ENZYMATIC MANAGEMENT OF HIGH pH GRAPE JUICE AND MUST

&

THE EFFECTS OF CROP THINNING ON GRAPE YIELD AND WINE QUALITY

A Thesis

by

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ABSTRACT

High pH winemaking is a common problem all across the state of Texas. Texas is a warm growing climate with temperatures reaching above 100°F (38°C) during the summer. In warm weather growing climate, such as Texas, grapes ripen more quickly, leading to higher soluble solids and lower acidity. These analytical parameters in grapes at harvest, along with other parameters such as potassium and pH, directly affect the wine made from these grapes. If these parameters fall outside of their desired ranges it can lead to serious quality issues in the finished wine such as instability and undesirable flavors. For the first study, the enzymes glucose oxidase (GOx) and catalase were used as a potential pH lowering pre-fermentative treatment on Tempranillo juice and must. The effects of GOx and catalase were observed and recorded on juice and must chemistry prior to fermentation as well as in the finished wine. Parameters measured on the grape juice and must during GOx treatments include pH, TA, glucose, and gluconic acid. Parameters measured on the resulting finished wine include pH, TA, alcohol %, Free SO_2, and volatile acidity (VA). Using GOx with catalase at a rate of 1.0(g/L) was most effective in juice which lowered pH from an average of 4.6 to 3.8, while increasing TA from an average of 3.13(g/L) to 7.86(g/L). The resulting wines had a lower pH and higher TA, but were less alcoholic and did not hold free SO_2 as well as the control wines.

The effects of crop thinning using a mechanical harvester on grape yield and composition and wine quality was also evaluated. Crop thinning and pruning treatments were carried out on Tempranillo and Mourvèdre grapevines at four different levels: vines pruned to two buds per spur and then shoot thinned and crop thinned using a mechanical harvester (2BFT), vines pruned to three buds per spur and then crop thinned using a mechanical harvester

(3BFT), vines pruned at two buds per spur (2B), and vines pruned at three buds per spur (3B). Data was recorded for berry composition at harvest and for the finished wines nine months after bottling. The parameters analyzed at harvest include pH, titratable acidity, Brix, potassium, and yeast assimilable nitrogen (YAN). After the wines spent nine months in bottle, they were then analyzed for pH, titratable acidity, alcohol percentage, free SO_2, volatile acidity, malic acid, lactic acid, tartaric acid, and color. The finished wines from the Tempranillo treatments were also judged blindly and scored based on preference by an untrained consumer panel. Scored data by the panelist include wine taste, wine aroma, wine appearance, and wine color. The data revealed that soluble solids (Brix), pH, and potassium were all higher in crop thinned treatments than non-crop thinned treatments for both Mourvèdre and Tempranillo at harvest. The resulting wines from both varieties were also higher in alcohol and redder in color. Data from the consumer panelist showed that the taste of Tempranillo wine made from treatment 2BSFT was most preferred, but not statically preferred from treatment 2B. Crop thinned wines were also statically preferred for appearance and color by consumer panelist.

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INTRODUCTION

The Texas Wine Industry

The Texas Wine Industry has seen remarkable growth in the past twenty years from 46 license wineries in 2001 to 812 in 2020 (Alcohol and Tobacco, Tax and Trade Bureau). Grape acreage in the state has increased as well in order to keep up with the demand for Texas grapes to supply to Texas wineries. There are currently eight American Viticultural Areas (AVA) in the state of Texas: Texas High Plains, Texas Hill Country, Texoma, Fredericksburg, Texas Davis Mountains, Escondido Valley, Mesilla Valley, and Bell Mountain. As of 2019 the majority of grapes grown in the state are grown in the Texas High Plains AVA (National Agriculture Statistics Services, USDA).

Growing grapes in Texas poses its own set of challenges, as fruit maturation and time of ripening are greatly accelerated in a warm growing climate (Goldammer, Grape Grower's Handbook, 2015). Optimal temperature for ripening grapes is between 20°C – 32°C (Goldammer, Grape Grower's Handbook, 2015) whereas in Texas the temperatures during harvest can reach above 38°C. Warm growing temperatures, such as the ones in Texas, hasten berry maturity by increasing soluble solids and lowering acidity in grapes during ripening (Goldammer, Grape Grower's Handbook, 2015).

Determining Grape Berry Maturity

The maturity of grapes is usually based on three parameters: sugar content, titratable acidity, and pH (Goldammer, Grape Grower's Handbook, 2015). Glucose and fructose are the two most abundant types of sugars found in grapes (Pickering, 2000) and are usually present in

equal amounts during ripening at a 1:1 ratio (Goldammer, Grape Grower's Handbook). Sugar content, which is measured as total soluble solids (TSS) in Brix, is most commonly used for determining when to harvest grapes (Goldammer, Grape Grower's Handbook, 2015). Sugar is important due to its impact on fruit quality and its role in alcoholic fermentation (Goldammer, Grape Grower's Handbook, 2015). The alcohol content in a finished wine is directly related to the amount of sugar initially present in the grapes (Goldammer, Grape Grower's Handbook, 2015).

Titratable acidity (TA) measures the quantity of grape acids, and is the total proton concentration which is expressed as grams of tartaric acid equivalents per liter (Margalit, Concepts in Wine Chemistry, 2004; Goldammers, Grape Grower's Handbook, 2015) whereas pH reflects the free proton concentration in solution (Margalit, Concepts in Wine Chemistry, 2004). As grapes ripen, sugar levels rise and acid levels fall, making grape ripeness the optimum cross-over point where sugars and acids are both high enough to allow for good wine-making (Goldammer, Grape Grower's Handbook, 2015)

It is also equally important to assess the qualitative parameters of wine grapes such as fruit integrity, color intensity of skin, seed coat color, and the degree of tannin (Goldammer, Grape Grower's Handbook, 2015). Total soluble solids, organic acids, polyphenols, and flavor compounds, determine technological maturity of fruit at harvest (Mattivi et al, 2006).

Grape and Wine Acidity

Next to sugars, organic acids are the most abundant solids present in grape juice (Goldammer, Grape Grower's Handbook, 2015). Acids give crispness and brightness to wines and are essential components of balance in a fine wine (Goldammer, Grape Grower's Handbook, 2015). Acid adjustments to juice or must can be made whenever the TA and/or the pH fall outside the desirable ranges (Margalit, Concepts in Wine Chemistry, 2004). It is quite common in warm climates that when desired sugar levels are reached, total acidity is low, and the pH is high (Margalit, Concepts in Wine Chemistry, 2004). Tartaric acid, malic acid, citric acid, lactic acid, acetic acid, and succinic acid are the major acids in wine (Margalit, Concepts in Wine Chemistry, 2004), the first three are formed in grapes in the vineyard, while the other three are products of fermentation (Margalit, Concepts in Wine Chemistry, 2004). Tartaric acid and malic acid account for over 90% of the total acids present in the berry (Goldammer, Grape Grower's Handbook, 2015). Tartaric acid accumulates in the early stages of berry development (Goldammer, Grape Grower's Handbook, 2015) and remains relatively consistent throughout the ripening process (Chidi et al, 2018). Tartaric acid is not metabolized by yeast or other microorganism, but both tartaric and malic acid are lost through the biochemical process of cellular respiration in warm climates (Chidi et al,2018). Before the color change of grape berries at veraison, malic acid can be found up to 25(g/L) before declining to 2.0(g/L) – 6.5(g/L) at berry maturity in warm growing regions (Chidi et al, 2018). Malic acid can be metabolized by *Oenococcus* and *Lactobacillus* bacteria to produce lactic acid, which is much softer on the palette (Chidi et al, 2018). Berries begin to deacidify after veraison as they become soft, ripe, and accumulate more sugars (Goldammer, Grape Grower's Handbook, 2015). It is well

established that fruit acidity at harvest is negatively correlated with increased temperatures during the ripening period, that is as temperatures increase berry acidity decreases (Goldammer, Grape Grower's Handbook, 2015). Acidity can be manipulated in the cellar through the process of adding natural acids to juice or must or wine in order to increase total acidity (Margalit, Concepts In Wine Chemistry, 2004). During alcoholic fermentation, acids produced by yeast and other microorganism, can also change the acidity which may result in a lower or higher totally acidity of the wines (Chidi et al, 2018). Lactic acid is an example, as well as succinic acid, which develops during fermentation due to yeast metabolism (Chidi et al, 2018).

Wine pH

pH is related to the concentration of Hydrogen protons in solution and is perhaps the most important measure of juice and wine acidity (Boulton, 1980). The pH in wine has a controlling influence over a number of factors including microbial spoilage, malolactic fermentation, sour taste, and color (Boulton, 1980). Proper pH range for red wine is between 3.3 – 3.7 (Margalit, Concepts in Wine Chemistry, 2004). Excessive grape acidity can lead to tart, acidic wines (Goldammer, Grape Grower's Handbook, 2015), whereas low grape acidity with an elevated pH will result in a flabby, unbalanced wine (Goldammer, Grape Grower's Handbook, 2015). Higher pH wines are more prone to oxidation in which ethanol is oxidized into acetaldehyde which can further be oxidized into acetic acid, will facilitate oxidation at a faster rate, and the protection provided by the use of sulfur dioxide is more difficult to manage (Margalit, Concepts in Wine Chemistry, 2004; Goldammer, Grape Grower's Handbook, 2015).

The color of wine itself is a remarkable phenomenon that depends upon pH and the stability of chemical compounds known as anthocyanins, which belong to the class of flavonoids (Tang et al, 2019; Margalit, Concepts in Wine Chemistry, 2004). Anthocyanins are water soluble natural pigments and are responsible for a wide variety of colors, such as red, purple, and blue. (Tang et al, 2019). The color of anthocyanins is related to the its structural formation that is transformable and reversable depending upon the pH value (Tang et al, 2019). In acidic solutions, flavylium cations predominate the anthocyanin structure, which causes a red color (Tang et al, 2019). An increase of pH up to 4.0 causes a rapid proton loss which leads to the formation of a purple or blue quinonodial base. (Tang et al, 2019). A further increase in pH to 6.0 causes the flavylium cation to become hydrated to produce the colorless carbinol pseudobase (Tang et al, 2019). After which there is a shift toward anhydro bases as the pH increase above 7.0, and further increasing the pH above 8.0 gives rise to yellow chalcone structures that are predominated with anhydro bases (Tang, et al, 2019). The color of anthocyanin solutions is reversed by changing the pH from alkaline back to acidic (Tang et al, 2019). A wine solution between a pH of 2.0 – 4.0 will produce colors of red hues, whereas in pH values of 8.0 – 9.0 anthocyanins will produce colors of blue hues (Tang et al, 2019).

Buffer Capacity in Wine

Buffer capacity is the resistance of a solution to pH change. The buffering capacity of wine is a combined result of the various acids it contains and also the result of the water-alcohol mixture of wine (Margalit, Concepts in Wine Chemistry, 2004). Buffer capacity depends on the concentration of acids and alkaline metal ions (Margalit, Concepts in Wine Chemistry,

2004). Alkaline metal ions such as potassium (K) and sodium (Na) can bind to weak organic acids present in the wine making it a buffer to pH change (Margalit, Concepts in Wine Chemistry, 2004).

Glucose Oxidase (GOx)

Glucose oxidase is an enzyme that is produced naturally by the fungus *Aspergillus niger,* as well as other certain fungi and insects (Wong, Wong, Chen, 2008). Glucose oxidase is Generally Regarded As Safe (GRAS) and is available in bulk for use in the food industry as an additive (Wong, Wong, Chen, 2008). Glucose oxidase, along with catalase, catalyzes the oxidation of glucose into gluconic acid by a two-step process (Pickering et al, 1998). Glucose oxidase is an aerobic dehydrogenase that catalyzes the oxidation of glucose to gluconolactone in the presents of molecular oxygen (Pickering, 2000). In a subsequent step, gluconolactone is hydrolyzed non-enzymatically to gluconic acid (Pickering, 2000). Aeration is required, specifically oxygen, as GOx reactions are dependent on O_2 concentrations (Pickering et al, 1999). Glucose oxidase is an oxygen scavenger and the catalase enzyme associated with GOx is a hydrogen peroxide scavenger (Wong, Wong, Chen, 2008). The oxidation of glucose into D-gluconolactone by GOx produces hydrogen peroxide as a byproduct, which results in D-gluconolactone + H_2O_2 (Wong, Wong, Chen, 2008). The catalase enzyme then decomposes the excess hydrogen peroxide into water and oxygen (Wong, Wong, Chen, 2008). The water molecule then hydrolyzes with D-gluconolactone non-enzymatically to yield gluconic acid (Pickering, 2000; Wong, Wong, Chen, 2008). Any excess of hydrogen peroxide can potentially

oxidize ethanol into acetaldehyde (McLeod and Ough, 1970). Evidence from another study stated that hydrogen peroxide was reduced by sulfur dioxide (Ough, 1975).

Optimum temperatures for GOx activity have been reported to be 30°C (86°F) (Ekinci et al, 2007). A further increase of temperature above 40°C (104°F) results in a decrease of GOx activity due to the altered enzyme conformation (Ekinci et al, 2007). Glucose oxidase and catalase are both exothermic reactions (Pickering et al, 1999) therefore, the experiment should take place in a controlled temperature environment.

Viticultural Practices

Vineyard management is critical for achieving optimal fruit maturity (Zhuang et al, 2014) and there are numerous viticulture practices that can influence berry development and quality. Grape yield and final berry quality are strongly determined by climate, soil, and cultural management practices which include crop thinning, shoot positioning, and leaf removal (Zhuang et al, 2014; Susaj et al, 2013). Irrigation practices and pruning practices have also show to impact final berry quality (Goldammer, Grape Grower's Handbook, 2015). Proper nutrition is a key requirement for the reliable production of grapevines, and even the vineyard site selection itself is probably the most important, fundamental, irreversible decision for the life of a vineyard (Goldammer, Grape Grower's Handbook, 2015).

Grapevine Phenology

Grapevine phenology is the study of the relationship between climate and the natural seasonal phenomena of a grapevine (Tomasi et al, 2011). The leaves of a grapevine provide

nourishment for the plant though the process of photosynthesis (Goldammer, Grape Grower's Handbook, 2015). The carbon fixed during photosynthesis is allocated to expanding leaves (Fisher, 2009) and as the leaf matures it becomes the primary source for exporting carbohydrates into expanding its growth, fruit development, new roots, or storage within the roots or truck (Fisher, 2009).

Flowering in Texas occurs in the Springtime with light exposure playing a very import role. Light intensity and light quality are critical for fertility, and an axial bud that is grown in the shade will be much less fruitful (Fisher, 2009). Many other conditions affect successful flowering including the nutrition status of the vine, soil water availability, previous winters cold temperatures, and the current seasons weather during blooming (Fisher, 2009).

Fruit set will begin just a few weeks after flowering, and once fruit set has begun, there are three steps or "developmental phases" that occur before the grapes are ready for harvest. Once berries have set, they go through a period of very rapid cell division, followed by lag phase, which is followed by cell expansion (Fisher, 2009; Goldammer, Grape Grower's Handbook, 2015). At this point, berry color and texture begin to change which is marked by the term "veraison". Veraison is a universal term that signifies that the grape berries are beginning to ripen (Fisher, 2009). At various times during veraison, many compounds are synthesized and stored in the berry, making them sweeter, less acidic, tannins and other complex phenols accumulate, and the berries gain more color as well (Fisher, 2009). It is during this time that the grape grower and winemaker must chose when to harvest, as harvesting wine grapes is one of the most crucial steps in the process of winemaking (Goldammer, Grape Grower's Handbook, 2015)

Crop Thinning

Crop thinning, also called cluster thinning, is the intentional action of removing fruit from the vine to obtain a balance between fruit and canopy in order to achieve optimum ripeness (Goldammer, Grape Grower's Handbook, 2015). Removal of grape clusters from the vine leads to a decrease in the number of berries that receive nutrients and photosynthates from the vine, which ends up improving the overall quality of the remaining crop (Kamas, 2019). The timing for crop thinning can be anywhere from pre-bloom to harvest (Goldammer, Grape Grower's Handbook, 2015) although thinning at or near fruit set has shown to increase metabolites in the berry by harvest (Goldammer, Grape Grower's Handbook, 2015). Fruit thinning at or near the end of veraison can still be beneficial but less effective as both vine energy and day length begin to decline later into the growing season (Kamas, 2019).

How Wines Are Affected by Different Crop Loads

Traditionally, low yielding vineyards have been associated with higher quality wines (Ross, 1999). Crop load is a common measure of yield relative to the size of the producing grapevine. More grapes produce more wine, yet the quality of the grapes is also an important component (Chapman et al, 2004). Pruning, shoot thinning, and crop thinning are all cultural practices that affect yield, but greater wine quality associated with lower yields is still questioned by some. Six studies (Ewart et al. 1985, Freeman et al. 1980, Ough and Nagaoka. 1984, Reynolds et al. 1986, Sinton et al. 1978, Zamboni et al. 1996) found no effect, or no consistent effects of yield on wine quality (Chapman et al, 2004). However, studies performed by (Bravdo et al, 1984; Bravdo et al, 1985) did report that lower wine-quality scores were

associated with high-yielding vines, and that a higher crop load above 10 on the Ravaz Index found to negatively affect wine quality. In order for wines to be identified as superior quality over other wines made from the same vineyard and variety, there has to be objective characterizations of aroma and flavor differences caused by viticultural treatments (Chapman et al, 2004). In one study performed on Cabernet Sauvignon, pruned and cluster thinned treatments resulted in wines that differed in aroma and taste attributes (Chapman et al, 2004). In that same study, wines made with vines pruned to 24 buds/vine were significantly higher in black pepper aroma, astringency, and veggie by mouth than the wines made from vines with 48 buds/vine (Chapman et al, 2004).

Sensory Analysis

Sensory analysis is increasingly viewed as a way to explain consumer preferences (Delarue and Sieffermann, 2003) as well as characterize products, and also to detect and describe differences between products. Changes in the formulation of a product may produce desirable or undesirable results that must be assessed, analyzed, and then interpreted in meaningful ways (Savits, 2014). Other academic studies have used sensory analysis in wine evaluation to describe the impact of cluster-thinning or the use of glucose oxidase as a pre-fermentation additive (Zhuang et al, 2014; Pickering et al, 1999). An untrained sensory consumer panel was used in this study to evaluate Tempranillo wines made from the crop thinning experiment.

Sensory Consumer Panel

A sensory panel can be classified as either trained or consumer. A consumer panel is used to test if the product will be accepted by the consumer. A ballot is used in conjunction with consumer panelist so that a score can be kept using a preference scale. Annex 1 shows the ballot that was used during the consumer panel study for the Tempranillo wine made from the crop thinning experiment. For this study, 101 untrained consumer panelists were screened and selected to judge blindly the Tempranillo wines that were made form the crop thinning experiment. The objective of this consumer panel study was to see how vineyard treatments affected wine taste, wine aroma, wine appearance, and wine color, and which wine consumers preferred the most.

CHAPTER I – ENZYMATIC MANAGMENT OF HIGH pH TEMPRANILLO JUICE AND MUST

Abstract

In this study, glucose oxidase with catalase was used to lower pH and increase titratable acidity (TA) in grape juice and must by oxidizing glucose into gluconic acid. GOx/catalase was dosed directly into the grape juice and must as a pre-fermentation treatment. Measurements were taking ever 4 hours for 24 consecutive hours and included pH, titratable acidity (TA), glucose levels, and gluconic acid levels. Using GOx/catalase in Tempranillo grape juice showed to lower pH by 0.8 units while increasing TA by 4.7(g/L) in laboratory trials. When scaling up to larger volumes of grape juice or must, it was found that more oxygen was needed to carry out the enzyme reaction. After vinification and 9 months in bottle, the wines were evaluated for pH, titratable acidity (TA), alcohol percentage, Free SO_2, and volatile acidity (VA). It was found that pH, final alcohol percentage, and Free SO_2 levels were all lower in the GOx wines than control wines. A slight color change was observed when using GOx in grape juice, but this color change was reversed by placing grape skins back into the juice and the start of fermentation.

Introduction & Literature Review

This study uses glucose oxidase with catalase as a pre-fermentative treatment to acidulate high pH Tempranillo juice and must. Previous studies using glucose oxidase with catalase have only shown to be used for the purpose of producing lower-alcohol wines (Pickering, 2000) and as an oxygen scavenger for food and drink preservation (Wong et al, 2008; McLeod and Ough, 1970). The work here in this manuscript, however, shows the use of

glucose oxidase with catalase as a way to lower the pH in grape juice and must which also simultaneously increase titratable acidity.

Glucose oxidase (GOx) is an enzyme that is produced naturally by the fungus *Aspergillus niger* and catalyzes the oxidation of D-glucose into D-gluconolactone, during which hydrogen peroxide is produced (Wong, Wong, Chen, 2008). GOx is generally regarded as safe (Wong, Wong, Chen 2008) and has found several commercial applications including glucose removal from dried egg; improvement of color, flavor, and shelf life of food materials; oxygen removal from fruit juices, canned beverages, and from mayonnaise to prevent rancidity. Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen which rapidly breaks down hydrogen peroxide (H_2O_2) into water and oxygen (McLeod and Ough, 1970) during which D-gluconolactone non-enzymatically binds with water to form gluconic acid.

The production of gluconic acid using glucose oxidase has been shown to increase acidity in grape juice/must (Pickering et al, 1999). (Pickering et al, 1999) also noted that GOx treated wines had approximately two to three times higher titratable acidity at bottling than control wines made without using GOx. The cause for this large increase in titratable acidity could be the fact that Pickering et al focused on lowering glucose, rather than increasing acidity, which required longer treatment times (up to 72 hours).

One observation in a study done by (Valencia et al, 2017) using glucose oxidase with catalase was that there was a browning effect on the *Carmenere* must that was caused by aerating the must. It should be noted that the experiment performed by Valencia et al lasted for 48 hours, whereas in this manuscript all experiments using the GOx/Catalase enzyme

system were concluded after 24 hours. The product Catazyme 25L, which contains both glucose oxidase and catalase, was used for all experiments in this manuscript.

Materials & Methods

The materials used for these experiments included the following: Tempranillo grapes with a pH of either 4.6 or 4.0 and a titratable acidity of either 4.4(g/L), 3.2(g/L), or 8.2(g/L) depending upon the experiment, Novozymes Catazyme 25L (Copenhagen, Denmark), Antifoam, Aqua Culture aquarium air pumps, hoses, sparging stones, 5-gallon buckets, destemmer & crusher, basket press, pH meter & Titralyser (Dujardin-Salleron, Noizay, France), Gallery Discrete Analyzer (Thermofisher Scientific, Waltham, Massachusetts), sodium hydroxide (NaOH), distilled water, beakers, hydrometers, yeast and yeast nutrients.

There were four different treatments for the laboratory experiments. These treatments include a control, an aeration treatment where an air pump supplied oxygen into the bucket of grape juice or must, a 0.5 GOx treatment in which 0.5(g/L) of Catazyme 25L was poured into the buckets of grape juice or must, and a 1.0 GOx treatment in which 1.0(g/L) of Catazyme 25L was poured into the buckets of grape juice or must. Catazyme 25L is a viscous liquid product.

The first experiment, *Batch 1*, had Catazyme 25L poured directly into the buckets of crushed and destemmed grape must. Measurements were taken every 4 hours for 24 consecutive hours and the fermented to dryness. The seconded experiment, *Batch 2 Method*, had Catazyme 25L poured directly into the buckets of Tempranillo juice (skins removed). Once the treatment was complete and measurements were taken (24 hrs) the grapes skins were added back into the juice and fermentation started. The Batch 2 method was conducted to

observe the effectiveness of glucose oxidase in the juice phase and then grape skins were added back to observe any possible color reversal during or after fermentation. The juice was then fermented to dryness.

The third experiment, *Industrial-Size*, was carried out in stainless steel fermentation tanks in which Catazyme 25L was poured directly into crushed and destemmed grape must. Measurements were taken every 4 hours for 24 consecutive hours. Scaling up the experiment to larger volume gave practical insight into using glucose oxidase with catalase large-scale.

Experimental Design

Outlined below are the procedures used for each of the experiments:

Batch 1

Four treatments:

1) Control
2) Aeration
3) GOx (0.5g/L) + aeration
4) GOX (1.0g/L) + aeration

Each treatment ran in duplicate for a total of eight individual fermentation replicates. 2019 Tempranillo grapes were crushed and destemmed and then divided into 12-liter batches; 5-gallon buckets were used. 1) Two control buckets contained only 12 liters of must each and 2 drops of Antifoam were added. 2) Two aeration buckets received 12 liters of must each with an aqua pump supplying air through two hoses and sparging stones, 2 drops of Antifoam were added. 3) Two buckets contained 12 liters of must each and were dosed with 6 grams of Catazyme 25L (0.5g/L), an aqua pump supplied air through two hoses and sparging stones, 2 drops of Antifoam were added. 4) Two buckets contained 12 liters of must each and were dosed with 12 grams of Catazyme 25L (1.0g/L), an aqua pump supplied air through two hoses and sparging stones, 2 drops of Antifoam were added.

The experiment lasted for 24 hours with parameters observed and recorded every 4 hours. Hypothetical outcomes for these trials were that: 1) pH will drop significantly; 2) 1.0g/L of GOx will be more effective than 0.5g/L of GOx at converting glucose into gluconic acid; 3) the aeration trial will make the least attractive wine on account of the excess of oxygen.

The Batch 2 Method

Four treatments:

1) Control
2) Aeration
3) GOx (0.5g/L) + aeration
4) GOx (1.0g/L) + aeration

Each treatment ran in duplicate for a total of eight individual fermentation replicates.
The main difference in this experiment was that the grape skins were removed during GOx treatment and added back into the juice once fermentation began. 2019 Tempranillo grapes were crushed, destemmed, and pressed. The juice was collected and divided into 10-liter batches; 5-gallon buckets were used. 1) Two control buckets contained 10 liters of juice per bucket and 2 drops of Antifoam added. 2) Two aeration buckets contained 10 liters of juice per bucket and had an aqua pump supplying air through two hoses and sparging stones, 2 drops of Antifoam were added. 3) Two buckets contained 10 liters of juice per bucket and were dosed with 5 grams of Catazyme 25L (0.5g/L), aeration supplied by an aqua pump with two hoses and sparging stones, 2 drops of Antifoam were added. 4) Two buckets contained 10 liters of juice per bucket and were dosed with 10 grams of Catazyme 25L (1.0g/L), aeration supplied by an aqua pump with two hoses and sparging stones, 2 drops of Antifoam were added.

The experiment lasted 24 hours with parameters observed and measured every 4 hours. Hypothetical outcomes were that: 1) pH will drop significantly; 2) GOx will be more effective in the juice phase, 1.0g/L being more effective than 0.5g/L; 3) any browning that may occur will be

remedied by the addition of the skins back into the juice at fermentation; 4) the aeration trials will make the least favorable wine because of the excess exposure to oxygen.

Industrial Size

Four treatments:

1) Control

2) 24 hr. GOx

3) Target pH of 3.4

4) Co-fermentation

- The co-fermentation treatment was dosed with Catazyme 25L and simultaneously inoculated with activated yeast.

Each treatment ran in duplicate for a total of eight fermentation replicates.

The equipment, a Titralyzer used for measuring pH and TA, Catazyme 25L, fish pumps with hoses and sparging stones for aeration, eight stainless steel tanks, and laboratory glassware, was all transported to Square Cloud Winery in Gunter, Tx where 91% 2020 Tempranillo grapes, 4% 202 Dornfelder grapes, and 5% 2020 Syrah grapes were all crushed, destemmed, homogenized, and the must was separated into 51-liter batches; 30-gallon stainless steel tanks were used. 1) Two control tanks contained 51 liters of must each, nothing else was added. 2) Two tanks contained 51 liters of must each and had 51 grams of Catazyme 25L added to them along with two aeration pumps each with hoses and sparging stones which supplied oxygen to each tank. This treatment was to be monitored for a full 24 hours in order to observe the effects of the enzymes. 3) Two tanks contained 51 liters of must each and had 51 grams of

Catazyme 25L added to them along with two aeration pumps each with hoses and sparging stones which supplied oxygen to each tank. This treatment was to be monitored until the must reached a target pH of 3.4 and then the aeration was to be halted. 4) Two tanks contained 51 liters of must each and had 51 grams of Catazyme 25L added to them along with two aeration pumps each with hoses and sparging stones which supplied oxygen to each tank. This treatment was then inoculated QA23 *Saccharomyces cerevisiae* yeast in order to observe the effects of the enzymes together with active yeast.

Vinification

After the 24-hour pre-fermentation research was concluded, the laboratory experiments of Batch 1 and Batch 2 Method were then inoculated with Viti Levure MT *Saccharomyces cerevisiae* yeast a rate of 1.67g/L for each bucket and also given a 1.2g/L addition of Go-Ferm. Each bucket was fermented to dryness and then pressed into glass carboys using a bladder press. A 60ppm addition of potassium metabisulfite (KMBS) was added to each carboy at this time for Batch 1. A 70ppm addition of KMBS was added to each carboy for Batch 2 Method. The wines were then sparged with Argon gas and sealed. Wines were racked twice and a one-time 70ppm addition of KMBS was added to Batch1, while a one-time 40ppm KMBS addition was given to Batch 2 Method. Wines were then bottled and stored in a 55° (10°C) chiller. Batch 1 wines were stored for three months before being chemically analyzed, while Batch 2 Method wines were stored for eight months before being chemically analyzed. There was no vinification for the Industrial-size experiment.

Statistical Analysis

Statistical data was generated using XLSTAT (Addinsoft, Paris, France). A one-way ANOVA was used to find any statistical differences with a p-value <0.05. Tukey's HSD was then used to separate treatment means that were statistically different from each other. This statistical method of using a one-way ANOVA followed by Tukey's HSD was used for every treatment within every experiment throughout the chapter of this study for both pre-fermentative composition and final wines.

Results & Discussion

Effects of GOx on pH and Titratable Acidity (TA)

pH and titratable acidy (TA) are indicators of the number of protons that are present in grape juice, must, or wine. pH is a measurement of the free proton ions in wine, whereas TA is a measurement of the total proton concentration. *Batch1, Batch 2 Method*, and *Industrial-size* experiments all showed a drop in pH that was statistically different between at least two different treatments. Only the Batch 1 and Batch 2 Method experiments showed a more dramatic drop in pH along with a simultaneous increase in titratable acidity. Using GOx in the juice phase seem to be more effected as Batch 2 Method pH dropped by 0.84 units compared to a drop of 0.7 pH units for Batch 1. Titratable acidity increased by 4.65(g/L) in Batch 2 Method compared to 3.65(g/L) increase in Batch 1. pH did show to be statistically different for GOx treatments in both Batch 1 and Batch 2 when compared to control treatment. Titratable acidity was only statistically different for Batch 2 Method.

The *Batch 2 Method* laboratory experiment using Catazyme 25L with Tempranillo juice showed the largest change in pH from an average of 4.6 to 3.8 when dosing GOx at a rate of 1.0(g/L). The pH change of the treatments that received Catazyme 25L showed to be statistically different from the control or aeration treatments in which Catazyme 25L was not used, table 1 shows statistical differences for pH. Figure 1 shows a visual representation of the pH of the Tempranillo juice from different treatment averages during the Batch 2 Method experiment. As expected, the treatments that were dosed with Catazyme 25L resulted in a drop in pH.

Titratable acidity increased as well in Batch 2 Method from an average of 3.13(g/L) to 7.86(g/L) when GOx was dosed at a rate of 1.0(g/L). Titratable acidity also showed to be statistically different for treatments that received Catazyme 25L as opposed to those treatments that did not, table 2 shows statistical differences for TA. Figure 2 shows a visual representation of the titratable acidity of Tempranillo juice from different treatment averages during the course of the experiment. The production of gluconic acid from using Catazyme 25L is responsible for the increase in titratable acidity in both the 0.5 GOx and 1.0 Gox treatments.

Tempranillo must in the *Batch 1* experiment displayed similar results under laboratory conditions with an average pH drop from 4.6 to 3.9 when dosing GOx at 1.0(g/L). This too showed to be statistically different as revealed in table 3. Figure 3 shows a visual representation of the pH of Tempranillo must from different treatment averages during the course of the experiment using Catazyme 25L.

Titratable acidity in the *Batch 1* experiment showed an increase from an average of 4.38(g/L) to 7.59(g/L). Surprisingly, titratable acidity in the Batch 1 experiment did not show to be statistically different as seen in table 4. Figure 4 shows a visual representation of TA of Tempranillo must form different treatment averages during the course of the experiment. The increase in TA for both the control and aeration treatments may be due to the onset of spontaneous fermentation while the production of gluconic acid caused by using Catazyme 25L is responsible for the increase in titratable acidity for the 0.5 GOx and 1.0 GOx treatments.

The *Industrial-size* experiment was run similarly to the laboratory experiments except that all treatments, except the control, were all dosed with 1.0g/L of Catazyme 25L. Each GOx treatment then had a different variable, that is either a target time, a target pH, or was co-fermented whilst using GOx. The Industrial-size experiment began to show similar results as that of the laboratory experiments, however, after about 4 hours pH no longer declined and began to level off in all of the treatments except for the co-fermentation treatment. Interestingly enough, the pH of the co-fermentation treatment slowly declined all the way until hour 20 and also proved to be statistically different than the control treatment, table 5. There was no statistical difference for TA amongst treatments, table 6. Figure 5 shows a visual representation of pH in Tempranillo must during the *Industrial-size* experiment, while figure 6 shows titratable acidity. The random jumps in titratable acidity could be a matter of sampling error or equipment misreadings, as grape must is much harder to work with than grape juice.

For Industrial-size experiment, the conclusion was that not enough oxygen was being delivered to the Tempranillo must in order for the GOx enzyme to properly carry out the reaction. This conclusion is based on a study stating that the glucose oxidase reaction is dependent upon O_2 concentrations (Pickering, 1999). In small-scale laboratory trials, aquarium tank fish pumps were enough to provide sufficient oxygen into the buckets of juice and must, but when scaling up to stainless still tanks the fish pumps simply could not provide enough oxygen needed to carry out the reaction. As for the co-fermentation treatment, the CO_2 gas that was produced by the yeast may have been enough to continuously bubble the must in order to expose the enzymes to just enough oxygen for the reaction to continue. Further studies need to be investigated in order to confirm or deny this hypothesis.

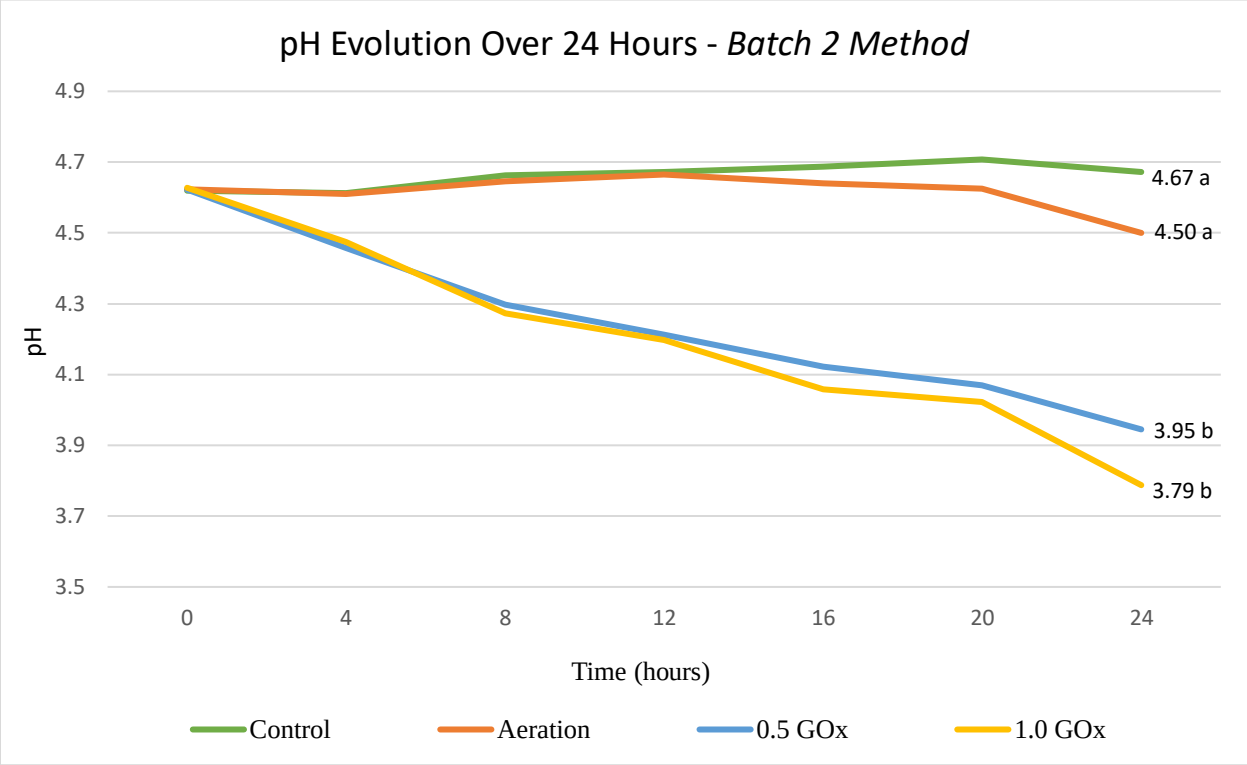

Figure 1. The effect of Catazyme 25L on pH in Tempranillo juice, Batch 2 Method.

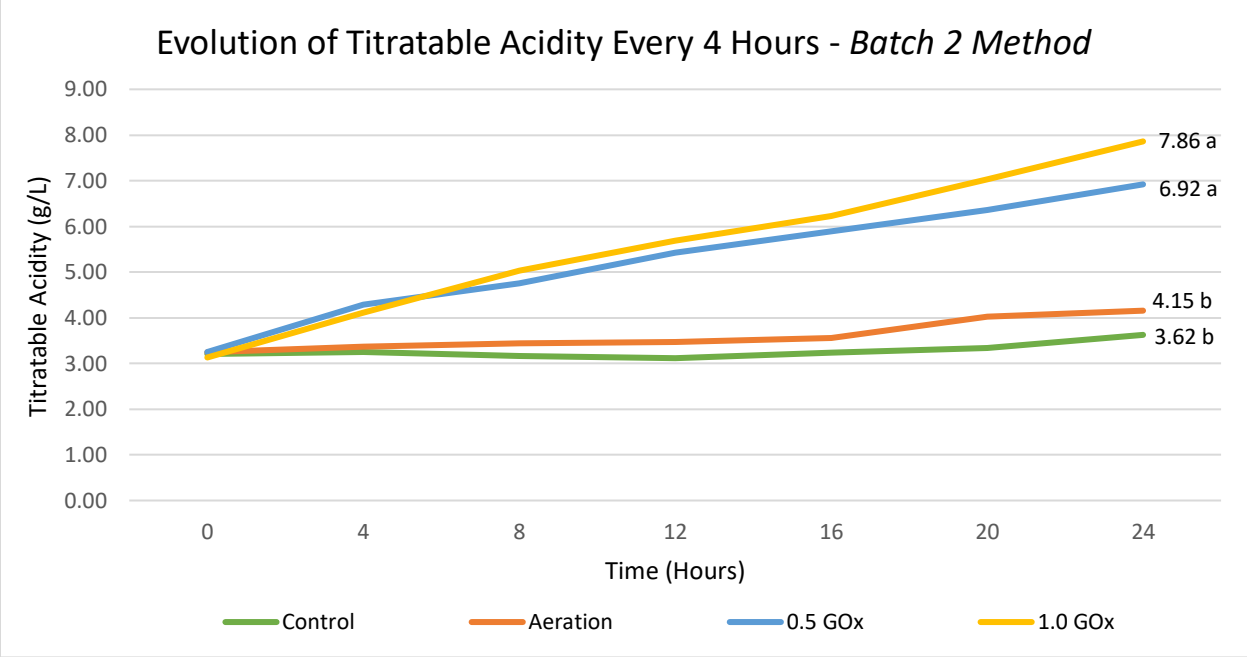

Figure 2. The evolution of titratable acidity (g/L) using Catazyme 25L in Tempranillo juice, Batch 2 Method.

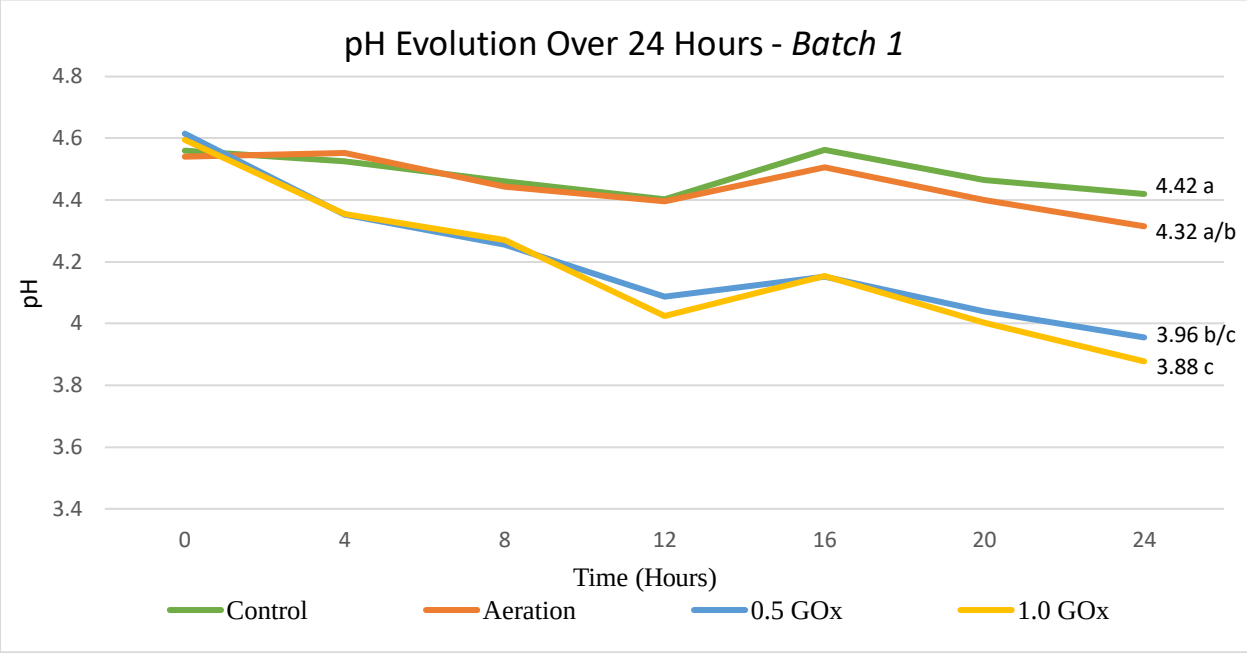

Figure 3. The effect of Catazyme 25L on pH in Tempranillo must, Batch 1.

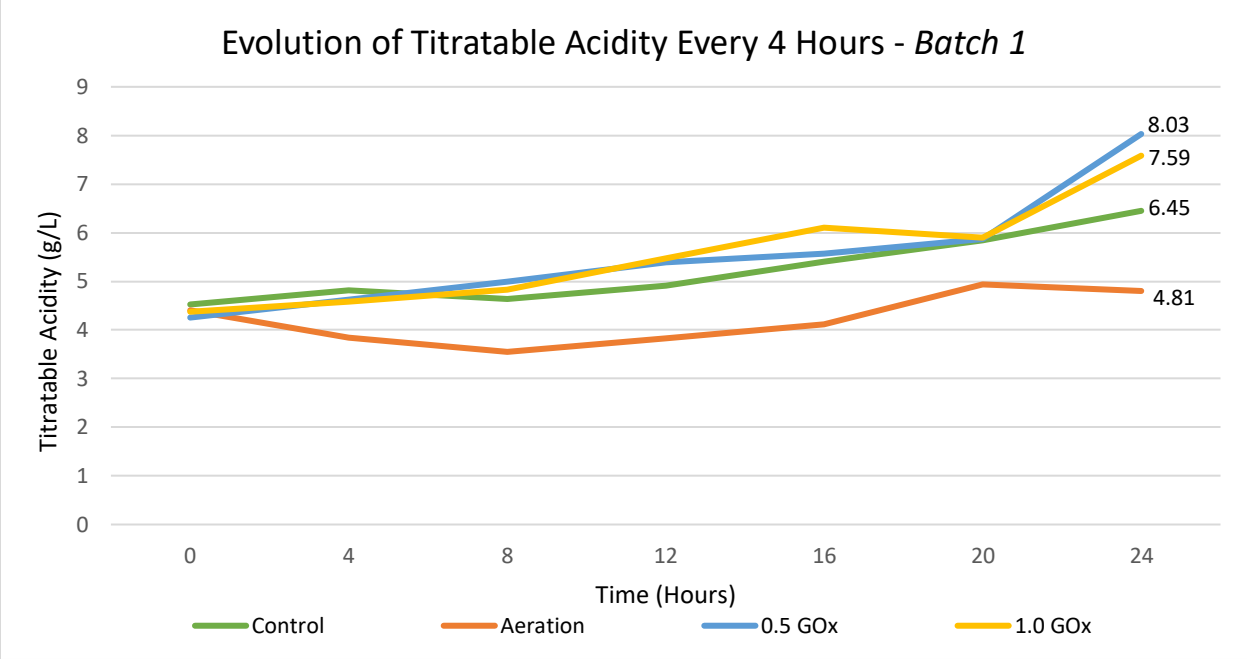

Table 4. The evolution of titratable acidity (g/L) using Catazyme 25L in Tempranillo must, Batch 1.

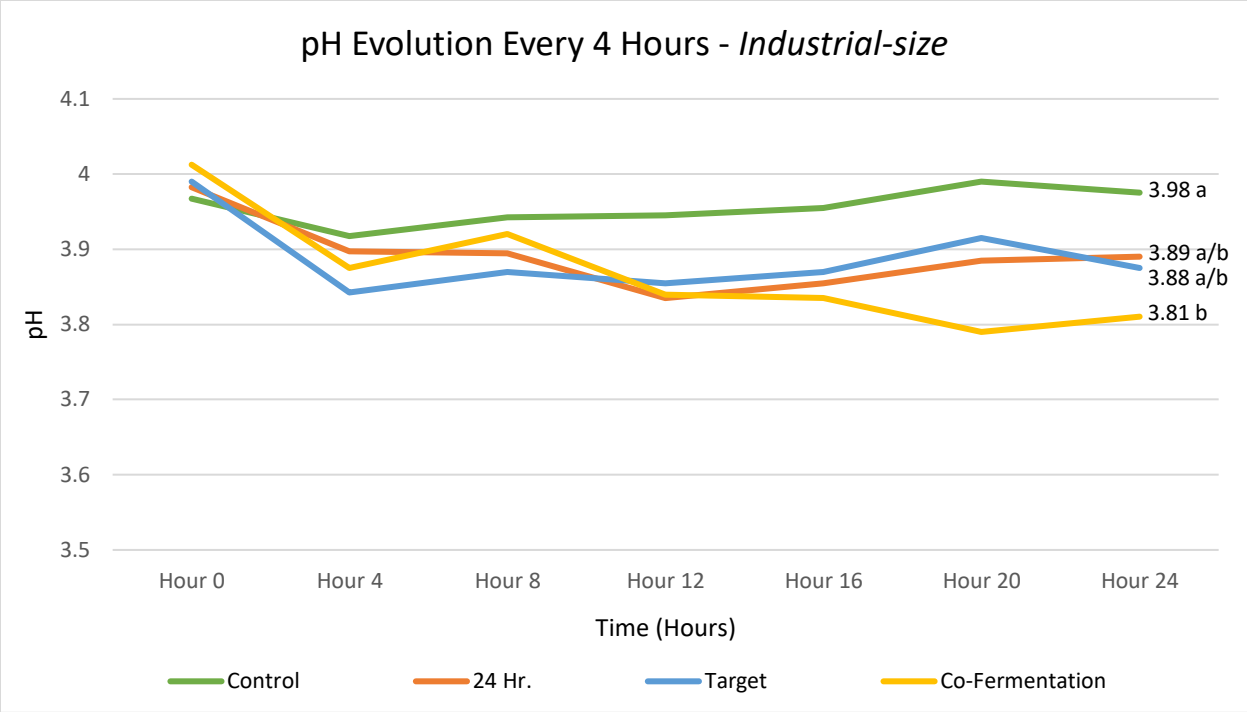

Figure 5. The effect of Catazyme 25L on pH in Tempranillo must, Industrial-size experiment.

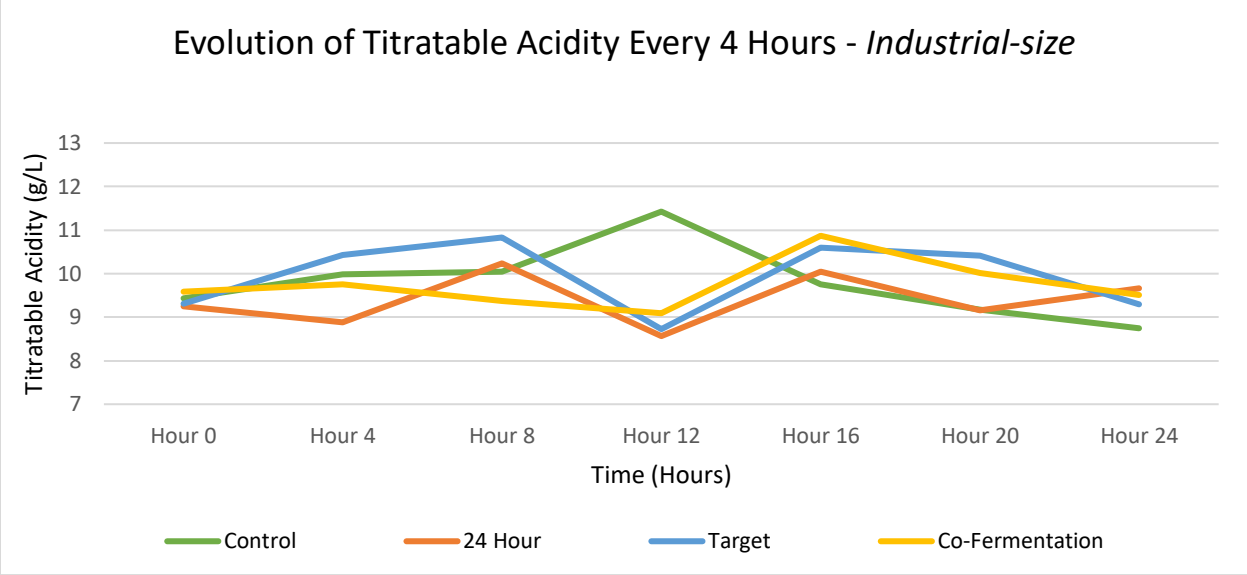

Figure 6. The evolution of titratable acidity (g/L) using Catazyme 25L in Tempranillo must, Industrial-size experiment.

pH – *Batch 2 Method*			
Treatment	Difference	CV	Significant?
C vs. A	0.0475	0.266	NO
C vs. 0.5	0.41607143	0.266	YES
C vs. 1.0	0.45714286	0.266	YES
A vs. 0.5	0.36857143	0.266	YES
A vs. 1.0	0.40964286	0.266	YES
0.5 vs. 1.0	0.04107143	0.266	NO

Table 1. Statistical differences for pH in the Batch 2 Method. Tukey's HSD following a one-way ANOVA.

TA – *Batch 2 Method*			
Treatment	Difference	CV	Significant?
C vs. A	-0.3307143	1.512	NO
C vs. 0.5	-1.9917857	1.512	YES
C vs. 1.0	-2.3060714	1.512	YES
A vs. 0.5	-1.6610714	1.512	YES
A vs. 1.0	-1.9753571	1.512	YES
0.5 vs. 1.0	-0.3142857	1.512	NO

Table 2. Statistical data for titratable acidity in the Batch 2 Method. Tukey's HSD following a one-way ANONA.

pH – *Batch 1*			
Treatment	Difference	CV	Significant?
C vs. A	0.035	0.249	NO
C vs. 0.5	0.27678571	0.249	YES
C vs. 1.0	0.30214286	0.249	YES
A vs. 0.5	0.24178571	0.249	NO
A vs. 1.0	0.26714286	0.249	YES
0.5 vs. 1.0	0.02535741	0.249	NO

Table 3. Statistical data for pH in Batch 1. Tukey's HSD following a one-way ANOVA.

TA – *Batch 1*			
Treatment	Difference	CV	Significant?
C vs. A	1.015	1.371	NO
C vs. 0.5	-0.3053571	1.371	NO
C vs. 1.0	-0.3232143	1.371	NO
A vs. 0.5	-1.3203571	1.371	NO
A vs. 1.0	-1.3382143	1.371	NO
0.5 vs. 1.0	-0.0178571	1.371	NO

Table 4. Statistical data for titratable acidity in Batch 1. Tukey's HSD following a one-way ANOVA.

pH – *Industrial-size*			
Treatment	Difference	CV	Significant?
C vs. 24h	0.06464286	0.0827	NO
C vs. T	0.06785714	0.0827	NO
C vs. Co	0.08714286	0.0827	YES
24h vs. T	0.00321429	0.0827	NO
24h vs. Co	0.0225	0.0827	NO
T vs. Co	0.01928571	0.0827	NO

Table 5. Statistical data for pH in the Industrial-size experiment. Tukey's HSD following a one-way ANOVA.

TA - *Industrial-size*			
Treatment	Difference	CV	Significant?
C vs. 24h	0.39628571	1.195	NO
C vs. T	-0.1447857	1.195	NO
C vs. Co	0.05271429	1.195	NO
24h vs. T	-0.5410714	1.195	NO
24h vs. Co	-0.3435714	1.195	NO
T vs. Co	0.1975	1.195	NO

Table 6. Statistical data for titratable acidity in Industrial-size. Tukey's HSD following a one-way ANOVA.

Effects of GOx on Glucose and Gluconic Acid

Glucose is important in grape juice and must as this will directly determine the potential alcohol of the resulting finished wine. Gluconic acid, though not naturally found in grapes, was produced by the enzymatic reaction from the GOx/Catalase system and is measured in this study to determine the titratable acidify of these treatments. Both the *Batch 1* and *Batch 2 Method* laboratory experiments in this study showed a decrease in glucose along with a simultaneous increase in acidity after using Catazyme 25L. Gluconic acid was highest in Batch 2 Method where grape juice was used.

Data from the Batch 2 Method experiment showed that glucose levels dropped an average of 14.1g/L in the 1.0 GOx treatment over a 24-hour period followed closely by the 0.5 GOx treatment in which glucose levels dropped by an average of 13.1g/L in the same time interval. These drops in glucose did not show to be statistically different, table 7. Figure 7 shows a visual representation of glucose levels of each treatment in Batch 2 Method over 24 hours. There was also a slight decrease in glucose levels for the control and aeration experiments but this may be due to the onset of spontaneous fermentation.

Batch 1 data showed similar results as glucose levels dropped an average of 11.9g/L in the 1.0 GOx treatment over a 24-hour period and the 0.5 GOx treatment showed an average drop of 11.3g/L. Table 8 shows that there was no statistical difference amongst treatments for glucose levels in Batch 1. Both the control and aeration treatments also showed slight decreases in glucose level as this is a result of spontaneous fermentation. There is a great deal of variation in the control treatment for glucose levels and it is also important to note that sampling grape must is much more cumbersome than sampling grape juice. Sampling error and

grape skin contact with some of the equipment could explain the variable readings of glucose levels that are seen in figure 8.

Gluconic acid production was highest when using only grape juice. Batch 2 Method treatment 1.0 GOx showed a final average gluconic acid level of 12.6g/L compared to only 0.12g/L in the control treatment, and treatment 0.5 GOx showed a final average gluconic acid level of 10.3g/L compared to the 0.12g/L in the control treatment. Table 9 shows the statistical differences amongst treatments. Gluconic acid is not a natural acid found in grapes or wine but can be caused by the infection of certain fungi such as Botrytis or Aspergillus. Fungus infected fruit may be the best explanation for the tiny traces of gluconic acid in both the control and aeration treatments. Figure 9 shows a visual representation of the production of gluconic acid in the Batch 2 method experiment over 24.

The Batch 1 experiment showed similar results although gluconic acid production was lower most likely as a result of treating must instead of juice. Treatment 1.0 GOx still had the highest average production of gluconic acid at 7.18g/L while treatment 0.5 GOx had a final average gluconic acid level of 5.62g/L. Control and aeration treatments had tiny measurements of gluconic acid as well, and as stated before, this is likely the result of fungal infected fruit. Table 10 shows that there were statistical differences amongst treatments. Figure 10 shows a full visual representation of gluconic acid production for the Batch 1 experiment.

It is no surprise that GOx treatments for both experiments show a vast increase in gluconic acid levels, as this seems to be solid evidence that the GOx/Catalase enzyme system works to effectively oxidize glucose into gluconic acid. It is also important to remember that the

GOx/Catazyme enzyme system only works in the presence of oxygen. Glucose and gluconic acid levels was not measured for the Industrial-size experiment.

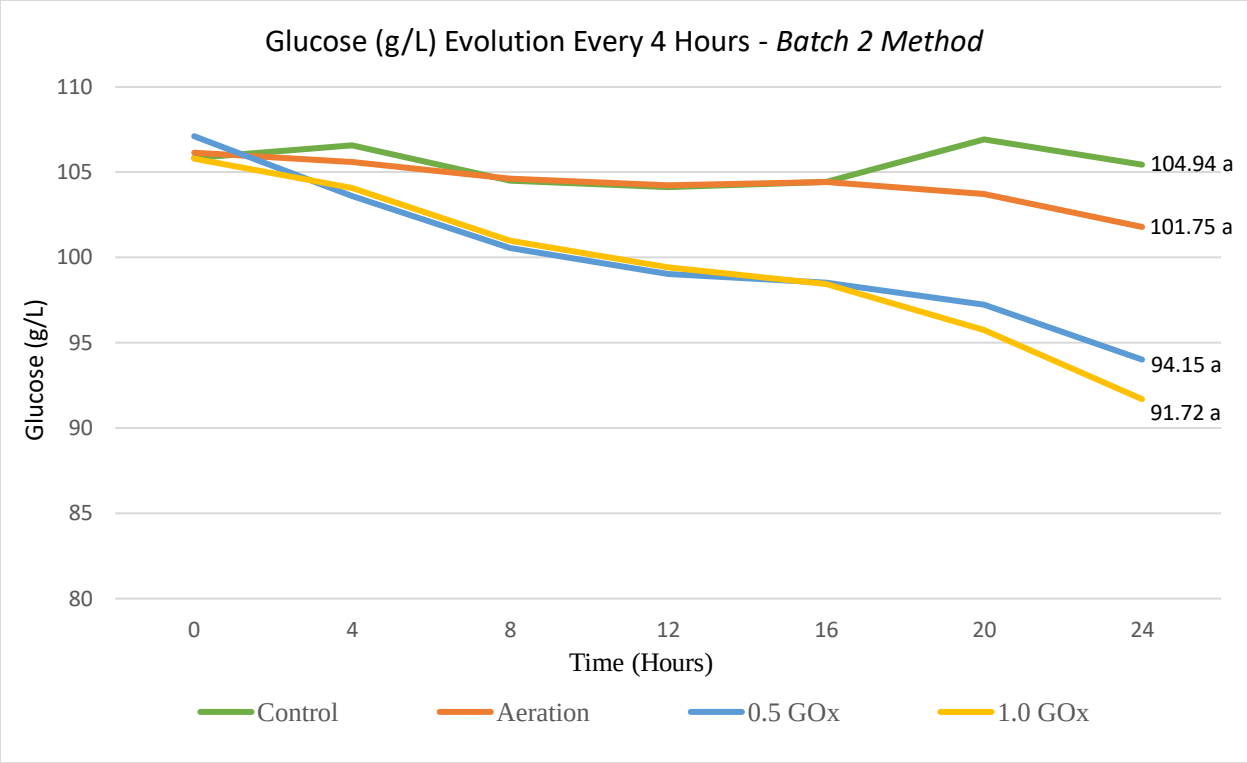

Figure 7. Glucose(g/L) of Tempranillo juice over a 24-hour period when using Catazyme 25L.

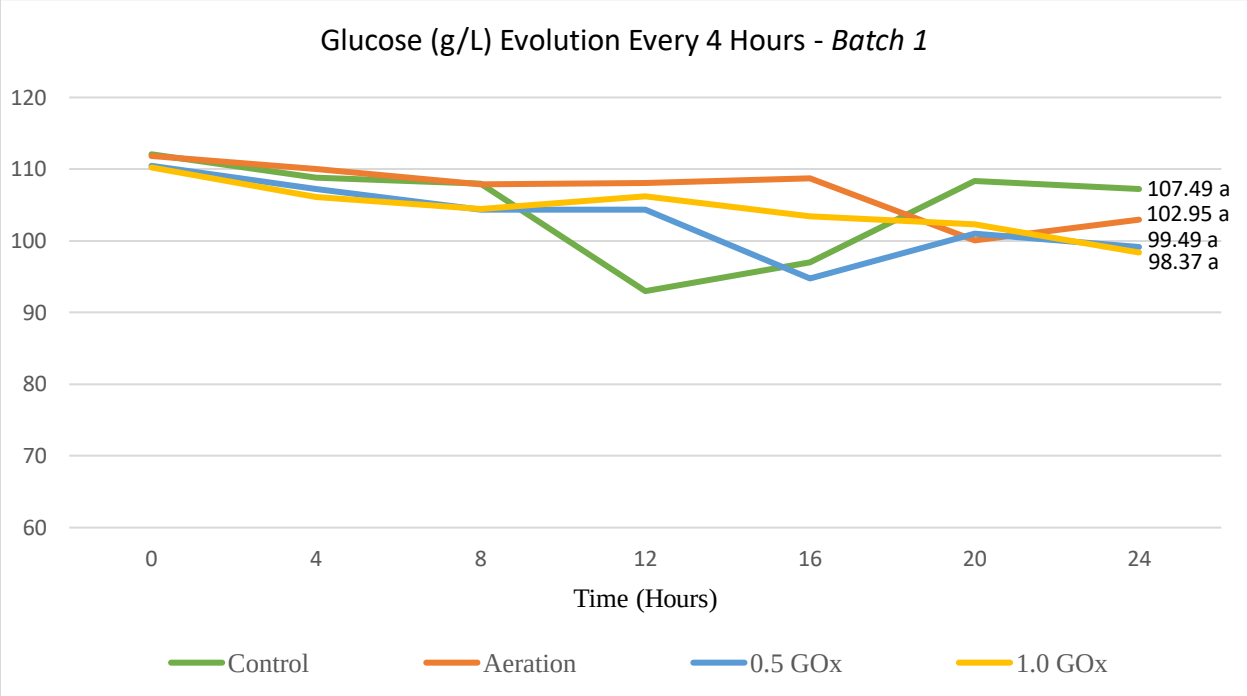

Figure 8. Glucose(g/L) of Tempranillo must over 24 hours when using Catazyme 25L

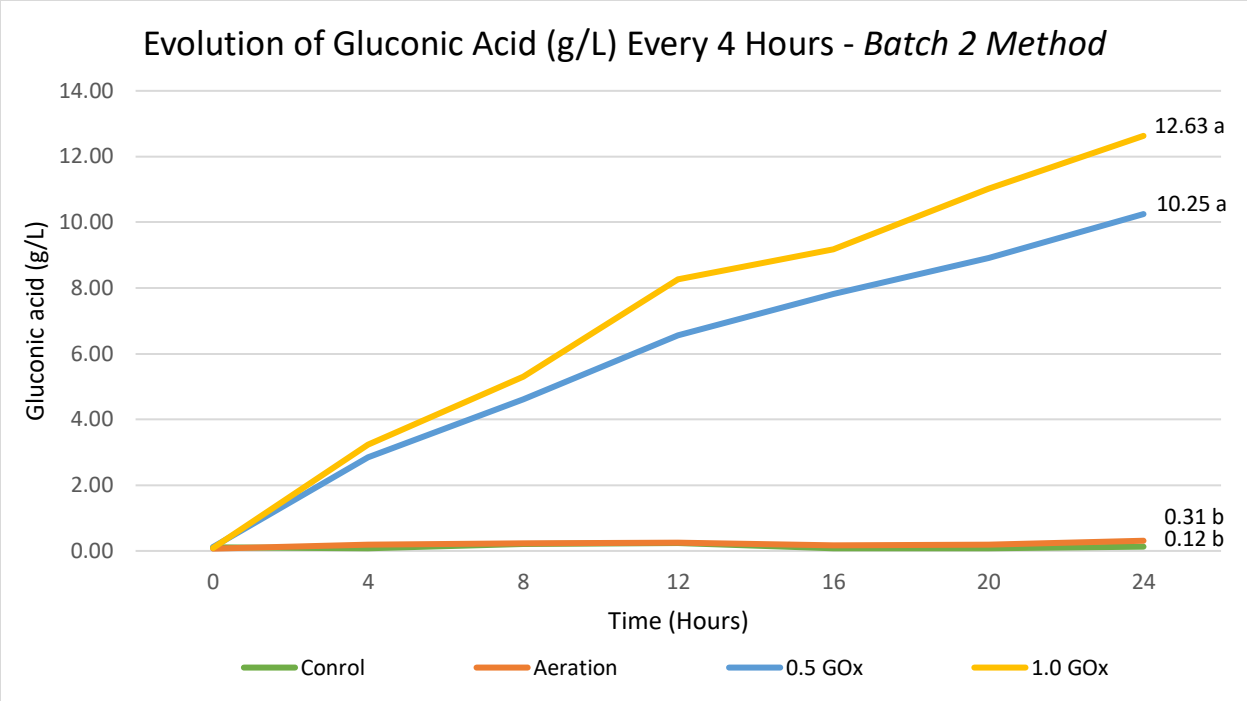

Figure 9. The production of gluconic acid(g/L) using Catazyme 25L in Tempranillo juice.

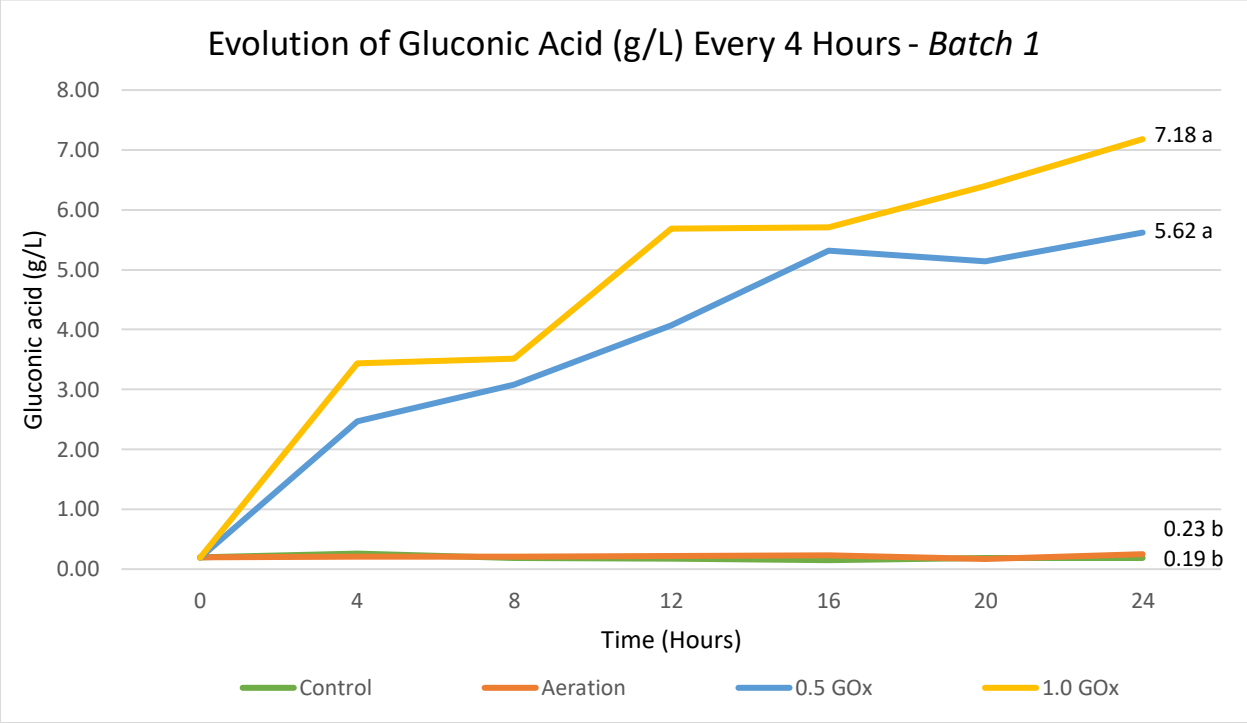

Figure 10. The production of gluconic acid(g/L) using Catazyme 25L in Tempranillo must.

Glucose – *Batch 2 Method*			
Treatment	Difference	CV	Significant?
C vs. A	0.97678571	17.63	NO
C vs. 0.5	11.765	17.63	NO
C vs. 1.0	5.88107143	17.63	NO
A vs. 0.5	10.7882143	17.63	NO
A vs. 1.0	4.90428571	17.63	NO
0.5 vs. 1.0	-5.8839286	17.63	NO

Table 7. Statistical data of glucose in Batch 2 Method. Tukey's HSD following a one-way ANOVA.

Glucose – *Batch 1*			
Treatment	Difference	CV	Significant?
C vs. A	7.69507143	31.34	NO
C vs. 0.5	18.0718571	31.34	NO
C vs. 1.0	10.3193571	31.34	NO
A vs. 0.5	10.3767857	31.34	NO
A vs. 1.0	2.62428571	31.34	NO
0.5 vs. 1.0	-7.7525	31.34	NO

Table 8. Statistical data of glucose in Batch 1. Tukey's HSD following a one-way ANOVA.

Gluconic Acid – *Batch 2 Method*			
Treatment	Difference	CV	Significant?
C vs. A	-65.504786	4077	NO
C vs. 0.5	-5738.5714	4077	YES
C vs. 1.0	-6971.1429	4077	YES
A vs. 0.5	-5673.0666	4077	YES
A vs. 1.0	-6905.6381	4077	YES
0.5 vs. 1.0	-1232.5714	4077	NO

Table 9. Statistical data of gluconic acid in Batch 2 Method. Tukey's HSD following a one-way ANOVA.

Gluconic Acid - *Batch 1*			
Treatment	Difference	CV	Significant?
C vs. A	-11.857143	2196	NO
C vs. 0.5	-3501.2857	2196	YES
C vs. 1.0	-4388.75	2196	YES
A vs. 0.5	-3489.4286	2196	YES
A vs. 1.0	-4376.8929	2196	YES
0.5 vs. 1.0	-887.46429	2196	NO

Table 10. Statistical data of gluconic acid in Batch 1. Tukey's HSD following a one-way ANOVA.

Effects of GOx on Wines After Bottle Aging

Reported in this section are parameters including pH, titratable acidity (TA), alcohol %, Free SO_2, and volatile acidity (VA) that were measured in the experimental wines after bottle aging. As mentioned earlier, pH and titratable acidity are indicators of proton concentration in wines. Alcohol percentage is the amount of ethanol alcohol present in the finished wine. Free SO_2, or free sulfur dioxide, is a common preservative added into wine to protect the wine from microbial spoilage and oxidation. The most common form of sulfur dioxide for wine preservation is known as potassium metabisulfite (KMBS) which comes in powder form. If free SO_2 levels in wine are high, then the wine is better protected from spoilage. If free SO_2 levels are low or absent, then the wine is more likely to foster acetobacter which produces acetic acid in the presence of oxygen that can spoil wine as well as other potential spoilage microorganisms. Volatile acidity is the measurement of acetic acid and is usually represented in grams per liter (g/L). Batch 1 spent three months in bottle before being analyzed for pH, TA, and alcohol %, while Batch 2 Method spent eight months in bottle before being analyzed for pH, TA, VA, alcohol %, and free SO_2. No data is shown for Industrial-size as it was not bottled.

For Batch 1, the pH, TA, and alcohol % all showed to be statistically different amongst treatments after three months of bottle aging. Table 11 shows the final average values of pH, TA, and alcohol percentage for Batch 1 Tempranillo wines. For pH, treatment 1.0 GOx had the lowest average pH at 4.01, followed by treatment 0.5 GOx at 4.11, control treatment at 4.66, and aeration treatment at 4.83. While the pH of the control and aeration treatments are outrageously high, it is not uncommon to see an average pH of 4.01 in Texas wines, as is for treatment 1.0 GOx, though a pH of 4.01 still falls outside of desired red wine pH ranges. Table

12 shows the statistical data for these treatments regarding pH. Treatment 0.5 GOx measured against treatment 1.0 GOx was not statistically different regarding pH.

Titratable acidity for Batch 1, which was not statically different when measured during the pre-fermentation experiment, did show to be completely statistically different across every treatment, table 13. Even when measuring control treatment against aeration treatment and 0.5 GOx treatment against 1.0 GOx treatment, every treatment showed to be statistically different from one another for TA post-bottling. Just as for pH, treatment 1.0 GOx had the highest average titratable acidity at 8.08(g/L), followed by treatment 0.5 GOx at 7.14(g/L), control treatment at 4.79(g/L), and aeration treatment at 3.77(g/L).

Alcohol percentage was another parameter that showed to be statically different amongst all treatments except for the control treatment against aeration treatment, table 14. Control treatment had the highest average alcohol concentration at 11.36%, followed by aeration treatment at 11.27%, treatment 0.5 GOx at 10.76%, and treatment 1.0 GOx at 10.62%. Since both GOx treatments lost glucose during the enzymatic reaction, their alcohol percentages show a decrease when compared against control and aeration treatments. Although the glucose lost by the GOx treatments did not show to be statically different at pre-fermentation, the resulting alcohol percentages were statistically different when compared to the control and aeration treatments.

For Batch 2 Method, the pH, titratable acidity, volatile acidity, alcohol percentage, and free SO_2 were all measured eight months after bottling, table 15 shows final parameter

averages. Of these parameters, volatile acidity was the only measurement that was not statically different amongst treatments.

The average pH for Batch 2 Method showed to be lowest in treatment 1.0 GOx at 3.97. Treatment 0.5 GOx had the second lowest average pH at 4.08, followed by control treatment at 4.63, and aeration treatment at 4.64. Dosing GOx into grape juice rather than grape must shows to be slightly more effective as Batch 2 treatment 1.0 GOx was 0.04 pH units lower than Batch 1 1.0 GOx treatment. Although a Tempranillo wine pH of 3.97 is still slightly high, it is exciting to see the GOx/Catalase enzyme system work to lower grape juice pH. These pH measurements all showed to be statistically different from one another except for when comparing control treatment to aeration treatment. Table 16 shows the statistical data for pH amongst Batch 2 Method treatments.

Titratable acidity for Batch 2 Method eight months post bottling revealed that treatment 1.0 GOx had the highest average TA at 8.50(g/L) followed closely by treatment 0.5 GOx with an average TA of 7.90(g/L). Control and aeration treatments had the lowest TA's with 3.60(g/L) and 4.45(g/L), respectively. Grape juice also showed to be better suited for the GOx/Catalase enzymes as treatment 1.0 GOx TA was 0.42(g/L) higher than in Batch 1 treatment 1.0 GOx. The same comparison can be made for treatment 0.5 GOx which was 0.76(g/L) higher for TA in juice and in must. Titratable acidity was statistically different eight months post bottle just as it was statistically different when measured pre-fermentation. The only statistical change was that control treatment against aeration treatment was statically different post bottle, whereas control treatment against aeration treatment was not statically different pre-fermentation. Neither 0.5 GOx or 1.0 GOx treatments were statistically different form one

another either post bottle or pre-fermentation. Table 17 shows the statistical data for titratable acidity post bottle.

When comparing alcohol percentage, it was found that control treatment had the highest alcohol concentration at 11.65%. Aeration treatment had an alcohol percentage of 11.51%, followed by treatment 0.5 GOx at 11.10%, and treatment 1.0 GOx at 10.59%. As expected, and seen in Batch 1, the treatments in Batch 2 Method that were dosed with Catazyme 25L contained less glucose sugar, thus resulted in a lower final alcohol percentage in the finished wine. Alcohol percentage was also shown to be statistically different amongst all treatments, except when comparing control treatment to aeration treatment. Table 18 shows the statistical data when comparing alcohol percentages amongst treatments.

Free SO_2 data of the wines made from the Batch 2 Method revealed that GOx treatments were less likely to hold potassium metabisulfite than the non-GOX treatments. The data shows that treatments 0.5 GOx and 1.0 GOx held average free SO_2 levels of 6.4ppm and 8.0ppm, respectively. Control treatment held an average free SO_2 level of 18.0ppm while aeration treatment held an average free SO_2 level of 26.4ppm, all eight months after bottling. As mentioned earlier, free sulfur dioxide is important to preserve wine and prevent wine from spoiling. The fact that both GOx treatments held significantly less free SO_2 is alarming. This would call for further GOx testing to see if free SO_2 levels could be maintained throughout bottle aging. Free SO_2 levels in Batch 2 Method wines were statistically different across half of the treatments. Table 19 shows statistical data for free SO_2 levels in Batch 2 Method Tempranillo wines.

Volatile acidity (VA) for Batch 2 Method wines was one parameter that was not statistically different across any treatment. The data shows that aeration treatment had the highest average levels of VA with 1.272(g/L) of acetic acid. Treatment 1.0 GOx had the next highest average VA levels with 1.206(g/L) of acetic acid. Control treatment had the next highest average VA levels with 1.119(g/L) of acetic acid, and treatment 0.5 GOx had the lowers average levels of VA with 1.002(g/L) of acetic acid. Although volatile acidity was not found to be statistically different, it is odd that aeration treatment had the highest levels of VA while also holding the highest free SO_2 level. Potassium metabisulfite, which is a common granular form of SO_2, is added to wines to help protect against the onset of acetic acid production. Table 20 shows the statistical data.

Batch 1 Tables:

Final Average Parameters - Batch 1 Wines			
	pH	**TA(g/L)**	**Alcohol %**
Control	4.66 a	4.79 a	11.36 a
Aeration	4.83 b	3.77 b	11.27 a
0.5 GOx	4.11 c	7.14 c	10.76 b
1.0 GOx	4.01 c	8.08 d	10.62 c

Table 11. Final average parameters for Batch 1 Tempranillo wines 3 months after bottling. pH, titratable acidity (g/L), and alcohol percentage.

pH - *Batch 1* - 3 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	-0.1725	0.111	YES
C vs. 0.5	0.5475	0.111	YES
C vs. 1.0	0.65	0.111	YES
A vs. 0.5	0.72	0.111	YES
A vs. 1.0	0.82	0.111	YES
0.5 vs. 1.0	0.11	0.111	NO

Table 12. Statistical data for pH in Batch 1 Tempranillo wines 3 months after bottling. Tukey's HSD following a one-way ANOVA.

TA (g/L) - *Batch 1* - 3 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	1.0275	0.745	YES
C vs. 0.5	-2.35	0.745	YES
C vs. 1.0	-3.2825	0.745	YES
A vs. 0.5	-3.3775	0.745	YES
A vs. 1.0	-4.31	0.745	YES
0.5 vs. 1.0	-0.9325	0.745	YES

Table 13. Statistical data for titratable acidity(g/L) for Batch 1 Tempranillo wines 3 months after bottling. Tukey's HSD following a one-way ANOVA.

Alcohol % - *Batch 1* - 3 Months After Bottle			
Treatment	Difference	CV	Significant?
C vs. A	0.08916667	0.11	NO
C vs. 0.5	0.59916667	0.11	YES
C vs. 1.0	0.73666667	0.11	YES
A vs. 0.5	0.51	0.11	YES
A vs. 1.0	0.6475	0.11	YES
0.5 vs. 1.0	0.1375	0.11	YES

Table 14. Statistical data for alcohol percentage for Batch 1 Tempranillo wines 3 months after bottling. Tukey's HSD following a one-way ANOVA.

Batch 2 Method Tables:

Final Average Parameters – Batch 2 Method Wines					
Wine/Treatment	**pH**	**T.A. (g/L)**	**Alcohol %**	**Free SO2**	**V.A. (g/L)**
Control	4.63 a	3.60 a	11.65 a	18.0 a/b	1.119 a
Aeration	4.64 a	4.45 b	11.51 a	26.4 a	1.272 a
0.5 GOx	4.08 b	7.90 c	11.10 b	6.4 c	1.002 a
1.0 Gox	3.97 c	8.50 d	10.59 c	8.0 b/c	1.206 a

Table 15. Final average parameters for Batch 2 Method Tempranillo wines 8 months after bottle. pH, titratable acidity (g/L), alcohol percentage, free SO_2 (ppm), and volatile acidity (g/L).

pH - *Batch 2* - 8 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	-0.0075	0.0349	NO
C vs. 0.5	0.545	0.0349	YES
C vs. 1.0	0.66	0.0349	YES
A vs. 0.5	0.5525	0.0349	YES
A vs. 1.0	0.6675	0.0349	YES
0.5 vs. 1.0	0.115	0.0349	YES

Table 16. Statistical data for pH for Batch 2 Method 8 months after bottling. Tukey's HSD following a one-way ANOVA.

TA (g/L) - *Batch 2* - 8 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	-0.85	0.603	YES
C vs. 0.5	-4.3	0.603	YES
C vs. 1.0	-4.9	0.603	YES
A vs. 0.5	-3.45	0.603	YES
A vs. 1.0	-4.1	0.603	YES
0.5 vs. 1.0	-0.6	0.603	NO

Table 17. Statistical data for titratable acidity(g/L) for Batch 2 Method 8 months post bottling. Tukey's HSD following a one-way ANOVA

Alcohol % - *Batch 2* – 8 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	0.14	0.2576	NO
C vs. 0.5	0.545	0.2576	YES
C vs. 1.0	1.055	0.2576	YES
A vs. 0.5	0.41	0.2576	YES
A vs. 1.0	0.92	0.2576	YES
0.5 vs. 1.0	0.51	0.2576	YES

Table 18. Statistical data for alcohol percentage for Batch 2 Method 8 months post bottling. Tukey's HSD following a one-way ANOVA.

Free SO_2 - *Batch 2* - 8 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	-8.4	10.874	NO
C vs. 0.5	11.6	10.874	YES
C vs. 1.0	10	10.874	NO
A vs. 0.5	20	10.874	YES
A vs. 1.0	18.4	10.874	YES
0.5 vs. 1.0	-1.6	10.874	NO

Table 19. Statistical data for free SO2(ppm) in Batch 2 Method Tempranillo wine 8 months after bottling. Tukey's HSD following a one-way ANOVA.

VA (g/L) - *Batch 2* - 8 Months Post Bottle			
Treatment	Difference	CV	Significant?
C vs. A	-0.153	1.029	NO
C vs. 0.5	0.117	1.029	NO
C vs. 1.0	-0.087	1.029	NO
A vs. 0.5	0.270	1.029	NO
A vs. 1.0	0.066	1.029	NO
0.5 vs. 1.0	-0.204	1.029	NO

Table 20. Statistical data of volatile acidity(g/L) from Batch 2 Method Tempranillo wines 8 months after bottling. Tukey's HSD following a one-way ANOVA.

Effects of GOx on Color Change

The color of red wine is attributed to the presence of polyphenols such as anthocyanins and tannins (Valencia et al, 2017) that are found in grape skins. However, when juice is pressed off of its skins shortly after harvest, there is far less of both anthocyanins and tannins in the remaining juice. Oxidation of these polyphenolic compounds makes the juice more susceptible to browning. During the Batch 2 Method experiment with Tempranillo juice, it was found that the aeration provided for the GOx/Catalase enzyme system caused the juice to turn brown during treatment. The placement of skins back into the juice during fermentation allowed for red color to reappear and eliminated the browning color that had occurred. No color change was observed during the Batch 1 experiment or the Industrial-size experiment.

Conclusion

The use of glucose oxidase with catalase was very effective at lowering the pH of grape juice and must when it's used as a pre-fermentative treatment. The titratable acidity of grape juice and must is also simultaneously increased when using glucose oxidase with catalase as a pre-fermentative treatment. Grape juice or must that contain very high sugar levels and very low titratable acidity could benefit from a pre-fermentative treatment of glucose oxidase with catalase if a higher titratable acidity was desired in the finished wine. What needs to be further investigated is how well these wines that are made using glucose oxidase with catalase hold up after bottling. If free SO_2 levels quickly diminish after bottling when using glucose oxidase with catalase, then this treatment could not be feasibly used for practical winemaking. Another variable that needs to be further explored is providing enough oxygen to larger volumes of

grape juice or must when using glucose oxidase with catalase on a larger scale. If sufficient oxygen cannot be provided in order to carry out the GOx/Catalase enzymatic reaction with larger volumes of grape juice or must, then using glucose oxidase with catalase would not be feasible for industrial winemaking. The fact that glucose sugar is consumed by the GOx and catalase enzymes is something that would need to be addressed prior to fermentation, as reduced alcohol wines will be the result. Inoculating grape juice or must simultaneously while using glucose oxidase with catalase is something that needs to be studied further. If the growth and reproduction of active yeast cells, along with daily punch-downs or pump-overs, is enough to provide sufficient oxygen for the GOx/Catalase enzyme reaction to work, then co-fermentation with glucose oxidase and catalase could be a feasible way to use this enzyme system.

A proper sensory panel should be employed to determine the wine taste, wine aroma, wine appearance, and wine color after using GOx with Catalase. Having an untrained consumer panel judge wines made from using Catazyme 25L would help determine the perceived preference of the gluconic acid produced in the wine.

CHAPTER III – CROP THINNING ON GRAPE YEILD AND WINE QUALITY

Abstract

Viticultural practices of pruning and crop thinning were conducted in four different treatments on 2019 Mourvèdre and 2019 Tempranillo grapevines in order to observe their effects of grape yield and wine quality. Treatments include: vines pruned to 2 buds and then shoot and crop thinned (2BSFT), vines pruned to 3 buds and crop thinned (3BFT), vines pruned to 2 buds only (2B), and vines pruned to 3 buds only (3B). Each treatment was harvested and vinified. Data was collected for both Mourvèdre and Tempranillo grapes at harvest and then again later 9 months after bottling the wines.

Data taken at harvest of the must composition showed that crop thinning for both varieties caused a higher accumulation of soluble solids (Brix), potassium, and a higher pH. Data form the Mourvèdre wines revealed that alcohol percentages and color were statistically different due to crop thinning, and that the onset of malolactic fermentation can increase pH post bottle, which did show be statistically different between two treatments. Tempranillo wines were also found to be statistically different for alcohol percentage and color, along with volatile acidity (VA). The pH of the Tempranillo wine increased post bottle across all treatments despite only two of the treatments going through what looks to be malolactic fermentation.

A consumer sensory panel was used to evaluate the taste, aroma, appearance, and color of the Tempranillo wines. Treatments 2BSFT was preferred for taste followed closely by treatment 2B, these two treatments were not statistically different from one another. Appearance and color for crop thinning treatments showed to be statistically preferred by panelists, aroma was not statistically preferred.

Introduction & Liturature Review

Cluster thinning is a practice used to adjust fruit yields in order to obtain balance between fruit and canopy and to achieve optimum ripeness (Goldammer, Grape Grower's Handbook, 2015). This chapter covers an experiment where Tempranillo and Mourvèdre grapevines were pruned and crop thinned to four different levels in order to investigate their resulting grape quality and yield while also observing the quality of the respective finished wines. Tempranillo and Mourvèdre grapevines where pruned and thinned to four levels: vines pruned to 2 buds and then shoot thinned and fruit thinned (2BSFT), vines pruned to 3 buds and then fruit thinned (3BFT), vine pruned to 2 buds only (2B), and vine pruned to 3 buds only (3B).

One study by (Ross, 1999) stated that lower yielding vineyards have traditionally been associated with higher quality wines, while (Chapman et al, 2004) found no effect, or no consistent effect, of grape yield on wine quality. The effects of crop thinning on wine quality is a debated topic that may even be subjective to the palette of the wine taster. Vineyard crop thinning on wine quality could be specific to certain grape varietals or certain grape species.

Materials & Methods

A mechanical harvester set at a ground speed of 3 km/hr and a shaker speed of 315 bpm was used to crop thin selected treatments. Crop thinning was done 30 days after anthesis. All treatments were harvested on September 6th, 2019 and berry must composition was measured using a Winescan (FOSS, Hilleroed, Denmark) at Texas A&M viticulture lab. After the grape must was analyzed it was then inoculated at a rate of 2.5g/gallon with Alchemy I *Saccharomyces cerevisiae* yeast and fermented to dryness at 21°C. Wines were then pressed using a bladder

press, and then racked, filtered, and bottled for sensory evaluation at a later date. Both the Tempranillo and Mourvèdre wines were bottled but only the Tempranillo wines were used in the consumer preference sensory portion of the study.

After 9 months in bottle, both Mourvèdre and Tempranillo wines were chemically analyzed to measure pH, titratable acidity (TA), alcohol percentage, free SO2, volatile acidity (VA), malic acid, lactic acid, tartaric acid, and color. The equipment used for these measurements are as follows: A Vinmetrica-SC 300 (Vinmetrica, Carlsbad, California) for pH and TA, an Anton Paar Alcolyzer (Anton Paar, Graz, Austria) for alcohol %, an SO_2 apparatus (Adams & Chittenden Scientific, Berkeley, California) using the oxidation-aspiration method with an Accuflow 5000 pump (Nitto Kohi USA, Roselle, Illinois) for free SO_2, an RD-80 cash still (Adams & Chittenden Scientific, Berkeley, California) for VA, and a Gallery Discrete Analyzer (Thermofisher Scientific, Waltham, Massachusetts) for malic acid, lactic acid, tartaric acid, and color.

Experimental Design

Crop Thinning

The experiment was performed on both Tempranillo and Mourvèdre grapevines of the 2019 vintage located in a vineyard in Brownfield, Tx. Planting density was 2700 vines per hectare with a vine spacing of 3 meters between rows and 1.2 meters between vines. The training system was bilateral cordon with 5 spurs per cordon. Four treatments were preformed to mimic viticultural practices: vines pruned to 2 buds per spur and then shoot and crop thinned (2BFT), vines pruned to 3 buds per spur and then crop thinned (3BFT), vines pruned to 2 buds

per spur only (2B), and vines pruned to 3 buds per spur only (3B). Each treatment was repeated in triplicate with twelve vines per repetition.

Vinification

For both Mourvèdre and Tempranillo, each repetition of each treatment was crushed and destemmed and separated into two fermentation replicates. Each replicate was inoculated with 2.5g/gal of Alchemy I *Saccharomyces cerevisiae* yeast and fermented to dryness at 70°F (21°C). After fermentation, each replicate was pressed off into carboys using a bladder press, followed by a 60 ppm addition of potassium metabisulfite (KMBS). Wines were then sparged with Argon gas and closed with stoppers. Wines were racked twice and then given another addition of potassium metabisulfite (KMBS) at a rate of 40 ppm before being filtered and bottled. Wines were filtered using a Buon Vino Mini Jet filter with course number 1 filter pads. Once bottled, wines were transported and stored in a chiller at 55°F (12.8°C) at Texas A&M University.

Sensory Consumer Panel

The Tempranillo 2019 wines were analyzed by an untrained sensory consumer panel for aroma, taste, appearance, and color. To distinguish between wine appearance and wine color, the appearance of the wine encompasses any haziness that may be present, the hue of the wine, the intensity of the wine color itself, and how the meniscus of the wine appearance to the consumer. Wine color simply implies the color alone. IRB 2020-1262, Effect of Cluster Thinning on Grape and Wine Quality, allowed for consumer panelist to analyze wine treatments side-by-

side so that each wine was scored in the categories of aroma, taste, appearance, and color. A scale of 1-9 was used with 1 being least preferred and 9 being most preferred. Each treatment of wine was assigned a numbered label and presented in random orders to panelist so that treatment 2BFT was not always presented first and treatment 3B was not always presented last. Wines were pulled from a 12.8°C chiller and allowed to warm up for one hour before being poured into standard ISO wine glasses at a rate of one ounce pours per treatment. Wines were then covered with watch glasses. Panelist judged aroma and taste under a red light so that the color of the wines would not influence their perception. After the panels scored the wines for aroma and taste they were then given fresh one ounce pours per treatment and asked to score the wines appearance and color under a white light.

Statistical Analysis

For the chemical data statistical analysis was performed using XLSTAT (Addinsoft, Paris, France). One-way ANOVA followed by Tukey's HSD was calculated using Mourvèdre and Tempranillo must composition data, as well as for chemical analysis data on the finished wine of each varietal. T-tests were used to generate statistical differences amongst treatments for the consumer preference data.

Results & Discussion

Data from the must composition showed that crop thinning increases accumulation of soluble solids, potassium, and pH as well. Yeast assimilable nitrogen (YAN) showed to accumulate more in Mourvèdre than Tempranillo, although crop thinning for Mourvèdre lead to less YAN accumulation than the non-crop thinned Mourvèdre treatments. Tempranillo showed to accumulate more YAN for their respected crop thinned treatments. Total yield was less for crop tinned treatments than non-crop thinned treatments for both varieties.

Mourvèdre wines held a lower pH than Tempranillo wines, but the average titratable acidity was almost identical between the two varietals. Final alcohol percentages were lower for non-crop thinned treatments for both varieties. The crop thinned treatments for both varieties also had a more intense color than the non-crop thinned treatments.

Consumer preference data from the Tempranillo wines showed that treatment 2BSFT had the highest preferred average score for taste, although it was not statistically different from treatment 2B.

Mourvèdre, 2019

Berry Must Composition at Harvest

Six points of data were chosen from the Mourvèdre must which include pH, titratable acidity (TA), soluble solids (Brix), potassium, yeast assimilable nitrogen (YAN), and total yield. Of these six data points only soluble solids (Brix) was found to be significantly different by treatment, table 22. pH, titratable acidity, and soluble solids (Brix) are the three main parameters used when decided when to harvest grapes for winemaking. Potassium is important

as it can increase pH and also affect the buffer capacity of wines. Yeast assimilable nitrogen (YAN) is important for proper yeast growth and reproduction during fermentation. Berry composition data averages at harvest for the 2019 Mourvèdre must can be viewed in table 21.

Data from the Mourvèdre must at harvest revealed Brix to be higher in crop thinned treatments than non-crop thinned treatments. Treatment 2BFST had the highest Brix average at 23.6 followed by treatment 3BFT which had an average Brix of 22.4. Treatment 3B had the next highest Brix with an average of 20.8, while treatment 2B had the lowest average Brix at 20.6. Statistical data for Brix can be seen in table 22.

For pH, the non-crop thinned treatments held a lower average pH than the crop thinned treatment. Treatment 3B had the lowest average pH at 4.00 followed by treatment 2B which had an average pH of 4.13. Treatments 3BFT and 2BSFT were both sequentially higher with an average pH of 4.17 and 4.19, respectably. Here, lower pH is associated with non-crop thinned treatments, although it was not shown to be statistically significant.

Titratable acidity did not inversely correlate with pH in Mourvèdre must, as treatment 3BFT had the highest average TA with 3.27(g/L) followed by the non-crop thinned treatment 3B at 3.13(g/L). The crop thinned treatment 2BSFT had the second lowest TA with an average of 3.07(g/L), while treatment 2B showed to have the lowest average TA at 2.97(g/L). Since both a crop thinned and non-crop thinned treatment held the highest average titratable acidities, no correlation or statistical data could be inferred to whether a crop thinned grapevine improves titratable acidity.

When comparing potassium, the crop thinned treatments accumulated the most potassium with treatments 2BSFT and 3BFT having potassium levels with an average of

2140(mg/L) and 2057(mg/L), respectively, compared to non-crop thinned treatments which accumulated lower levels of potassium with treatment 2B having an average potassium level of 1910(mg/L) and treatment 3B having the lowest average potassium level at 1697(mg/L). This is positively correlated with the pH of each treatment, table 21. Treatment 2BSFT, which had the highest pH, also had the highest potassium levels. Similarly, treatment 3B, which had the lowest pH, also had the lowest potassium level.

Yeast assimilable nitrogen (YAN) was lowest in crop thinned treatments with treatments 2BSFT and 3BFT having an average YAN of 261(mg/L) and 252(mg/L), respectively. The non-crop thinned treatments 2B and 3B had higher average YAN levels of 287(mg/L) and 274(mg/L). YAN accumulation in Mourvèdre treatments was not statistically significant. It should also be noted that higher YAN levels were recorded in crop thinned treatments for the Tempranillo trials. Total yield was lowest for the crop thinned treatments as treatment 2BSFT had the lowest yield weight of 71.3 pounds followed by to treatment 3BFT which had a total yield of 88.7 pounds. Treatment 2B and 3B had total yield weights of 111.1 pounds and 114.1 pounds, respectfully. No statistical data is present for total yield.

Wine Composition

The wines made from the four Mourvèdre treatments were chemically analyzed nine months after bottling. Parameters measured include pH, titratable acidity, alcohol percentage, free SO2, volatile acidity, malic acid, lactic acid, tartaric acid, and color. The pH, titratable acidity, alcohol percentage, and color all showed to be statistically different between at least

two treatments. Table 23 shows parameter averages of each treatment for the Mourvèdre wines.

The pH of the finished wines was an interesting phenomenon which did not correlate with the pH of the must composition at harvest. The crop thinned treatments possessed both the highest and lowers pH after bottling with treatment 3BFT having the lowest average pH of 4.15 while treatment 2BSFT had the highest average pH of 4.40. These two treatments showed to be the only treatments that were statistical different amongst all Mourvèdre treatments regarding pH. The non-crop thinned treatments of 3B and 2B held an average pH of 4.20 and 4.24, respectively. Table 24 shows statistical data for pH.

Titratable acidity for the Mourvèdre wines were highest in treatment 3B with an average TA of 4.85(g/L) followed by treatment 3BFT with an average TA of 4.10(g/L). Treatment 2BSFT had the next highest average TA at 3.75(g/L) and treatment 2B had the lowest average TA of 3.48(g/L). The only statistical difference in TA was between treatments 3B and 2B, see table 25.

Alcohol percentage varied greatly amongst treatments and were statically different from one another, except between treatments 2B and 3B. This was expected as Brix levels directly translates into final alcohol percentage. Treatment 2BSFT had the highest average alcohol at 13.10% followed closely by treatment 3BFT which had an average alcohol of 12.01%. These two treatments were statically different from one another. The non-crop thinned treatments of 2B and 3B had average alcohol percentages of 10.81% and 10.72%, respectively, and were not statically different from one another. Table 26 shows statical differences by treatment.

The color of the wines shown to be statistically different when comparing crop thinned treatments against non-crop thinned treatment, see table 27. A numeric system was used where a higher number indicates a redder color, generated by a Gallery Analyzer. Treatment 3BFT, followed very closely by treatment 2BSFT, had red wine color averages of 19.5au and 19.4au, respectively. Treatments 2BFT and 3BFT both had a deeper, redder color hue, while treatments 2B and 3B had a paler brick-red color. The average red color for treatments 2B and 3B were 12.8au and 11.7au, respectively.

As for malic acid, lactic acid, and tartaric acid, there was not statistical difference for these parameters amongst treatment. Statistical data for Free SO_2 and volatile acidy was not generated for lack of sufficient repetition. Table 23 shows the overall averages of all Mourvèdre parameters analyzed after bottling. Please keep in mind that Free SO_2 and VA has few counts when averages were calculated.

Tempranillo, 2019

Berry Must Composition at Harvest

The same six data points where chosen in the Tempranillo must which were pH, titratable acidity (TA), soluble solids (Brix), potassium, yeast assimilable nitrogen (YAN), and total yield. Soluble solids (Brix), pH, and potassium were all found to be statically different by treatment for the Tempranillo must at harvest time, tables 29, 30, & 31. The data also revealed Brix, pH, potassium, and YAN to be higher in crop thinned treatments than non-crop thinned treatments. Table 28 shows the treatment averages of the must composition at harvest.

For Brix, the crop thinned treatments of 2BSFT and 3BFT both shared the highest Brix averages with 23.7 a piece. Treatments 2B and 3B had average Brix levels of 19.83 and 18.60, respectively. These Brix averages would suggest a direct relationship between crop thinning and sugar accumulation, which was also the case in the Mourvèdre treatments. The Brix of the Tempranillo must was statistically different when comparing crop thinning treatments to non-crop thinned treatments, see table 29.

Tempranillo must pH was higher in crop thinned treatments with 2BSFT and 3BFT having an average pH of 4.00 and 4.07, respectively, while the non-crop thinned treatments of 2B and 3B both shared the same lowest average pH of 3.84. Higher average pH levels in the Tempranillo grape must was associated with crop thinning treatments, as was the same case with Mourvèdre must. pH was shown to be statistically different in Tempranillo when comparing crop thinned treatments against non-crop thinned treatments as seen in table 30.

Titratable acidity varied by treatment with 2B having the highest average TA at 3.10(g/L) and treatment 3BFT having the lowest average TA at 2.77(g/L). Treatments 2BSFT and 3B, fell in

between with TA's of 2.93(g/L) & 2.87(g/L), respectively. No statistical differences were found for titratable acidity nor did crop thinning seem to influence the titratable acidity of the grape must at harvest, when compared to pH, the average TA's for all treatments did not follow the common inverse relationship that pH and TA usually have. This was also observed in the Mourvèdre treatments.

Potassium accumulation was highest in the crop thinned treatments than in the non-crop thinned treatments. Treatment 3BFT had the highest average potassium at 2030(mg/L) followed by treatment 2BSFT with an average potassium of 1903(mg/L) The non-crop thinned treatments of 2B and 3B had the lowest average levels of potassium at 1553(mg/L) and 1360(mg/L), respectively. Potassium did show to be statically different when comparing crop thinned treatments to non-crop thinned treatments, see table 31.

Yeast assimilable nitrogen (YAN) was highest in Tempranillo crop thinned treatments with treatments 2BSFT and 3BFT having average YAN levels of 205(mg/L) and 193(mg/L), respectively. Treatments 3B and 2B had average YAN levels of 177(mg/L) and 174(mg/L), respectively. YAN accumulation did not show to be statistically different by treatment for Tempranillo. Interestingly, the average YAN accumulation levels for the Tempranillo vineyard treatments were found to be opposite of those in the Mourvèdre treatments. For Tempranillo, more YAN showed to accumulate in crop thinned treatments, whereas in Mourvèdre more YAN showed to accumulate in non-crop thinned treatments.

Total yield was lowest in the crop thinned treatments. Treatment 3BFT had the lowest total yield with 64.7 pounds, the other crop thinned treatment of 2BSFT had a total yield of

113.8 pounds. Treatment 2B and 3B had the highest total yield of 181.2 pounds and 171.7 pounds. There is no statistical data for Tempranillo berry weight.

Wine Composition

The wines made from the four Tempranillo treatments were chemically analyzed nine months after bottling. Parameters measured include pH, titratable acidity (TA), alcohol percentage, free SO_2, volatile acidity (VA), malic acid, lactic acid, tartaric acid, and color. Of these parameters, alcohol percentage, color, and volatile acidity (VA) were found to be statistically different when comparing at least two treatments. Table 32 shows parameter averages for each treatment of the Tempranillo wines.

The average pH of the finished wines after bottling was found to be lowest in treatment 3B at 4.30. The second lowest average pH was found in treatment 2BSFT at 4.39, followed by treatment 2B with an average pH of 4.49. Treatment 3BFT had the highest average pH at 4.59. Every treatment, except for treatment 2B, correlated its average wine pH with its average must pH at harvest. No statistical differences were found for final wine pH.

Titratable acidity (TA) had increased across all treatments from must composition to final wine analysis after bottle aging. The crop thinned treatments displayed higher TA averages, although this was not found to be statistically different. Treatment 2BSFT had the highest average titratable acidity of 4.80(g/L) followed closely by treatment 3BFT with an average TA of 4.32(g/L). Treatment 3B had next highest average TA at 3.82(g/L), and treatment 2B had the lowest average TA at 3.23(g/L).

Alcohol percentages were highest in treatments 3BFT and 2BSFT with averages of 13.9% and 13.4%, respectively. These two treatments were not statistically different from one another; however, alcohol percentages did show to be statistically different when comparing crop thinned treatments to non-crop thinned treatments. Treatments 2B and 3B had the lowest average alcohol percentages with 10.0% and 8.9%, respectively. Statistical data can be view in table 33.

Average free SO_2 levels in the finished wines were found to be highest in treatment 2B at 11.2(ppm) followed by treatment 2BSFT with an average free SO_2 of 8.8(ppm. Treatment 3B was next with an average free SO_2 level of 8.3(ppm), and treatment 3BFT had the lowest average free SO^2 level of 2.7(ppm). These free SO_2 values did not show to be statistically different by treatment.

Volatile acidity (VA) did show to be statistically different but only between treatments 2BSFT and 3B, see table 34. Treatment 2BSFT had the lowest average VA at 0.611(g/L), whereas treatment 3B had the highest average VA at 1.197(g/L). Treatments 2B and 3BFT had average VA levels of 0.959(g/L) and 1.005(g/L), respectively.

The color of the wines showed to be statistically different when comparing crop thinned treatments against non-crop thinned treatments, table 35. A numeric system was used where a higher number indicates a redder color, generated by a Gallery Analyzer. Treatments 3BFT and 2BSFT had a redder color average of 31.1au and 27.7au, whereas treatments 2B and 3B had a less red color average of 12.2au and 12.0au. Treatments 3BFT and 2BSFT both held a deeper red color compared to the treatments 2B and 3B which held a more brick-red color. Figures 11, 12, 13, and 14 show color photographs of these wines.

Consumer Preference

One hundred and one consumer panelist blindly judged and scored each treatment of the Tempranillo wine in categories of aroma, taste, appearance, and color. A scale of 1-9 was used to score wines in each category with 1 being least preferred and 9 being most preferred. Table 36 shows consumer preference average by wine treatment and category. T-tests were used to generate any possible statistical differences amongst treatments, table 37.

For wine aroma, the data did not reveal any statistical differences. Treatment 2BSFT had the highest average score of 5.73 followed closely by treatment 2B with an average score of 5.70. Treatments 3B and 3BFT had average preference scores of 5.60 and 5.46, respectively.

For wine taste, treatment 2BSFT had the highest preference average score of 5.06 while treatment 3BFT had the lowest preference average score of 4.35. Treatment 2B and 3B had average taste preference scores of 4.82 and 4.40, respectively. There was statical differences between treatment 2BSFT and 3BFT and then again between treatments 2BSFT and 3B. This would imply that the taste of 2BSFT was significantly favored over the others. There were no other statistical differences amongst treatments. Table 37 shows the statistical data.

Appearance of the wines and color of the wines were both scored in the same order of preference for all of the treatments. To clarify, wine appearance encompasses the hue of the wine, any haziness that may be associated with the wine, intensity of the color itself, and how the edge of the meniscus appears. The color for the wine simply deals with the wine color alone (i.e., Ruby Red, Brick Red, Brown). Treatment 3BFT was most preferred for appearance and color followed by 2BSFT, 3B, and 2B. The appearance of the wines was all statistically different except between treatments 2B and 3B which scored the lowest for appearance. Color

preference was statistically different when comparing crop thinned treatments to non-crop thinned treatments only. Treatments 2BSFT and 3BFT were not statistically different by color just as treatments 2B and 3B were not statistically different for color.

Conclusion

The must composition at harvest for both Mourvèdre and Tempranillo shows a strong correlation between crop thinning and more sugar accumulation in the berries. Higher sugar accumulation in the berry directly translates into higher potential alcohol in the finished wine.

The pH for both Mourvèdre and Tempranillo musts at harvest would suggest that higher pH is associated with crop thinning, as this was shown to be statistically different in the Tempranillo experiment. However, the pH of the musts at the time of harvest did not directly correlate with the pH of the finished wines after nine months of bottle aging.

Titratable acidity (TA) did not show any correlation to crop thinning, nor was TA significantly different at the time of harvest. This was also the case for the finished wine after bottle aging.

Alcohol percentage in the finished wines directly correlated to the Brix levels in the musts at harvest. This is a well understood correlation as yeast convert sugar into ethanol during fermentation.

Potassium, like Brix and pH, accumulated more the crop thinning treatments for both the Mourvèdre and Tempranillo experiments. Potassium accumulation was statistically different in the Tempranillo musts at harvest but not for the Mourvèdre.

Yeast assimilable nitrogen (YAN) was an interesting phenomenon as Mourvèdre crop thinned treatments showed to have less YAN accumulation than the non-crop thinned

treatments, although this was not statistically different. However, for the Tempranillo must at harvest, the crop thinned treatments showed a greater accumulation of YAN than the non-crop thinned treatments, although this too was not statistically different. It is possible that YAN accumulation could be grape variety and/or rootstock dependent. Further experimentation would need to occur in order to observe the effects of YAN accumulation.

There was little variation, and fewer statistical differences, in the finished wines of both Mourvèdre and Tempranillo regarding pH, TA, VA, free SO_2, malic acid, lactic acid, and tartaric acid. Alcohol percentage and color showed to substantially influenced by vineyard treatment as these two parameters had large average variations and great statistical differences.

Consumers seems to prefer the taste of treatment 2BSFT over any other treatments for the Tempranillo wines, even though treatment 2BSFT and treatment 2B were not statically different regarding taste, table 37. Both crop thinned treatments of 2BSFT and 3BFT also scored higher by consumers for wine appearance and wine color as well.

2019 MOURVÈDRE MUST AVERAGES AT HARVEST

Treatment	pH	TA(g/L)	Brix	Potassium(mg/L)	YAN(mg/L)	Total Yield (lbs.)
Blue (2BSFT)	4.19	3.07	23.6	2140	261	71.28
Green(3BFT)	4.17	3.27	22.4	2057	252	88.72
Orange (2B)	4.13	2.97	20.8	1910	287	111.13
Yellow (3B)	4.01	3.13	20.6	1697	274	114.10

Table 21. pH, titratable acidity (TA), Brix, potassium, yeast assimilable nitrogen (YAN), and total yield (lbs.) of 2019 Mourvèdre must at harvest.

Brix - Mourvèdre, 2019 - Must Composition

Treatment	Difference	CV	Significant?
2BSFT/3BFT	1.2	2.856	NO
2BSFT/2B	2.8	2.856	NO
2BSFT/3B	3.0	2.856	YES
3BFT/2B	1.6	2.856	NO
3BFT/3B	1.8	2.856	NO
2B/3B	0.2	2.856	NO

Table 22. Statistical data of pH for 2019 Mourvèdre must at harvest. Tukey's HSD following a one-way ANOVA.

2019 MOURVÈDRE WINE PARAMETER AVERAGES BY TREATMENT

Treatment:	pH	TA (g/L)	Alcohol %	Free SO2	VA (g/L)	Malic Acid (g/L)	Lactic Acid (mg/L)	Tartaric Acid (g/L)	Color (au)
Blue(2BSFT)	4.40	3.75	13.70	22.5	0.885	0.053	985.33	1.378	19.4
Green(3BFT)	4.15	4.10	12.01	6.8	0.734	0.555	556.33	1.328	19.5
Orange (2B)	4.24	3.48	10.81	8.53	0.565	0.020	817.83	1.255	12.8
Yellow (3B)	4.20	4.85	10.72	15.73	0.627	0.013	739.25	1.393	11.7

Table 23. Mourvèdre wine analysis averages for pH, titratable acidity (TA), alcohol percentage, free sulfur dioxide (ppm), volatile acidity, malic acid, lactic acid, tartaric acid, and color 9 months post bottle.

pH - Mourvèdre, 2019 - Post Bottle

Treatment	Difference	CV	Significant?
2BSFT/3BFT	0.255	0.208	YES
2BSFT/2B	0.1641	0.208	NO
2BSFT/3B	0.2066	0.208	NO
3BFT/2B	-0.0908	0.208	NO
3BFT/3B	-0.0483	0.208	NO
2B/3B	0.0425	0.208	NO

Table 24. Statistical data of pH in 2019 Mourvèdre wine 9 months after bottling. Tukey's HSD following a one-way ANOVA

Titratable Acidity - *Mourvèdre, 2019* - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	-0.3833	1.326	NO
2BSFT/2B	0.266	1.326	NO
2BSFT/3B	-1.1	1.326	NO
3BFT/2B	0.65	1.326	NO
3BFT/3B	-0.7166	1.326	NO
2B/3B	-1.366	1.326	YES

Table 25. Statistical data for 2019 Mourvèdre wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

Alcohol % - *Mourvèdre, 2019* - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	1.685	1.138	YES
2BSFT/2B	2.884	1.138	YES
2BSFT/3B	2.975	1.138	YES
3BFT/2B	1.199	1.138	YES
3BFT/3B	1.29	1.138	YES
2B/3B	0.9	1.138	NO

Table 26. Statistical data for alcohol percentage in 2019 Mourvèdre wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

Color - Mourvèdre, 2019 - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	-0.0833	5.693	NO
2BSFT/2B	6.5833	5.693	YES
2BSFT/3B	7.75	5.693	YES
3BFT/2B	6.667	5.693	YES
3BFT/3B	7.833	5.693	YES
2B/3B	1.166	5.693	NO

Table 27. Statistical data of color for 2019 Mourvèdre wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

2019 TEMPRANILLO MUST AVERAGES AT HARVEST

Treatment	pH	TA(g/L)	Brix	Potassium(mg/L)	YAN(mg/L)	Total Yield (lbs.)
Blue (2BSFT)	4.00	2.93	23.7	1903	205	113.78
Green(3BFT)	4.07	2.77	23.7	2030	193	64.65
Orange (2B)	3.84	3.10	19.8	1553	174	181.18
Yellow (3B)	3.84	2.87	18.6	1360	177	171.65

Table 28. pH, titratable acidity (TA), Brix, potassium, yeast assimilable nitrogen (YAN), and total yield (lbs.) of 2019 Tempranillo must at harvest.

Brix - *Tempranillo, 2019* - Must Composition

Treatment	Difference	CV	Significant?
2BSFT/3BFT	0.0	1.946	NO
2BSFT/2B	3.9	1.946	YES
2BSFT/3BFT	5.1	1.946	YES
3BFT/2B	3.8	1.946	YES
3BFT/3B	5.1	1.946	YES
2B/3B	1.2	1.946	NO

Table 29. Statistical data of Brix in 2019 Tempranillo must at harvest. Tukey's HSD following a one-way ANOVA.

pH - *Tempranillo, 2019* - Must Composition

Treatment	Difference	CV	Significant?
2BSFT/3BFT	-0.07	0.0769	NO
2BSFT/2B	0.1566	0.0769	YES
2BSFT/3BFT	0.1633	0.0769	YES
3BFT/2B	0.2266	0.0769	YES
3BFT/3B	0.2333	0.0769	YES
2B/3B	0.006	0.0769	NO

Table 30. Statistical data of Brix in 2019 Tempranillo must at harvest. Tukey's HSD following a one-way ANOVA.

Potassium - *Tempranillo, 2019* - Must Composition

Treatment	Difference	CV	Significant?
2BSFT/3BFT	-126.67	195.09	NO
2BSFT/2B	350	195.09	YES
2BSFT/3BFT	563.33	195.09	YES
3BFT/2B	476.67	195.09	YES
3BFT/3B	690	195.09	YES
2B/3B	213.33	195.09	YES

Table 31. Statistical data of potassium in 2019 Tempranillo must at harvest. Tukey's HSD following a one-way ANOVA.

TEMPRANILLO WINE PARAMETER AVERAGES BY TREATMENT									
Treatment:	pH	TA (g/L)	Alcohol %	Free SO2	VA (g/L)	Malic Acid (g/L)	Lactic Acid (mg/L)	Tartaric Acid (g/L)	Color
Blue(2BSFT)	4.39	4.80	13.37	8.8	0.611	0.516	776.25	1.798	27.7
Green(3BFT)	4.59	4.32	13.88	2.7	1.005	1.819	272.50	1.715	31.1
Orange (2B)	4.49	3.23	10.00	11.2	0.959	0.317	836.75	1.616	12.2
Yellow (3B)	4.30	3.82	8.97	8.3	1.197	1.148	302.08	1.549	12.0

Table 32. Tempranillo wine analysis averages for pH, titratable acidity (TA), alcohol percentage, free sulfur dioxide (ppm), volatile acidity, malic acid, lactic acid, tartaric acid, and color 9 months post bottle.

Alcohol % - *Tempranillo, 2019 Wine* - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	-0.509	1.507	NO
2BSFT/2B	3.3675	1.507	YES
2BSFT/3	4.4	1.507	YES
3BFT/2B	3.8766	1.507	YES
3BFT/3B	4.91	1.507	YES
2B/3B	1.0333	1.507	NO

Table 33. Statistical data of alcohol percentage in 2019 Tempranillo wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

VA - Tempranillo, 2019 Wine - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	-0.266	0.376	NO
2BSFT/2B	-0.348	0.376	NO
2BSFT/3B	-0.586	0.376	YES
3BFT/2B	-0.082	0.376	NO
3BFT/3B	-0.32	0.376	NO
2B/3B	-0.238	0.376	NO

Table 34. Statistical data of volatile acidity (VA) in 2019 Tempranillo wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

Color - *Tempranillo, 2019 Wine* - Post Bottle			
Treatment	Difference	CV	Significant?
2BSFT/3BFT	-3.4166	6.75	NO
2BSFT/2B	15.5	6.75	YES
2BSFT/3	15.667	6.75	YES
3BFT/2B	18.916	6.75	YES
3BFT/3B	19.083	6.75	YES
2B/3B	0.1667	6.75	NO

Table 35. Statistical data for color in 2019 Tempranillo wines 9 months after bottling. Tukey's HSD following a one-way ANOVA.

CONSUMER PREFERENCE AVERAGE SCORES OF 2019 TEMPRANILLO WINES

AROMA		TASTE		APPEARANCE		COLOR	
Treatment	**Mean**	**Treatment**	**Mean**	**Treatment**	**Mean**	**Treatment**	**Mean**
2BSFT	5.73	2BSFT	5.06	2BSFT	6.64	2BSFT	6.86
3BFT	5.64	3BFT	4.35	3BFT	7.14	3BFT	7.28
2B	5.70	2B	4.82	2B	5.00	2B	4.95
3B	5.60	3B	4.40	3B	5.49	3B	5.39

Table 36. Consumer preference average scores (scale of 1-9) for wine aroma, wine taste, wine appearance, and wine color in 2019 Tempranillo wines.

T-TEST AMONGST TREATMENTS FOR 2019 TEMPRANILLO WINES

AROMA		TASTE		APPEARANCE		COLOR	
T-test	**p-value**	**T-test**	**p-value**	**T-test**	**p-value**	**T-test**	**p-value**
2BSFT/3BFT	0.32117641	2BSFT/3BFT	0.02390401	2BSFT/3BFT	0.032073615	2BSFT/3BFT	0.054871553
2BSFT/2B	0.9133612	2BSFT/2B	0.41662585	2BSFT/2B	2.85126E-09	2BSFT/2B	3.61011E-12
2BSFT/3B	0.64028878	2BSFT/3B	0.0268304	2BSFT/3B	1.46375E-05	2BSFT/3B	2.59241E-08
3BFT/2B	0.36547284	3BFT/2B	0.11526558	3BFT/2B	2.06893E-15	3BFT/2B	3.14897E-17
3BFT/3B	0.59009976	3BFT/3B	0.84916807	3BFT/3B	1.43518E-10	3BFT/3B	8.61918E-13
2B/3B	0.71328809	2B/3B	0.13744578	2B/3B	0.081845023	2B/3B	0.128486009

Table 37. T-tests amongst treatments for wine aroma, wine taste, wine appearance, and wine color for 2019 Tempranillo wines. P-values less than 0.05 signify a statistical difference between treatments.

PHOTOGRAPHS OF THE 2019 TEMPRANILLO WINES

Figure 11. (2BSFT)

Figure 12. (3BFT)

Figure 13. (2B)

Figure 14. (3B)

Figure 11. 30mL of 2019 Tempranillo wine from treatment 2BSFT.

Figure 12. 30mL of 2019 Tempranillo wine from treatment 3BFT.

Figure 13. 30mL of 2019 Tempranillo wine from treatment 2B.

Figure 14. 30mL of 2019 Tempranillo wine from treatment 3B.

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APPENDIX A

Appendix A. Below is the two-page consumer ballot that was used to score Tempranillo wines for consumer panelists.

Date _____ Respondent Number

Session _____ **Sample Number** _____

Please take a bite of cracker followed by a sip of water prior to evaluating the wine sample.

Place a mark in the box that represents your answer for each of the following questions.

1. How much do you **like or dislike** the OVERALL APPEARANCE of this wine?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

2. How much do you **like or dislike** the COLOR of this wine?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

Date _____
Session _____

Respondent Number
Sample Number _____

3. How much do you **like or dislike** the OVERALL AROMA [smell] of this wine?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

4. How much do you **like or dislike** the OVERALL FLAVOR [taste] of this wine?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

5. How much do you **like or dislike** this wine OVERALL?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely — Neither Like or Dislike — Like Extremely

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