO₂ according to supplementation type. The wine made with 100 mg/L nitrogen and the wine made with a total of 250 mg/L nitrogen had different concentrations of both free SO₂ and total SO₂. The wine made with no supplementation and the wine made with a total of 250 mg/L had a different concentration of free SO₂. In the wines made from cold stored juice, the type of supplementation did not affect the concentration of free and total SO₂.

The free SO₂ was higher in the cold stored juice than the ambient stored juice. However, the ambient stored juice had a higher concentration of total SO₂. The wines made from both ambient and cold stored juice had the same average percent decrease in free SO₂ of 84%, compared to the starting amount in the juice (not diluted). The wines made from ambient stored juice had an average decrease of 41% in total SO₂, while the wines made from cold stored juice had only a 27% average decrease in total SO₂.

Sensory: Table 7 (page 25) shows that the first three wines, the wine made from cold stored juice with 100 mg/L nitrogen, the wine made from cold stored juice with a total of 250 mg/L

nitrogen and wine made from the ambient juice with a total of 250 mg/L nitrogen were statistically different in panelist's preference from the wines made from the ambient stored juice without any supplementation and the wine made from ambient stored juice with complex nutrition. There were no other statistically significant differences based on panelist's preference.

These results show a couple of different things. First, either nitrogen supplementation method seems to be preferred over no supplementation or complex nutrition. Even though complex nutrition adds other nutrients, the fermentation was not improved when compared to a straight addition of DAP. There were no statistical differences based on panelist's preference between the wines made from cold stored juice. Further sensory tests would be needed to tell if there are slight differences in panelist's preference for those wines. However, the implication is that a cold storage method produces the more consistent wine. Supplementation would be much more important in wines made from ambient stored juice.

Phenolics: There were some statistical differences in the concentrations of phenols in the different types of wine. For gallic acid, there were no statistical differences between the different wine types. The average percent decrease in all the wines compared to the starting amount in the juice was 1%.

The type of supplementation did not affect the gallic acid concentration in the wines made from ambient stored juice. For wines made from cold stored juice, the concentration of gallic acid was dependent on the type of supplementation used. In the wines made from ambient stored juice, the percent change ranged from -1% to 5%. In the wines made from cold stored juice, all the wines showed an increase in the concentration of gallic acid. The concentration of gallic acid in wines made from complex supplementation with cold stored juice was statistically

lower than the other three types of wine. This indicates that the additional supplements added during the complex supplementation were causing less gallic acid to form during fermentation.

For chlorogenic acid in wines made from ambient stored juice, only the wine made with a total of 250 mg/L nitrogen and wine made with complex supplementation were statistically the same. All the other wines were statistically different. The wines had an average percent decrease of 44% compared to the starting amount in the juice. For caffeic acid in wines made from ambient stored juice, the only statistical difference was between the wine made without supplementation and wine made with complex supplementation. The concentration of caffeic acid decreased by an average of 33% during fermentation. No wine supplementation type produced detectable amounts of coumaric acid.

Gallic acid levels in the wines made from cold stored juice generally showed statistical differences. The wine made without supplementation and the wine made with a total of 250 mg/L nitrogen supplementation were statistically the same; and the wine made with 100 mg/L nitrogen and the wine made with a total of 250 mg/L nitrogen were statistically the same. All the other wines were statistically different. The wine made with 100 mg/L nitrogen and the wine made with a total of 250 mg/L nitrogen had the same concentration for chlorogenic acid as well. The only other two wines statistically the same for chlorogenic acid were wines made without supplementation and wines made with complex supplementation. For caffeic acid, the only statistical difference in concentration was between the wine made from a total of 250 mg/L nitrogen and the wine made from complex supplementation. No wine supplementation type produced a detectable amount of coumaric acid. Compared to the starting concentration in the juice, there was an average 281% increase in gallic acid, an average 50% decrease in chlorogenic acid, and an average 42% decrease in caffeic acid.

When the gallic acid concentrations of wines made from ambient stored juice were compared to the juice, only the wine with no supplementation had a different concentration from the juice. The concentration was lower in the wine without supplementation. This could indicate that there is some reaction going on during the fermentation that is consuming gallic acid when there was no supplementation added. Compared to the cold stored juice, all the wines had a different concentration.

The concentration of chlorogenic acid was dependent on the type of supplementation used for the wines made from ambient and cold stored juice. The variability in the concentration was consistent between wines made from cold and ambient stored wine. The percent decreases ranged from 39% to 47% in the wines made from ambient stored juice and from 47% to 54% in the wines made from cold stored juice. The concentration of chlorogenic acid of all the wines was lower than the starting concentration in the juice.

The concentration of caffeic acid was only slightly dependent on the type of supplementation for both the ambient and cold stored juice. The majority of the wine types had the same concentration. For both the ambient and cold stored juice, there was only one pair of wine types that was not statistically the same: the wine made by complex supplementation and one of the other types of wine. The range in percent decreases was small in wines made from both ambient and cold stored juice. In wines made from ambient stored juice, the percent decrease ranged from 31% to 35% and in the wines made from cold stored juice, the percent decrease ranged from 41% to 43%. In all the wines, the concentration of caffeic acid was lower than the starting concentration in the juice.

The starting concentrations of phenols in the ambient stored juice were higher than in the cold stored juice for all the phenols. Therefore, it makes sense that the concentrations of the

phenols in the wines were higher in the wines made from ambient stored juice than for the cold stored juice. Figure 16 compares the percent difference between the cold and ambient stored juice and wine. Gallic acid concentration was much higher in the starting juice than in the wines. Chlorogenic acid and caffeic acid concentrations were only slightly higher in the wines than in the juice.

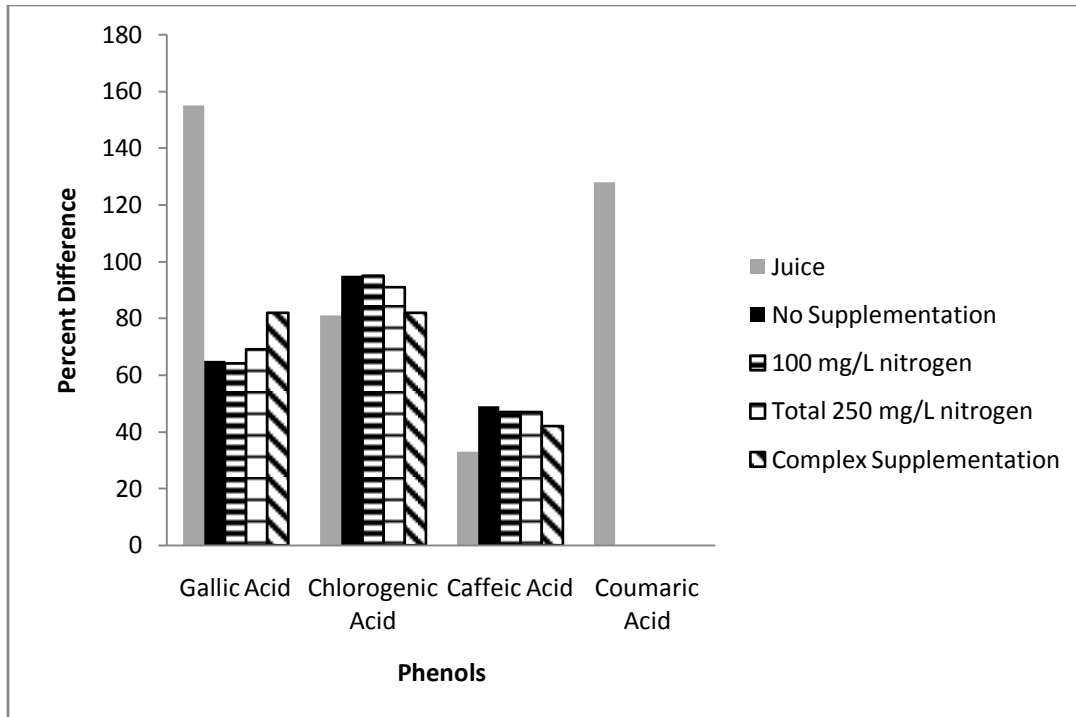


Figure 16. Comparisons of phenols between ambient and cold stored juice and wine

The type of supplementation and the type of juice used did affect the concentrations of phenols. Chlorogenic acid had the biggest difference in concentration in the different wine supplementations. Gallic acid had the biggest difference in concentration between the two juice types.

Organic Acids: Citric, tartaric, and malic acids concentrations were statistically the same for all the wine types made from ambient stored juice. The concentration of citric acid had the lowest average percent decrease, 38%, when compared to the starting concentration in the juice. The concentration of tartaric acid had the largest average percent decrease, 48%. The concentration of malic acid had an average percent decrease, 46%.

The concentrations of acetic acid in ambient stored juice, the wine made with no supplementation and the wine made with 100 mg/L nitrogen were statistically the same. The only other wine pair that was statistically the same was the wine made with no supplementation and the wine with a total of 250 mg/L nitrogen. The wine made with complex supplementation had the highest amount of acetic acid and the wine made with a total of 250 mg/L nitrogen had the lowest concentration. Of all the organic acids analyzed, only the concentration of acetic acid depended on the type of supplementation in wines made from ambient stored juice.

However, there were some differences when looking at the wine made from cold stored juice. Only malic acid had concentrations that were statistically the same for all the wine supplementations. The wine made with no supplementation and the wine made with a total of 250 mg/L nitrogen had concentrations of citric, tartaric, and acetic acids that were statistically different. There was an additional difference in citric acid concentration between the wine made with no supplementation and wine made with 100 mg/L of nitrogen. The type of supplementation was more important with respect to the concentration of organic acids for the wines made from cold stored juice than the wines made from ambient stored juice.

The starting concentrations of citric, tartaric, and malic acid in the ambient stored juice were all statistically higher than their final concentrations in the wines. Tartaric acid had the greatest decrease in the wines, followed by malic acid. For cold stored juice, concentrations of

tartaric and malic acid in the wines were lower than the concentrations in the juice. Neither acetic nor citric acid were present in the juice, but were present in the wines, indicating fermentation reactions are forming these two compounds.

The starting concentrations of tartaric and malic acids were higher in the cold stored juice than the ambient stored juice. Therefore, it makes sense that the concentration of tartaric and malic acids are higher in the wines made from cold stored juice. However, there was a greater decrease compared to the starting concentrations in the wines made from ambient stored juice.

Neither type of juice had detectable amounts of acetic acid. The wines made with cold stored juice had a higher concentration of acetic acid. Only the ambient juice had detectable concentrations of citric acid. The concentration of citric acid decreased in the wines made from ambient stored juice and increased in the wines made from cold stored juice.

For organic acids, both the type of juice used to make the wines and the type of supplementation used had an effect on the concentrations. Malic acid was the only organic acid that only depended on the type of juice used to make the wine. There was something different going on between the wine made without supplementation and the wine made with a total of 250 mg/L nitrogen for wines made with cold stored juice. This wine pair was the only one that had different concentrations for tartaric and acetic acids and was one of two pairs that had different concentrations for citric acid. There was no wine pair in the wines made from ambient stored juice that had concentration differences for more than one organic acid.

The ratio of malic to tartaric acid was compared. In the wines made from ambient stored juice, the ratio showed no statistical difference due to the supplementation types. The average ratio of all the supplementations was 1.3 ± 0.2 . This ratio was also the same as the starting ratio in the juice, which was 1.25. When the wines were made from ambient stored juice, the wines

made without supplementation and the wines made with 100 mg/L nitrogen had a different malic to tartaric acid ratio. The rest of the supplementations had the same ratio. The average ratio for all the supplements was 1.3 ± 0.1 . The cold stored juice had a ratio of 1.86, which was higher than the ratios in the wines.

The concentration of acetic acid was measured by both use of a Cash still and by HPLC. The concentration determined by the HPLC was lower in the juice and the wines made with a supplementation types. In fact, the percent difference was at least 93% between the two methods. Since the HPLC is a more accurate way to measure the concentration of acetic acid, the assumption that acetic acid was the volatile acid with the greatest concentration was flawed. Formic acid, butyric acid, and propionic acid are other volatile acids that are found in wines. (2) Since these acids were not analyzed in this experiment, their concentration was unknown, but there is a high likelihood that they are present in a significant concentration.

Table 10 shows the amounts of titratable acids using both the automatic titrator and the HPLC. The total amount of titratable acids measured using the automatic titrator was higher in all cases.

Table 10. Comparison of Titratable Acids

	Automatic Titrator (g/L)	HPLC (g/L)
Ambient Stored Juice	12.6	9.498
Wines made from Ambient Stored Juice	9.0 ± 0.3	5.3 ± 0.2
Cold Stored Juice	16.7	13.531
Wines made from Cold Stored Juice	11.2 ± 0.2	8.1 ± 0.1

Tartaric and malic acids usually have the greatest concentrations. Since citric, lactic, malic, and tartaric acids were the only titratable acids analyzed by the HPLC, either the automatic titrator was not accurate in measuring the acid content or there were other titratable acids with significant concentrations. There was also a larger percent difference between the two methods for the ambient stored juice and the wine made from ambient stored juice when compared to the wines made from cold stored juice.

There were no statistical differences between the different supplementation types for total concentration of phenols and the total concentration of organic acids. In the wines made from ambient stored juice, the total concentration of phenols was 6.9 ± 0.1 mg/L and the total concentration of organic acids was 5.3 ± 0.3 g/L. There was a 32% decrease in the phenolic concentration and a 61% decrease in organic acid concentration compared to the starting concentrations in the juice. For wines made from cold stored juice, the total concentration of phenols was 3.78 ± 0.08 mg/L and the total concentration of organic acids was 8.4 ± 0.3 g/L. There was a 61% decrease in total phenolic concentration and a 31% decrease in total organic acid concentration compared to the starting concentrations in the juice.

The total phenolic concentration was higher in the wines made from ambient stored juice. The total organic acid concentration was higher in the wines made from cold stored juice. The wines made from ambient stored juice had a higher percent decrease in total phenolic and total organic acid concentration than the wines made from cold stored juice.

One question that recurred throughout the research was, “Why did all the wines have stuck or sluggish fermentations?” DAP, Fermaid-K, and Go-Ferm were supposed to prevent this in fermentations, but clearly something else was going on. In a study performed by L. Blateyron and J.M. Sablayrolles, it was found that adding DAP and oxygen decreased the incidence of

stuck or sluggish fermentation. They noted that while DAP did help increase the rate of fermentation, oxygen helped the fermentation at the end by reducing the toxic effect of ethanol on the yeast. (Blateyron & Sablayrolles, 2001) This may help explain why some of the wines with DAP in this experiment still experienced stuck or sluggish fermentation as they got closer to completion.

Oxygen deficiency has been known to cause stuck or sluggish fermentations. Lipid biosynthesis is inhibited when there is a lack of oxygen. Lower levels of production of sterols and fatty acids decrease the yeast's ability to tolerate high levels of ethanol. Aeration of the must (juice recently pressed that still contains the solid particles) is often performed by winemakers to increase the amount of oxygen before fermentation (Alexandre & Charpenter, 1998)

The timing of the additions of DAP and oxygen also appear to be important. In another study by J.M. Sablayrolles et. al, it was found that adding nitrogen before oxygenation did not affect the rate of fermentation, but when nitrogen was added at the same time as oxygenation or after oxygenation, the rate of fermentation increased. However, further research would need to be performed to see if adding oxygen would improve the rate of fermentation. The research done on oxygen and nitrogen supplementation was performed on other types of grape varieties so there is some uncertainty as to what the results would be if Concord grapes were used instead (Sablayrolles et. al., 1996).

Conclusion

We have found that differences in the mode of storage of Concord grape juice prior to fermentation, as well as differences in the various forms of nitrogen supplementation used during fermentation, produce only small differences in the chemical composition of wine. Statistically

significant differences were detected for residual nitrogen after fermentation, volatile acidity, free and bound SO₂, phenolics, and organic acids for both the type of juice storage and the type of nitrogen supplementation during fermentation. The type of supplementation played a bigger role in the organic acids present in wines made from cold stored juice. There were no differences detected in pH levels and only the type of juice storage affected the level of TA. The results of sensory evaluation showed slight differences in panelists' preferences. However, even though differences were detected, no one type of wine appeared to be significantly better than the others.

This study brought up some possible areas of research for further study. The wines produced in this study were not aged and aging is known to change both the color and the taste of the wine. In addition, possible differences in the metabolic pathways of yeast as nitrogen supplementation is changed arose as a possible explanation for differences in phenolic and organic acids. To our knowledge, this factor has not been evaluated. Finally, additional sensory tests should be done to refine our understanding of the panelists' preferences for the difference wines.

Acknowledgements

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Department at the College at Brockport, specifically Dr. Stephen Godleski, for his help in writing this thesis. And finally, I'd like to thank the Western NY IFT for funding provided to the NYS Agricultural Experiment Station to support this project.

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Appendix A – Phenolics HPLC Chromatograms

Table 8. Phenolics Standards

Phenol	Maximum Wavelength (nm)	Average Retention Time (min)
Gallic Acid	280	10.688
Catechin	280	17.769
Chlorogenic Acid	320	18.131
Caffeic Acid	320	20.730
Epicatechin	280	21.403
Coumaric Acid	320	26.978
Ferulic Acid	320	29.362

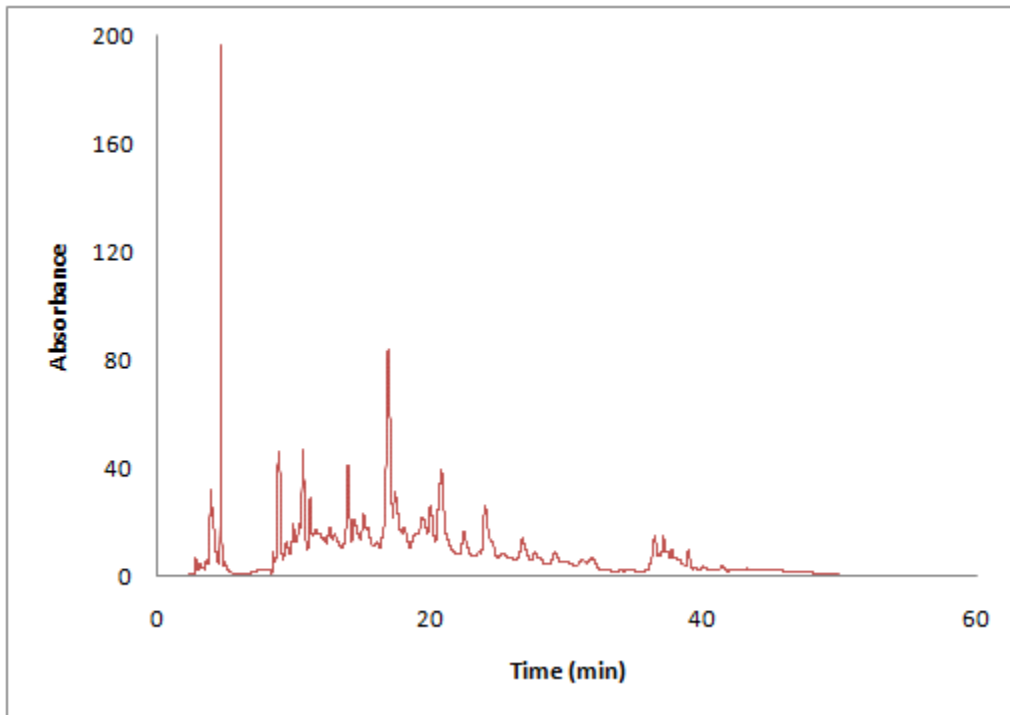


Figure 17. Ambient Stored Juice at 280 nm

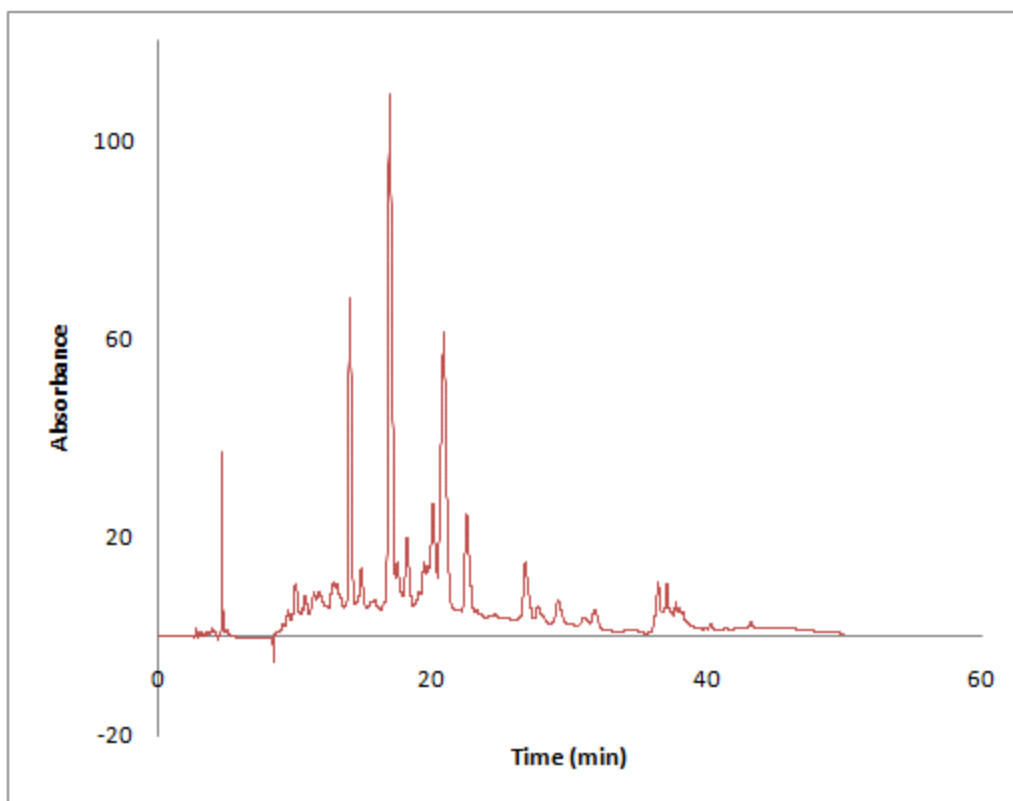


Figure 18. Ambient Stored Juice at 320 nm

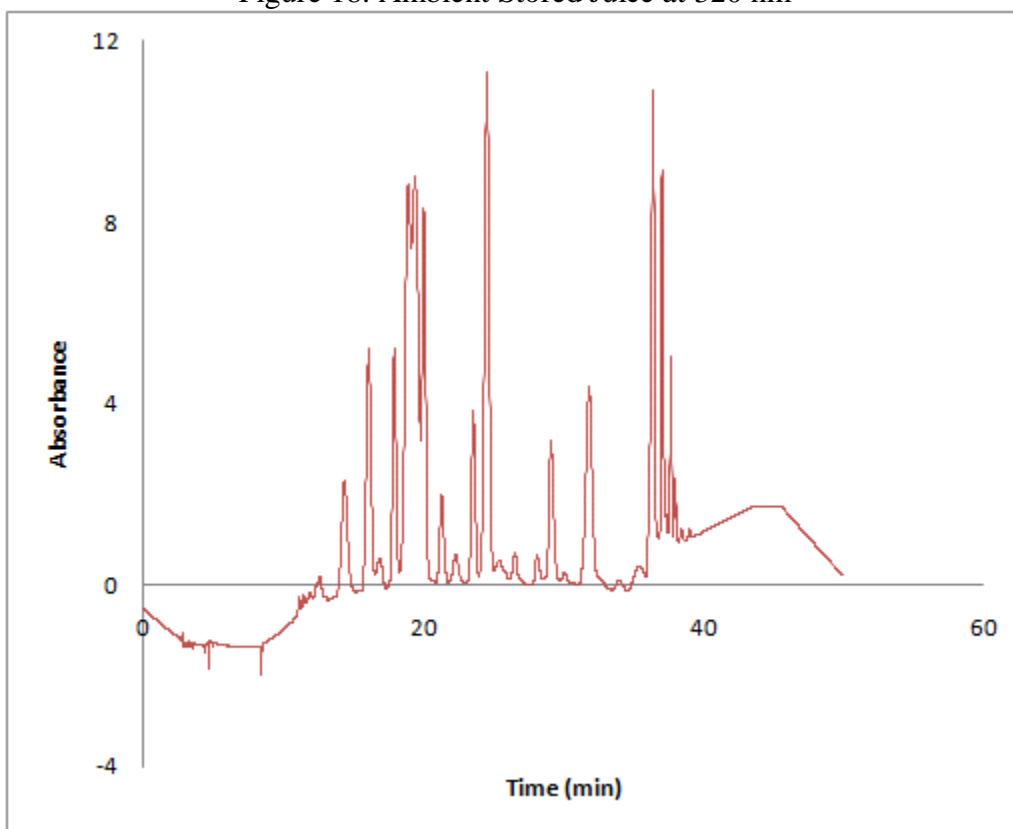


Figure 19. Ambient Stored Juice at 520 nm

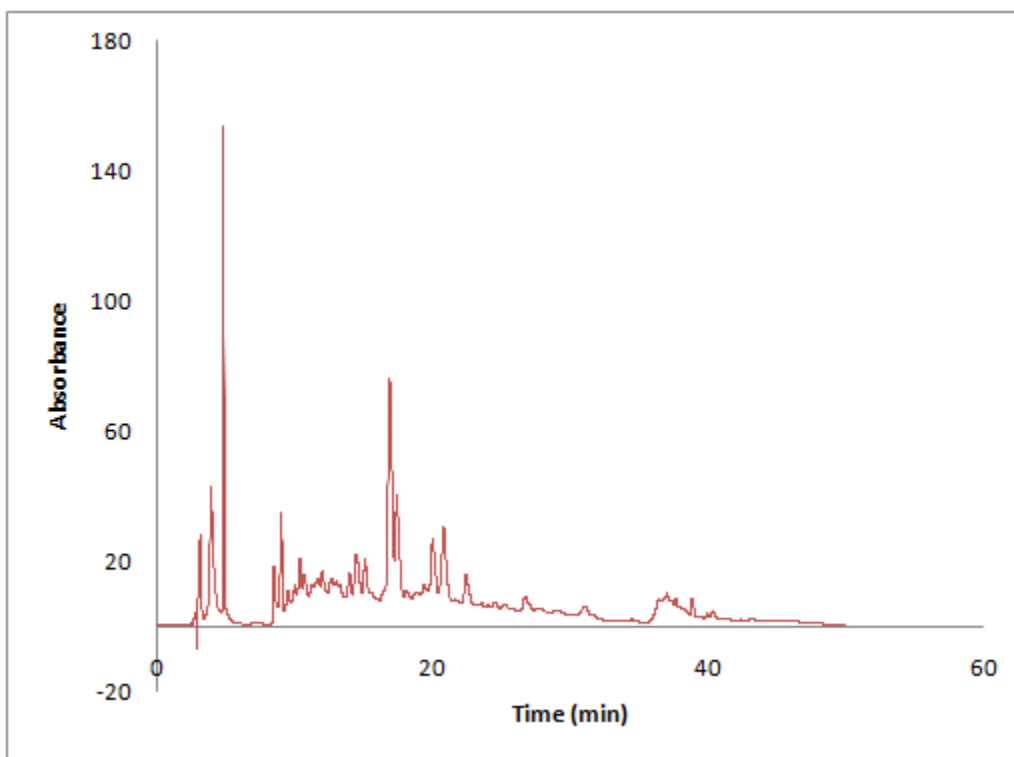


Figure 20. Cold Stored Juice at 280 nm

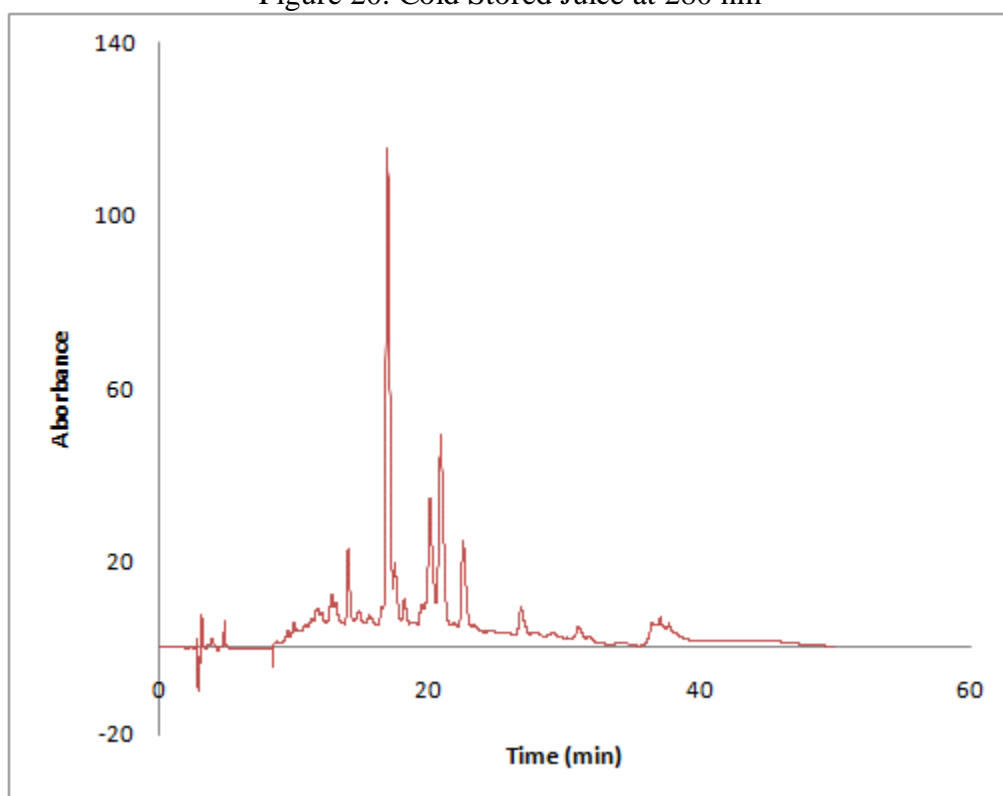


Figure 21. Cold Stored Juice at 320 nm

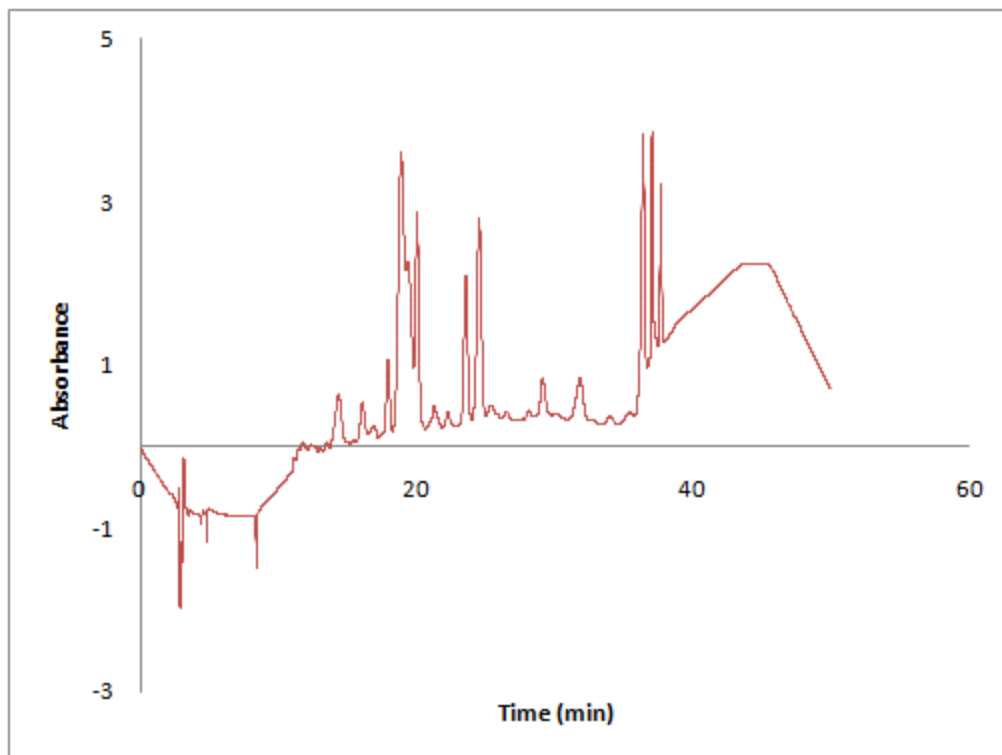


Figure 22. Cold Stored Juice at 520 nm

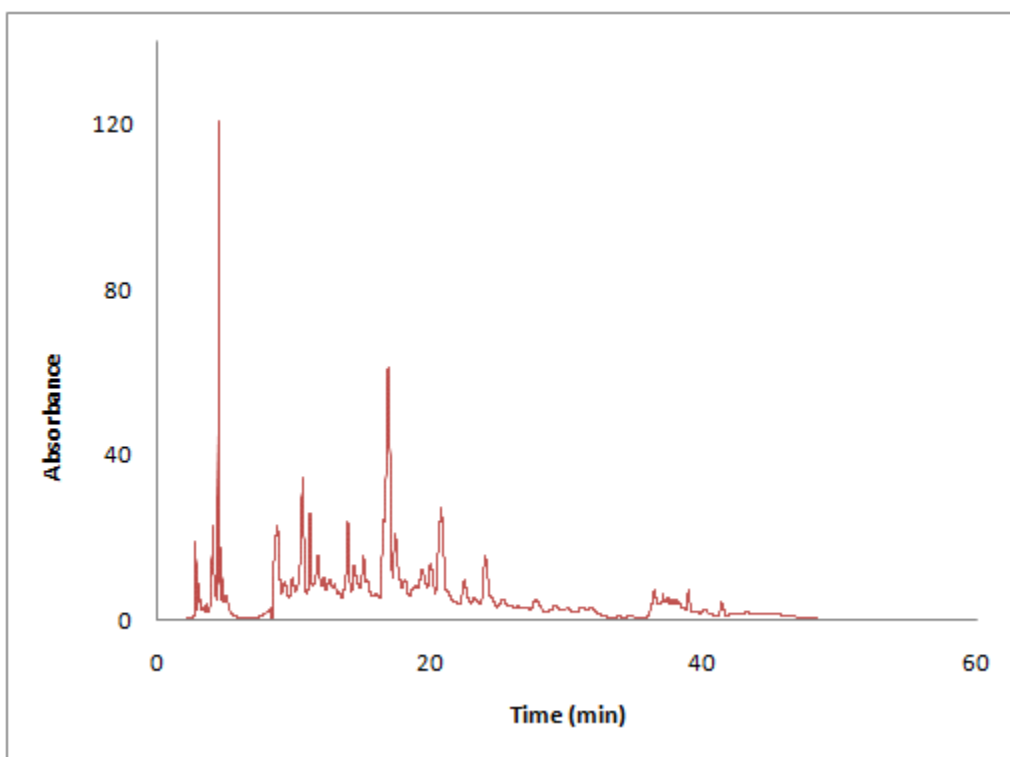


Figure 23. Wine with No Supplementation Made with Ambient Stored Juice at 280 nm

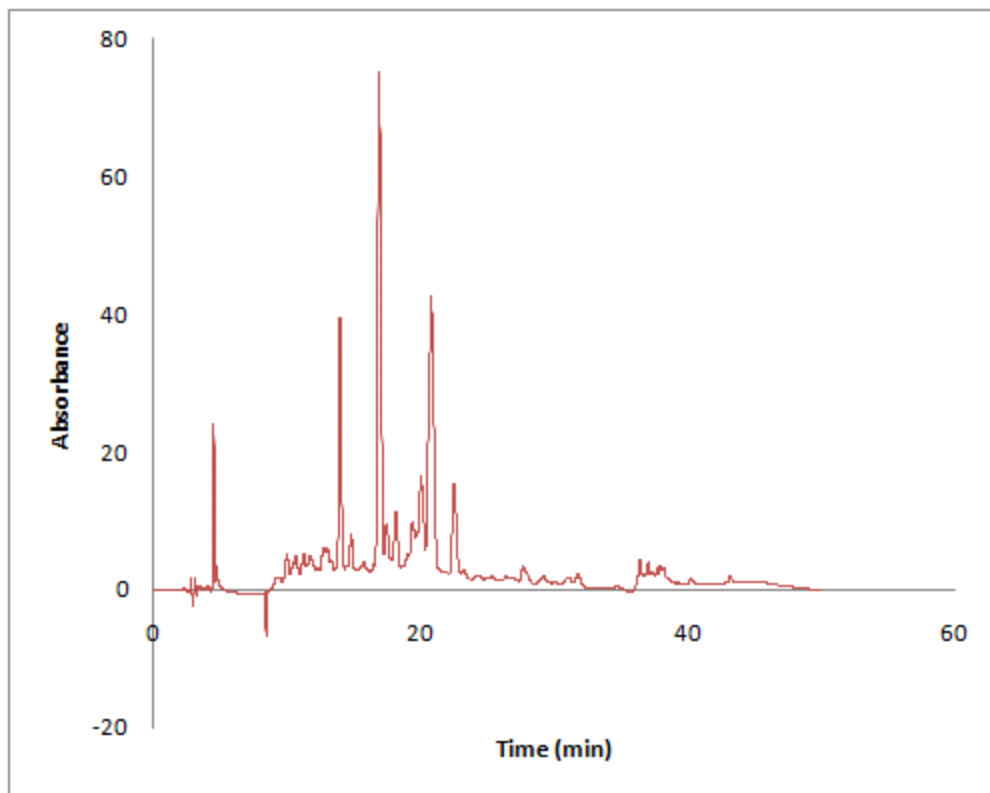


Figure 24. Wine with No Supplementation Made with Ambient Stored Juice at 320 nm

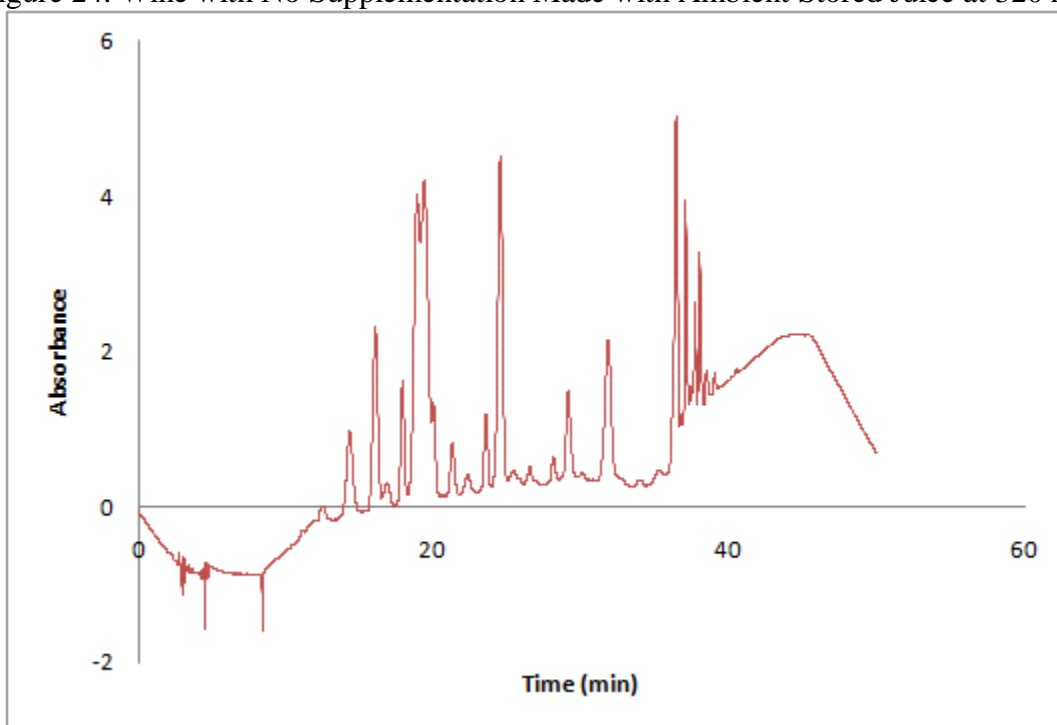


Figure 25. Wine with No Supplementation Made with Ambient Stored Juice at 520 nm (baseline corrected +0.5)

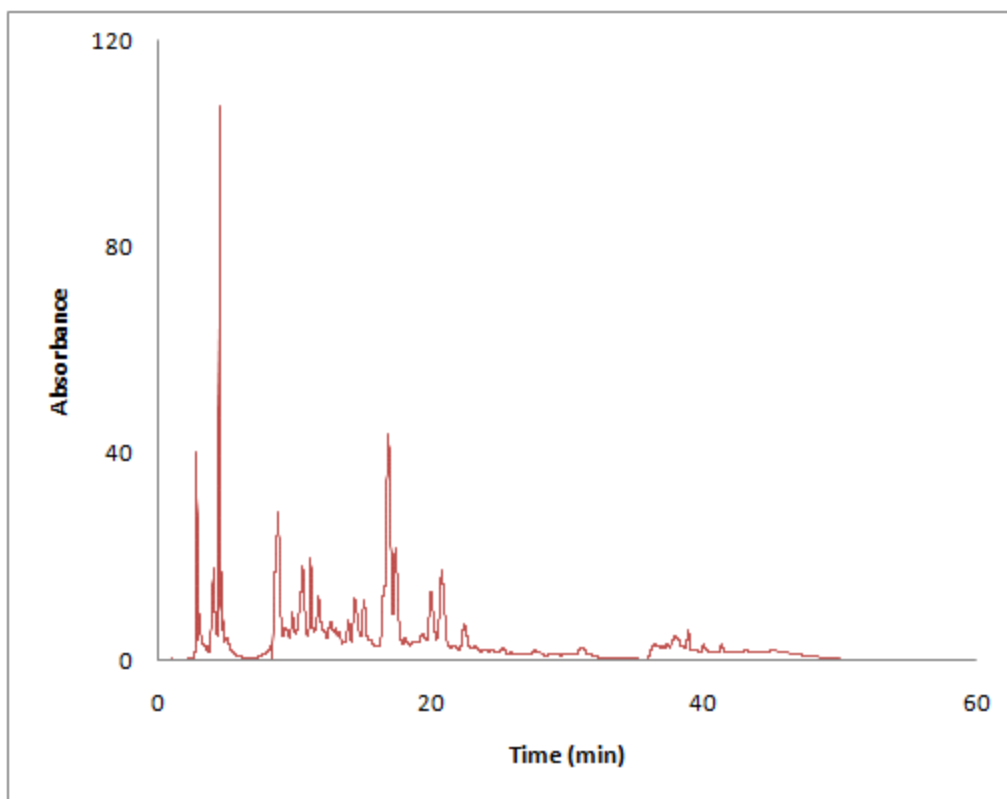


Figure 26. Wine with No Supplementation Made with Cold Stored Juice at 280 nm

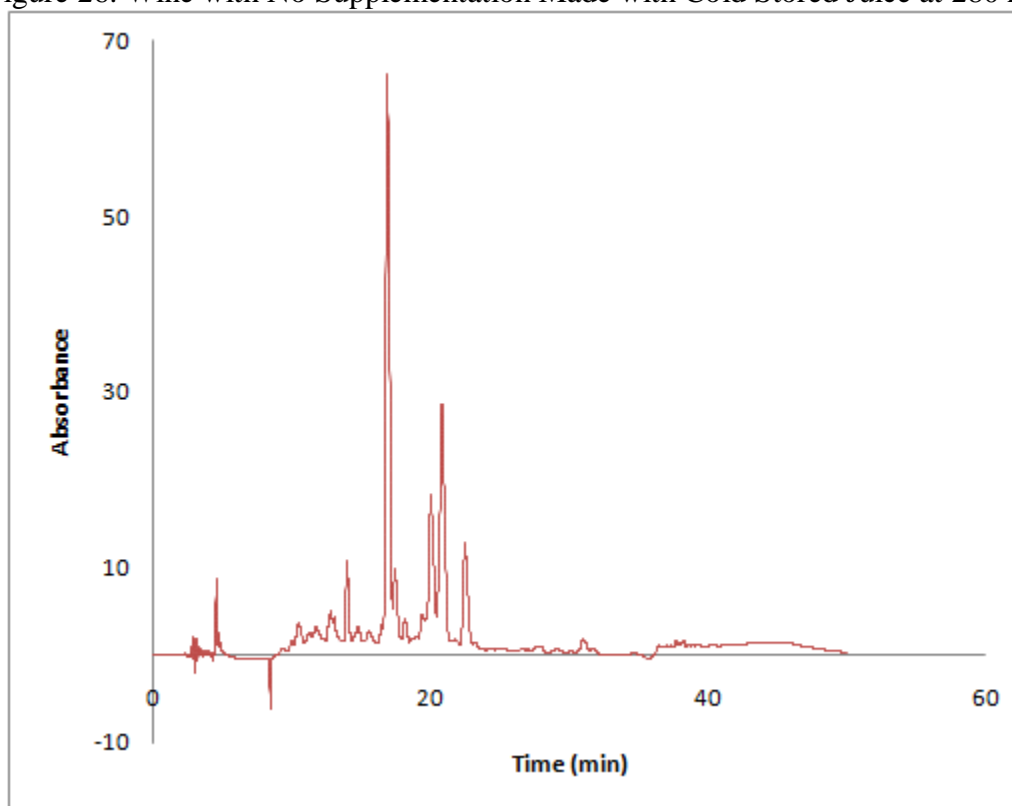


Figure 27. Wine with No Supplementation Made with Cold Stored Juice at 320 nm

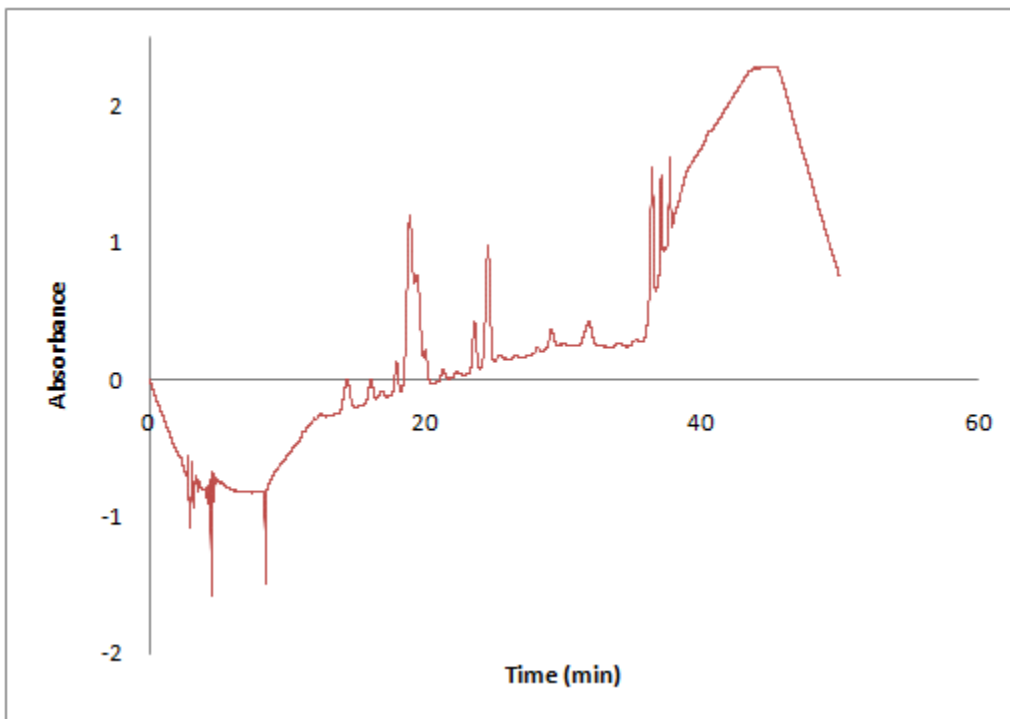


Figure 28. Wine with No Supplementation Made with Cold Stored Juice at 520 nm (baseline corrected + 0.55)

Appendix B – Phenolics Standard Curves

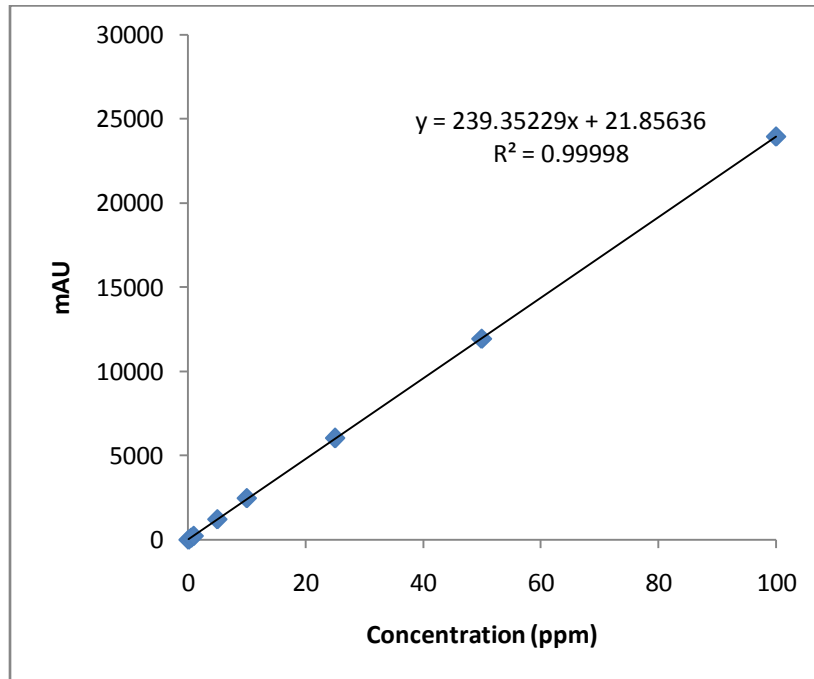


Figure 29. Caffeic Acid Standard Curve

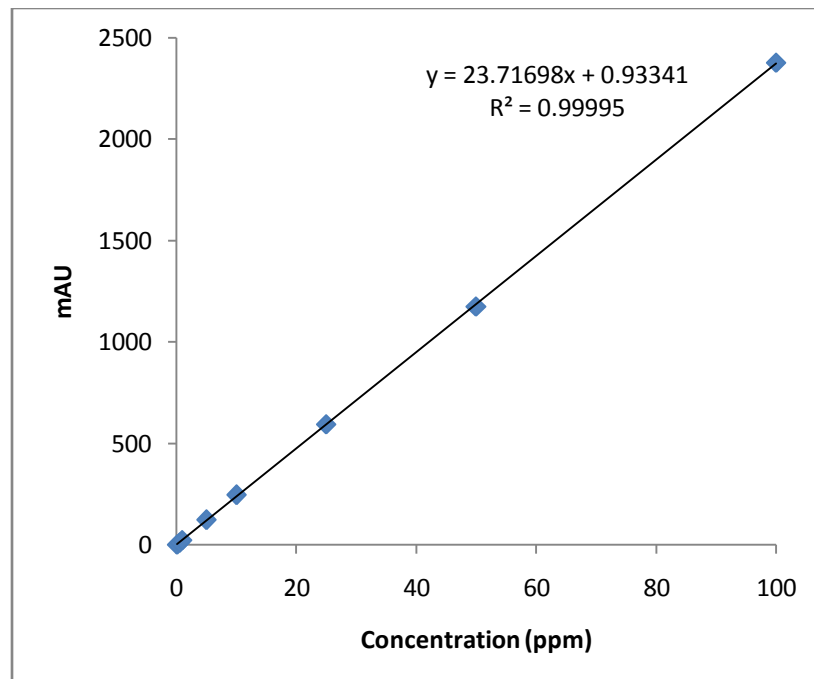


Figure 30. Catechin Standard Curve

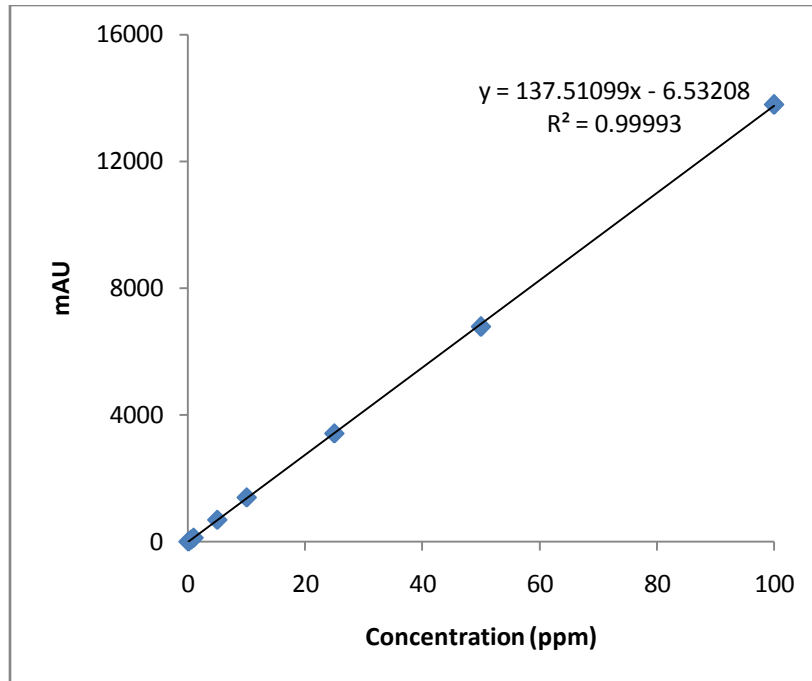


Figure 31. Chlorogenic Standard Curve

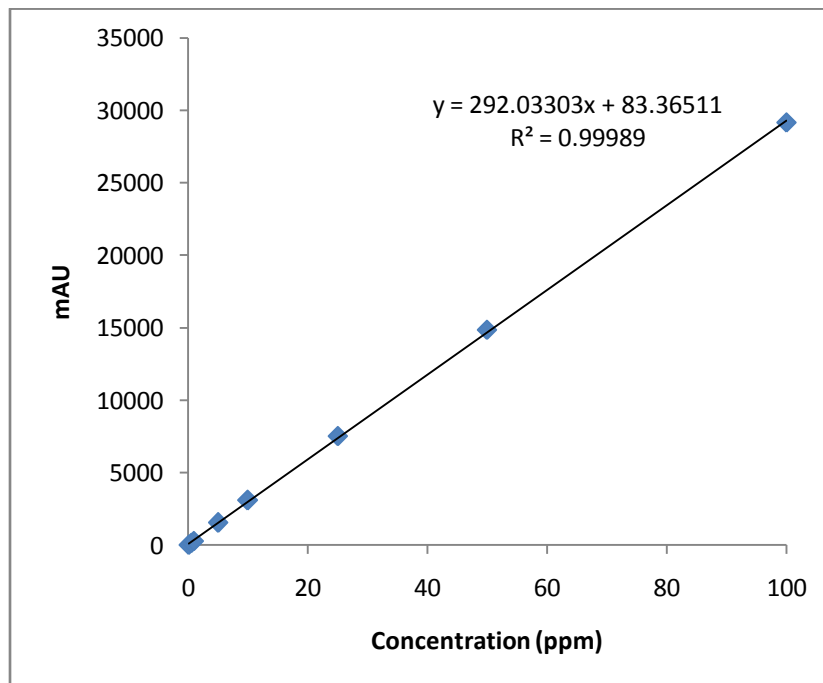


Figure 32. Coumaric Acid Standard Curve

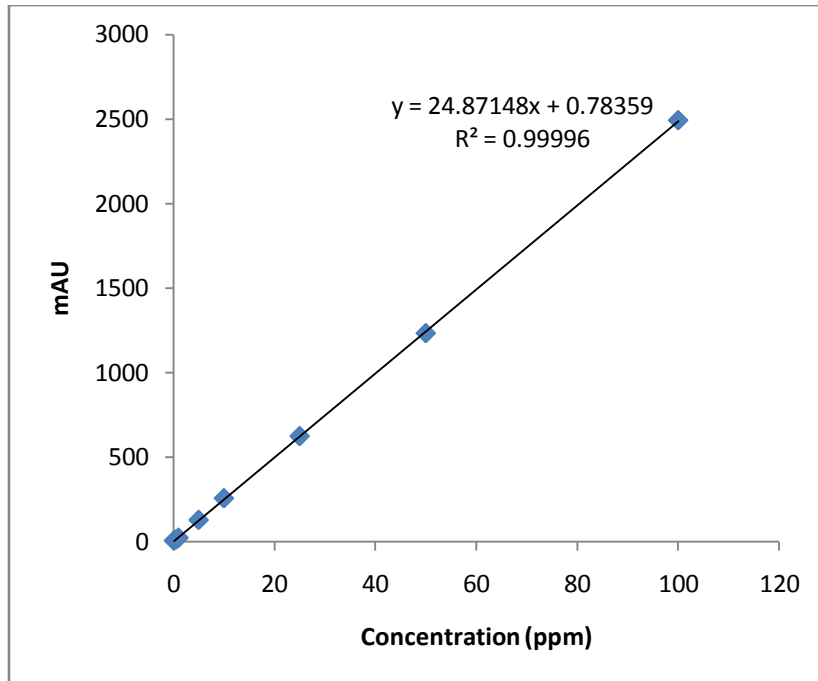


Figure 33. Epicatechin Standard Curve

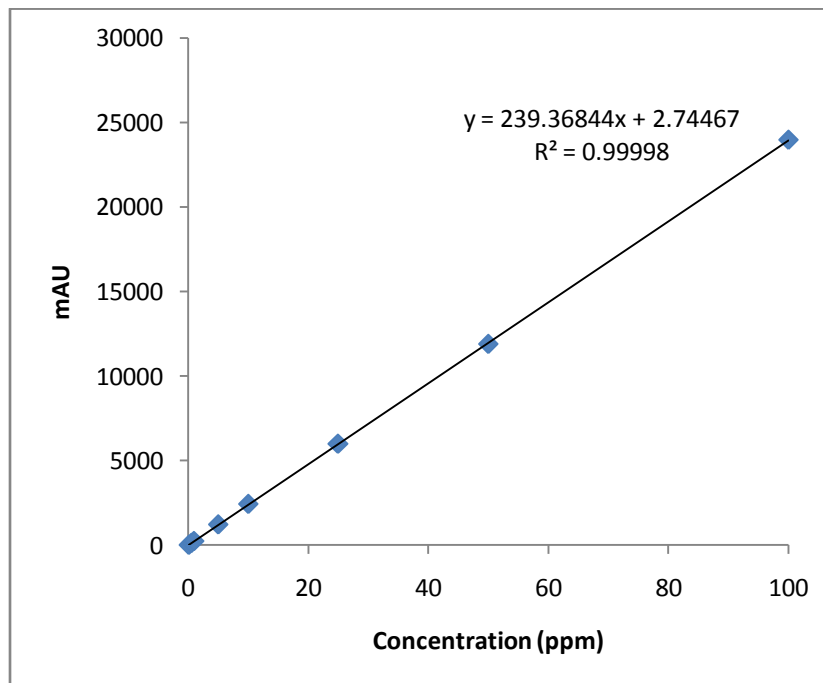


Figure 34. Ferulic Acid Standard Curve

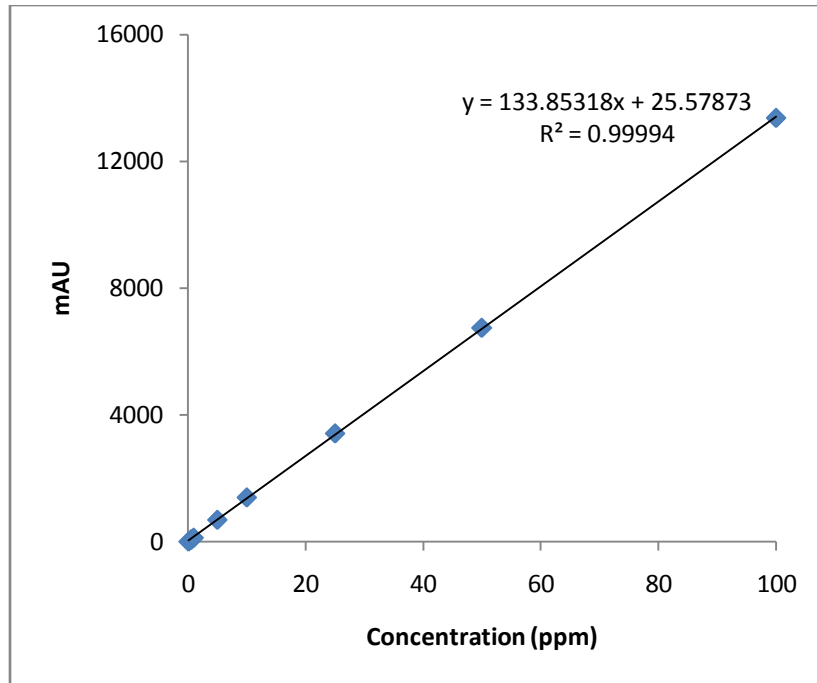


Figure 35 Gallic Acid Standard Curve

Appendix C – Organic Acid HPLC Chromatograms

Table 9. Organic Acid Retention Times

Organic Acid	Average Retention Time (min)
Citric Acid	9.427
Tartaric Acid	10.238
Malic Acid	11.101
Lactic Acid	14.451
Acetic Acid	17.057

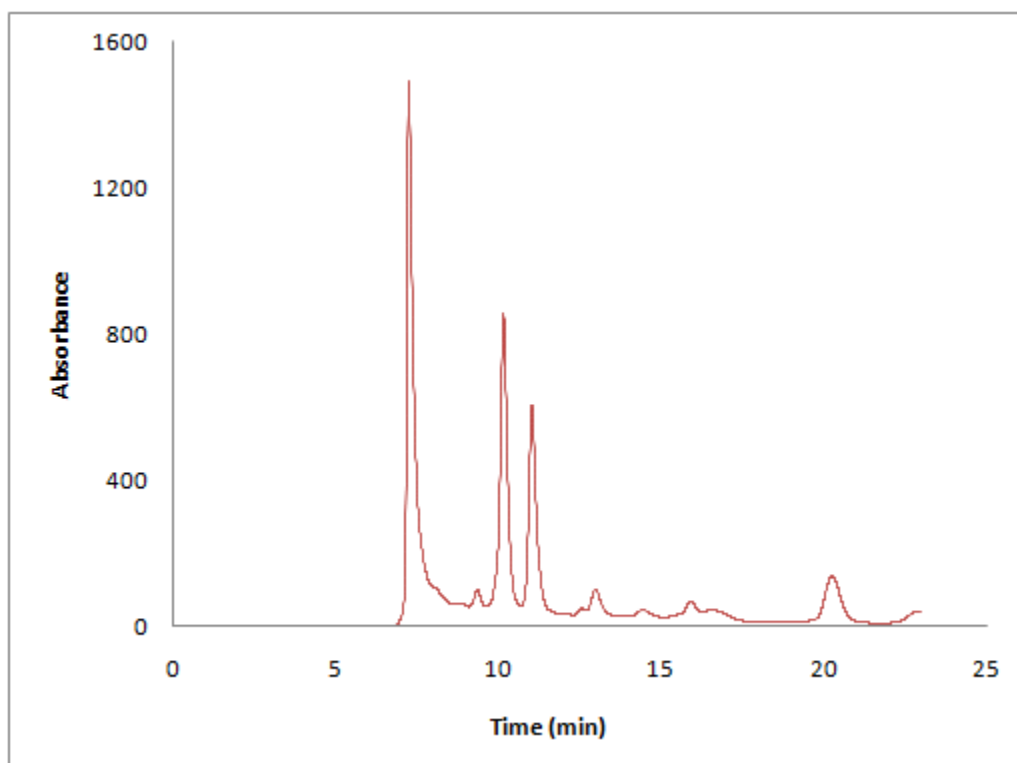


Figure 36. Ambient Juice HPLC Chromatogram

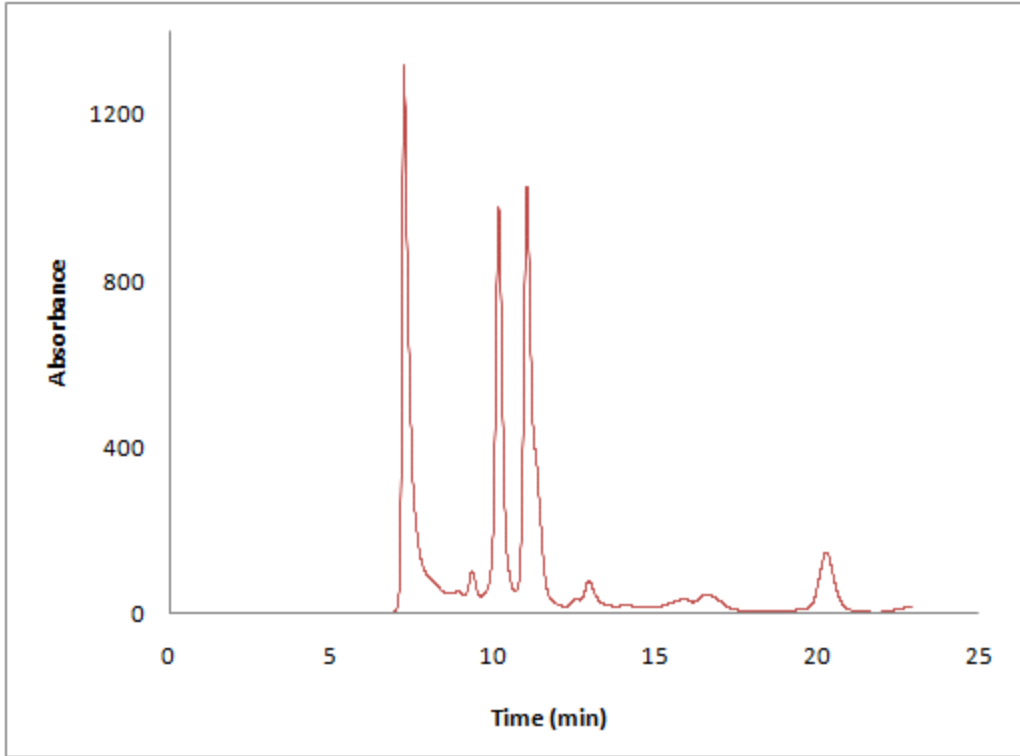


Figure 37. Cold Juice HPLC Chromatogram

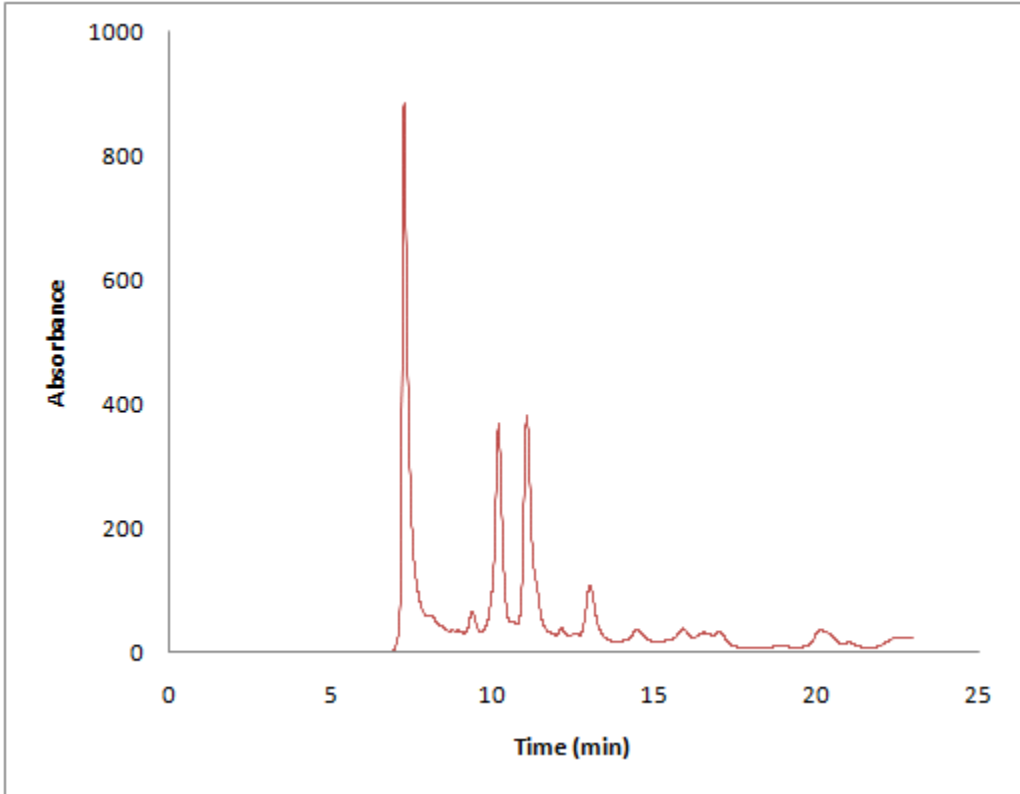


Figure 38. Wine with No Supplementation Made with Ambient Stored Juice

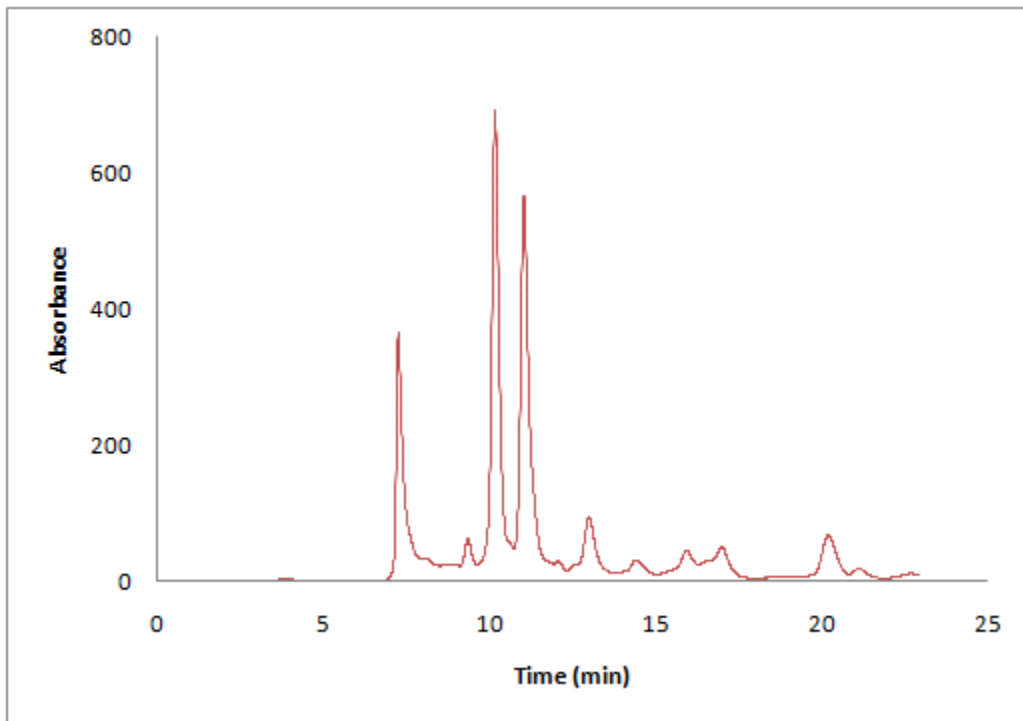


Figure 39. Wine with No Supplementation Made with Cold Stored

Appendix D – Organic Acid Standard Curves

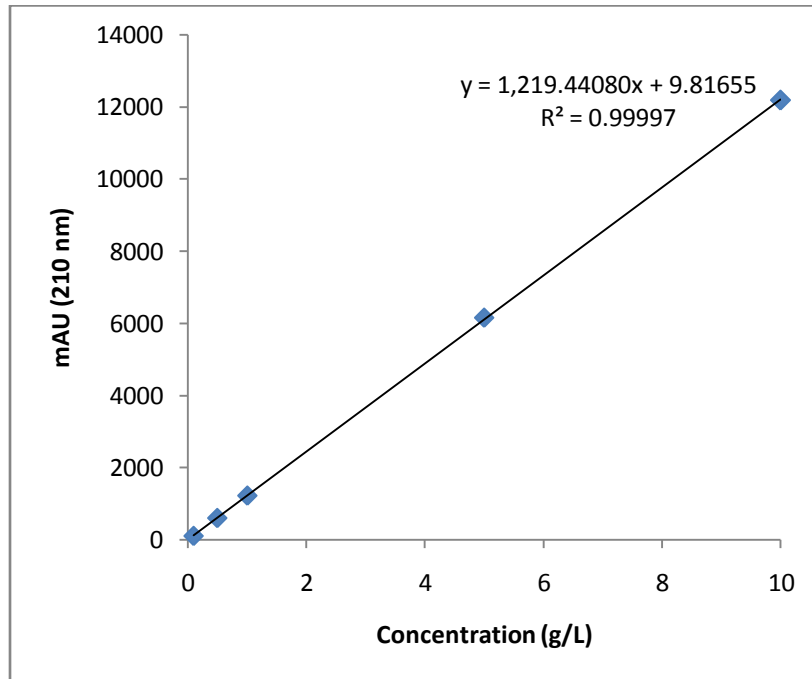


Figure 40. Acetic Acid Standard Curve

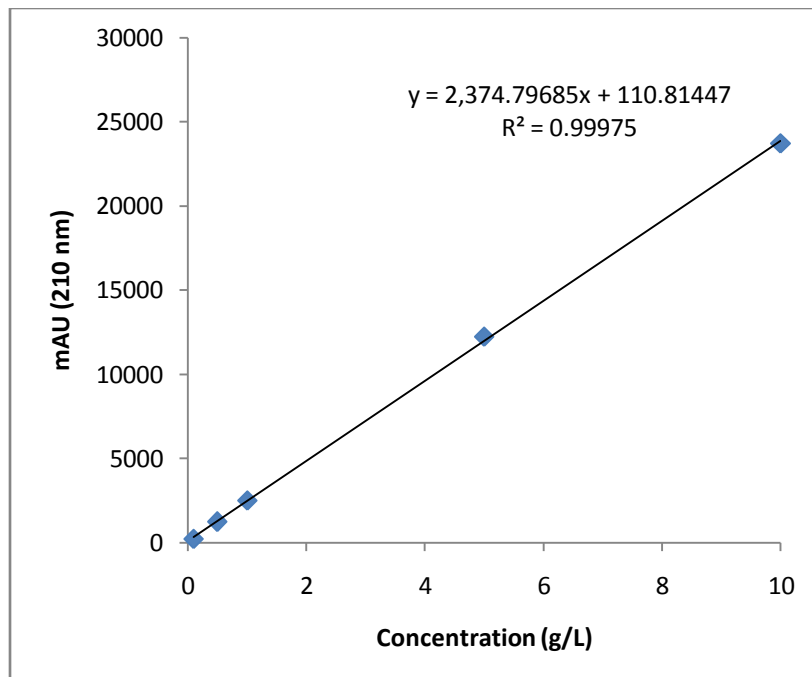


Figure 41. Citric Acid Standard Curve

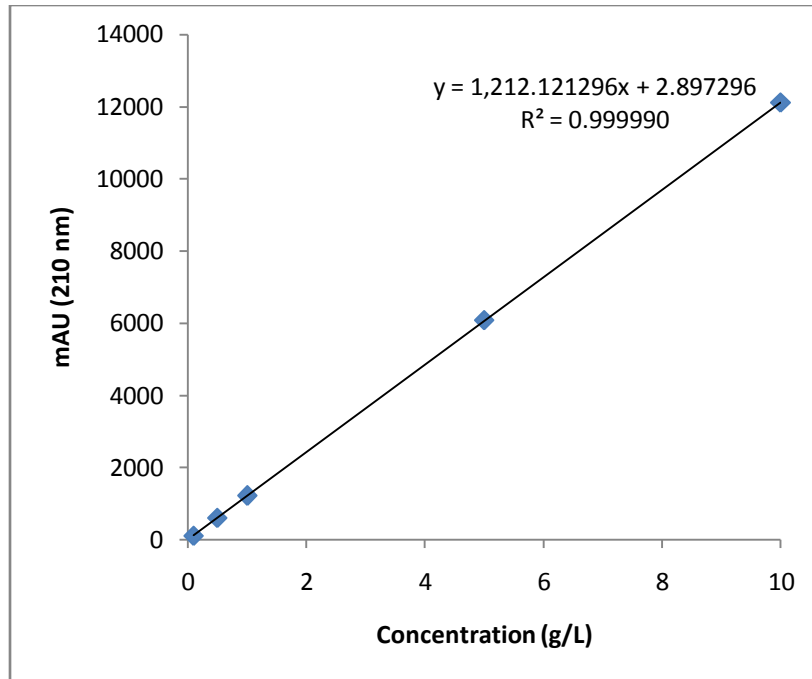


Figure 42. Lactic Acid Standard Curve

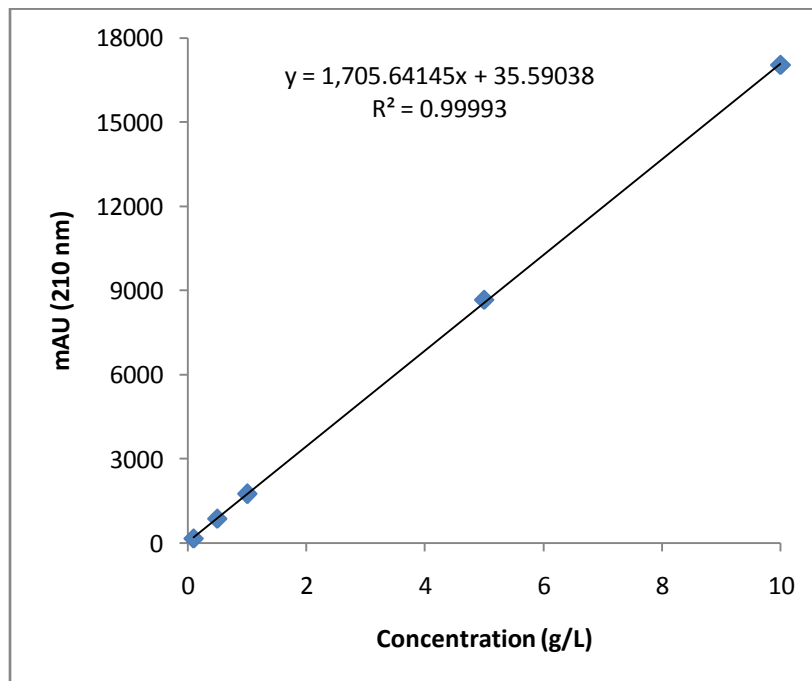


Figure 43. Malic Acid Standard Curve

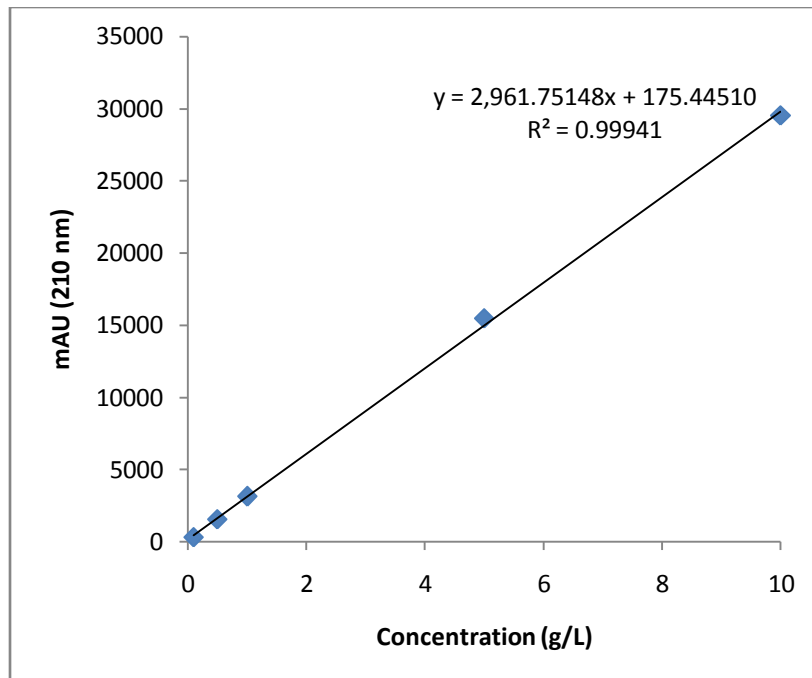


Figure 44. Tartaric Acid Standard Curve