STRATEGIES FOR INCREASING THE RELEASE OF PIGMENTS IN RED WINE

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ABSTRACT

Strategies for Increasing the Release of Pigments in Red Wine

Briana Heywood

The perception of wine's quality is directly influenced by its color. Anthocyanin molecules are responsible for imparting color to red wines. They are extracted from grape skins during alcoholic fermentation. This work compares the effects of three parameters: berry integrity, enzyme addition, and fermentation temperature, on phenolic compound extraction (total phenol, tannin and anthocyanin) during the production of Paso Robles' Cabernet Sauvignon wine. Analyses on phenolic compounds were completed during alcoholic fermentation and barrel aging over the course of eighteen months. Berry integrity compared the degree of berry crushing (whole destemmed berries versus fully crushed berries). Results showed that phenolic compound content after alcoholic fermentation seem to be unaffected by this parameter, while minor increases in total phenol concentration (3%) and tannin concentration (3%) during barrel aging were observed. Adding pectinase-rich macerating enzymes increased the total phenols by 8.7 and 21.0% to the 2010 and 2011 vintage, respectively, and tannin concentrations by 20.8 and 48.8%, respectively, during barrel aging. Alcoholic fermentation temperature of 25.0°C was compared to a fermentation temperature of 32.2°C in the 2011 vintage. When fermented at 32.2°C, concentrations of total phenol and tannin were significantly increased (20.6% and 28.9%, for the 2010 and 2011 vintages, respectively) when compared to 25.0°C. A cooler fermentation temperature led to 57.5% greater anthocyanin concentration throughout barrel aging. The results suggested that fermenting berries at a cooler temperature (25.0°C) increased anthocyanin levels and decreased total phenol and tannin concentration, which are desired outcomes for Paso Robles' Cabernet Sauvignon wine quality.

Keywords: Anthocyanin, tannin, total phenol, Adams-Harbertson assay, extraction, maceration, fermentation, red wine, enzyme, temperature

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

1.1 Importance of the project

The perception of a wines' quality is directly influenced by its color (Bichescu et al. 2013, Escott et al. 2016, Morrot et al. 2001). Grapes sourced from the Paso Robles American Viticultural Area (AVA) contain ample amounts of tannin. The struggle winemakers' have is extracting anthocyanin molecules. Anthocyanins are monomers responsible for providing color to red wine. One way for color to remain stable in wine is to form polymeric pigments. Precipitation and degradation of anthocyanin during fermentation and aging can be avoided when they complex with tannins. A typical red berry alcoholic fermentation begins with great color and tannin extraction. The more anthocyanin and tannin extracted from the berry skin during the maceration process, the greater the opportunity for complexing reactions between anthocyanin and tannin. Polymeric pigments are formed when a monomeric anthocyanin molecule binds with a tannin, creating a polymeric pigment resistant to precipitation.

Winemakers could maximize color extraction during alcoholic fermentation by promoting anthocyanin extraction. When red berries are harvested, they are left in contact with skins to maximize extraction of anthocyanin, tannin, flavan-3-ols, and various acids and polyphenolics (Fig 1).

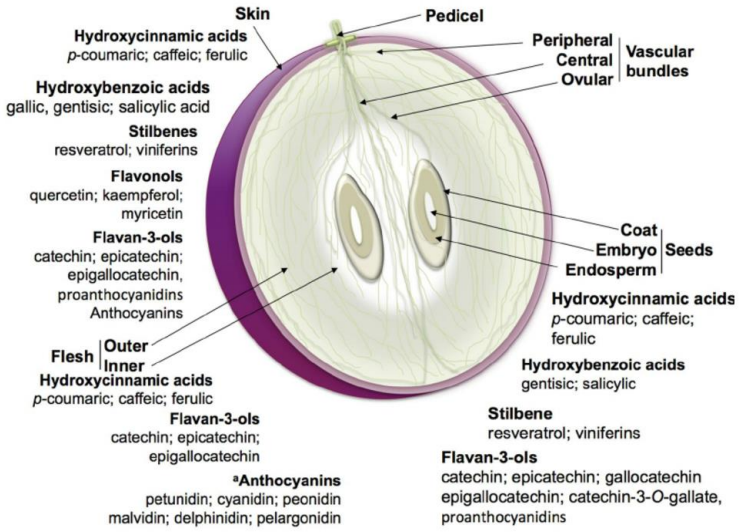

Figure 1. Structure of a ripe berry and pattern phenolics biosynthesis distribution between several organs and tissues. Copyrighted with permission by Teixeira et al. 2013.

Alcoholic fermentation typically lasts one to two weeks where skin and juice contact is encouraged by punch downs and pump overs (Section 3.5). Glucose and fructose, the native sugars found in berries, are converted by yeast to produce ethanol. Once the sugars are consumed, the wine is pressed off the skins, and additional color extraction after this step is not possible. By encouraging more anthocyanin extraction from berry skins, a deeper, darker wine can be obtained. Wines containing more color saturation are associated with higher quality (Escott et al. 2016, Morrot et al. 2001). The objective of this research was to identify extraction processes that can be applied pre-fermentation or during alcoholic fermentation. Fermentation treatments included fermenting crushed versus destemmed berries, adding pectinase-rich macerating enzymes, and cool versus hot fermentation temperatures in efforts to impact pigment release and anthocyanin stabilization (Bichescu et al. 2013, Escott et al. 2016).

1.2 Statement of hypotheses

Commercial pectinase-rich enzyme preparations have shown to increase anthocyanin and phenolic extraction during alcoholic fermentation of red berries. The increased extraction of anthocyanin monomers will complex with tannin to form stable polymeric pigments. This greater anthocyanin concentration would be persistent through aging in barrel. Anthocyanin extraction would increase according to the concentration of enzyme preparation.

Fermenting at cooler temperatures (25.0°C) will lead to greater anthocyanin extraction during alcoholic fermentation of red berries when compared to a hot fermentation temperature (32.2°C). When anthocyanin molecules are exposed to high temperatures, color may decrease quickly and irreversibly by degradation of monomeric anthocyanin molecules (Hillmann et al. 2011). Anthocyanin extraction would increase at lower fermentation temperatures and be persistent through maturation in barrel.

Crushed berries will have greater extraction of anthocyanin versus whole destemmed berries due to the increase of exposed surface. Anthocyanin molecules, which are located in the skin layer, will be more easily extracted from berries that have been crushed and destemmed. The increased extraction of anthocyanin and tannin molecules will complex to form stable polymeric pigments that will not precipitate. Anthocyanin extraction will be persistently greater in berries that have been crushed and destemmed throughout barrel aging.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of red winemaking

In a typical fermentation, red berries are crushed and destemmed, adjusted with acid, pumped into fermentation tanks, inoculated with yeast and nutrients, pressed off their skins, inoculated with malolactic bacteria, preserved with potassium metabisulfite, and barrel aged (Appendix A).

2.2 Overview of phenolic compounds

Phenolic compounds are a vast group of compounds that can be altered by various viticultural and enological practices thereby affecting color, bitterness and astringency of wine. Phenolic compounds are naturally present in the berry, but they can also be introduced through various oak-derived adjuncts during the winemaking process. Phenolic compounds consist of a benzene ring with at least one hydroxyl group attached. These naturally occurring phenols are classified into two groups; flavonoid and non-flavonoid phenolics.

Flavonoid phenolics represent 80 to 90% of the phenolic content of conventionally produced red wine (Zoecklin et al. 1995). These flavonoids are located in the seeds, skin and stems of grapes (Zoecklin et al. 1995). Flavonoid rings contain two benzene rings linked by a chain containing three carbon atoms (Moreno and Peinado 2012). They can exist free, or polymerize with either another flavonoid, sugar, nonflavonoid, or a combination of these compounds (Zoecklin et al. 1995). Anthocyanin, tannin, and flavanols are phenolic flavonoid compounds. Anthocyanin monomers are found in berry skin; they are the principal pigmentation source of red wine (Section 2.2.2). Tannin directly effects mouthfeel,

imparting bitterness and the sensation of astringency (Section 2.2.3). Tannin can bind to an anthocyanin to create a stable polymeric pigment. Flavanols are found in the epidermis skin layer of the berry; they influence flavors (Fig. 1). Catechin is a compound belonging to the subfamily of flavon-3-ols. They specifically impart bitter flavors sourced from grape seeds and stems (Cheynier et al. 2006, Harbertson 2007, Lorrain et al. 2013).

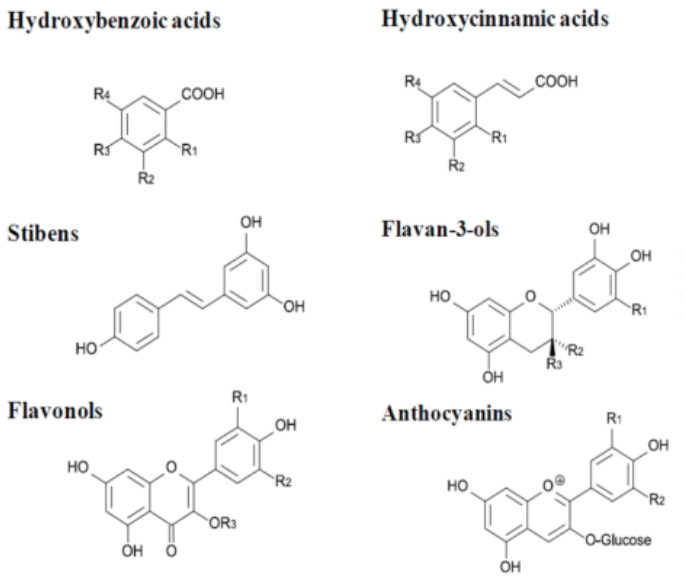

Figure 2. Structures of important monomeric phenolic compounds in grapes and wines. Phenolics are displayed as a hydroxyl group (-OH) bonded to an aromatic hydrocarbon group. Copyrighted with permission from Lorrain et al. 2013.

Non-flavonoids have two sources of origin: grape non-flavonoids and non-grape non-flavonoids. Grape non-flavonoids are sourced from the berry, and non-grape non-flavonoids are sourced from oak adjuncts. Grape non-flavonoids consist of hydroxycinnamates, stilbenes, and gallic acid. Hydroxycinnamates are a class of aromatic acids found in the berry pulp layer (Fig. 1). The oxidation of these hydroxycinnamates can contribute to the browning of must during prefermentation if esterified with tartaric acid (Kennedy et al. 2006). Stilbenes are also located in grape skins, and are produced by grapevines during ripening as a defense response to environmental stressors; they have anticarcinogenic and

antioxidative properties (Moreno-Arribas and Polo 2009). Gallic acid is found in grape seed extract. Gallic acids are antioxidants; they prevent oxidation reactions that can lead to browning (Moreno-Arribas and Polo 2009).

Non-grape non-flavonoids come from oak products used in the winemaking process, such as oak chips, powders, and barrels. Ellagitannin and vanillin are examples of non-grape non-flavonoids present in red wine, sourced from oak barrels (Harbertson 2007, Zoecklin et al. 1995).

Table 1. Phenolic levels in a "typical" *Vitis vinifera* red wine. Adapted from Singleton and Noble 1976.

Phenol Type	Concentration (mg/L)
Nonflavonoids	200
Flavonoids	
Anthocyanin	150
Condensed tannin	750
Other flavonoids	250
Flavanols	50

2.2.1 Importance of phenolics

Wine phenolics affect the color, taste, mouthfeel and structure of a wine (Kennedy et al. 2006). Variations in wine style are due to concentration and composition of phenolics, among other factors (Table 2) (Zoecklin et al. 1995).

Table 2. Extractable low-molecular-weight phenolic compounds (mg kg^{-1}) of Sauvignon Blanc (SB), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace. n=3. Statistical significance between cultivars ($p<0.05$). Adapted from de la Cerda-Carrasco et al. 2015.

Polyphenol	SB	CS	CA
Gallic acid	25.9±2.3b	13.2±0.5b	19.9±5.3ab
Caftaric acid	ND	2.6±0.1a	2.9±0.2a
Procyanidin B1	22.5±3.6b	10.6±0.9a	11.9±2.3a
Procyanidin B2	80.7±6.4a	ND	ND
Procyanidin B3	40.8±4.9c	9.2±3.4a	18.8±3.9ab
Procyanidin B4	59.1±7.7c	17.0±1.0a	17.7±3.0a
(+)- Catechin	477.3±36.8b	87.7±3.3a	178.3±22.2b
(-)- Catechin	506.1±67.0b	68.4±5.1a	130.9±22.5a
Flavonols	ND	121.1±4.1b	75.6±9.9a

To better understand phenolic complexity, it is necessary to consider the development of phenolic compounds in the vineyard, their extraction and modification during fermentation, and their fate during aging (Kennedy et al. 2006). The latter sections will divulge on each topic.

2.2.2 Anthocyanin

Anthocyanins are the main source of pigmentation in red wine. They have no flavor nor organoleptic properties. Anthocyanins are found in berry skins, with the exception of *Vitis vinifera* Tenturier varieties. Once extracted, anthocyanins can react with other must components to form anthocyanin derived pigments (Harbertson 2007). Wine color stability, as determined by stable polymeric pigments, can be directly affected by interactions with other polyphenolic compounds, proteins and polysaccharides. During fermentation, yeast can release secondary metabolic products that react with anthocyanin monomers.

Anthocyanin concentration can also be altered by potassium metabisulfite additions and pH values. Lower pH wines contain more purple and ruby tones, and less brown and brick hues. Monomeric anthocyanin molecules can also react via self-association (monomeric anthocyanins reacting with other monomeric anthocyanins) and co-pigmentation (monomeric anthocyanins reacting with other phenolics) (He et al. 2012).

2.2.2.1 Development

Anthocyanin concentrations gradually accumulate during berry ripening (Rolle et al. 2009). The biosynthesis of anthocyanin is regulated by the enzyme phenylalanine ammonia-lyase. This enzyme increases activity at the start of veraison, creating berries of greater anthocyanin accumulation in higher ambient temperatures (Moreno and Peinado 2012). During veraison, the period between berry growth and berry ripening, cells are rapidly growing and expanding, and berry skin color changes to dark red and purple. Anthocyanin monomers begin to accumulate in the hypodermal cell layer approximately two weeks before color development (Australian Wine Research Institute 2010). All color extracted from red berry fermentations come from anthocyanin monomers, with the exception of *Vitis vinifera* Teinturier varieties (Ribereau-Gayon et al. 2000). Accumulation of anthocyanin increases during the period of veraison, peaks, and then begins to decrease immediately before harvest (Fig. 3) (Ribereau-Gayon et al. 2000).

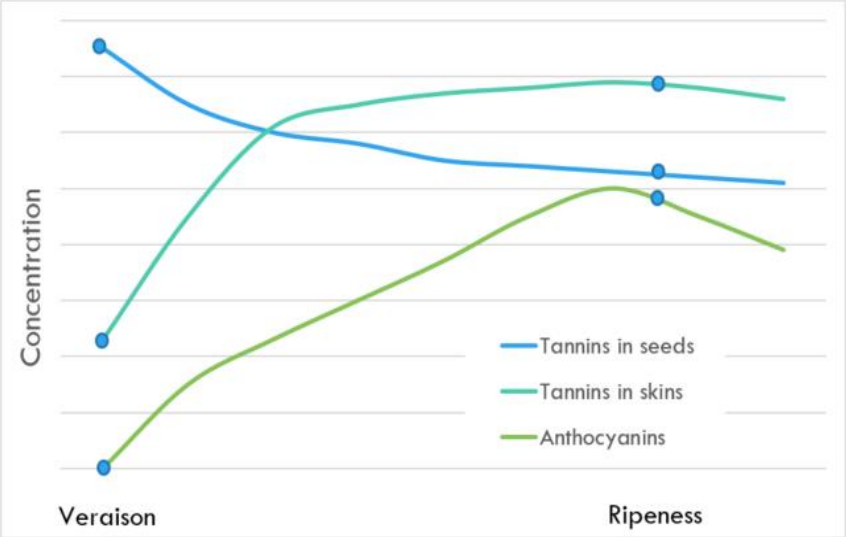

Figure 3. Anthocyanin and tannin concentrations over the course of grape ripening. Adapted from Moreno and Peinado 2012.

The extractability of anthocyanins increases through grape ripening due to cell wall degradation by naturally occurring pectolytic enzymes (El Darra et al. 2016, Rolle et al. 2009).

Several factors affect berry anthocyanin concentration in the vineyard, including temperature, sun exposure, and seasonal conditions. Temperature influences the accumulation of anthocyanins, depending on a region's growing degree days and diurnal temperature swings. Greater anthocyanin concentration was observed at 20°C (controlled growing temperature) compared to 30°C, with the most sensitive timing for maximum anthocyanin concentration occurring 1-3 weeks after the start of veraison (Yamane et al. 2006). Sun exposure, determined in part by row orientation, height of canopy, and leaf thinning practices, influence anthocyanin accumulation. Seasonal conditions can influence the quantity of anthocyanins, but not the general distribution of the different phenolic compounds (Yamane et al. 2006). Berry anthocyanin content is genetically predetermined, and concentration varies greatly amongst cultivars (Rolle et al. 2009).

2.2.2.2 Types

There are five main types of anthocyanins; cyanidin, delphinidin, malvidin, peonidin and petunidin (Table 2). All anthocyanin molecules are bound to a glucose or fructose molecule via a glycosidic bond (Moreno and Peinado 2012).

Table 3. Chemical structures of anthocyanidins. Adapted from Moreno-Arribas and Polo 2009.

Anthocyanidins	R1	R2
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Malvidin is the most abundant anthocyanin across all grape cultivars. Concentration of the anthocyanidins present in fermenting juice and wine varies amongst cultivars (Fig. 4) (Moreno-Arribas and Polo 2009).

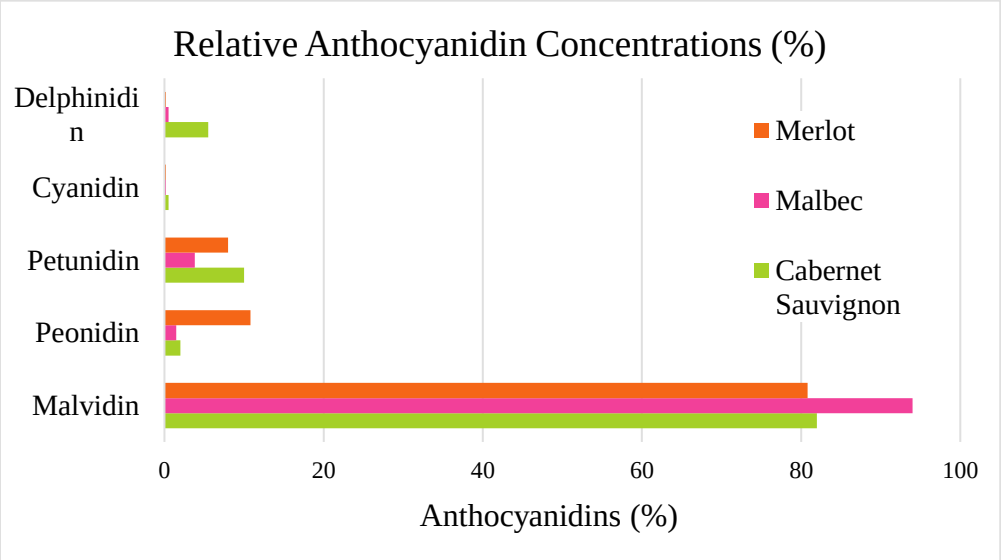

Figure 4. Relative anthocyanidin composition of six grape varieties. Adapted from Moreno and Peinado 2012.

2.2.2.3 Extraction

Wine color is the result of various molecular interactions of free monomers, anthocyanins, and polymeric pigments (Versari et al. 2007). Monomeric anthocyanins in young red wine are the largest contributors to color (He et al. 2012). In young wines, anthocyanin reactions are readily reversible. Stable pigmented polymers depend on complexing reactions, self-association, and co-pigmentation for color stabilization. The fermentation process extracts phenolic substances by macerating berries, whereby anthocyanin monomers from the hypodermal skin layer are released. Anthocyanin extraction can be promoted through pectolytic enzyme treatments (Section 2.3), fermentation temperatures (Section 2.4), must freezing, and extended contact time (Ribereau-Gayon et al. 2000, Sacchi et al. 2005).

Table 4. Anthocyanin composition post maceration of 4 different treatments. V1: crushed grapes, fermented in wooden casks. V2: crushed grapes, fermented in a rotating tank. V3: 70°C thermovinification maceration. V4: -20°C for 24 hours pre-fermentation. Different subscripts in the same row indicate significant differences ($p<0.05$). Adapted from Bichescu et al. 2013.

Anthocyanins, ppm	Variant 1	Variant 2	Variant 3	Variant 4
Cyanidin	3.6 a	9.7 b	4.6 c	0.2 a
Delphinidin	22.2 a	26.5 a	24.1 c	0.4 a
Petunidin	23.4 a	30.4 b	31.2 a	0.9 a
Peonidin	25.7 c	37.2 c	29.1 c	1.9 a
Malvidin	140.6 a	139.4 ab	125.5 ab	37.0 b
Total anthocyanins (ppm)	233.4 a	284.5 ab	236.1 ab	44.1 ab

Breaking the cap is another effective way to increase anthocyanin extraction during alcoholic fermentation by promoting skin to juice hydrophilic interactions. The cap is a thick layer of grape solids, typically skins, seeds and stems (when present), that floats to the surface of a fermenting vessel due to the carbon dioxide produced during alcoholic

fermentation. Breaking a fermenting cap can be done by hand or pneumatic tools (punch down), or by pulling juice from the bottom of a tank and pumping it over in efforts to break the cap (pump over) (He et al. 2012). Anthocyanin concentration is significantly influenced by grape maceration method (Ribereau-Gayon et al. 2000, Sacchi et al. 2005).

Anthocyanin levels accumulate during the first days of alcoholic fermentation due to their solubility in aqueous solutions (Gomez-Miguez and Heredia 2004, Romero-Cascales et al. 2005). Concentration of anthocyanin peaks and begins to decline during alcoholic fermentation, whereas tannin accumulation continues to rise (Fig. 5).

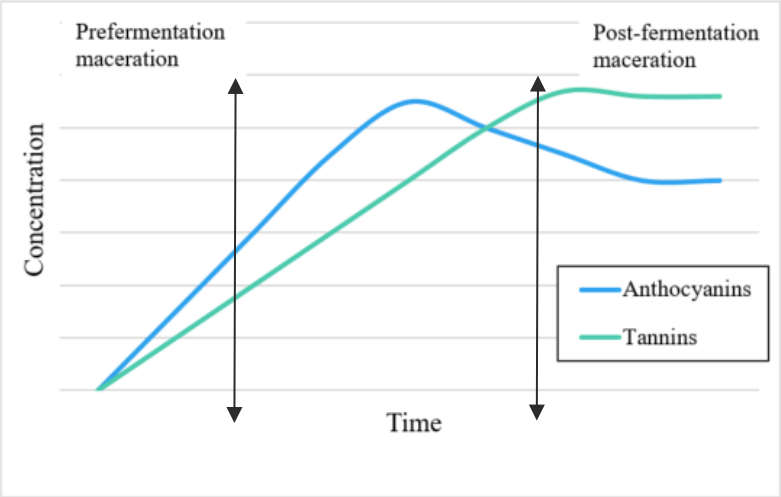

Figure 5. Relationship between fermentation time and extraction of tannin and anthocyanin. Adapted from Moreno and Peinado 2012.

Phenolic extraction is largely influenced by variety and the physiological maturity of the berry. Color extraction can be modified by enological processes; the length and type of maceration can alter anthocyanin extraction (Fig. 6) (Casassa and Harbertson 2014, Ribereau-Gayon et al. 2000, Versari et al. 2007).

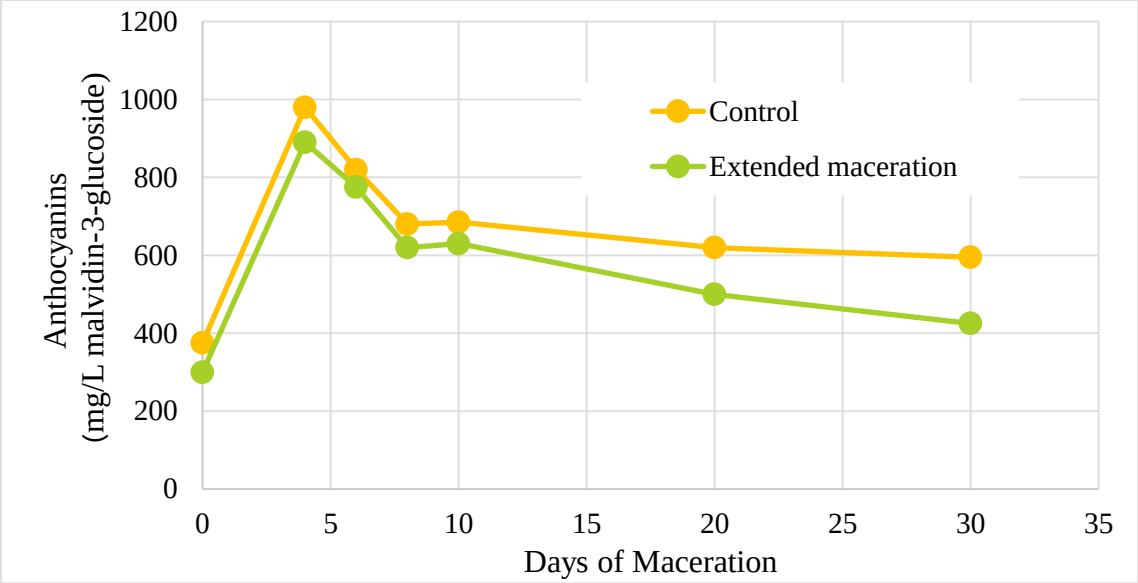

Figure 6. Total anthocyanin levels for a control fermentation and an extended maceration during maceration. Adapted from Casassa and Harbertson 2014.

During traditional winemaking, about 40% of anthocyanins and 20% of tannins present in berry skins are successfully transferred into the resultant wine (Boulton 2001). It is imperative to extract the maximum amount of anthocyanin in efforts to create stable polymeric pigments.

2.2.2.4 Complexing reactions

Anthocyanin can complex with tannin and other phenolic acids to create polymeric pigments. Anthocyanin can also react with polyphenols and other non-desirable compounds (pyruvic acid and acetaldehyde) to form long term color pigments (El Darra et al. 2016, Harbertson et al. 2002). Research has shown anthocyanin condensing with other phenolic compounds including acetaldehyde, keto-acids, and cinnamates to form polymeric pigments (Harbertson 2007). Polymeric pigments are a stable source of color in wine (El Darra et al. 2016, Singleton and Trousdale 1992). Bonds are formed quickly during alcoholic fermentation in the abundance of monomeric anthocyanin. Increasing tannin concentrations,

either through increased extraction or additions, can result in greater amounts of pigmented phenolic polymers (Singleton and Trousdale 1992). Complexing reactions between anthocyanin and tannin impact astringency, color stability and quality of red wines (El Darra et al. 2016, Singleton and Trousdale 1992).

2.2.2.5 Aging

Anthocyanin levels decline during aging for several reasons: they can adsorb onto yeast cell walls and lees (Table 4), precipitate with tartrate salts, or they can be eliminated during fining or filtration processing. Anthocyanin levels also decrease due to the incorporation of monomeric pigments into polymeric pigments. (Moreno-Arribas and Polo 2009, Vasserot et al. 1997). Yeast strains can modify anthocyanin concentration during alcoholic fermentation; if an anthocyanin molecule does not complex with a phenolic compound immediately, the yeast will adsorb the anthocyanin monomer (Medina et al. 2005). Total anthocyanin concentration decreased an average of 17% post fermentation, with no significant differences among yeast strains.

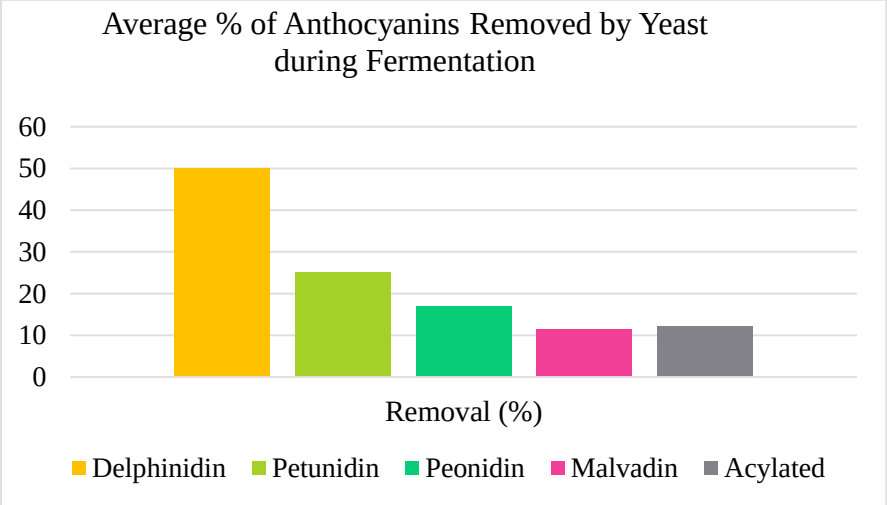

Figure 7. Average percentage of anthocyanins removed during fermentation using different yeast strains. Adapted from Medina et al. 2005.

2.2.3 Tannin

2.2.3.1 Development

Contradictory results have been published regarding peak tannin accumulation pre-harvest. Some studies suggest tannin accumulation increases at fruit set and declines at verasion, as illustrated in Fig. 8 (Hanlin and Downey 2009). Other studies suggest tannin increases before verasion, reaching a maximum content per berry, before decreasing pre-harvest (Harbertson et al. 2002). Differences are likely related to environmental conditions between vineyard sites and vineyard management (Hanlin and Downey 2009). Environmental drivers that could affect tannin concentration are temperature, soil type, irrigation practices, health and vigor of the vine, nutrition, and viticulture management practices (Hanlin and Downey 2009, Harbertson et al. 2002).

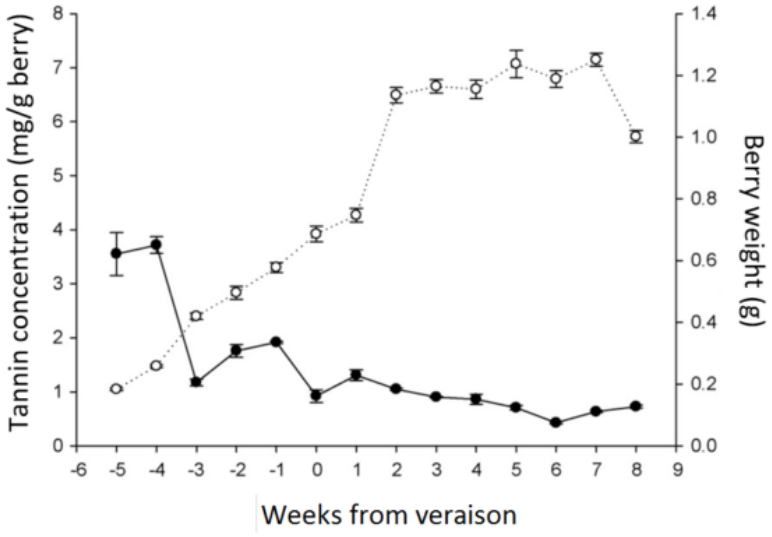

Figure 8. Pattern of tannin accumulation expressed in mg/g fresh berry weight and fresh berry weight expressed in grams (±SE, n=3) extracted from the skin of Cabernet Sauvignon during berry development. Reproduced from Hanlin and Downey 2009.

2.2.3.2 Type

All tannins precipitate with proteins (Smith et al. 2015). Tannin is classified as either condensed and hydrolyzable. Condensed tannin is readily extracted from grape seeds, stems, and skins during maceration (Zoecklin et al. 1995). Condensed tannin contribute the majority of total tannin concentrations to red wine (up to 4 g/L) (Smith et al. 2015). Seed tannin becomes soluble in solution when the seed cuticle has been dissolved by ethanol produced during alcoholic fermentation. Condensed tannin are polymeric flavan-3-ols that contain monomers of catechin, epicatechin, epigallocatechin, or epicatechin gallate; they are large macro-molecules formed by polymerization (Harbertson et al. 2002, Sarni-Manchado et al. 1999, Smith et al. 2015). Condensed grape tannin is converted to more complex wine tannin during fermentation and aging. Tannin is expressed in catechin equivalents (mg/L catechin equivalents) (Harbertson et al. 2002). Understanding wine tannin is less clear than grape tannin chemistry due to modifications by yeast, enzymes, and other by-products (Smith et al. 2015, Harbertson et al. 2012).

Hydrolyzable tannin is absent in grapes. Hydrolyzable tannin is introduced with oak additives or holding vessels such as oak barrels, chips and powder (Hanlin and Downey 2009). They exist as esters. Their structure consists of a glucose molecule acylated with galloyl groups (Smith et al. 2015). Hydrolyzable tannin is a derivative of gallic acid, composed of polyols (glucose and quinic acid) linked to one or more gallotannin or ellagic tannin. They are easily decomposed by hydrolysis (Gil-Munoz et al. 2009, Smith et al. 2015). Most enological tannin additives contain between 12% and 48% tannin, implying the manufacturer's recommended doses are too insignificant to make an impact (Harbertson et al. 2012).

2.2.3.3 Important sensory properties of wine

Variations in tannin content, composition, and polymer length contribute to mouthfeel and aging properties of wine by affecting astringency, bitterness, color stability and aging potential (Zoecklin et al. 1995). During aging, tannin levels decrease from oxidation and precipitation with protein (Zoecklin et al. 1995). Most tannin is highly unstable and undergo various reactions as wine ages, changing the structural composition of the wine to yield new compounds and structures (Moreno-Arribas and Polo 2009). Understanding the fluctuation of tannin composition allows the winemaker to manipulate tannin to meet winery specifications (Hanlin and Downey 2009). If a wine is too astringent or tannic, remediation can be done with fining agents, specifically gelatins, in efforts to remove excessive tannin (Zoecklin et al. 1995). Gelatins preferentially remove high molecular weight grape tannin by adsorption, followed by settling or precipitation.

2.2.3.4 Reactions

The perception of astringency results from the interaction of tannin and salivary rich proteins, where tannins complex with proteins (Sarni-Manchado et al. 1999). Astringency is described as a tactile sensation where salivary proteins are precipitated, reducing mouth lubrication and increasing perception of roughness and dryness in the mouth. The level of astringency is related to tannin concentration, and increases with molecular weight (Cheynier et al. 2006). Bitterness is a perception of taste. Small molecules enter the taste receptor to activate the signal transduction process.

Polymeric pigments are products formed from reactions of anthocyanins with tannin. Reactions of tannin and anthocyanin yield both large polymeric pigments (LPP's) and small polymeric pigments (SPP's) (Cheynier et al. 2006). LPP's precipitate proteins, while SPP's cannot precipitate proteins. Some reactions produce colorless, low molecular weight

compounds that do not involve tannin (Cheynier et al. 2006). These reactions are outside the scope of this thesis, therefore will not be discussed. It has been reported that there is no relationship between total tannin per berry and the amount of tannin extracted in the resulting wine (Harbertson et al. 2002). Our research aims to provide insight to grape tannin concentrations and different fermentation processes in efforts to modify tannin levels for the winemaker's benefit.

2.3 Macerating enzymes

2.3.1 Background

Macerating enzymes help hydrolyze polysaccharides in efforts to extract phenolic compounds from grape skins. In an aqueous wine solution, hydrolysis changes the permeability of the cell wall to promote the extraction of phenolic compounds from the grape cell wall (Li et al. 2015). The degree of hydrolysis is widely influenced by the grape varietal, and the type, concentration and purity of the enzyme(s) applied (Li et al. 2015, Sacchi et al. 2005). During pre-fermentation maceration, tannin and monomeric anthocyanin have different degrees of solubility. Anthocyanin and tannin derived from the berry skin are extracted first, and seed tannin is extracted afterwards. Macerating enzymes have shown to accelerate this process of extraction by increasing speed of phenolic extraction, which may have the potential to decrease overall maceration time (Romero-Cascales et al. 2012). Commercial enzyme preparation addition extracted phenolic compounds by approximately 3 days quicker when compared to the control in the study of Romero-Cascales et al. 2012 (Table 5).

Table 5. Chromatic parameters during the maceration process with a commercial enzyme. C = control wine, E = wine with added enzyme. Different letters within the same column have significant differences ($p<0.05$). Reformatted from Romero-Cascales et al. 2012.

Time of Maceration	Total Phenolics	Total Tannin (mg/L)	Total Anthocyanin (mg/L)
2 days C (n=9)	19ab	221.3a	31.4a
2 days E (n=9)	18.4a	234.3a	32.2a
5 days C (n=9)	39.5b	559.5b	195.1b
5 days E (n=9)	42.8c	599.3c	287.6e
7 days C (n=6)	45.4d	672.2d	284.1e
7 days E (n=6)	50.4f	716.4e	435.9e
10 days C (n=6)	47.7e	674.4d	333.7d
10 days E (n=6)	54.4g	731.9e	528.1f
15 days C (n=3)	47.5e	587.3bc	260.5e
15 days E (n=3)	56.6h	666.6d	572.2f

Commercial pectinases are generally sourced from *Aspergillus sp*., less commonly *Trichoderma harzianum*, with special interest rising over pectolytic yeasts such as *Kluyveromyces marxianus* (Piemolini-Barreto et al. 2014). Commercial pectinases usually consist of several enzymes, mostly cellulases, hemicellulases and pectinases. Cellulase activity breaks down cell walls to free trapped phenolic compounds. Pectinases act on pectic substances, mostly pectin. These pectic substances have high molecular weights, negative charges, acidic properties; they are glycosidic macromolecules (polysaccharides) (Jayani et al. 2005). β-Glucosidase activity releases bound aromatic compounds important to aromatic white winemaking. Contamination of commercial enzyme treatments by β-glucosidase activity can cleave the glucose moiety from the anthocyanin, creating a free anthocyanin that will readily decompose (Di Profio et al. 2011).

2.3.2 Types of enzymes

Commercial macerating enzymes typically serve two primary functions; color extraction and clarification (Revilla and Gonzalez-San Jose 2003, Haight and Gump 1994). Specific to red winemaking, macerating enzymes are added to increase wine color by breaking down skin cell walls to allow greater anthocyanin and tannin extraction (Sacchi et al. 2005). Macerating enzyme preparations contain pectinase activities, cellulases and hemicellulases. Pectinase-rich macerating enzymes are most commonly used in winemaking (Haight and Gump 1994). A list of common commercial pectinase-rich enzymes can be found in Table 6. Pectolytic enzymes break down pectin, a polysaccharide responsible for binding plant cell wall material. By attacking the pectic substances that bind the middle lamella and primary grape skin wall, anthocyanin and tannin are more readily extracted from the skin layer in the hypodermal tissue (El Darra et al. 2016, Haight and Gump 1994).

Table 6. Commercial pectinase preparations commonly used in winemaking. Adapted from Moreno-Arribas and Polo 2009.

Commercial preparation to increase extraction of color and aroma compounds:	
Vinozyme FCE G (Novo Nordisk)	Lallyzyme EX-V (Lallemand)
Vinozyme Vintage FCE (Novo Nordisk)	Red-style (Lallemand)
Rapidase Ex color (DMS)	Endozyme Rouge (Lallemand)
Rapidase X Press (DMS)	ColorPro (Scott Labs)
Lallyzyme EX (Lallemand)	Color X (Scott Labs)
Commercial pectinase preparations for clarification and filtration of juice and wine:	
Ultrazyme 100G (Novo Nordisk)	Zimopec PX I (Perdomini)
Novoclair speed (Novo Nordisk)	Endozyme Active (AEB)
Rapidase Filtration (DMS)	Endozyme Glucalyse (AEB)
Rapidase Vinosuper (DMS)	Endozyme Glucapec (AEB)
Vinoflow G (Novo Nordisk)	Endozyme ICS 10 (AEB)
Rapidase CB (DMS)	Endosyme TMO (AEB)
Rapidase CR (DMS)	White-style (Lallemand)
Lallyzyme C (Lallemand)	

In white winemaking, enzymes are generally added to increase juice yield and reduce turbidity (Revilla and Gonzalez-SanJose 2003). The effect of clarifying enzymes on wine fermented and aged on heavy lees in efforts to increase the formation of polysaccharides and mannoproteins has been reported (Revilla and Gonzalez-San Jose 2003). The effects of three enzymes and their concentrations was determined to be statistically significant on total juice yield compared to the control in Rubired fruit.

2.3.3 Effect of enzymes on color of wines

Conflicting results have been reported on the use of color enhancing enzymes. Commercial pectinase-rich macerating enzymes have been reported to promote color extraction in red grapes and wine products (Bakker et al. 1999, Romero-Cascales et al. 2012, Kelebek et al. 2007, Li et al. 2015). Others have reported pectinase-rich macerating enzymes negatively affect or diminish anthocyanin extraction (Bautista-Ortin et al. 2005, El Darra et al. 2016, Wightman et al. 1997). One paper had conflicting results with vintage to vintage variation (Revilla and Gonzalez-SanJose 2003).

Often, the effect of pectinase-rich macerating enzymes on wine phenolics are published immediately after alcoholic fermentation, not depicting if the enzymatically-treated grapes had statistically significant phenolic concentrations at the time of bottling. Using conventional winemaking techniques, approximately 40% of anthocyanin molecules and 20% of tannin molecules are transferred into the resultant wine through vinification (El Darra et al. 2016). Companies that formulate and sell pectinase-rich macerating enzymes claim to increase anthocyanin and phenolic extraction. There seems to be more skepticism over advocacy of enzymes in the current enological world. The active ingredients which make up the numerous enzyme trademarks are largely proprietary, and little in-house investigation nor research can be found in published papers (Di Profio et al. 2011).

2.3.3.1 Successful color enhancement

When comparing different pre-treatment techniques, one study found that a macerating enzyme increased color intensity (measured by *L*a*b**) by 22% over the control when using a Cabernet Sauvignon model wine solution immediately after alcoholic fermentation (Fig. 9) (El Darra et al. 2016).

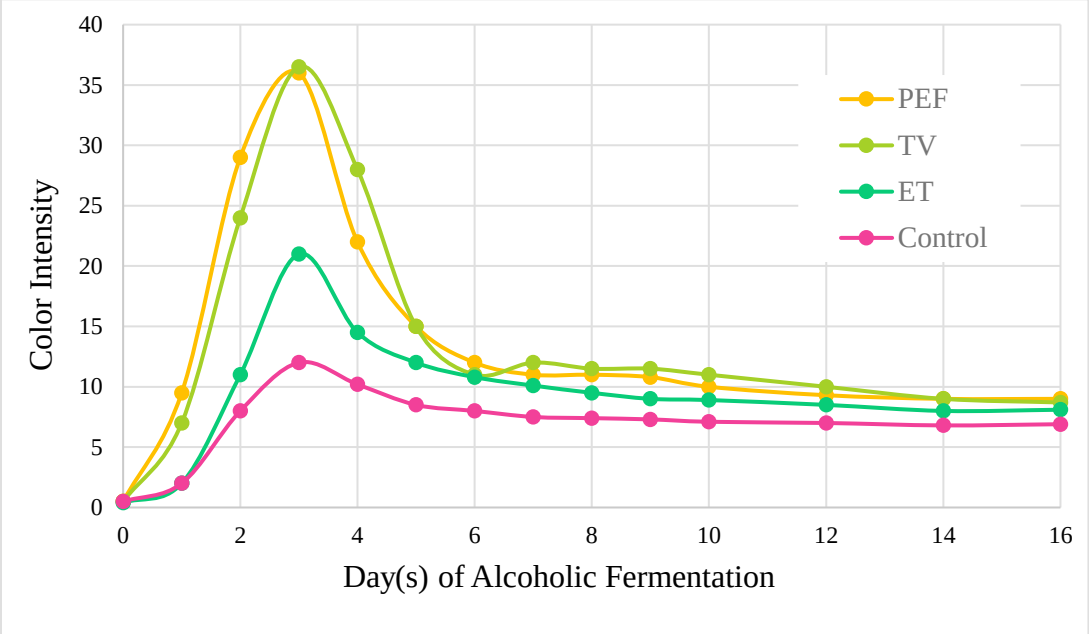

Figure 9. Color Intensity (CI) of Cabernet Sauvignon grape must versus days of alcoholic fermentation when comparing three pretreatments: control, enzyme treatment (ET), thermovinification (TV), and pulsed electric field (PEF). Adapted from El Darra et al. 2016.

The use of pectolytic enzymes, with rates varying from 0.01 g/L to 0.05 g/L at 20°C, gave Tinto Fino wines' better chromatic characteristics that were more stable over time than their control wines throughout two years of storage (Revilla and Gonzalez-San Jose 2003). Another study reported the effect of two commercial pectinase-rich enzymes on phenolic composition; both enzyme preparations (3 g/L) improved the extraction of anthocyanin concentration, total phenolics, and tannin (Table 7) (Kelebek et al. 2007).

Table 7. General composition of phenolic compounds, 6 months after alcoholic fermentation. n=3. Different subscripts in the same row indicate statistical differences (p<0.01). Adapted from Kelebek et al. 2007.

Analysis	Control	Rapidase Ex Color	Vinozyme G
Total Phenolics (280A)	65.2±0.02a	73.1±0.05b	75.4±0.06b
Tannin (g/L)	4.3±0.01a	4.5±0.00a	5.0±0.02b
Color Intensity	0.986±0.04a	1.062±0.06b	1.105±0.05c

Although the addition of enzymes extracted greater color intensity than the control, neither enzyme addition produced one specific anthocyanin concentration to be more pronounced (Kelebek et al. 2007).

Total phenols were reported to increase 19% by the end of the maceration process (day 15), with the greatest extraction of phenolics occurring between day 7 and 15 of alcoholic fermentation. The addition of the enzyme preparation (Lafase Grand Cru, Laffort Oenology, Bordeaux, 3 g/100kg berries) led to anthocyanin concentrations 6-8% higher than corresponding controls (Table 5) (Romero-Cascales et al. 2012).

2.3.3.2 Non-successful color enhancement

Pectinases was reported to not significantly increase anthocyanin extraction, but have been found to statistically increase other phenolic compounds, such as tannin and polyphenolics (Sacchi et al. 2005, Bautista-Ortin et al. 2005). One study using pectinase-rich macerating enzymes produced statistically different results over the course of 2 vintages; increased anthocyanin and tannin concentrations resulted from the addition of two enzymes (2 g/hL) during the first vintage of experimentation, while the second vintage depicted no such benefit (Revilla and Gonzalez-SanJose 2003). Two different pectinase-rich enzymes,

ColorPro® and Color X® (Laffort, Petaluma CA, 100 mL/1000 kg berries) found a nil or negative effect on must anthocyanin concentration after fourteen days of alcoholic fermentation (Di Profio et al. 2011). Adding the macerating enzyme Enozyme Vintage (Agrovin, Spain, 5 g/100 kg berry weight) did not produce statistically significant wines in the first 10 days of alcoholic fermentation at 25°C when compared to the control vinification (Table 9) (Busse-Valverde et al. 2011).

Table 8. Anthocyanin composition during fermentative maceration of four vinification treatments; control vinification, must freezing with dry ice, low temperature prefermentative maceration and vinification with a commercial maceration enzyme. Different letters within the same column indicate significant differences ($p<0.05$). Adapted from Busse-Valverde et al. 2011.

	Total Anthocyanins (mg/L)		
	Day 2	Day 6	Day 10
Control vinification (CW)	274.2a	561.2a	721.1a
Must freezing with dry ice (DIW)	469.1b	715.6c	802.0b
Low temperature prefermentative maceration (LTPW)	438.1b	651.5b	789.6ab
Commercial maceration enzyme	325.5a	561.4a	755.5ab

2.4 Fermentation temperature

2.4.1 Berry development

The effects of temperature on berry phenolic composition begins during veraison. Use of a low temperature (20°C) on berries resulted in significantly higher anthocyanin concentrations, specifically when applied one to three weeks after verasion in a greenhouse setting when compared to 35°C (Yamane et al. 2006). High temperatures (max 35°C) during berry development has shown to reduce anthocyanin concentration to less than half in

Cabernet Sauvignon skins when compared to a control 6 weeks after veraison when grown in a phytotron (max 25°C) (Mori et al. 2007). The concentration of individual anthocyanin (delphinidin, cyanidin, petunidin, and peonidin) decreased significantly 6 weeks after veraison with the exception of malvidin derivatives when experimenting with Cabernet Sauvignon (Mori et al. 2007).

The expression of anthocyanin biosynthesis is strongly affected by temperature, with lower temperatures causing an increase in the transcript levels. Higher growing temperatures (35°C) increase the degradation rate of anthocyanin by inhibiting their accumulation (Mori et al. 2007). Vineyard temperatures will become increasingly important as global warming alters the ambient temperatures of revered wine producing regions around the world.

2.4.2 Pre-fermentation temperatures

Anthocyanin monomers are easily soluble in aqueous solution, whereas tannin is readily soluble in alcoholic solutions (Fig. 5). This extraction order allows anthocyanin to be released from the skins first, followed by tannin extraction once fermentation has started to convert grapes' natural sugars (glucose and fructose) into ethanol. Pre-fermentative cold maceration (cold soaking) consists of holding the fruit at a low temperature for several days before the must is inoculated (Gil-Munoz et al. 2009, Cheynier et al. 2006). Conflicting results suggest the effect of cold maceration on the extraction concentration of anthocyanin is dependent on variety, vintage, temperature and skin contact time (Gil-Munoz et al. 2009, Cheynier et al. 2006).

Table 9. Anthocyanin concentration at the end of alcoholic fermentation. Different letters within the same row indicate significant differences ($p<0.05$). Adapted from Gil-Munoz et al. 2009.

Anthocyanin concentration (mg/L)

Varietal	Control	Frozen grapes	Dry ice	Cold maceration	Enzyme
Cabernet Sauvignon	776.4a	809.1ab	894.2b	1,027.2c	894.7b
Syrah	468.4a	668.4b	905.4c	547.4ab	522.1ab

Anthocyanin concentration in Cabernet Sauvignon significantly increased with treatments of dry ice (100 kg dry ice/ 20 kg berries), cold maceration (10°C for 10 days), and commercial enzymes (Enozyme Vintage, Agrovin, Spain, 5 g/100 kg berries) by 15.2%, 32.2%, and 15.2%, respectively when compared to the control treatment (Gil-Munoz et al. 2009).

Malvidin 3-glucoside was extracted more rapidly as temperature increased from 20°C to 30°C in *Vitis vinifera* Pinot Noir wine (27.4% increase) during alcoholic fermentation. At the time of bottling, treatment temperatures of 20°C and 30°C were not statistically significant, yet the hot temperature, short time treatment was statistically significant from the other two treatments (Gao et al. 1997).

2.4.3 Effects of fermentation temperatures

During alcoholic fermentation of red wine, must temperatures have the greatest effect on seed and skin derived phenolics (Lerno et al. 2015). Total phenols and tannin are extracted by diffusion in alcoholic solutions. Fermentation temperature directly affects the rate at which alcoholic fermentation occurs; changing the fermentation temperature is an effective method for influencing extraction of polyphenolic compounds (Gil-Munoz et al. 2009). Wines from *Vitis vinifera* Cabernet Sauvignon and Pinot Noir grapes have

significantly higher tannin concentration post fermentation with prefermentative low temperature treatments (freezing of grapes) and macerating enzyme addition (Enozyme Vintage, Agrovin, Spain, 5 g/100 kg berries), 200% and 54.6% respectively (Gil-Munoz et al. 2009).

Higher temperatures generally lead to increased phenolic extraction due to the increased permeability of the hypodermal cells and solubility of certain phenolics (Sacchi et al. 2005). Fermenting at higher temperatures favor phenolic extraction but may affect the ability of a fermentation to successfully complete if yeast cannot survive in the stressful conditions created by hotter temperatures (Zoecklin et al. 1995). Temperature affects the rate of extraction but not the final concentration of skin phenolics (Zoecklin et al. 1995).

Fermentation temperatures significantly affected volatile acidity, pH, and alcohol content post alcoholic fermentation when comparing treatments of 15°C to 25°C (Sener and Yildirim 2013). Post alcoholic fermentation, hot fermentation temperature treatments (25°C) resulted in less desirable wines with higher volatile acidity, tartaric acid, and alcohol (Sener and Yildirim 2013). Adequate tannin must be present in the beginning stages of fermentation to bind with anthocyanin; if tannin concentration is lacking, less polymeric pigment will be in the resultant wine (Sacchi et al. 2005). The disappearance of monomeric anthocyanins and increase of polymeric pigments during wine aging has been confirmed to increase as fermentation temperature rose from 20°C to 30°C (Gao et al. 1997).

2.5 Berry processing

2.5.1 Destemming berries

Destemming berries, also called destalking, is common procedure in red winemaking. Stems increase astringent and bitter tannins, contribute to ‘stemmy’ flavors, and may cause

significant color loss. Removing stems before maceration can help mitigate these undesirable contributions (Pascual et al. 2016). The destemming process typically occurs before crushing to reduce the chance of stem material passing through the crusher. Crushing and destemming fruit is completed by a crusher/destemmer unit. Berries, stems, rachis, and seeds enter the destemmer (Fig. 10), allowing berries to fall through the exit holes of the destemmer, into the crusher hopper (Fig. 11). The remaining material other than grapes (MOG) exits through the back of the destemmer to be disposed of or recycled via a trash auger.

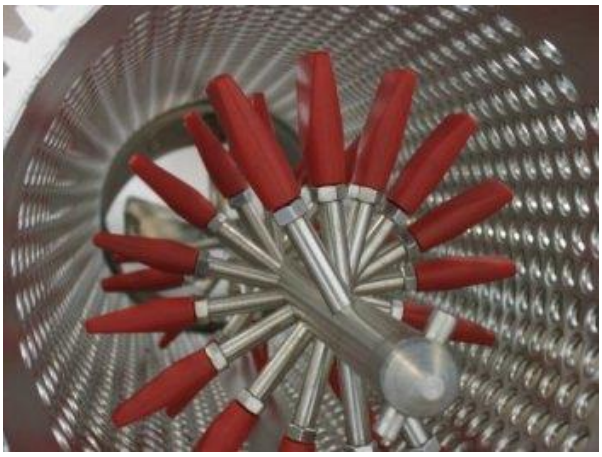

Figure 10. Internal picture of a destemmer. Copyrighted with permission by J. Lohr Vineyards & Wines 2017.

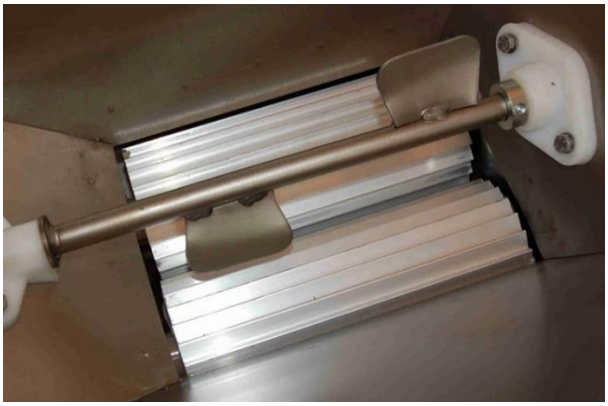

Figure 11. Internal picture of a crusher's rollers. Copyrighted with permission by J. Lohr Vineyards & Wines 2017.

2.5.2 Crushing berries

Berries are traditionally crushed to immediately release the pulp and juice from within an individual grape to facilitate fermentation and maximize phenolic extraction. Benefits of crushing berries include: immediate exposure to oxygen, more homogenous protection from microbial contamination by potassium metabisulfite addition, and immediate start of phenolic extraction (Ribereau-Gayon et al. 2000). Two types of crushers exist; roller crushers and wall crushers. Roller crushers are typically coated in plastic, spinning in opposite directions (Fig. 11). Spacing is adjustable to allow for whole crushing of the berries, or complete bypass from crushing. High speed perforated wall crushers eject grapes against a perforated wall, and the grapes burst open, thoroughly macerating the berry (Fig. 12). Crushing intensity is controlled by an external motor, where higher speeds decrease berry intactness.

Figure 12. A high speed perforated wall crusher. Copyrighted with permission by Pellenc USA, 2017.

2.5.3 Comments on extent of research on berry integrity

There is a lack of documented production scale experimentation on berry integrity. Typical research fermentations are completed on a laboratory scale, these experiments

require less volume of wine, so each treatment can be performed in triplicate for statistical significance. These laboratory scale experiments are less costly, but less accurate. A laboratory scale fermentation is generally not applicable to what happens in the cellar; a red wine production fermentation allows for continuous contact between fermenting juice and the cap to allow for greater phenolic extraction.

To date, there are no published studies investigating the effect of crushed berries versus whole berries on phenolic concentration on a production scale magnitude. One study investigated the effect of whole berry and whole cluster phenolic compound extraction compared to a control treatment (500 L ferments, 25°C). It is impossible however to draw statistical differences from the results published since no replicate experimentations were performed (Pascual et al. 2016) (Table 10). Preliminary findings suggested whole destemmed berries extracted more phenolic compound than did the destemmed crushed berries.

Table 10. Effect of prefermentative cluster treatment on the composition of phenolic compounds. Adapted from Pascual et al. 2016.

Parameter	Control	Whole Berry	Whole Cluster
Ethanol (v/v %)	16.6	16.5	16.1
Volatile acidity (g/L)	0.51	0.49	0.49
Total polyphenols (A280)	38.3	45.7	51.1
Tannins (mg/L)	300	371	474
Total anthocyanins	236	331	297
Total flavonols	15	24	22
Non-flavonoids	79	119	72

Another study investigated the effect of crushed fruit percentage (25% increments, 5 replicates) on proanthocyanidin concentration on *Vitis vinifera* Merlot. The microscale fermentations were maintained at 25°C, and the caps were kept submerged throughout

alcoholic fermentation. The highest proanthocyanidin concentration for skin and seed (435 mg/L and 344 mg/L, respectively) was observed for the 75% crushed berry treatment at pressing (17 days).

Published works on the effect of crushed versus whole berry fermentation focused on terpene extraction in white wine. Pomace maceration and berry maceration during alcoholic fermentation negatively affected the aromatic compound composition as determined by a trained sensory panel. Negative impacts by pomace maceration and berry maceration in white varieties investigated produced lower contents of esters and less expressed fruitiness when fermentations were completed on a 2 L scale (Bavcar et al. 2011).

2.6 Summary

A wine's perceived quality is directly influenced by its color. It is imperative to foster the extraction and retention of phenolic compounds in red winemaking to enhance anthocyanin stability. Accumulation of phenolic compounds starts in the vineyard, and several viticultural management practices have shown to affect accumulation; temperature, sun exposure, seasonal conditions, row orientation and canopy aspect. Extraction of anthocyanin and tannin from berry skin and seeds begins immediately after harvesting. Different extraction techniques affect total phenol, tannin, and anthocyanin concentrations. This literature review suggests phenolic composition can be altered by altering berry integrity, adding pectinase-rich macerating enzymes, and modifying alcoholic fermentation temperatures.

CHAPTER 3

MATERIALS AND METHODS

3.1 Grapes and experimental design

Two vintages (2010 and 2011) of *Vitis vinifera* Cabernet Sauvignon donated by J. Lohr Vineyards & Wines (Paso Robles, CA) were used to perform our experiments. For the 2010 and 2011 vintage, 73 tons and 129 tons of *Vitis vinifera* Cabernet Sauvignon grapes were donated, respectively.

3.2 Chemicals

Bovine serum albumin (BSA, Fraction V powder, catalog #A3803), sodium dodecyl sulphate (SDS, catalog #L-5750), triethanolamine (TEA, catalog #T-1377), ferric chloride hexahydrate (catalog #F-2877), 37% hydrochloric acid (catalog #435570), sodium hydroxide pellets (catalog #S8263), sodium chloride (catalog #S98888), maleic acid (catalog #M153), and glacial acetic acid (catalog #A6283) were purchased from Sigma Aldrich (St. Louis, MO).

The chemical composition of ColorPro® is a proprietary blend. The following ingredients were listed on the Safety Datasheet (Table 11).

Table 11. Ingredients of ColorPro® and their percentages. (http://www.scottlab.com/uploads/documents/technical-documents/1275/SCOTTZYME%20COLOR%20PRO%20MSDS%202015.pdf)

Ingredient	% of blend
Glycerol	30%
Polygalacturonase	10-15%
Protease	5-10%
Pectin esterase	3-8%
Pectin lyase	3-8%
Diammonium phosphate	5%
Potassium sorbate	0.14%

3.3 Equipment

A Genesys® 10 UV spectrophotometer (Thermo Electron Corporation, Madison, WI) was used for UV and VIS spectral readings. A Fisher Scientific Accumet AE150 Orion Meter was used for determining pH values (Fisher Scientific, Waltham, MA). A large Thermo Scientific centrifuge, capable of spinning twenty-eight 50 mL samples at 4,000 rotations per minute (RPM) (Thermo Electron Corporation, Madison, WI) was used for initial separation of solids. A Thermo Scientific micro-centrifuge, capable of spinning twenty-one 1.5 mL samples at 14,000 RPM (Thermo Electron Corporation, Madison, WI) was used for further separation.

3.4 Description of fruit handling

Harvesting in 2010 and 2011 was done in a mechanized homogenous manner to ensure minimal berry variance in the Estrella District American Viticultural Area (AVA). *Vitis vinifera* Cabernet Sauvignon arrived in the early morning hours to J. Lohr Vineyards &

Wines. A representative juice sample was collected and analyzed for titratable acidity, pH, BRIX (soluble solids), and yeast assimilable nitrogen (YAN) upon arrival to the winery. Each truckload of fruit received 50 ppm of potassium metabisulfite (KMBS), the production facility's standard addition rate. Titratable acidity was adjusted by adding a 40% tartaric acid solution to achieve a 3.45 pH for alcoholic fermentation.

The experiments performed in 2010 investigated the effects of pectinase-rich macerating enzymes at two different concentrations (60 mL/ton and 100 mL/ton) on phenolic compounds extracted from *Vitis vinifera* Cabernet Sauvignon. The experiments performed in 2011 investigated the effects of destemming and crushing fruit, adding pectinase-rich macerating enzymes, and alternating fermentation temperatures on phenolic compounds extracted from *Vitis vinifera* Cabernet Sauvignon.

In vintage 2010, the control received no enzyme treatment. The low dose treatment experiment received 60 mL/ton enzyme (ColorPro®, Scott Labs, Petaluma, California) based on the lowest recommended manufacturer's concentration. The high dose treatment received 100 mL/ton enzyme, the highest recommended manufacturer's concentration. In vintage 2011, the high dose treatment (100 mL/ton ColorPro®) was compared to the control (no enzyme); there was no low dose treatment as there was in 2010.

The fruit was destemmed and roller crushed by a Vaslin Bucher® Delta E8, capable of 70-80 tons maximum output per hour (Fig. 13). For the enzyme experimentations of the 2010 and 2011 vintages, all berries were destemmed and crushed. For the fermentation temperature experiments of the 2011 vintage, all berries were destemmed and crushed.

Figure 13. The Vaslin Bucher Delta E8 destemmer / crusher (left), and the rollers as viewed from below the destemmer / crusher (right). Copyrighted with permission by J. Lohr Vineyards & Wines, 2017.

For the experimentation on the effect of crushed versus whole berries, the control consisted of destemming and crushing the berries; the treatment consisted of destemming the berries and completely bypassing the crusher (Fig. 14).

Figure 14. Berry integrity - crushed and destemmed berries (left) and destemmed only berries (right). Copyrighted with permission by J. Lohr Vineyards & Wines, 2017.

3.4.1 Pectinase-rich macerating enzymes addition design

Experiments of 2010 and 2011 added different concentrations of pectinase-rich macerating enzymes (ColorPro®). Dosage rates of 60 mL/ton and 100 mL/ton were based on the manufacturers' recommended low and high doses for red crushed berries to increase extraction of anthocyanin, polymeric phenols and tannin concentrations.

3.5 Processing procedures

Post SO_2 and tartaric acid adjustments (Section 3.4), the berries were pumped through 4-inch hard lines into 24-ton stainless steel fermenting jacketed vessels via a food-grade Waukesha pump. Fermentation temperatures were set (Table 13) according to the treatment immediately after fruit entered the tank by digital temperature faceplates located on each tank.

Table 12. Fermentation temperatures of the 2010 and 2011 vintages.

Experiment / Treatment	Set Point °Celsius
2010 Enzyme –	
Control, 60, and 100 mL/ton	29.4°C
2011 Enzyme –	
Control and 100 mL/ton	29.4°C
2011 Alcoholic fermentation	
Cool fermentation	25.0°C
Hot fermentation	32.2°C
2011 Berry integrity	
Whole berry	32.2°C
Crushed berry	32.2°C

Temperatures were monitored via TankNET®, a remote program that monitors and records a temperature data point every 5 minutes. The fruit was inoculated within 24 hours of being received by the winery at a rate of 25 g/hL by *Saccharomyces cerevisiae* IVC D254 (Lallemand, Blagnac, Sedex), diluted at a 1:10 ratio as suggested by the manufacturer with well water (40°C). IVC D254 optimum fermentation temperature range is 15-30°C (www.lallemandwine.com). Startup®, a combination of yeast products, minerals and vitamins, (BSG, Napa) was added the day after inoculation, at a rate of 25 g/hL to stimulate yeast growth. On day two, nitrogen content was adjusted to 250 ppm in the form of powdered diammonium phosphate based on initial YAN content of juice (Scott Labs®, Sonoma).

Pump overs were automatically actuated by TankNET®, the same remote program that monitored, recorded, and changed primary fermentation temperatures. Pump overs were scheduled every 4 hours for 6 pump overs each day. Each tank had a 2” hardline and a 1.5 horsepower pump (Fig. 15).

Figure 15. A fermenting tank with a designated pump for automated pump overs. Copyrighted with permission by J. Lohr Vineyards & Wines, 2017.

3.6 Monitoring

3.6.1 Alcoholic fermentation

Production scale wineries inoculate freshly crushed and destemmed juice with *Saccharomyces cerevisiae* yeast. These yeasts ferment the naturally present sugars found in berries (glucose and fructose) into ethanol, heat and CO_2 as by-products of their fermentation. Alcoholic fermentation is the first of two fermentations. Alcoholic fermentation lasted 9 and 14 days (respectively) for the 2010 and 2011 vintages. Daily BRIX readings and temperatures were recorded every morning using a DMA35® (Anton Parr, Ashland, Vermont). Once the DMA35® BRIX reading fell below 0.00, we could assume most glucose and fructose molecules had been consumed by yeast.

To confirm a wine was "dry" (< 0.200 g/100mL residual sugars), an enzymatic assay confirmed summation of residual sugar concentrations were below 0.200 g/100mL (Randox, Kearneysville, West Virginia). Once a wine was determined to be "dry", 4 barrels of each

treatment were free drained into neutral American oak barrels (neutral barrels are ≥ 3-year-old barrels). The Adams-Harbertson Assay was performed daily during alcoholic fermentation to determine the fermenting wine's polyphenolic profile (Appendix C) (Harbertson et al. 2003).

3.6.2 Malolactic fermentation

Secondary fermentation is also known as malolactic fermentation (MLF). Malolactic bacteria convert malic acid into lactic acid and CO_2 as biproducts of their fermentation. Each barrel was inoculated with 2.4 grams of CH-16 freeze-dried malolactic bacteria (Chr. Hansen, Sonoma, California). A wine is considered malolactic complete (MLC) when malic acid concentration is < 0.200 g/L. To confirm a wine had completed malolactic fermentation MLC), an enzymatic assay confirmed malolactic acid concentration was below 0.200 g/L (Randox, Kearneysville, West Virginia). Once a treatment completed malolactic fermentation, each barrel was racked into an empty neutral barrel, leaving the heavy lees behind. Each barrel had 40 ppm potassium metabisulfite added once MLF was complete.

Table 13. Malolactic fermentation of the 2010 and 2011 vintages.

Vintage	Tank	Treatment	# of Neutral barrels	Date of Drain	Days of Fermentation
2010	1	Control – No ColorPro®	4	11/5/2010	9
2010	2	60ml/ton ColorPro®	4	11/5/2010	9
2010	3	100ml/ton ColorPro®	4	11/5/2010	9
2011	1	Control – No ColorPro®	4	11/16/2011	14
2011	2	100ml/ton ColorPro®	4	11/16/2011	14
2011	1	90°F Fermentation	4	11/16/2011	14
2011	2	77°F Fermentation	4	11/16/2011	14
2011	1	Destemmed only berries	4	11/16/2011	14
2011	2	Destemmed and Crushed berries	4	11/16/2011	14

Additional wine parameters were analyzed on a WineScan® (FOSS, Denmark), using Fourier transform infrared detection (FTIR) to calculate wine constituents including pH, titratable acidity, glucose plus fructose, malic acid, alcohol, volatile acidity, and different absorbance wavelengths to determine a tannin index (280A) and color density (420A + 520A).

3.7 Determination of phenolic compounds

3.7.1 Adams-Harbertson assay

The Adams-Harbertson (AH) Assay is based on a tannin precipitation assay originally developed by Hagerman and Butler (1978). The AH assay uses protein precipitation with BSA whereby multiple classes of phenolic compounds can be quantified and identified: pigmented polymers, tannin (catechin equivalents), non-tannin iron reactive

phenolics, and anthocyanin. Combining precipitation of proteins and bisulfite bleaching, monomeric anthocyanin can be differentiated from polymeric pigments. Of these polymeric pigments, small polymeric pigments (SPP's) cannot precipitate with protein, and large polymeric pigments (LPP's) can. The sum of LPP's and SPP's make up the potassium metabisulfite (SO_2) resistant pigmented polymers (Australian Wine Research Institute 2015). The AH Assay can be performed on a microplate reader or an individual spectrophotometer; its range of application throughput makes it adaptable on a small or large scale (Harbertson et al. 2003, Mercurio and Smith 2008).

3.7.2 Buffer preparation

For a complete list of buffer solutions and procedures, refer to Appendix B.

3.7.3 Procedure

Please refer to the published Adams-Harbertson Assay (Harbertson et al. 2003).

For the complete Adams-Harbertson assay procedure, refer to Appendix C.

3.7.4 Calculations

For the complete list of calculations, refer to Appendix D.

3.8 Statistical analysis

This study could not run any statistical analysis since each treatment was only performed once.

Figure 16. Schematic – Effect of berry integrity on phenolic compounds in the 2011 vintage. LPP's – long polymeric pigments, SPP's – short polymeric pigments, IRP's – iron reactive phenolics

Figure 17. Schematic – Effect of enzyme application on phenolic compounds in the 2010 vintage. LPP's – long polymeric pigments, SPP's – short polymeric pigments, IRP's – iron reactive phenolics

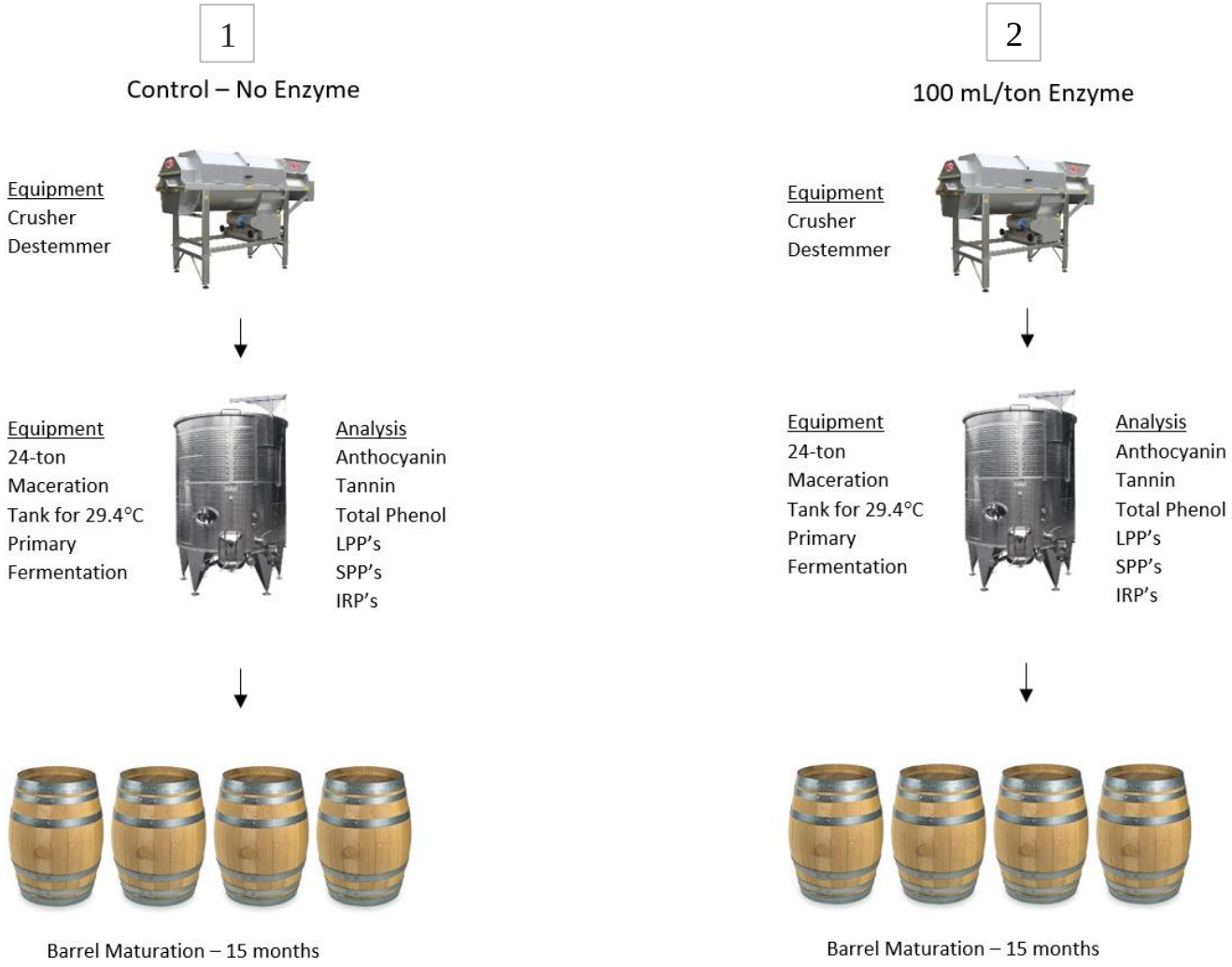

Figure 18. Schematic – Effect of enzyme application on phenolic compounds in the 2011 vintage. LPP's – long polymeric pigments, SPP's – short polymeric pigments, IRP's – iron reactive phenolics

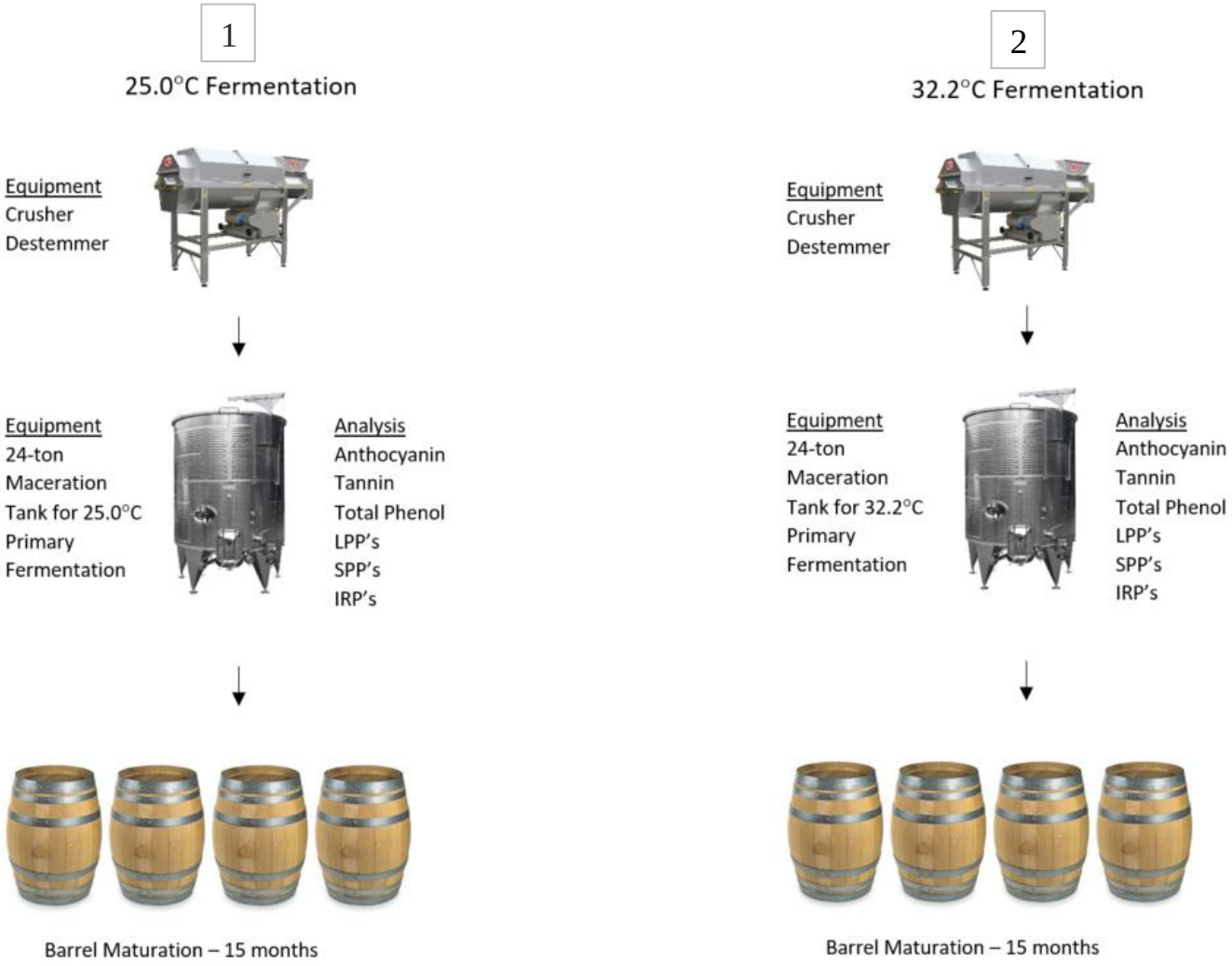

Figure 19. Schematic – Effect of alcoholic fermentation temperature on phenolic compounds in the 2011 vintage. LPP's – long polymeric pigments, SPP's – short polymeric pigments, IRP's – iron reactive phenolics

CHAPTER 4

RESULTS AND DISCUSSION

In this work, two vintages (2010 and 2011) were investigated. Paso Robles Cabernet Sauvignon grapes have ample amounts of readily extractable tannin. The inherent struggle of this region is successfully extracting anthocyanin from skins, and having these anthocyanin monomers not degrade or precipitate during the aging process. A typical fermentation begins with an abundance of anthocyanin and tannin extraction. A monomeric anthocyanin can bind with tannin, creating a stable color complex. Pectinase-rich macerating enzymes can break down the cellular structure of berry skins, thereby releasing anthocyanin and tannin more readily than traditional maceration procedures. The more anthocyanin and tannin concentration present during vinification, the more likely a wine is to develop and retain stable color complexes. The research from vintages 2010 and 2011 aim to understand the long-term effects of a pectinase-rich macerating enzyme on *Vitis vinifera* Cabernet Sauvignon fruit from Paso Robles. To verify our hypothesis (Section 1.2), we began experimentation on the impact of enzyme concentration in vintage 2010. Based on the preliminary results, we then began the second stage of our experimental design by repeating a segment of vintage 2010's enzyme treatment. Furthermore, we explored the effect of fermentation temperature and berry maceration on phenolic extraction in the 2011 vintage.

4.1 Fruit composition

Table 14. Receiving fruit analysis – 2010 tank data, day 0.

FOSS Analysis	Average	Standard Deviation
Brix	24.40	±0.06
pH	3.63	±0.06
TA (g/L)	4.98	±0.17
YAN (mg/L)	176	±4
Malic (g/L)	1,594	±158
Potassium (g/L)	1,863	±81

TA = titratable acidity. YAN = yeast assimilable nitrogen.

Table 15. Receiving fruit analysis – 2011 tank data, day 0.

FOSS Analysis	Average	Standard Deviation
Brix	24.00	±0.74
pH	3.65	±0.07
TA (g/L)	5.80	±0.41
YAN (mg/L)	110	±15
Malic (g/L)	1,419	±134
Potassium (g/L)	1,726	±269

TA = titratable acidity. YAN = yeast assimilable nitrogen.

4.2 Crushed versus whole berries

Table 16. Effect of berry integrity on phenolic compound concentrations at the end of alcoholic fermentation 2011.

Treatment	Total Phenols (mg/L)	Tannin (mg/L)	Anthocyanin (mg/L)
Crushed Berries	2,147±3	905±202	589±11
Whole Berries	2,203±13 +2.6%	1,116±10 +23.3%	598±1 +1.5%

Table 17. Effect of berry integrity on mean phenolic compound concentrations during barrel aging 2011.

Treatment	Total Phenols (mg/L)	Tannin (mg/L)	Anthocyanin (mg/L)
Crushed Berries	2,137±43	1,163±14	358±49
Whole Berries	2,096±38 +2.0%	1,132±16 +2.7%	368±51 +2.7%

4.2.1 Total phenol concentration

Crushing berries did not greatly increase total phenolic concentration during the first two weeks of alcoholic fermentation. Total phenol concentration was an average of 56 mg/L greater in whole berries versus crushed berries (2.6% higher) (Table 16). On average, wine made from crushed berries was higher in total phenolic concentration versus whole berries throughout barrel aging. The mean total phenolic concentration for crushed berries was 2.0% greater compared to the whole berry treatment (Table 17).

4.2.2 Tannin concentration

Berry integrity did not affect tannin concentration during the first two weeks of alcoholic fermentation. Tannin concentration was an average of 211 mg/L higher (23.3%

greater) in whole berries versus crushed berries during alcoholic fermentation (Table 16). There was an increase in mean tannin concentration for crushed berries when compared to whole berries. The mean tannin concentration for the crushed berry and whole berry treatments during barrel maturation was 1163 mg/L (3% greater) and 1132 mg/L, respectively (Table 17).

4.2.3 Anthocyanin concentration

Berry integrity did not affect anthocyanin concentration when compared to whole berries during the first two weeks of fermentation. Anthocyanin concentration was an average of 43 mg/L greater (7.8% higher) in whole berries compared to crushed berries during alcoholic fermentation (Table 16). There were not great differences in mean anthocyanin concentration for crushed berries versus whole berries. The mean anthocyanin concentration for the crushed berry and whole berry treatments during barrel aging were 358 mg/L and 368 mg/L (3.0% greater), respectively (Table 17).

4.2.4 Discussion – Effect of berry integrity

The effect of berry integrity on the color of red wine was investigated. We hypothesized (Section 1.2) that crushed berries would have greater anthocyanin extraction versus whole berries during alcoholic fermentation, and wine made from crushed berries would contain greater anthocyanin concentrations through barrel aging when compared to wine made from whole berries. There is not enough evidence to prove our hypothesis with the data collected and analyzed during vintage 2011. Concentrations of total phenols, tannin and anthocyanin did not differ greatly between crushed and whole berries during alcoholic fermentation. Traditional vinification procedures crush berries for immediate extraction of anthocyanin content from their skins. Contrary to published claims, our results did not

substantiate an immediate color release during alcoholic fermentation (Ribereau-Gayon et al. 2000). Our experiment's conclusions suggested that berry integrity did not affect anthocyanin extraction during the first two weeks of alcoholic fermentation.

After fifteen months of barrel aging, mean concentrations of total phenols in wines made from crushed berries were greater than whole berry wines. Tannin concentrations increased in wines made from crushed berries when compared to wines made from whole berries throughout barrel aging. Anthocyanin concentrations remained unchanged during barrel aging. Crushing berries did not lead to greater color extraction in the vintage of 2011.

There have been a variety of studies that have investigated the effect of berry integrity on total phenols, tannin and anthocyanin concentrations (Cerpa-Calderon and Kennedy 2008, Pascual et al. 2016). However, in our experiment, the effect of berry integrity on total phenols, tannin and anthocyanin concentrations have been monitored through aging on a commercial scale.

A highest tannin concentration was observed in 75% crushed fruit on day 17, statistically higher than all other treatments in the study of Cerpa-Calderon and Kennedy (2008). The larger structures of proanthocyanidins are tannins; we measured tannin concentration (catechin equivalents). Our experiment produced different results; there was no difference in tannin concentrations (mg/L) after 14 days of alcoholic fermentation. This could be due to varietal differences; we experimented on Cabernet Sauvignon, and Cerpa-Calderon and Kennedy (2008) experimented on Merlot. In addition, the authors stated the Merlot was "...under-ripe from a commercial standpoint". Physiologically, the experiments are very different. In our experiment, we pressed the grapes once alcoholic fermentation was complete (day 14); the experiment by Cerpa-Calderon and Kennedy (2008) left the skins and seeds in contact for an additional 4 days after alcoholic fermentation was complete. This extended skin contact could provide insight as to why there was a difference between

concentration of berry intactness post alcoholic fermentation; the longer the wine is in contact with skins and seeds, the greater the amount of phenolic extraction (Moreno and Peinado 2012).

Other findings suggest whole destemmed berries extracted more phenolic compounds than did the destemmed crushed berries and whole cluster fruit (Pascual et al. 2016). The authors suggested whole destemmed berries had more total polyphenolic, anthocyanin, and tannin concentration that did the destemmed, crushed berries (control) after 6 months of barrel aging, although no statistics were published. After 15 months of barrel aging our Cabernet Sauvignon wines, there was a difference in total phenols and tannin, but not in anthocyanin concentration. The two experiments differed drastically. The experiment by Pascual et al. (2016) fermented 500 L of each treatment at 25°C. Our experiment fermented approximately 13,000 L per treatment (whole vs. crushed) at 32.2°C. Our experimentation was conducted on Cabernet Sauvignon, and Pascual et al. (2016) experimented on Grenache Noir.

Winemakers sourcing fruit from the Paso Robles AVA are typically looking for increased anthocyanin extraction and decreased tannin concentration. The results of our investigation suggest that the act of crushing berries creates wines with greater tannin and phenolic structure, without impacting anthocyanin concentration. The act of crushing berries in our experiment did not increase anthocyanin concentration during alcoholic fermentation. The act of crushing berries takes an extra step in red wine production that is both time and energy consuming. Bypassing the crusher apparatus on the crusher/destemmer will hypothetically allow a greater processing limit for an industrial winery; production speed will not be hindered by slower processing times mandated by implementing the crusher rollers.

Results from the 2011 vintage hint that wines made from whole berries contain a greater anthocyanin content than do wines made from crushed berries.

It is recommended to repeat this production scale experiment to see if the results obtained in 2011 are replicated. In a commercial production setting, whole berries experience a small amount of crushing as they are removed from the jacks. After the berries are destemmed, they fall though a hopper, then are transported to the fermentation vessel via 4-inch hard lines. These activities are affected by gravity and pump pressure, and a minimal amount of crushing results. Due to the various movements through the cellar, in addition to pump overs of berries throughout fermentation, it is fair to assume that "whole berries" lose their integrity as soon as they are received at a large-scale production winery. This would not be the case for a smaller, boutique winery processing three tons of fruit at a time. Minimal movement, gravity affect, tank size, and pump overs would greatly affect the level of naturally present "whole berries".

In addition, the resultant wines created from whole and crushed berries should be tasted by an expert sensory panel.

4.3 Enzyme application

Table 18. Effect of enzyme addition on phenolic concentration at the end of alcoholic fermentation in the 2010 and 2011 vintages.

Vintage	Concentration of enzyme	Total Phenols (mg/L)	Tannin (mg/L)	Anthocyanin (mg/L)
2010	Control	1,739±10	743±4	885±5
2010	60 mL/ton enzyme	1,894±1 +8.9%	875±10 +17.8%	872±3 -1.5%
2010	100 mL/ton enzyme	1,939±28 +11.5%	945±2 +27.2%	902±15 +1.9%
2011	Control	1,945±18	988±4	650±6
2011	100 mL/ton enzyme	2,307±27 +18.6%	1,263±6 +27.8%	754±26 +16.0%

4.3.1 Total phenol concentration

Table 19. Effect of enzyme addition on mean phenolic concentration during barrel aging in the 2010 and 2011 vintages.

Vintage	**Concentration of enzyme**	**Mean Total Phenolic concentration (mg/L)**
2010	Control	992±39
2010	60 mL/ton enzyme	1,113±52 +12.2%
2010	100 mL/ton enzyme	1,201±55 +21.1%
2011	Control	1,647±35
2011	100 mL/ton enzyme	1,791±47 +8.7%

Adding a pectinase-rich macerating enzyme increased total phenols during alcoholic fermentation in both the 2010 and 2011 vintages during barrel aging. The treatment of 60 mL/ton and 100 mL/ton enzyme increased total phenols by 8.9% and 11.5%, respectively, with the 2010 vintage at the end of alcoholic fermentation (Table 18). The treatment of 100

mL/ton enzyme increased total phenols by 18.6% (Table 18) in the vintage of 2011 at the end of alcoholic fermentation.

On average, the treatment of 60 mL/ton and 100 mL/ton enzyme increased total phenol concentration by 12.2% (121 mg/L) and 21.1% (209 mg/L) during barrel aging in the 2010 vintage. With the 2011 vintage, the treatment of 100 mL/ton increased total phenol concentration by 8.7% (144 mg/L) during barrel aging (Table 19).

4.3.2 Tannin concentration

Table 20. Effect of enzyme addition on mean tannin concentration during barrel aging in the 2010 and 2011 vintages.

Vintage	Concentration of enzyme	Mean Tannin concentration (mg/L)
2010	Control	377±68
2010	60 mL/ton enzyme	481±71 +27.6%
2010	100 mL/ton enzyme	561±73 +48.8%
2011	Control	773±25
2011	100 mL/ton enzyme	934±22 +20.8%

Adding pectinase-rich macerating enzymes affected tannin concentration during alcoholic fermentation in both the 2010 and 2011 vintages. The treatment of 60 mL/ton and 100 mL/ton enzyme increased tannin concentration by 17.7% and 27.0%, respectively (Table 18) in the 2010 vintage at the end of alcoholic fermentation. The treatment of 100 mL/ton enzyme increased tannin by 27.8% (Table 18) in the vintage of 2011 at the end of alcoholic fermentation.

Tannin concentration was associated with greater enzyme concentration during barrel aging in the 2010 and 2011 vintages. On average, enzyme treatments of 60 mL/ton and 100

mL/ton increased tannin concentration by 27.6% (104 mg/L) and 48.8% (184 mg/L), respectively in 2010 (Table 20). In 2011, the enzyme treatment of 100 mL/ton increased average tannin concentration by 20.8% (161 mg/L) (Table 20).

4.3.3 Anthocyanin concentration

Table 21. Effect of enzyme addition on mean anthocyanin concentration during barrel aging in the 2010 and 2011 vintages.

Vintage	Concentration of enzyme	Mean Anthocyanin concentration (mg/L)
2010	Control	327±64
2010	60 mL/ton enzyme	308±67 -5.8%
2010	100 mL/ton enzyme	346±63 +5.8%
2011	Control	394±43
2011	100 mL/ton enzyme	456±68 +15.7%

Different concentrations of pectinase-rich macerating enzymes did not influence anthocyanin concentration during alcoholic fermentation in 2010. The 60 mL/ton enzyme addition decreased anthocyanin by 5.8% (19 mg/L), while 100 mL/ton increased anthocyanin by 5.8% (18 mg/L) in 2010 (Table 18). The addition of enzyme during alcoholic fermentation of 2011 increased total anthocyanin concentration by 16% (104 mg/L) when compared to the control (Table 18).

There was no apparent correlation between anthocyanin concentration and enzyme treatment during barrel aging in 2010 (Table 21). Greater anthocyanin concentration can be explained by enzyme concentration during barrel aging in 2011. On average, anthocyanin concentrations were approximately 16% higher in the resultant wine treated with enzyme when compared to no enzyme treatment while maturing in barrel (Table 21).

4.3.4 Discussion – Effect of enzyme application

There has been a variety of studies claiming pectinase-rich macerating enzymes have effectively shown to increase anthocyanin content of red wines (Bakker et al. 1999, Kelebek et al. 2007, Li et al. 2015). Typically, these scientific papers involving the use of pectinase-rich macerating enzymes publish results immediately after alcoholic or malolactic fermentation. However, in our studies, the effect of pectinase-rich macerating enzymes on total phenols, tannin and anthocyanin were observed through 15 months of barrel aging on a commercial scale. The scale of our fermentations (20+ tons per fermentation), as well as the length of analyses (18 months total), make this experiment unique. Current research is limited by magnitude or timeline (Table 22).

Table 22. Scale of production and timeline on published papers on the effect of polymeric pigments by enzyme application.

Author(s)	Weight (kg) of Grapes Fermented	Results Reported (timeline)
Bautista et al. 2005	20	8 weeks
El Darra et al. 2016	50	2 weeks
Li et al. 2015	11,800	3+ weeks
Revilla and San Jose 2003	10	104 weeks
Romero-Cascales et al. 2012	140	2+ weeks
Wightman et al. 1997	4	26 weeks

In our 2010 experiment, the data collected suggested total phenolic concentration and tannin were correlated to concentration of enzyme (60 mL/ton and 100 mL/ton), while concentration of anthocyanin was not. With both vintages, increased amounts of tannin were extracted from the berries with the use of pectinase-rich macerating enzymes. If Paso Robles *Vvitis vinifera* Cabernet Sauvignon berries naturally contain ample amounts of tannin

extractable by traditional maceration techniques, it hinders wine quality to extract more tannin. Short monomeric compounds (monomers, dimers and trimers) are bitter; they cannot precipitate proteins. As winemakers, we want to limit any excessive extraction of tannin.

Based on the composition of Cabernet Sauvignon harvested in Paso Robles, adding pectinase-rich macerating enzymes to the berries in 2010 did the opposite of what we were aiming to prove (Section 1.2). In our 2011 experiment, the data collected suggested that all three measured phenolic compounds (total phenols, tannin and anthocyanin concentrations) greatly increased with the addition of 100 mL/ton enzyme application. We cannot presume to understand every chemical and biological reaction in the 2010 and 2011 vintages; several explanations could support our findings for vintage variations. Original berry chemistry (Tables 14 & 15) depicts both vintages' tank chemistry on day 0. Appendix E depicts the 2010 vintage total phenols, tannin and anthocyanin on day 0, and Appendix H depicts the 2011 vintage day 0 concentrations. Weather conditions, such as temperature or precipitation throughout the growing season, might have impacted the anthocyanin accumulation during veraison (Ortega-Regules et al. 2008). Vineyard management decisions such as pruning, leaf removal, dropping fruit, or irrigation practices, could have impacted anthocyanin development (Chorti et al. 2016, Guidoni and Hunter 2012). The fruit chemistry was different among the vintages, and this initial difference in phenolic concentrations could account for the different results.

In 2010, enzyme addition did not affect mean anthocyanin concentration, vindicating previously published papers that concluded enzyme application was of no statistical significance (Kelebek et al. 2007, Romero-Cascales et al. 2012). In 2011, 100 mL/ton enzyme addition greatly increased the mean anthocyanin concentration on average by 16%. This increase in anthocyanin concentration corresponds with what other scientific papers have concluded (El Darra et al. 2016, Kelebek et al. 2007, Romero-Cascales et al. 2012).

Macerating enzymes have shown to increase color intensity by 22% immediately after alcoholic fermentation when compared to the control (El Darra et al. 2016). Color intensity is a colorimetric coordination of 3 absorbances; clarity, red/green, and blue/yellow color components (*L*a*b**). Our experiment measured pigment release by assaying anthocyanin content (monomeric pigments). The basis by which color was measured and reported in the two experiments is different; therefore, it is not possible to compare color conclusions. The authors (El Darra et al. 2016) used a model wine Cabernet Sauvignon solution. The raw grapes were stored for approximately 1 week at 4°C before being processed. Our literature review suggests near freezing temperatures of berries prefermentation extract greater concentrations of anthocyanin during alcoholic fermentation (Table 9) (Busse-Valverde et al. 2011). These factors, along with magnitude (5 L ferments) and lack of pump overs, make the experiments quite different. Laboratory studies do not conduct normal winemaking pump overs, which is how anthocyanin molecules are typically extracted from the hyperdermal layer of the skin cell. Laboratory studies either have the cap submerged in the fermenting wine for the duration of alcoholic fermentation (greater anthocyanin extraction), or the caps will not be broken by a pump over (less anthocyanin extraction).

When maceration time was looked at, the extraction of tannin and anthocyanin concentration was faster when the enzyme was added when compared to the control (Bautista et al. 2005, Romero-Cascales et al. 2011). The application of the enzyme preparation led to higher anthocyanin concentrations (6-8%) that remained stable throughout aging when compared to the control. Equivalent results of greater anthocyanin extraction in the 100 mL/ton enzyme application was observed during our 2011 enzyme experiment when compared to the control. The berries used in our experimentation were Cabernet Sauvignon,

(Romero-Cascales et al. 2011) experimented with *Vitis vinifera* Monastrell berries. Our study did not induce wines that extracted tannin and anthocyanin concentrations faster with the use of a pectinase-rich macerating enzyme when compared to a control.

Cellulases and hemicellulases, both common in commercial enzyme preparations, could also assist in degrading the structure of the berry cell wall; these activities could increase extractable concentrations of phenols, tannins and anthocyanins (Gump and Haight 1995). We did not run chemical analysis on the enzyme used for our experiments (ColorPro®) to determine if there was cellulases or hemicellulases present. This could be a factor when trying to compare different macerating enzymes.

Color macerating enzymes are expensive. J. Lohr Vineyards & Wines® spends more than $25,000 annually on ColorPro®, a mixture of pectinase-rich macerating enzymes. If adding these enzymes to the berries does not extract more pigment that is persistent throughout barrel aging, the monetary investment is lost. Our hypothesis regarding the addition of color macerating enzymes (Section 1.2) suggested pigment release would be persistent through aging in barrels. In addition, pigment release would increase accordingly to the concentration of the enzyme preparation.

No conclusive results on the effect of pectinase-rich macerating enzymes versus anthocyanin concentration can be determined. It is recommended to conduct more experimentation comparing the effect of enzyme addition on anthocyanin concentration by repeating the experimental design of the 2011 vintage. The effectiveness of pectinase-rich macerating enzymes largely depends on the grape cultivar, the enzyme type, and the enzyme addition rate (Li et al. 2015). Our experiments used two different vineyards, which implies different clones, soil aspect, vine integrity, health, nutrients, etc. could have led to potential differences in grape chemistry.

4.4 Alcoholic fermentation temperature

Table 23. Effect of alcoholic fermentation temperature on phenolic compound concentrations at the end of alcoholic fermentation for the 2011 vintage.

Treatment	Total Phenols (mg/L)	Tannin (mg/L)	Anthocyanin (mg/L)
25.0°C	2,013±25	988±4	747±11
32.2°C	2,613±13 +29.8%	1,435±1 +45.2%	595±15 -20.3%

Table 24. Effect of alcoholic fermentation temperature on mean phenolic compound concentrations during barrel aging for the 2011 vintage.

Treatment	Total Phenols (mg/L)	Tannin (mg/L)	Anthocyanin (mg/L)
25.0°C	1,577±40	759±31	545±54
32.2°C	1,902±56 +29.8%	978±46 +45.2%	346±37 -36.5%

4.4.1 Total phenol concentration

Fermenting berries at 32.2°C during alcoholic fermentation was associated with an increase in total phenol concentration. Total phenol concentration was 600 mg/L higher (29.8% greater) in fruit fermented at 32.2°C than fruit fermented at 25.0°C by the end of alcoholic fermentation (Table 23). Wine fermented at 32.2°C remained greater in total phenol concentration from alcoholic fermentation throughout barrel aging (Table 24). On average, berries fermented at 32.2°C had increased total phenolic concentrations when compared to berries fermented at 25.0°C during barrel aging (Table 24). The mean total phenol concentration for berries fermented at 32.2°C and 25.0°C was 1,902 mg/L (20.6% greater) and 1,577 mg/L, respectively (Table 24).

4.4.2 Tannin concentration

Alcoholic fermentation carried out at 32.2°C resulted in an increased tannin concentration. Total tannin concentration was 447 mg/L higher (45.2% higher) in fruit fermented at 32.2°C than fruit fermented at 25.0°C by the end of alcoholic fermentation (Table 23). Wine fermented at 32.2°C continued to have greater tannin concentrations throughout barrel aging (Table 24). There was an increase amongst mean tannin concentration in berries fermented at a hot temperature (32.2°C) versus berries fermented at a cool temperature (25.0°C) during barrel aging. On average, berries fermented at 32.2°C had increased tannin concentrations when compared to berries fermented at 25.0°C throughout barrel aging. The mean tannin concentration for berries fermented at 32.2°C and 25.0°C was 978 mg/L (28.9% greater) and 759 mg/L, respectively (Table 24).

4.4.3 Anthocyanin concentration

Fermentation temperatures (32.2°C and 25.0°C) did not affect anthocyanin concentrations during the first two weeks of fermentation. Anthocyanin concentration was an average of 152 mg/L greater (25.5% higher) when fermented at 25.0°C (Table 23). There was an increase in anthocyanin concentration in berries fermented cool (25.0°) versus berries fermented hot (32.2°C) during barrel maturation. The mean anthocyanin concentration for berries fermented at 32.2°C and 25.0°C was 346 mg/L and 545 mg/L (57.5% higher), respectively (Table 24).

4.4.4 Discussion – Effect of temperature

There has been in the past a variety of studies that have looked at total phenols, tannin and anthocyanin concentrations on the effect of alcoholic fermentation temperature

(Sacchi et al, 2005, Sener and Yildirim 2016, Yamane et al 2006). However, in our study, the effect of alcoholic fermentation temperatures has been followed through barrel aging on a commercial magnitude. The effect of fermentation temperature on the color of red wine was investigated in the 2011 vintage. Our hypothesis (Section 1.2) stated fermenting at cooler temperatures (25.0°C) will lead to a greater anthocyanin extraction during alcoholic fermentation of red grapes when compared to hotter alcoholic fermentation temperatures (32.2°C). There was no difference when fermenting at 25.0°C when compared to 32.2°C on anthocyanin concentrations during alcoholic fermentation, whereas total phenol and tannin concentrations were greater during the first fourteen days of alcoholic fermentation. By the end of alcoholic fermentation at 32.2°C, total phenol and tannin concentrations increased by 29.8% and 45.2%, respectively, when compared to 25.0°C alcoholic fermentation temperatures.

At the end of barrel aging, increased extraction in total phenol and tannins was still evident with 32.2°C alcoholic fermentation temperatures. Total phenol concentrations were 21% greater and tannin concentrations were 29% greater in wines resulting from hot (32.2°C) fermentation temperatures. There was a difference in anthocyanin concentration levels between berries fermented at 25.0°C versus berries fermented at 32.2°C during barrel aging; on average, anthocyanin concentration was 36.5% higher in the wine resulting from berries fermented cooler versus berries fermented hot.

Our results corroborate with other published studies where fermentation temperatures were altered to increase phenolic extraction (Lerno et al. 2015, Gil-Munoz et al. 2009). Fermenting berries at cooler temperatures (25.0°C) produced wine with greater anthocyanin concentration, and less total phenol and tannin concentrations when compared to hot (32.2°C) fermentation temperatures. Extraction of monomeric anthocyanin concentrations were

increased with higher fermentation temperatures (Gao et al. 1997). This could be due to varietal differences; our experimentation was on Cabernet Sauvignon, and Gao et al. (1997) experimented on Pinot Noir. Our results support our hypothesis stating fermenting at cooler temperatures will create a wine with greater anthocyanin concentrations.

Temperature affected the rate of extraction, but not the final concentration of phenolic extraction (Lerno et al. 2015). This experiment, like ours, used Cabernet Sauvignon grapes. Lerno et al. (2015) had the fruit hand-picked, minimizing any additional extraction of polyphenolics while the fruit was transported from Lodi to Davis, CA. The fruit used for our experimentation was machine picked due to the sheer volume of the experiment. The Lodi fruit went through an extended maceration of four days; ours did not. When juice is in contact with skins, the fermenting juice will continue to extract polyphenolics, specifically tannin.

Changing the temperature during alcoholic fermentation is an effective way to influence polyphenolic extraction; temperature affects cell and membrane permeability (Gil-Munoz et al. 2008). Using cold maceration techniques, Gil-Munoz et al. (2008) could obtain the highest concentrations of anthocyanins using Cabernet Sauvignon grapes. These results were similar to the results obtained with cooler (25.0°C) fermentation temperatures.

In a production environment, cooler fermentation temperatures are more difficult to deliver than hotter fermentation temperatures. It presents a challenge to keep fermentation temperatures cool during harvest when yeast exert heat as a biproduct of their fermentation. Tank space is typically maximized, and glycol chilling systems are working on extreme overload to keep up with cooling glycol jacket demands. In an experiment done on a production scale such as our study, it was easier for glycol systems to keep temperatures at 32.2°C opposed to cooler 25.0°C temperatures.

A winery needs to consider how important is it to have less tannin and total phenol concentrations, and more anthocyanin concentrations in their wines. It might make the most sense for winemakers to identify highly tannic, low color wines, and adapt cooler fermentation temperatures to these specific wines. If current glycol systems are not able to obtain cooler fermentation temperatures, a winery would need to determine if expanding or replacing the current glycol system would be worth the financial investment. Would the winery be able to increase profitability to pay for the newly expanded cooling infrastructure? Adding or amending a glycol system is extremely costly. A more cost friendly way to mitigate excessive amounts of tannin and total phenols is done with fining agents, either during aging or before bottling.

In our experiment, the tanks received six pump overs per day. By increasing the number of pump overs over traditional winemaking techniques, the cap remained relatively cool. As a result, neither the fermenting juice nor the berries were hot. Typically, wineries manually complete two pump overs or punch downs per day. The heat released by the fermentation is trapped in the cap, increasing the temperature 10-14°C higher than the fermenting juice below (Schmid et al. 2009). This large temperature difference between the cap and the fermenting juice can create a wine that is ridden with problems; the yeast can become stressed and produce off flavors and aromas, or can die from the environment. In our experiment, the tank tops were visually and aromatically inspected three times per day, and special attention was paid to ensure there no off aromas. Fermenting at hotter temperatures (32.2°C) can impact wine quality. Make sure to maintain homogenous tank temperatures, pump over the fermenting juice frequently, and maintain glycol jacketed tanks so they work properly.

It is recommended to repeat this production scale experiment to see if the results obtained in 2011 can be replicated. It is also recommended to publish large polymeric pigments (LPP's) and small polymeric pigments (SPP's) to see if monomeric anthocyanin concentrations were shifting into polymeric stable pigment compounds. The resultant wines created from different fermentation temperatures should be tasted by an sensory expert panel.

4.5 Conclusion

Experiments of the 2010 and 2011 vintages analyzed Paso Robles Cabernet Sauvignon on an actual production scale (24 tons). Each fermentation contained a minimum of 20 tons of fruit. The experiments were an accurate representation of a large production facility. In addition, this study investigated total phenol, tannin, and anthocyanin concentrations through the various life stages of the wine.

It is insignificant if berry integrity, adding pectinase-rich macerating enzymes, or fermenting at different temperatures helped to extract greater anthocyanin concentrations during alcoholic fermentation. It is only relevant if an anthocyanin molecule binds with a (tannin) molecule to form a stable polymeric pigment, and this greater pigmented wine is greater than its control. If the anthocyanin concentration is not significantly different from a control treatment when the wine is ready to bottle, all efforts and money towards extracting and stabilizing polymeric pigments are wasted.

The trends of this study were obtained at a large scale on Estrella district Cabernet Sauvignon. Our study's trends could be different if wine production is performed in different conditions, including magnitude and varietal(s).

4.6. Future Studies

It is recommended to repeat each experiment. It is strongly advised to use the same vineyard used in the 2011 experiments, as well as have duplicate experiments, to conclude statistical significance amongst treatments.

It is further recommended to experiment with other varietals. Each varietal has a different range of phenolic concentrations. We only experimented with *Vitis vinifera* Cabernet Sauvignon.

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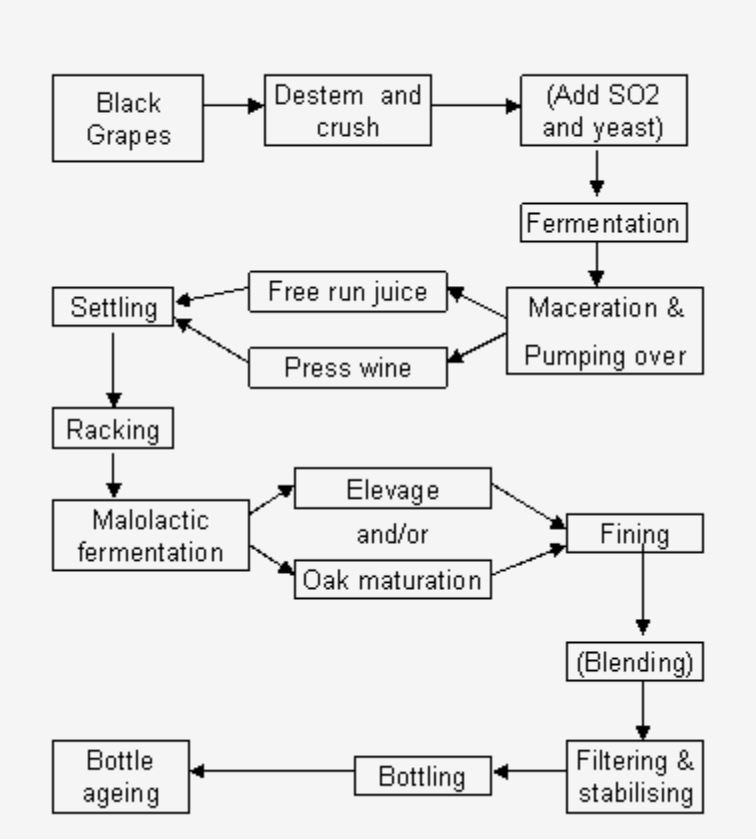
APPENDICES

Appendix A. Red winemaking flowchart



http://www.execellars.co.uk/making_wine.html

Appendix B. Buffer solution preparation

Buffer A

6.0 ml of acetic acid was mixed with 4.97 grams of NaCl into 400 ml deionized water in a 500 mL graduated flask. 1N NaOH (4 g/100ml) was used to raise the pH to 4.9. Deionized water was added to the graduated flask to reach 500 mL. Buffer A was homogenized thoroughly by inversion. Buffer A had a shelf life of one month at room temperature.

Buffer B

2.5 grams potassium bitartrate (KHT) was added to 300 mL deionized water in a 500 mL graduated flask. 63 ml of 95% ethanol was added to the flask and mixed. 1N HCl (82 mL /1,000 ml) was then added (dropper increments) into the KHT solution to reach 3.3 pH. The solution was raised to 500 ml with deionized water. Buffer B was homogenized thoroughly. Buffer B had a shelf life of one month at room temperature.

Buffer C

25.0 grams of SDS was mixed into 400 mL deionized water using a 500 mL graduated flask. 25.0 ml of 5% TEA was added to the SDS solution with a magic pipette (viscous solution). 1N HCl was added (dropper increments) to the SDS solution to reach 9.4 pH. The solution was raised to 500 mL with deionized water, and homogenized thoroughly by inverting the graduated flask several times. Buffer C had a shelf life of one month at room temperature.

Buffer D

11.61 grams maleic acid and 4.97 grams NaCl were added to 400 mL deionized water using a 500 mL graduated flask. 1N NaOH was added (dropper increments) to the Buffer D

solution to reach 1.8 pH. The solution was raised to 500 mL with deionized water, and homogenized thoroughly by inversion. Buffer D had a shelf life of one month at room temperature.

Ferric Chloride

225 ml of 0.01N HCl (8.2 mL /1,000 mL) was transferred into a 250 mL graduated flask. 0.676 g (±0.003 grams) of ferric chloride was added to the HCl solution, and mixed thoroughly. The volume of the ferric chloride solution was increased to 250 mL with 0.01N HCl. The ferric chloride solution was homogenized completely by inversion. Ferric Chloride buffer had a shelf life of 1 week at room temperature.

Bleaching solution

3.95±0.05 g potassium metabisulfite (KMBS) was weighed and transfer into a 50 mL volumetric flask containing ~ 40 mL deionized water. The flask was mixed thoroughly to completely dissolve the KMBS, and the volume was brought up to 50 mL with deionized water. The solution was kept refrigerated. The bleaching solution had a shelf life of 1 day.

Protein solution

0.075±0.005 g of BSA was weighed and transferred into a 50 mL volumetric flask containing ~ 40 mL Buffer A. The flask was mixed thoroughly until the BSA was completely dissolved, and the volume was brought up to 50 mL with Buffer A. The solution was kept refrigerated. The protein solution had a shelf life of 1 day.

Appendix C. Adams-Harbertson procedure

Pigments (step 1/2)

300 µL Buffer B was pipetted into a 1.5 ml microfuge tube using a Magic Pipette®. To the microfuge tube, 200 µL of wine and 1 ml Buffer A was added. The content of the microfuge tube was mixed by inverting it up and down several times. The tube was incubated at room temperature for 10 minutes. The tube was opened, and 1 ml of the mixture was pipetted into a clean cuvette. The spectrophotometer was blanked with Buffer A. The samples were read and recorded at 520 absorbance for Reading A. The cuvettes were saved for pigments step 2/2.

Bleachable Pigments (step 2/2)

150 µL of bleaching solution was added to each cuvette made in step 1/2 (pigments). The cuvette was covered with parafilm paper, and inverted 2-3 times to properly homogenize. The cuvette was incubated at room temperature for 10 minutes. The spectrophotometer with blanked with Buffer A. The samples were read and recorded at 520 absorbance for Reading B. The cuvettes were dumped.

Tannin (step 1/3)

30 µL Buffer B was pipetted into a 1.5 ml microfuge tube using a Magic Pipette®. To the microfuge tube, 20 µL of wine and 1 ml BSA solution was added. Using a vortex mixer, the microfuge tubes were homogenized. The microfuge tubes were incubated at room temperature for 15 minutes, then centrifuged for 5 minutes at 14,000 RPMs. 1 ml of the supernatant (liquid) was pipetted into a clean cuvette. The centrifuge tubes were kept to the side for a later step. 50 µL bleach was added to each cuvette. The cuvettes were then homogenized using a vortex mixer, then incubated for an additional 10 minutes at room

temperature. The spectrophotometer with blanked with BSA solution. The samples were read and recorded at 520 absorbance for Reading C.

Tannin (step 2/3)

From the microfuge tubes, any supernatant (liquid) remaining was disgorged. 1 ml Buffer C was added to the microfuge tubes. The tubes were then incubated for 15 minutes at room temperature. The tannin pellet settled to the bottom of the microfuge tube was resuspended with the vortex, and the solution was incubated for another 5 minutes at room temperature. The entire contents from the microfuge tube was dumped into a clean cuvette. The spectrophotometer was blanked with Buffer C. The samples were read and recorded at 520 absorbance for the Tannin Background reading. The cuvettes were saved for tannin step 3 of 3.

Tannin (step 3/3)

150 µL ferric chloride was added to the cuvettes made in step 2. The cuvettes were thoroughly homogenized with the Vortex mixer. The samples were incubated for 10 minutes at room temperature. The spectrophotometer was blanked with Buffer C. The samples were read and recorded at 520 absorbance for the Tannin Final reading.

Anthocyanin

450 µL Buffer B was pipetted into a 1.5 ml cuvette using a Magic Pipette®. 50 µL wine and 1 ml Buffer D was added to the cuvette. Using the vortex mixer, the cuvette was homogenized. The cuvettes were incubated at room temperature for 10 minutes. The spectrophotometer was blanked with Buffer D. The samples were read and recorded at 520 absorbance for Reading D.

Total Phenolics (1/2)

100 µL wine was pipetted into a 1.5 ml cuvette using a Magic Pipette®. 1 ml Buffer C was added to the cuvette. A vortex mixer was used to homogenize the cuvette. The

cuvettes were incubated for 10 minutes at room temperature. The spectrophotometer was blanked with Buffer C. The samples were read and recorded at 520 absorbance for the Iron Reactive Phenolics (IRP) background reading. The cuvettes were kept for Total Phenolics part 2 of 2.

Total Phenolics (2/2)

150 µL ferric chloride was added to the IRP background cuvettes step 1. A vortex mixer was used to homogenize the solution after the ferric chloride was added. The cuvettes were incubated for 10 minutes at room temperature. The spectrophotometer was blanked with Buffer C. The cuvettes were read and recorded at 520 absorbance for the IRP Final reading.

Appendix D. Adams-Harbertson calculations

Anthocyanin calculations

((30 x Reading **D**) – (7.5 x Reading **A**) AU) / (0.0153) = mg/L malvidin-3-glucoside

Where 30 and 7.5 are dilution factors of

Polymeric Pigment calculations

1.15 x 1.33 x 7.5 x (Reading **B** – Reading **C)** = AU_{520} LPP

1.15 x 1.43 x 7.5 x (Reading **C**) = AU_{520} SPP

Where 1.15 accounts for the dilution due to sulfur dioxide addition, 1.33 is the empirical bleaching correction coefficient for LPP, and 1.43 is the empirical bleaching coefficient for SPP.

Tannin calculations

[5 x ((1.15 x Tannin $_{\mathbf{final}}$) – Tannin $_{\mathbf{background}}$ AU))] / 0.0052 mg $^{-1}$ L AU) = mg/L catechin equivalents

Where the absorbance of Tannin $_{\mathbf{final}}$ is multiplied by the dilution factor of 1.15 to account for the Ferric Chloride addition.

Total Phenolics calculations

[11 x ((1.136 x IRF $_{\mathbf{final}}$) – IRP $_{\mathbf{beginning}}$ AU)] / (0.0052 L mg $^{-1}$ L AU) = mg/L catechin equivalents.

Where the absorbance of IRF $_{\mathbf{final}}$ is multiplied by the dilution factor of 1.136 to account for the Ferric Chloride addition.

Appendix E. Receiving fruit analysis (2010)

Adams-Harbertson Data (Average of duplicates)

Adams Data	**PP/T**	**TP**	**Tannin**	**Anthos**	**PP**	**LPP**	**SPP**
10CSP4X1-230	1.94	94	7	22	0.14	-0.02	0.16
10CSP4X2-231	1.52	99	9	16	.014	-0.03	0.17
10CSP4X3-232	1.61	89	9	14	0.14	-0.06	0.20
Averages	1.69	94	8	17	0.098	-0.025	0.18

PP/T = Polymeric pigments / tannin. TP = Total phenols. Anthos = Anthocyanin. PP = Polymeric pigments. LPP = Large polymeric pigments. SPP = Small polymeric pigments.

Blend ID	**Tons**
10CSP4X1-230	23.82
10CSP4X2-231	26.48
10CSP4X3-232	22.76
Averages	24.35

.

Appendix F. Fermentation analysis (2010)

Tank	Blend ID	Date	CD	TI	Brix	Temp
336	**15CSP4X1-230**	10/31/2010			24.0	87
		11/1/2010	5.2	29.3	23.6	81
		11/2/2010	7.1	34.0	17.3	80
		11/3/2010	7.4	39.2	11.6	80
		11/4/2010	8.7	42.8	7.4	80

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
337	**15CSP4X2-231**	10/31/2010			24.3	80
		11/1/2010	5.4	29.3	22.8	80
		11/2/2010	7.2	35.2	16.0	78
		11/3/2010	7.8	42.3	10.4	80
		11/4/2010	8.8	41.7	6.3	81

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
300	**15CSP4X3-232**	10/31/2010			24.0	87
		11/1/2010	4.8	26.8	23.6	81
		11/2/2010	7.2	35.0	17.3	80
		11/3/2010	8.6	43.6	11.6	80
		11/4/2010	8.6	42.0	7.4	80

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Appendix G. Pressed wine analysis (2010)

Tank	Blend ID	CD press	TI press	CD/TI
BX661	10CSP4X1-230 Control	8.5	42.6	1.99
BX662	10CSP4X2-231 60 mL/ton	8.7	44.7	1.94
BX660	10CSP4X3-232 100 mL/ton	8.2	44.2	1.86
	Averages	8.5	43.9	1.93

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD/TI = Color density / tannin index.
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Appendix H. Receiving fruit analysis (2011)

Adams-Harbertson Data (Average of duplicates)

Adams Data	**PP/T**	**TP**	**Tannin**	**Anthos**	**PP**	**LPP**	**SPP**
11CSP14-3D1	1.54	415	18	271	0.28	-0.01	0.29
11CSP14-3D2	1.36	534	21	301	0.28	-0.05	0.33
11CSP14-3D3	1.83	520	19	312	0.36	-0.06	0.42
11CSP14-3D4	1.45	570	24	342	0.34	-0.06	0.41
11CSP14-3D5	1.56	499	23	278	0.35	-0.04	0.39
11CSP14-3D6	1.76	513	26	370	0.46	-0.07	0.53
Averages	1.58	509	22	312	.35	-0.05	0.40

PP/T = Polymeric pigments / tannin. TP = Total phenols. Anthos = Anthocyanin. PP = Polymeric pigments. LPP = Large polymeric pigments. SPP = Small polymeric pigments.

Blend ID	**Tons**
11CSP14-3D1	22.28
11CSP14-3D2	21.22
11CSP14-3D3	21.41
11CSP14-3D4	21.27
11CSP14-3D5	22.29
11CSP14-3D6	20.07
Averages	21.42

Appendix I. Fermentation analysis (2011)

Tank	Blend ID	Date	CD	TI	Brix	Temp
295	**11CSP14-3D1**	11/4/2011	1.8	18.3	25.6	61
		11/5/2011	2.9	22.3	25.5	61
		11/7/2011	3.8	27.3	20.3	69
		11/8/2011	4.8	29.9	16.9	72
		11/9/2011	5.5	37.6	12.8	83
		11/10/2011	5.9	38.5	9.5	84
		11/11/2011	6.5	38.7	8.7	85
		11/12/2011	6.7	39.6	7.8	92
		11/14/2011	7.3	44.1	7.2	76
		11/15/2011	8.5	45.6	6.9	74
		11/16/2011	7.7	47.3	6.5	73

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
296	**11CSP14-3D2**	11/4/2011	1.0	16.4	25.7	69
		11/5/2011	3.2	20.8	25.0	67
		11/7/2011	5.5	33.6	16.6	72
		11/8/2011	7.3	37.1	13.5	73
		11/9/2011	6.6	38.5	11.2	72
		11/10/2011	6.5	38.3	9.4	72
		11/11/2011	6.8	41.9	7.9	72
		11/12/2011	6.5	40.6	6.7	75
		11/14/2011	7.4	44.2	4.3	73
		11/15/2011	8.3	49.0	3.7	72
		11/16/2011	8.2	47.1	2.9	74

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
297	**11CSP14-3D3**	11/4/2011	1.1	16.4	26.3	64
		11/5/2011	3.9	24.5	24.9	69
		11/7/2011	6.5	38.9	14.6	79
		11/8/2011	6.3	39.1	11.2	80
		11/9/2011	7.2	43.2	8.7	84
		11/10/2011	6.8	41.0	6.9	84
		11/11/2011	6.6	46.3	5.6	82
		11/12/2011	6.0	40.1	4.7	85
		11/14/2011	7.0	49.7	4.2	83
		11/15/2011	8.0	51.1	4.1	84
		11/16/2011	8.6	51.9	4.3	81

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
298	**11CSP14-3D4**	11/4/2011	1.1	14.5	25.9	71
		11/5/2011	4.1	24.2	24.3	69
		11/7/2011	6.5	41.3	13.3	82
		11/8/2011	6.4	37.5	10.2	83
		11/9/2011	7.4	44.0	7.9	82
		11/10/2011	7.2	44.6	6.2	83
		11/11/2011	7.0	45.6	5.0	83
		11/12/2011	5.9	43.3	4.2	85
		11/14/2011	7.2	48.8	4.0	86
		11/15/2011	8.9	50.1	4.0	84
		11/16/2011	8.8	51.5	4.0	84

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
299	**11CSP14-3D5**	11/4/2011	2.2	17.9	26.0	72
		11/5/2011	4.1	25.3	24.9	71
		11/7/2011	6.2	36.7	16.4	72
		11/8/2011	6.5	36.5	13.6	72
		11/9/2011	6.6	36.5	11.7	72
		11/10/2011	6.5	38.4	10.2	72
		11/11/2011	8.1	42.3	8.4	73
		11/12/2011	6.6	38.4	7.2	74
		11/14/2011	7.3	45.9	4.8	74
		11/15/2011	7.9	46.3	4.0	73
		11/16/2011	7.8	45.7	3.1	73

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Tank	Blend ID	Date	CD	TI	Brix	Temp
300	**11CSP14-3D6**	11/4/2011	3.5	22.2	26.3	71
		11/5/2011	6.6	33.7	23.8	72
		11/7/2011	7.3	41.1	14.4	73
		11/8/2011	7.1	41.4	11.2	81
		11/9/2011	7.6	42.8	8.7	82
		11/10/2011	7.4	43.6	6.7	84
		11/11/2011	8.2	47.1	5.2	84
		11/12/2011	7.1	41.2	4.2	86
		11/14/2011	7.8	49.0	3.1	84
		11/15/2011	8.2	46.2	3.2	84
		11/16/2011	9.5	48.7	3.0	85

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD and TI were analyzed on a spectrophotometer (Average of duplicates)

Appendix J. Pressed wine analysis (2011)

Tank	Blend ID	CD press	TI press	CD/TI
329	11CSP14-3D1	9.0	53.2	1.70
331	11CSP14-3D2	9.1	53.9	1.70
335	11CSP14-3D3	11.1	68.7	1.61
291	11CSP14-3D4	11.5	69.9	1.64
295	11CSP14-3D5	9.6	54.8	1.76
296	11CSP14-3D6	11.2	64.6	1.74
	Averages	10.25	52.85	1.69

CD = Color density (420A + 520A). TI = Tannin index (280A).
CD/TI = Color density / tannin index.
CD and TI were analyzed on a spectrophotometer (Average of duplicates)