The influence of Cabernet Sauvignon grape maturity on the concentration and extraction of colour and phenolic compounds in wine

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Summary

Extended maturation of wine grapes is employed to achieve optimum berry flavour development and phenolic maturity for the desired wine style. While it has been suggested that fruit maturity may also influence the extraction efficiency of colour and mouthfeel compounds from grapes into wine during processing, this has not been thoroughly evaluated.

One aim of this research was to determine the impact of grape harvest date on the colour metrics and phenolic compounds in wines made from grapes harvested beyond historic or traditional maturity levels. To investigate this, berry phenolic composition and concentration were measured over two seasons (2008 and 2009) throughout post-veraison maturity of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes, along with the composition and concentration of colour and phenolics in the wines produced from these grapes.

The data did not support the notion of increased extractability of phenolic compounds with grape maturity. However, the relative wine phenolic concentrations themselves might be more commercially relevant than extractability. Based on the 2008 grape and wine phenolic data, concentrations in wine appeared directly related to the grape concentrations. Unfortunately, the trends were not as clear in 2009. Grape malvidin-3-glucoside and polymeric tannin concentrations increased with ripening and the wine concentrations trended similarly. Grape caftaric acid, catechin, epicatechin, and B2 dimer concentrations declined with ripening, and this was reflected in their concentrations in the wine.

Phenolic compounds were measured as they are known to provide colour, astringency and bitterness to wines. Descriptive analysis was performed in order to determine how grape ripeness affected the wines made from these grapes. Principal component analysis of the sensory data differentiated the wines by harvest week; however, the phenolic compounds measured did not fully explain the changes in wine sensory properties. Prediction models of sensory attributes describing colour and astringency were reasonable in 2008, but not 2009. This was likely due to the weaker chemical concentration trends in 2009. Additional metrics are likely needed to explain the complex nature of the wine.

Harvesting grapes at higher maturities also results in increased alcohol concentrations in the resulting wines. This can result in wines which possess undesirable sensory aspects such as excessive alcohol, as well as stuck fermentations due to alcohol inhibition of yeast growth. In some cases, incoming must may be diluted with water to adjust the final alcohol content of the wine to approximately 14% (v/v). To test the impact of dilution, wines were made from Chardonnay and Zinfandel grapes harvested at high sugar levels. The pre-fermentation sugar concentrations were lowered with water or dealcoholized wine, and compared to wines made with no sugar adjustment. The concentration of both the phenolic and aroma compounds of these wines was assessed and correlated to sensory data. Using PCA, the Chardonnay control wines were separated from the treatment wines based on phenolic chemistry and descriptive analysis, but the aroma compound concentrations were not diluted by the water or dealcoholized wine addition. In Zinfandel, PCA of the phenolic compound concentrations did not separate the control and water added treatment; however, the aromas were more similar between the control and dealcoholized wine treatment. Sensorially, the Zinfandel control wines could be separated from the treatments, which also differed from one another.

Declaration

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Statement of the contributions of jointly authored papers

1. Yonker, C.C., Ford, C.M., Dry, P.R., Dokoozlian, N.K. Fruit maturity influences the extraction of berry polyphenol compounds into Cabernet Sauvignon (*Vitis vinifera* L.) wines. Manuscript in preparation for submission to *Australian Journal of Grape and Wine Research*.

Author Contributions: CCY produced wines, conducted HPLC analysis, analysed the data and drafted/constructed the manuscript. CMF, PRD and NKD contributed to the research ideas and the editing of the manuscript.

2. Yonker, C.C., Bastian, S.E.P., Ford, C.M., Johnson, T.E., Dokoozlian, N.K. Impact of fruit maturity on polyphenol compounds in Cabernet Sauvignon (*Vitis vinifera* L.) wines and correlations to sensory perception. Manuscript in preparation for submission to *Australian Journal of Grape and Wine Research*.

Author Contributions: CCY produced wines, conducted HPLC analysis, assisted with sensory panel, analysed the data, and drafted/constructed the manuscript. SEPB and TEJ conducted wine sensory analysis. SEPB, CMF and NKD contributed to the research ideas and the editing of the manuscript. TEJ led descriptive analysis panel and assisted with sensory data analysis and editing of the manuscript.

3. Yonker, C.C., Ford, C.M., Bastian, S.E.P., Johnson, T.E., Dokoozlian, N.K. Impacts of water addition to facilitate fermentation on the concentration of polyphenolic compounds and sensory properties of wines. Manuscript in preparation for submission to *American Journal of Enology and Viticulture.*

Author Contributions: CCY produced wines, conducted HPLC analysis, assisted with sensory panel, analysed the data, and drafted/constructed the manuscript. SEPB and TEJ conducted wine sensory analysis. SEPB, CMF and NKD contributed to the research ideas and the editing of the manuscript. TEJ led descriptive analysis panel and assisted with sensory data analysis and editing of the manuscript.

The following authors agree that the Statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1, 2, and 3 and give consent to their inclusion in this thesis.

..... Yonker, C.C.

..... Ford, C.M.

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..... Bastian, S.E.P.

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Abbreviations

Abs420	absorbance at 420nm
Abs520	absorbance at 520nm
ANOVA	analysis of variance
AU	absorbance units
AWRI	Australian Wine Research Institute
BSA	berry sensory analysis
Con	experimental control (Chapter 4)
CRP	caftaric reaction product
DA	descriptive analysis
DAL	dealcoholized wine added to must pre-fermentation (Chapter 4)
DAP	diammonium phosphate
DE	diatomaceous earth
EI	extractability index
ETc	estimated daily water requirement
ETo	daily reference evapotranspiration value
FTIR	Fourier transform infrared spectroscopy
GAE	gallic acid equivalent

GC-MS	gas chromatography-mass spectrometry
GRP	grape reaction product
HPLC	high performance liquid chromatography
Hunter a	colour measured from green (-) to red (+)
Hunter b	colour measured from blue (-) yellow (+)
Hunter L	colour measured from black (0) to white/clear (100)
ITV	Institute Technique de la Vigne et du Vin
Kc	seasonal crop coefficient
LSD	least significant difference
mDP	mean degree of polymerization
MFA	multiple factor analysis
PB	dealcoholized wine added post-fermentation (Chapter 4)
PCA	principal component analysis
PLS	partial least squares
PLSR	partial least squares regression
RS	reducing sugar
SA	saignee followed by water addition to must pre-fermentation (Chapter 4)
SPME	solid phase micro-extraction

TA	titratable acidity
TSS	total soluble solids
UV	ultraviolet
VA	volatile acidity
WA	water added to must pre-fermentation (chapter 4)
YAN	yeast assimilable nitrogen

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1 Introduction

1.1 Introduction

Consumer preferences, along with the influence of the wine and food media, are the primary drivers of the wine styles produced for the U.S. market. Winemakers and grape growers must ensure that their products meet stylistic preferences desired by consumers. A desire for wines with strong fruit intensity and an absence of negative flavours, including green or vegetative aromas and flavours, has brought about a change in wine grape harvest criteria. The overall goal of production winemakers is to harvest at a point that the green compounds have degraded and the fruit flavours are prominent.

Historically, the harvest date of wine grapes was determined based on basic juice composition, including sugar, acid, and pH. However, more recently, additional metrics including berry colour and flavour have been used to indicate grape harvest quality. Coombe and Iland (2004) defined flavour ripening as the development of preferred berry flavour and used the term engustment to refer specifically to the aroma changes during ripening which can only be assessed by tasting (Coombe and McCarthy, 1997, Coombe and Iland, 2004). Le Moigne (2008) defined phenolic maturity as the time at which the anthocyanin concentration is greatest and the seed tannin contribution is low relative to the total tannins, and showed that a trained sensory panel could discriminate between grapes harvested on different dates using textural maturity attributes. Winter et al. (2004) defined protocols for Berry Sensory Analysis (BSA) to evaluate sugar concentration, aromas, colour, and phenolics. The goal is to ensure the negative aromas have decreased, that positive fruit aromas have developed, and that the tannins have matured to achieve the desired wine style.

The practice of maintaining the fruit on the vine beyond traditional harvest maturities (ca 24°Brix) is called extended maturation (Coombe and McCarthy, 1997). This technique is employed to achieve

optimum berry flavour development and phenolic maturity for the desired wine style. Desirable flavours may include a decrease in vegetal aromas (LeMoigne et al., 2008) and an increase in fruit characters (Winter et al., 2004). However, extended maturation may also lead to undesirable viticultural effects such as berry shrivel and the associated yield losses (McCarthy and Coombe, 1999, Rogiers et al., 2006).

Extensive analysis of grape berries sampled throughout the growing season over the past 4 years (ca. 10,000 samples total) by the Grape Analysis Laboratory at E. & J. Gallo Winery suggests that in many cases the composition and concentration of grape colour and phenolic compounds does not change dramatically over the course of the extended maturation period. However, wines produced from grapes harvested at later dates often contain increased amounts of colour and phenolic compounds. Price et al. showed (1995a) that Pinot Noir wines from shaded fruit had decreased anthocyanins, flavanols and phenolic polymers compared to wines from sun exposed fruit, although the grapes from these treatments did not differ significantly in these compounds. Thus, changes in the extraction efficiency of grape colour and phenolic compounds during winemaking may be at least partially responsible for changes in wine composition commonly observed as a result of extended grape maturation.

Little research has been published regarding the effects of extended maturation on the grape phenolic composition or the extractability of grape phenolic compounds into wine. Holt et al. (2010) reported on the total phenolics, total tannins, and total anthocyanins in Cabernet Sauvignon grapes during late stages of ripening, but extraction of these compounds was not assessed. In five samples taken between 22° and 37°Brix, Holt et al. (2010) observed that the total phenolic and total tannin

concentrations peaked at ~30°Brix while the total anthocyanin concentration peaked at ~27°Brix. Canals et al. (2005) and Fournand et al. (2006) are among the few who have studied extraction of phenolic compounds from grapes into wine at different stages of grape ripeness. Canals et al. (2005) showed improved extraction of anthocyanins and proanthocyanidins from riper Tempranillo grapes. However, the last harvest date was less than six weeks post-veraison and sugar content did not exceed 18°Brix (Canals et al., 2005). In contrast, Fournand et al. (2006) assessed grapes with sugar concentrations from 162.6-275.0 g/L and showed no changes in extraction of skin anthocyanins or proanthocyanidins due to increased sugar content.

Grape maturity is considered a factor in wine quality (Ough and Singleton, 1968, DuPlessis and VanRooyen, 1982). However, most studies focus on early fruit development or from veraison to commercial harvest. The present study was conducted to determine the effects of extended maturation on grape phenolic concentrations and the extraction of those compounds into wine.

1.2 General description of phenolic compounds in grapes and wines

Phenolic compounds are the third most abundant constituent in grapes and wine, following carbohydrates or the alcohol produced from them and the organic acids (Singleton, 1980). Phenols contribute to the colour and mouthfeel of the grapes and wines (Coombe and Iland, 2004) and may provide health benefits in the form of antioxidants (Scalbert et al., 2005, Prajitna et al., 2007).

Phenolic compounds are cyclic benzene compounds having one or more hydroxyl groups associated directly with the ring structure (Jackson, 2000). While they contain alcohol groups, they do not display the properties of an alcohol. Grape phenolics are primarily composed of two groups of compounds – nonflavonoids and flavonoids. Nonflavonoids are simpler structures based on a single phenol ring with a 1- or 3-carbon side chain (Figure 1.1), whereas, flavonoid structures consist of two phenols joined by a pyran carbon-ring structure (Jackson, 2000).

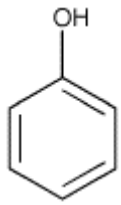

Figure 1.1 General phenolic structure

1.2.1 Nonflavonoids

Nonflavonoids include the hydroxycinnamic acids, hydroxybenzoic acids and their derivatives (Figure 1.2). They are found in both grapes and wine.

Hydroxycinnamic acids are found in grape pulp and juice, and are readily found in free-run grape juice (Singleton et al., 1978). They are the primary phenols in white wines not aged in oak. Hydroxycinnamates include caffeic, *p*-coumaric and ferulic acids. These acids are found in the grapes as esters of tartaric acid named caftaric, coutaric and fertaric acids, respectively (Singleton et al., 1978). Caftaric acid is the main substrate for enzymic oxidation in wine (Singleton et al., 1985, Singleton et al., 1986).

Benzoic acids are found at low levels in young wine, but concentrations increase with wine aging (Waterhouse, 2002). Gallic acid is the only hydroxybenzoic acid found in grapes (Monagas et al., 2005). The major source of gallic acid is likely to be hydrolysis of the epicatechin gallate which is found in grape seeds (Singleton et al., 1966). Ellagic acid is not found in grapes, and must be extracted during aging on oak (Quinn and Singleton, 1985).

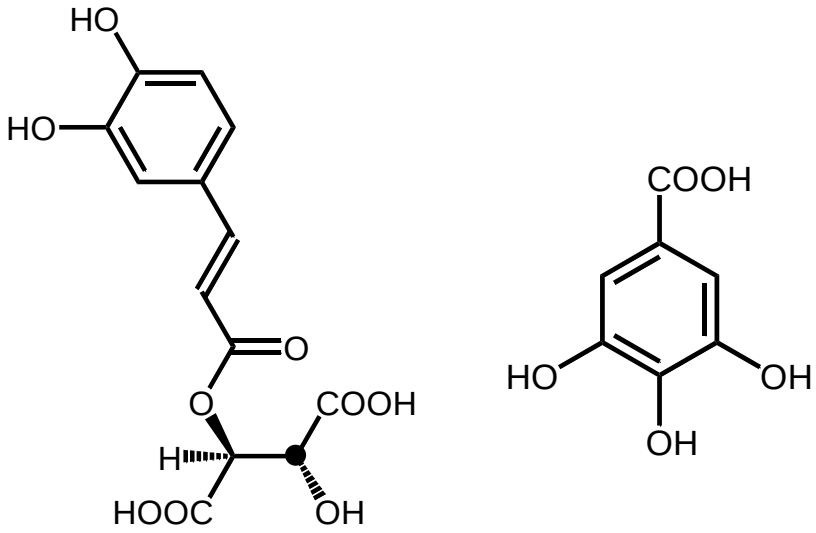

Figure 1.2 Nonflavonoid structures - caftaric acid, left; gallic acid, right

1.2.2 Flavonoids

Flavonoids account for the majority of phenols in grapes (Fulcrand et al., 2006, Kennedy et al., 2006b) and red wines (Downey et al., 2006). Flavonoids are a class of compounds that have a C6-C3-C6 skeleton (Figure 1.3). This class of compounds includes anthocyanins, flavonols, flavanols, and proanthocyanidins. Flavonoids are produced in the endoplasmic reticulum and stored in the vacuole of the producing cell (Moskowitz and Hrazdina, 1981, Hardie et al., 1996). They are found at high levels in the seeds and skins of red wine grapes. Flavonoids are frequently bound to sugars or

nonflavonoids - termed the glycosides and acyl derivates respectively, but may also exist in their free form. The glycosidic form increases solubility in water (Alonso et al., 1986) and enhances stability against enzymatic oxidation (Price et al., 1995b).

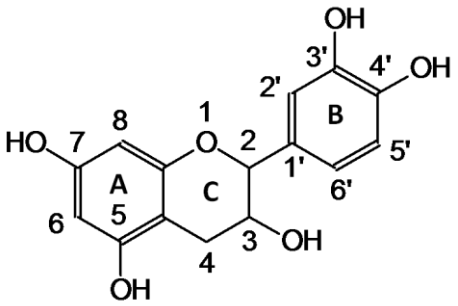

Figure 1.3 General flavonoid structure

1.2.2.1 Anthocyanins

Anthocyanins are the glucosides of anthocyanidins. The grape anthocyanidins are unstable, so they are found in only small quantities (Iacobucci and Sweeney, 1983). The sugar component increases stability and water solubility (Waterhouse, 2002). The anthocyanidin structure contains a phloroglucinoid ring A, a shikimate derived ring B showing p-coumaric, caffeic or gallic-type hydroxylation and an electron deficient pyrylium ring C (Iacobucci and Sweeney, 1983). Anthocyanins are classified by the position of the hydroxyl and methyl groups on the B ring of the anthocyanidin, and are called cyanidins, petunidins, peonidins, delphinidins, and malvidins (Figure 1.4). The hydroxylation pattern on the B ring affects hue and colour stability (Asen et al., 1972). Blue increases with the number of free hydroxyl groups while red increases with the degree of methylation (Winkler et al., 1974).

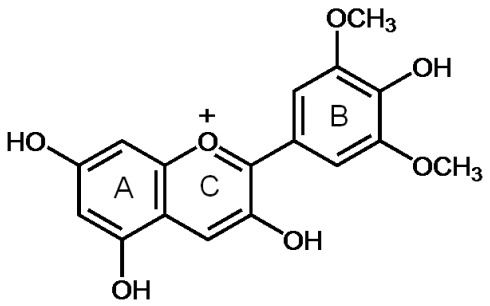

Figure 1.4 Anthocyanidin structure (Malvidin)

Anthocyanins are found in the skin of red and black grape varieties and provide the colour to red grapes and young red wine. Anthocyanin synthesis is due to expression of the UDP glucose flavonoid glucosyl transferase gene (UFGT) which is only found in red grape varieties (Boss et al., 1996). Anthocyanins can exist in various forms. Mono-glucoside, acetyl-glucoside, and coumaroyl-glucoside are the most abundant forms, though 3,5 diglucoside and caffeoyl-glucoside forms also exist. Thus, more than fifteen different anthocyanins may be present (Roggero et al., 1986). The amount and proportion of anthocyanin classes varies by cultivar and growing conditions. Most red varieties have a variable portion of acylated anthocyanins with Pinot Noir a notable exception (Cheynier et al., 2006). *Vitis vinifera* only produces monoglucosidic forms of anthocyanin, but hybrids may produce diglucosides (Singleton and Esau, 1969, Ribereau-Gayon et al., 2000). The main monomeric anthocyanins in red wine include cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside. Malvidin-3-glucoside is the most prominent anthocyanin in *V. vinifera* (Figure 1.5) (Winkler et al., 1974).

As anthocyanins are normally in the skin rather than the free-run juice (Winkler et al., 1974), with the exception of the red-fleshed teinturier varieties, skin contact is necessary to extract them through maceration and give the wine its red colour. Anthocyanins are necessary for red wine quality due to

their contribution to colour. However, they do not contribute much to wine taste (Brossaud et al., 2001).

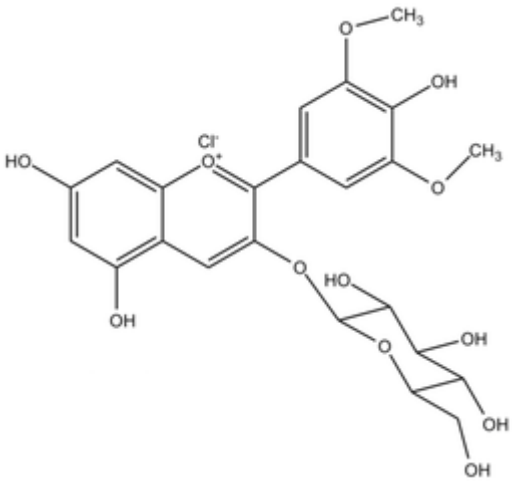

Figure 1.5 Anthocyanin structure (Malvidin-3-glucoside) (Polyphenols Laboratories AS, 2012)

Anthocyanins are unstable in wine. Long-term colour stability is dependent on interactions of anthocyanins with proanthocyanidins to form pigmented polymers (Brouillard et al., 1989, Boulton, 2001, Cheynier et al., 2006, Fulcrand et al., 2006). Anthocyanins can also exist in complexes with themselves (self-association) (Asen et al., 1972, Mazza, 1995) or with other compounds (copigmentation) (Boulton, 2001, Eiro and Heinonen, 2002). Both self-association and copigmentation increase light absorption and colour density in younger wines (Asen et al., 1972, Boulton, 2001).

Boulton (2001) suggested that copigment complexes are planar stacks caused by hydrophobic and π-π interactions rather than hydrogen bonding, as was previously considered significant (Asen et al., 1972, Timberlake and Bridle, 1976). Copigmentation may involve flavonoids (especially quercetin),

hydroxycinnamoyl esters and polyphenols, though most studies of copigmentation have focused on monomeric components as cofactors (Boulton, 2001). Copigmentation enhances the colour and shifts the wavelength of the absorbance maximum (Boulton, 2001). This is especially important in young wines. Copigmentation also results in greater extraction of anthocyanins from the skins by shifting the adsorption equilibrium (Boulton, 2001). Darias-Martin et al. (2001) found an increase in colour (AU at 520 nm) with the pre-fermentation addition of caffeic acid. The phenolic content of the musts and the copigmentation that occurs, affects extraction of anthocyanin and enhances wine colour (Darias-Martin et al., 2001).

Ethanol destabilizes pigment complexes. The loss of colour during vinification is due to the dissociation of the pigment complexes, not directly to a decline in anthocyanin content (Somers and Evans, 1979). This decrease in colour may result if the grape skins are not left in contact with the wine for a sufficient time. Factors that play a role in wine colour loss during storage and aging include pH, phenolic composition, and alcohol content of the wine (Brouillard and Dangles, 1994).

1.2.2.2 Flavanols

Flavanols can be divided into two groups – the monomeric flavanols (e.g. catechin and epicatechin), and polymeric proanthocyanidins, also known as condensed tannins (Cheynier et al., 2006).

1.2.2.2.1 Flavanol monomers

Flavanol monomers are an important subclass of flavonoids. The flavanol monomers contribute to the sensations of bitterness and astringency (Robichaud and Noble, 1990, Noble, 1994, Brossaud et al., 2001, Kennedy et al., 2006b) and play a role in wine anthocyanin polymerization during the first few

years of aging (Singleton and Trousdale, 1992, Vidal et al., 2004). The most prevalent monomers are catechin and epicatechin, with gallocatechins and epicatechin gallates present at lower levels (Figure 1.6) (Souquet et al., 1996). Flavanols may exist free, as dimers including dimers B1, B2, B3, and B4, or as oligomers and polymers – called proanthocyanidins. The flavanol monomers are located primarily in the grape stems and seed coat while flavanol polymers are found in the grape skins. Catechin monomers have also been found in the grape skin though the amount in the grape seed is far greater (Kennedy et al., 2002). Kennedy et al. (2002) found the total monomer concentration in Cabernet Sauvignon seed extracts to be approximately 50 times that found in the skin extracts at veraison (568µg/berry:12 µg/berry seed:skin), and approximately 14 times greater at harvest (70µg/berry:5 µg/berry seed:skin). This reflects the loss of monomers and increase in polymers with ripening.

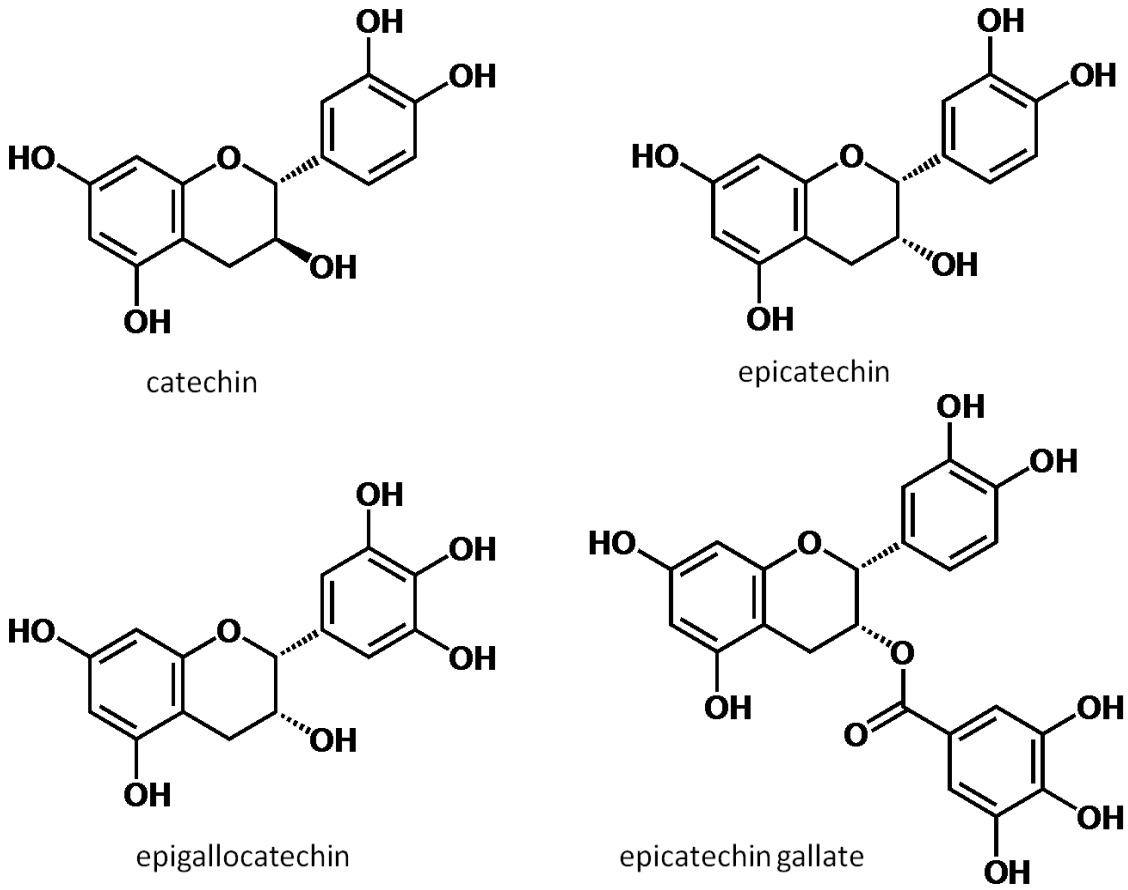

Figure 1.6 Four flavanol monomers commonly found in grape berries

1.2.2.2.2 Flavanol oligomers and polymers

Tannins are generally defined as phenolic compounds with certain chemical and physical properties such as water solubility, molecular weight in the 500-3,000 range and the ability to interact with proteins and polyamides (Ribereau-Gayon et al., 2000, Margalit, 2004). Tannins are so named due to their ability to tan hides to make leather. Tannins may be composed of flavonoids, termed condensed tannins or of nonflavonoids, termed hydrolysable tannins. Hydrolysable tannins are not found in grapes and, therefore, will not be discussed further in this text.

Flavanol oligomers and polymers are termed condensed tannins or proanthocyanidins (Cheynier et al., 2006, Downey et al., 2006, Fulcrand et al., 2006). The name 'proanthocyanidin' refers to the fact that these compounds release anthocyanidins when heated in acidic solution (Cheynier et al., 2006, Fulcrand et al., 2006). Two major groups of proanthocyanidins are found in grapes: procyanidins are polymers of catechin and epicatechin; and prodelphinidins are polymers of gallocatechin and epigallocatechin (Monagas et al., 2005). Subunits are linked by C4-C8 and, to a lesser extent, C4-C6 interflavan bonds (Figure 1.7) (Prieur et al., 1994, Souquet et al., 1996). Proanthocyanidin structures are characterized by their constituents and degree of polymerization (Prieur et al., 1994). The identity of proanthocyanidin terminal and extension subunits can be determined using acid catalysed degradation; the terminal units are released as flavanols while the extension subunits are released as benzylthioesters (Prieur et al., 1994).

The role of proanthocyanidins in grapes has not been conclusively determined, but tannins are thought to act as a feeding deterrent to animals and insects. The proanthocyanidins form before veraison in the hypodermal cells of the skin and soft parenchyma cells of the seed (e.g. the true seed

coat) (Adams and Scholz, 2008). In wine, proanthocyanidins contribute to mouthfeel and colour stability (Vidal et al., 2002, Downey et al., 2004).

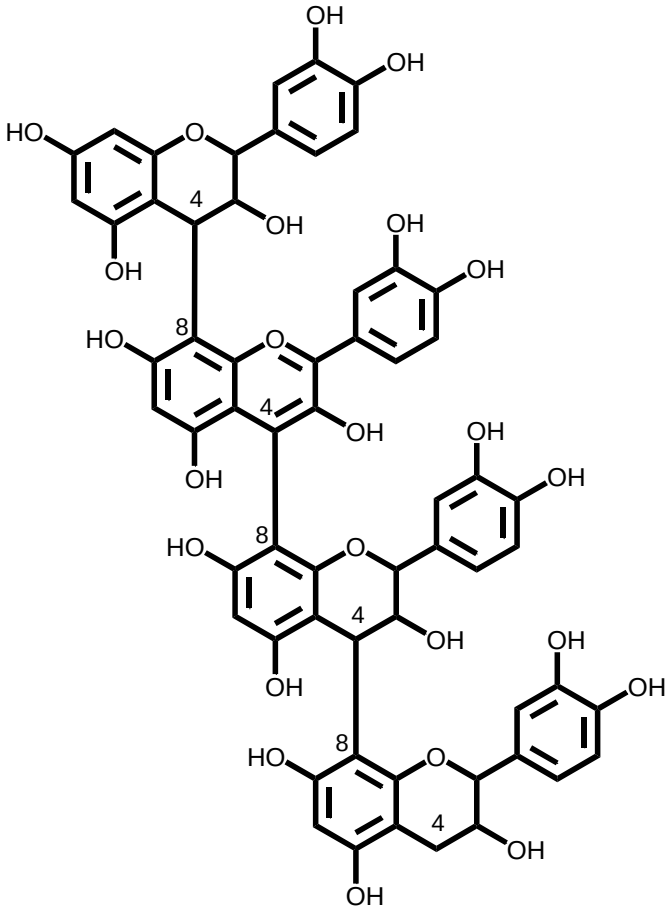

Figure 1.7 General condensed tannin structure (Polymeric tannin)

1.2.2.3 Flavonols

Flavonols are another subclass of the flavonoids. Flavonols are found in the grape skins, almost always in the glycosylated form (Cheynier and Rigaud, 1986). Quercetin-3-O-glucoside (Figure 1.8) and quercetin-3-O-glucuronide are the most common flavonols present in grape skins (Price et al., 1995b) though glycosides of kaempferol and myricetin are also present (Singleton, 1980). Flavonols (e.g. quercetin glycosides) absorb UV radiation thereby providing some protection from UV damage

(Price et al., 1995b). Flavonols are colourless, but serve as anthocyanin copigments (Asen et al., 1972, Kennedy et al., 2002, Downey et al., 2003a, Downey et al., 2004). Synthesis of flavonol is activated by direct exposure to UV (Price et al., 1995b). Downey et al. (2003b, 2004) showed that expression of the *VvFLS1* gene, which encodes for flavonol synthase is dependent on light exposure.

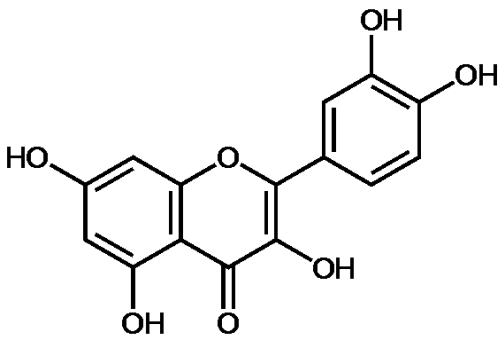

Figure 1.8 Quercetin glycoside structure

1.2.3 Phenolic composition and distribution of grape seeds, skins and pulp

Phenolic compounds are distributed differentially within the grape berry (Figure 1.9). The total phenolic distribution in red grape berries has been estimated as follows: pulp – 1%; juice – 5%; skin – 50%; and the remaining 44% in seeds (Singleton and Esau, 1969, Winkler et al., 1974, Sacchi et al., 2005).

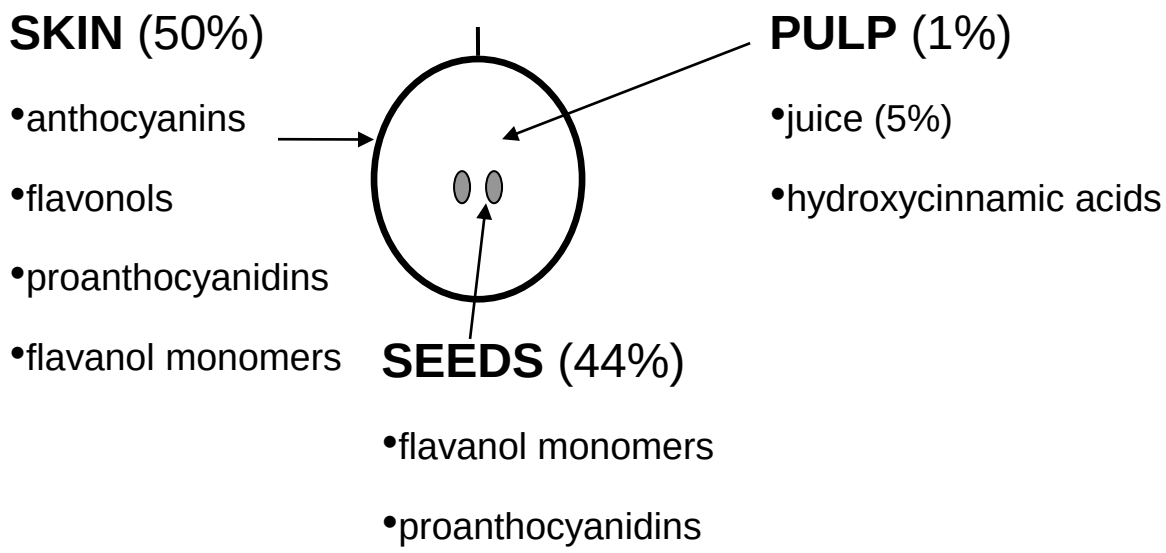

Figure 1.9 Location of phenolic compounds in the grape berry (Adapted from Adams, 2006)

Phenolic compounds found in the grape skin include the anthocyanins (Mazza, 1995), flavonols (Cheynier and Rigaud, 1986), flavanol monomers, and proanthocyanidins (Souquet et al., 1996). In most red grape varieties, anthocyanins and their copigment complexes are found only in the outer hypodermal layers of the skin; however, skin senescence may result in pigmentation in the flesh of the berry. The anthocyanins are believed to be produced in the cell cytoplasm and stored in the vacuole (Hollman and Arts, 2000, Romero-Cascales et al., 2005b). Skins also contain most of the flavonol glycosides and a portion of flavanols (Boulton et al., 1996). Skin proanthocyanidins are long polymers composed mainly of epicatechin subunits (Kennedy et al., 2001, Downey et al., 2003a, Downey et al., 2006). Proanthocyanidins from the skin tend to associate with cell wall constituents including polysaccharides, lignins and pectins (Saint-Cricq de Gaulejac et al., 1997, Bindon et al., 2010, Bindon and Kennedy, 2011).

The primary phenolic compounds found in grape seeds are monomeric flavanols and procyanidin dimers, especially B2 (Katalinic and Males, 1997). Proanthocyanins from grape seeds are composed

of equal portions of catechin and epicatechin subunits and usually exist in a free state rather than associated with other cellular constituents.

Proanthocyanidin composition is variable and differences are seen based on the location of the proanthocyanidins in the grape berry. Seed proanthocyanidins are galloylated procyanidins based on catechin, epicatechin and epicatechin-3-gallate subunits (Prieur et al., 1994, Souquet et al., 1996). The seed wall proathocyanidins contain greater proportions of epigallocatechin-3-gallate than found in the inner part of the seed (Geny et al., 2003). The increase in the number of gallic acid ester groups (or percent galloylation) enhances the binding efficiency between tannins and proteins (Spencer et al., 1988). Skin tannins also contain epigallocatechin subunits (Souquet et al., 1996, Brossaud et al., 2001, Cheynier et al., 2006, del Llaudy et al., 2008), which are generally absent from seed tannins. Seed proanthocyanidins have a lower average molecular weight and polymer length than skin proanthocyanidins (Hanlin et al., 2011). Downey (2003a) found Shiraz seed polymers to contain 5-6 subunits while skin polymers contained 25-40 subunits. Procyanidins from grape seeds are usually in their free state, while procyanidins in grape skins tend to assemble with cell wall compounds such as polysaccharides, lignins and proteins (Saint-Cricq de Gaulejac et al., 1997, Kennedy et al., 2000a, Bindon et al., 2010).

While the majority of grape phenols are found in the skins and seeds, they are also found in the pulp, juice, and rachis (Souquet et al., 2000). While trace amounts of flavanol monomers and proanthocyanidins have been reported in the pulp (Mane et al., 2007), juice phenols are almost entirely nonflavonoids (Margalit, 2004). The hydroxycinnamic acids and their derivatives are found primarily in the pulp and skins. The grape rachis contains large concentrations of catechin, caftaric acid, quercetin (Price et al., 1995b), and quercetin glycosides (Souquet et al., 2000).

1.2.4 Changes in phenolic composition during grape berry ripening and extended maturation

Grape phenolic composition and concentration change over the course of berry development. Grape berry growth follows a double sigmoid growth curve including two growth phases punctuated by a lag phase (Harris et al., 1968, Coombe, 1992). Harris et al. (1968) defined the phases of berry growth as follows: Phase 1 entails cell division and growth and occurs between anthesis and a lag in growth; Phase 2 entails cell enlargement only and occurs following veraison. Winkler et al. (1974) defined three phases of berry enlargement by separating the lag phase from phase 1 as defined by Harris (Harris et al., 1968). The second growth period includes a peak in acidity, the beginning of sugar accumulation and the loss of chlorophyll resulting in a colour change (Winkler et al., 1974).

Ristic and Iland (2005) defined 3 phases of seed growth: 1) seed growth defined by a steady increase in fresh and dry weight, the accumulation of flavanol monomers and tannins, and green appearance of the seed; 2) transition associated with maximum seed fresh and dry weights, maximum accumulation of flavanol monomers and seed tannins, the basal end of the seed continues to enlarge, and the onset of oxidation and a yellow appearance of the seed; and 3) seed drying and maturation associated with a decrease in fresh weight, oxidation of tannins and an overall brown appearance.

Based on changes in the grape berry, harvest date will yield variations in phenolic concentrations. Hydroxycinnamates (Singleton et al., 1986), monomeric flavanols (Lee and Jaworski, 1989, Jackson, 2000, Kennedy et al., 2000a, Kennedy et al., 2002, Pastor del Rio and Kennedy, 2006), and seed tannins (Herderich and Smith, 2005, Adams, 2006) develop early in berry development and then decline with ripening (Figure 1.10). The reduction may be due to increased fruit mass resulting in a decreased concentration, but decreases in absolute content may be attributed to oxidation as the

tannins become fixed to the seed coat (Kennedy et al., 2000a, Kennedy et al., 2000b, Ristic and Iland, 2005). Saint Cricq de Gaulejac et al. (1997) suggested that seed tannin levels did not decrease with maturity, but rather the procyanidins continue to polymerize causing the proanthocyanidins to be less extractable. Extractability of seed tannins decreases from veraison through ripening due to tannins binding to the fruit tissues (Kennedy et al., 2000b, Downey et al., 2003a). Skin proanthocyanidin content also appears to decrease over maturity. This may be due to the formation of associations with polysaccharides or proteins (Pastor del Rio and Kennedy, 2006, Bindon et al., 2010, Hanlin et al., 2010, Bindon and Kennedy, 2011). Compositional data determined from studies assessing changes in grape phenolics with ripening has been presented in several forms, including weight per berry (Downey et al., 2003a) or moles per seed (Kennedy et al., 2000a). This creates challenges when comparing results from different authors.

Polymerization increases with maturity. Dimer and trimer flavanol concentrations increase with ripening (Katalinic et al., 1997, Saint-Cricq de Gaulejac et al., 1997, Perez-Magarino and Gonzalez-San Jose, 2004, Downey et al., 2006). Proanthocyanidin composition also changes with grape maturity. Specifically, the mean degree of polymerization (mDP) (Kennedy et al., 2002, Fournand et al., 2006, Pastor del Rio and Kennedy, 2006) and the proportion of skin epigallocatechin extension subunits increases with berry ripening (Kennedy et al., 2001, Kennedy et al., 2002).

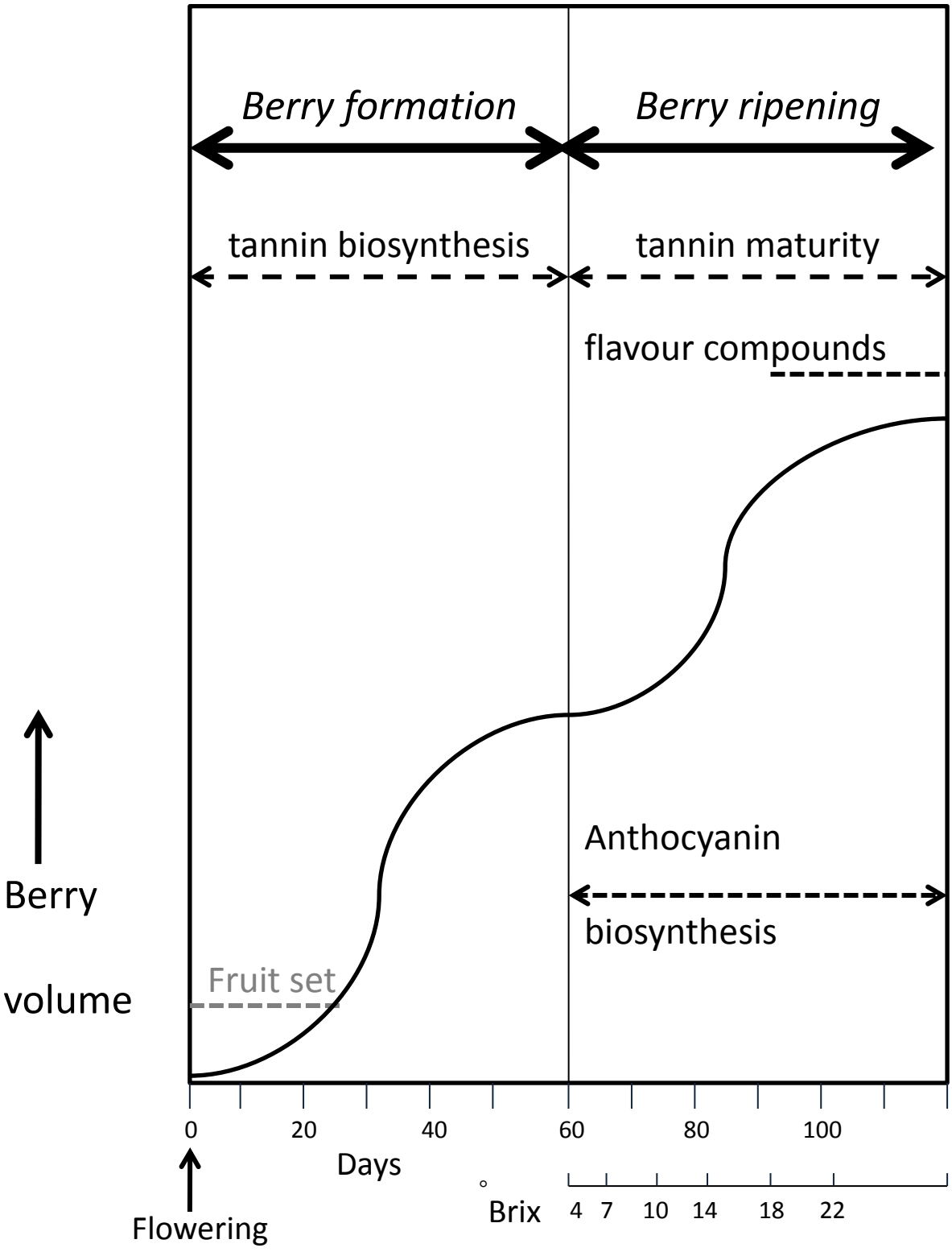

Figure 1.10 Berry formation and ripening and the associated biosynthesis of phenolic compounds (Adapted from Herderich et al., 2005). The solid line is the berry weight.

The initiation of colour development is a visual cue for berry ripening. Fruit anthocyanin concentration increases during ripening (Keller and Hrazdina, 1998, Matthews and Anderson, 1998, Burns et al., 2001, Kennedy et al., 2002, Ristic and Iland, 2005, Fournand et al., 2006) and the proportion of the individual anthocyanins present also changes. Delphinidin, cyanidin and petunidin derivatives reach a maximum earlier than peonidin and malvidin derivatives (Fournand et al., 2006). The anthocyanin content of grapes may decrease in ripe or over-ripe grapes, and anthocyanin content may decrease in berries warmed to simulate overripe conditions (Winkler et al., 1974, Keller and Hrazdina, 1998, Matthews and Anderson, 1998, Ryan and Revilla, 2003, Fournand et al., 2006).

1.2.5 Varietal differences in phenolic composition

Phenolic content and composition vary by variety (Table 1.1) (Singleton, 1966, Katalinic and Males, 1997, Mattivi et al., 2009). Nonflavonoids also differ by variety (Singleton, 1980). Ryan and Revilla (2003) showed that the distinct anthocyanin composition of each grape cultivar carries through all stages of grape ripening.

In contrast to most red grape varieties, Pinot Noir has no acylated pigments (Powers et al., 1980) and a lower concentration of anthocyanins. The lower concentration of anthocyanins in Pinot Noir grapes results in relatively low coloured wines - the best coloured Pinot Noir wines may have only one half to two thirds the colour density of Shiraz wines (Bissell et al., 1989).

Table 1.1 Average total phenol and anthocyanin content of the fruit of different grape varieties in the south of France (Adapted from Bourzeix et al., 1983)

	Level (mg/kg fresh weight)		Individual monoglucoside anthocyanins (%)		
Variety	Total Phenolics	Total Anthocyanins	Delphinidin	Petunidin	Malvidin and Peonidin
Pinot Noir	7722	631	4	12	77
Cabernet Sauvignon	6124	2339	17	8	48
Syrah (Shiraz)	6071	2200	11	12	45
Tempranillo	5954	1493	25	16	41
Grenache	3658	1222	7	10	63

1.2.6 Impact of viticultural practices on grape and wine phenolics

Viticultural practices can impact the phenolic composition of grapes. Factors that influence phenolic compositions include: grape variety (Singleton, 1966, Katalinic and Males, 1997); seasonal variation (Katalinic and Males, 1997); light exposure (Wicks and Kliewer, 1983, Price et al., 1995b, Bergqvist et al., 2001, Adams, 2006, Joscelyne et al., 2007); temperature (Buttrose et al., 1971, Bergqvist et al., 2001, Downey et al., 2006, Pastor del Rio and Kennedy, 2006); water deficits (Sipiora and Granda, 1998, Ojeda et al., 2002, Roby et al., 2004, Koundouras et al., 2006, Koundouras et al., 2009); training system (Peterlunger et al., 2002); pruning (Holt et al., 2008a, Holt et al., 2008b), and fruit maturity (Czochanska et al., 1979, Romeyer et al., 1986, Katalinic and Males, 1997, de Freitas et al., 2000, Kennedy et al., 2000a, Kennedy et al., 2001, del Llaudy et al., 2008, Holt et al., 2010).

Viticultural practices including canopy management and control of vine vigour can influence berry sun exposure. Kennedy et al. (2001) reported that grapes from low vigour Pinot Noir vines contained greater skin proanthocyanidin content, a greater proportion of epigallocatechin subunits, and an

increased degree of polymerization. Price et al. (1995a) showed that increased exposure to sunlight also increased the flavonol content of grape skins in Pinot Noir and Downey et al. (2004) showed that shading significantly reduced the level of flavonols in Shiraz skins. The shaded fruit coloured normally, but exhibited decreased proportions of malvidin, petunidin and delphinidin glucosides relative to peonidin and cyanidin glucosides (Downey et al., 2004). However, shading had no effect on the levels of proanthcoyanidins in skin or seeds (Downey et al., 2004). The difference in sun exposed fruit and shaded fruit is likely to be mediated by expression levels of the *VvFLS1* and *VvmybA1* genes, which are affected by sunlight (Downey et al., 2004) and temperature (Yamane et al., 2006), respectively. Other studies have also shown that wines made from shaded fruit contain lower total phenolics, anthocyanins, total tannin, and polymeric pigments (Joscelyne et al., 2007, Ristic et al., 2007) while wines made from fruits well exposed to sunlight contained greater levels of phenolics and colour (Mazza et al., 1999).

Several workers have reported that vine water deficits increase the tannin and anthocyanin concentration of grapes and wines (Nadal and Arola, 1995, Esteban et al., 2001, Koundouras et al., 2009). Water deficits also result in decreased berry size and weight (Roby et al., 2004). These reductions in berry size are considered to yield higher quality wines due to increases in the skin to juice ratio (Singleton, 1972, Romero-Cascales et al., 2005b). In contrast, Roby et al. (2004) asserted that the source of berry size variation is more important than berry size itself after showing skin tannin concentration remained essentially unchanged with increases in Cabernet Sauvignon berry size. Sipiora et al. (1998) reported that pre-veraison water stress increased wine total anthocyanin concentration though this was probably due to the smaller size of the berries from the water deficit treatment. The effects of water deficits on flavonoid biosynthesis were assessed by measuring flavonoid content as well as concentration. Strong pre-veraison water deficit decreased anthocyanin

and proanthocyanidin biosynthesis. However, strong post-veraison water deficit increased anthocyanin and proanthocyanidin biosynthesis (Ojeda et al., 2002).

1.2.7 Phenolic compositional differences between grapes and wine

The composition and content of phenols in the grape do not directly predict the composition and content of the resulting wine. This may be due to extraction, polymerization or chemical reactions occurring during fermentation and aging (Cheynier et al., 2006).

Phenolic compounds may change forms moving from grapes to wine. Flavanol monomers exist in grapes and wine, though the relative proportion of compounds and the polymer lengths differ from grapes to wine (Monagas et al., 2003). Grape flavonols are present as glycosides, but the sugar is cleaved during fermentation leaving a flavonol aglycone (Price et al., 1995b). Romero Cascales et al. (2005a) found fewer acylated anthocyanins in the wine compared to grapes and a difference in the proportion of individual anthocyanins. This may be due to enzymatic oxidation of the anthocyanins (Romero-Cascales et al., 2005a) or adsorption of anthocyanins by yeast cell walls (Morata et al., 2003).

In a review article, Herderich and Smith (2005) described the differences in grape and wine tannins. Grape tannins are synthesized during berry development. Grape tannins are colourless and must react with anthocyanins to become pigmented polymers (Herderich and Smith, 2005). In wine, these pigmented polymers, as well as anthocyanin copigmentation, stabilize the wine colour. The degree of polymerization of wine proanthocyanidins may increase or decrease with wine aging. Vidal et al. (2002) showed that proanthocyanidins can undergo cleavage of their interflavanic bonds in mildly

acidic conditions resulting in smaller compounds. However, flavanol monomers may also be added to proanthocyanidins thereby increasing the degree of polymerization (Vidal et al., 2002). Other reactions include oxidation of flavanol subunits which results in quinones that can polymerize further (Herderich and Smith, 2005), and aldehyde condensation reactions (Fulcrand et al., 2006). The various reactions that take place during winemaking and aging result in a large number of structurally diverse compounds, which further complicate comparisons between grape and wine tannins.

Attempts to correlate grape and wine composition have been conducted with mixed success. For example, Gonzalez-Neves et al. (2004) showed high correlations between grape and wine phenols and anthocyanins, and Harbertson et al. (2002) found the total tannins per berry did not correlate with wine tannins measured. Research designed to study the effects of viticultural practices also revealed disparities in results from grapes to wine. For example, Price et al. showed (1995a) that Pinot Noir grapes and wines from shaded fruit had decreased anthocyanins, flavonols and polymeric phenols compared to sun exposed fruit. However, the flavonol and anthocyanin profiles in the wines were different from those in the grapes. This may be due to differences in extraction and stability of individual anthocyanins (Price et al., 1995a). Diago et al. (2010) found that mechanical thinning of Tempranillo vines increased grape anthocyanin concentrations by up to 50% and also increased colour and total phenolics in the wine. However, the increase in ethanol in the wines made from mechanically thinned grapes might also have resulted in the higher total phenolics levels in the wine.

1.3 Role of phenolic substances in grape and wine quality

1.3.1 Sensory properties of grape and wine phenolic compounds

Grape phenolic compounds provide colour, UV protection, and protect the fruit from herbivores. Wine phenolic compounds are responsible for several important wine sensory factors including colour, bitterness and astringency (Noble, 1994, Gawel, 1998). Bitterness is a taste and is perceived though the taste receptors on the tongue and other components of the gastrointestinal system, while astringency is a tactile (trigeminal) sensation defined by dryness, puckering, and roughness in the oral cavity (Peleg et al., 1999). Reactions of phenolic compounds also contribute to the changes in wine during aging, including colour shifts and decreased astringency (Somers, 1971, Timberlake and Bridle, 1976, Cheynier et al., 2006, McRae et al., 2010). These attributes play an important role in consumer acceptance and preference of wines.

Flavanol monomers and proanthocyanidins have been shown to account for bitterness and astringency (Robichaud and Noble, 1990, Noble, 1994, Kennedy, 2002). The molecular size of polyphenolic compounds affects their relative bitterness. Monomers are reportedly more bitter than astringent, while large molecular weight derivatives are more astringent than bitter (Robichaud and Noble, 1990, Brossaud et al., 2001). Peleg et al. (1999) showed maximum astringency is greater in dimers and trimers compared to monomers. Increased flavanol size decreased the intensity and persistence of bitterness (Peleg et al., 1999); however, proanthocyanidin degree of polymerization did not affect bitterness perception (Vidal et al., 2003a). The perception of astringency may decrease with tannin polymerization since the increased intramolecular hydrophobic interactions which cause a compact tannin conformation will lead to fewer available binding sites (McRae et al., 2010).

Phenolic compound conformation can also impact the perception of bitterness and astringency. Epicatechin has a greater maximum intensity and longer persistence of bitterness and astringency than its stereoisomer catechin (Peleg et al., 1999). Vidal et al. (2003a) showed the percent

galloylation of proanthocyanidins impacts the perception of astringency. Degalloylated seed fractions were rated lower in dry, chalky, and coarse grain than the control with the same degree of polymerization.

Tannin concentration and quality are affected by seed number and fruit maturity (Kennedy et al., 2000b). Sensorially, tannin quality improves with time. This may be due to a decrease in low molecular weight proanthocyanidins and the associated decrease in 'harsh' tannins. This change may also be attributed to an increase in the amount of skin tannins versus seed tannins, thereby decreasing the bitterness (Kennedy et al., 2002, Kennedy, 2007). Vidal et al. (2003a) showed that the presence of epigallocatechin subunits in proanthocyanidins decreased the ratings for 'coarse grain' character in sensory trials; these subunits are found only in skin proanthocyanidins (Souquet et al., 1996). Ristic et al. (2002) found higher quality wines had greater amounts of anthocyanins and skin phenolics, and a lower amount of total flavanol monomers and seed procyanidins.

Anthocyanins provide red wine its colour and wine colour is perceived to be an indication of wine quality (Somers and Evans, 1974). The wine colour may be altered due to pH, free SO_2 level, and the age of the wine (Boulton et al., 1996). Colour stability corresponds to the degree of polymerization between phenolics and anthocyanins (Auw et al., 1996). The tannin-anthocyanin complexes are less sensitive to decolorization by either an increase in pH or the addition of bisufite (Ribereau-Gayon and Glories, 1986). Anthocyanins themselves do not contribute to mouthfeel, but may have a contribution when they are polymerized with tannins (Waters, 1997).

Phenolic compounds in wine are important for wine aging and oxidation. The capacity of wine to consume oxygen is roughly proportional to its total phenol content (Boulton et al., 1996). The phenolic content of wine decreases as the phenols are oxidized with aging (Boulton et al., 1996, Yokotsuka et al., 2000). The anthocyanin-tannin complexes that form will also aid in maintaining the colour. Therefore wines with a greater initial phenolic content are better suited for cellaring as oxidation will not have as great an effect.

1.3.2 Extraction of phenolic compounds during red winemaking

Grape phenolics must be extracted from the grape solids during red winemaking. The concentration of phenolic compounds in wine cannot exceed the amount present in the grapes (Price et al., 1995b, Katalinic et al., 1997). Fournand et al. (2006) showed extraction of skin phenolics was less than 38% for proanthocyanidins and 77% for anthocyanins. Kennedy (2007) showed the percentage of grape tannin found in Pinot Noir wine after juice skin and seed contact, or maceration (Figure 1.11).

Extraction rates are dependent upon the location of the phenolic components, their solubility, and the size of the molecules (Romero-Cascales et al., 2005a, Cheynier et al., 2006) (Figure 1.12). For example, prodelphinidins found in the skin may diffuse faster than the galloylated procyanidins in the seeds due to their location in the hypodermal layers of the grape skin or their higher hydrophilicity (Cheynier et al., 1999). Extraction may also be influenced by pH, sulphur dioxide content, ethanol content and processing parameters (Oszmianski et al., 1986, Romero-Cascales et al., 2005a). Grape variety can affect extraction rates (Romero-Cascales et al., 2005b, Mattivi et al., 2009).

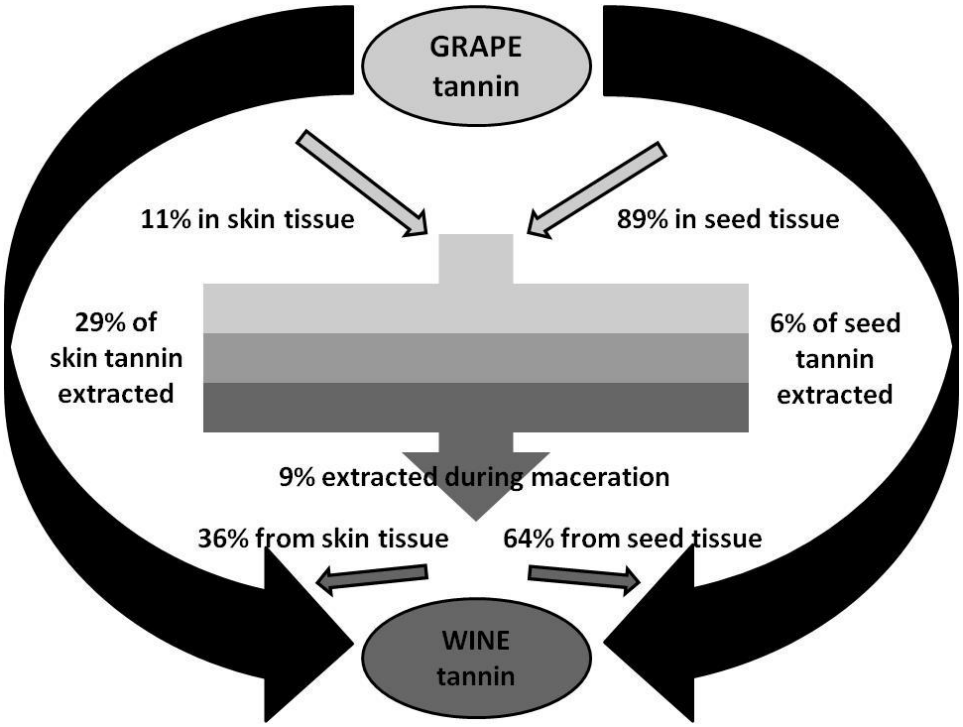

Figure 1.11 Percent skin and seed tannin extracted during maceration (Taken from Kennedy, 2007)

Phenolic location in the grape berry impacts extraction. Adams and Scholz (2008) found that 56% of the skin tannin and 4% of the seed tannins were extracted from Shiraz during fermentation. Compounds in the skin, such as anthocyanins and flavonols, are extracted more rapidly than flavanols in the seed (Cheynier et al., 1999, Koyama et al., 2007).

Pigments, which are mainly monomeric compounds, extract faster than polymeric tannins (Canals et al., 2005, Koyama et al., 2007). Yet, despite the rapid extraction, standard vinification results in approximately 20% extraction of anthocyanins present in the grape skins (Sipiora and Granda, 1998). This may be due to fixation of the anthocyanins to the grape solids and yeast cells (Ribereau-Gayon and Glories, 1986, Vasserot et al., 1997, Morata et al., 2003, Adams and Scholz, 2008, Bindon et al., 2010, Hanlin et al., 2010, Bindon and Kennedy, 2011) and/or polymerization of the anthocyanins with

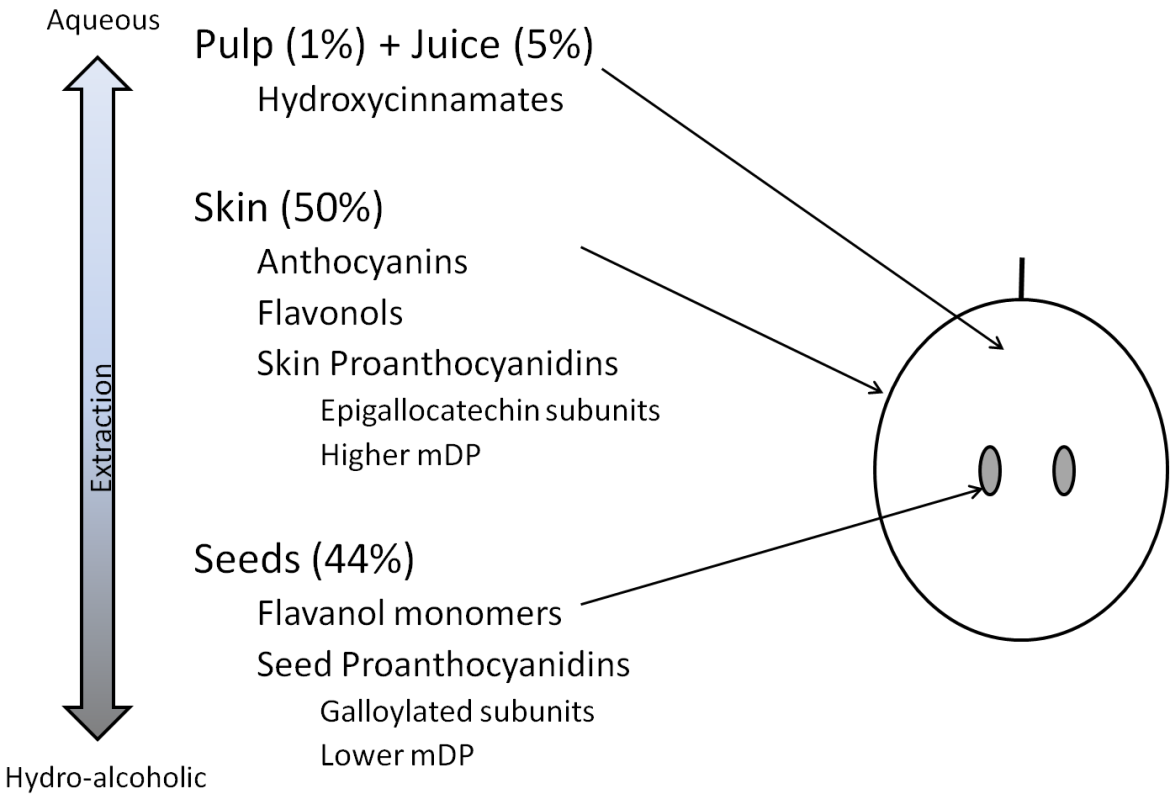

Figure 1.12 Location and extractability of grape phenolic compounds

tannins (Bakker et al., 1986, Pastor del Rio and Kennedy, 2006). The skin and mesocarp may bind significant amounts of the berry tannin. Copigmentation may increase the wine pigment by shifting the equilibrium of free anthocyanins in solution during fermentation thereby enabling a higher anthocyanin concentration in the wine. It has also been suggested that copigmentation may reduce the adsorption of pigment cofactors to grape solids and yeast cells (Boulton, 2001).

Extraction of low molecular weight flavanols from grape skins, including flavanol monomers and dimers, is dominant during the first days of maceration. For example, the maximum anthocyanin concentration typically occurs four days into the fermentation (Katalinic et al., 1997, Yokotsuka et al., 2000, Canals et al., 2005). However, seed based flavanols are slow to extract so a longer maceration

is required to yield a more complete extraction (Oszmianski et al., 1986, Kovac et al., 1992, Katalinic et al., 1997, Zimman et al., 2002, Harbertson et al., 2009). Extended maceration is used to increase the pomace contact time to enable the extraction of catechin, epicatechin and proanthocyanidin into wine (Auw et al., 1996, de Freitas et al., 2000, Gomez-Plaza et al., 2001, Zimman et al., 2002). Tannin extraction from seeds may be affected by the parenchyma cells outside the seed coat and the cuticle on the outer surface of the seed (Adams and Scholz, 2008).

The ethanol produced during fermentation aids in the extraction of some compounds. Seed proanthocyanidins are slower to extract than those found in skins, but increasing ethanol concentration of the solute will increase their extraction rate (Kovac et al., 1992, Zimman and Waterhouse, 2002, Gonzalez-Manzano et al., 2004, Canals et al., 2005, Cheynier et al., 2006, Harbertson et al., 2009). Recently, Hernandez-Jimenez et al. (2012) showed that seed proanthocyanidins may be extracted in the absence of ethanol, though extraction is faster in the presence of ethanol.

Grape maturity plays a key role in the extraction of grape tannins into wine. Grape seed tannins are more easily extracted from less ripe grapes. This may be due to proanthocyanidin polymerization with maturity and the associated decrease of solubility in an aqueous solvent (Saint-Cricq de Gaulejac et al., 1997, Herderich and Smith, 2005). Herderich et al. (2005) also suggested that cross-linking with other cell wall components later in maturity decreases extraction in more mature grapes. In contrast, Bindon and Kennedy (2011) found that increasing polymerization of proanthocyanidins resulted in a lower affinity for skin cell wall material in model solutions. Anthocyanin extraction also changes with grape maturity. Canals (2005) found increasing anthocyanin extraction with increasing ripeness in Tempranillo grapes, but found that extraction of anthocyanins levelled-off after 3 days maceration

regardless of grape maturity. In contrast, Fournand et al. (2006) showed that in Cabernet Sauvignon, anthocyanin extraction did not change with grape maturity. Sims and Bates (1994) showed that must derived from ripe muscadine fruit reached maximum colour within 4 days of fermentation; however, must colour increased through day 6 in fruit harvested two weeks later. Total anthocyanins did not however increase significantly between days 4 and 6 (Sims and Bates, 1994). The colour change shown may be due to increased polymerization in the riper fruit, which results in less loss of anthocyanins rather than further anthocyanin extraction.

1.3.3 Influence of fermentation parameters and winemaking practices on the extraction of phenolic compounds

Extraction is influenced by processing decisions during winemaking. Processing options that have been shown to impact wine phenolics include the following: temperature (Ough and Amerine, 1961, Singleton and Draper, 1964), cold soaks (Heatherbell et al., 1997, McMahon et al., 1999), skin contact time (Singleton and Draper, 1964, Singleton and Trousdale, 1983, Auw et al., 1996, Gomez-Plaza et al., 2001), mixing (Ough and Amerine, 1961, Cynkar, 2004) or processing that disrupts the cell wall (Cerpa-Calderon and Kennedy, 2008), must composition, fermentor geometry, post-fermentation extended maceration (Mayen et al., 1995, Gomez-Plaza et al., 2001, Harbertson et al., 2009), ethanol content (Oszmianski et al., 1986, Gonzalez-Manzano et al., 2004, Canals et al., 2005, Hernandez-Jimenez et al., 2012), commercial enzyme additions (Parley et al., 2001, Di Profio et al., 2011a), fining and must heating (Amerine, 1955, Wagener, 1981, Oszmianski et al., 1986). Fining, incomplete extraction, and reactions of compounds during fermentation result in a greatly reduced amount of colour and tannin in the wines versus in the juice (Kennedy, 2003).

Anthocyanins and tannins are contained in the plant cell vacuole. Extraction of these compounds can be enhanced by breaking the cell membrane, increasing exposed surface area and increasing dispersive currents (Kennedy, 2003, Cerpa-Calderon and Kennedy, 2008). Crushing effectiveness can be adjusted to extract these compounds. Destemming may also be used to exclude the stems, which may otherwise increase catechin and proanthocyanidin levels in the wine (Sun et al., 2001). Chemical additions to the must and compositional changes during vinification such as sulphur dioxide and ethanol in the must and wine may affect extraction (Oszmianski et al., 1986, Canals et al., 2005). Greater ethanol concentration increases the extraction of anthocyanins and proanthocyanidins, but decreases colour stability by affecting copigmentation (Canals et al., 2005). Higher concentrations of SO_2 increased the extraction of flavonoids from white (Singleton et al., 1980) and red grapes (Oszmianski et al., 1986). The simultaneous presence of SO_2 and ethanol increased seed catechin and epicatechin extraction over either treatment alone (Oszmianski et al., 1986). These increases in extraction may be due to disruption of the cell membranes thereby aiding extraction.

Cold maceration or cold soaking is the practice of allowing the skins and seeds to soak at 15-20°C for several days prior to fermentation to enhance aqueous extraction (Boulton et al., 1996, McMahon et al., 1999, Sacchi et al., 2005). Cold soaks may result in the breakdown of cell wall components, allowing greater extraction later in fermentation (Kennedy, 2003). Heatherbell et al. (1997) showed that the cold soak treatment increased colour, but that the improved colour did not persist with wine aging (Heatherbell et al., 1997). Similarly, McMahon et al. (1999) showed that cold soaks increased glycoside concentration and colour in must, but that this did not persist through to the end of fermentation (McMahon et al., 1999). An alternative strategy for colour improvement may be thermovinification, which destroys the skin cells thereby releasing colour and tannin (Wagener, 1981, Oszmianski et al., 1986, Margalit, 1996).

Temperature and pomace contact time are the major factors affecting polyphenol extraction (Sims and Bates, 1994, Auw et al., 1996, Yokotsuka et al., 2000, Gomez-Plaza et al., 2001). Higher temperatures increase the rate of extraction for all phenolic compounds (Boulton et al., 1996) and increase the contribution of copigmentation to colour (Zimman et al., 2002). Higher temperatures and contact time also resulted in increased amounts of total proanthocyanidins in the finished wines.

The duration of skin contact with the wine during fermentation and the associated timing of pressing the wine from the grape solids will change the level of extraction. Criteria to determine the time of pressing may include colour, tannin content, taste, sugar content and contact time. The time to pressing may be used for stylistic influence (Boulton et al., 1996). A short pomace contact time may be desirable for young wines, while a long pomace contact time might be used to produce wines intended to be aged.

The concentrations of total anthocyanins, monomeric anthocyanins, cinnamates and polymeric pigments in wine generally increase steeply with increased pomace contact time (Gomez-Plaza et al., 2001). However, Auw et al. (1996) showed that increased Cabernet Sauvignon skin contact time resulted in decreased colour intensity and Sims and Bates (1994) found a similar result using muscadine grapes. This may be due to binding of anthocyanins to cell wall material (Morata et al., 2003, Bindon et al., 2010). Yokotsuka et al. (2000) found red wines made with 4, 8 and 16 day pomace contact were judged to have higher complexity, acceptable bitterness and astringency and better appearance than wines made with fewer or more days pomace contact. Wines with 32 and 64

days of pomace contact had good colour, but had high bitterness and astringency and were not preferred (Yokotsuka et al., 2000).

Pomace contact time during fermentation and prior to pressing is necessary in red winemaking in order to extract colour and other phenolic compounds. Extended maceration is the process of prolonging the pomace contact beyond the completion of fermentation. Extended maceration is perceived to increase colour stability. Sipiora et al. (1998) found increased total phenol concentration and increased degree of colour stability but a lower total anthocyanin concentration with extended maceration. Zimman et al. (2002) showed an increase in polymeric pigments and proanthocyanidins with extended maceration. Yokotsuka et al. (2000) found that total phenol content increased with skin contact time. Extended maceration may result in increased extraction of seed tannin, though not skin tannins (Rossi and Singleton, 1966b).

Greater juice contact with the grape skins will increase the rate of extraction. Pumping the juice over the must during processing may favour extraction from the skins rather than seeds (Boulton et al., 1996). The pump-over operation increases the liquid contact with the skins while the seeds are relatively unaffected by the process due to their location on the bottom of the tank (Boulton et al., 1996). Submerged-cap is another cap management technique. With the free-run and press fraction combined, Bosso et al. (2009) found greater concentrations of anthocyanins, proanthocyanidins, and flavonoids, greater colour and a lower hue in the submerged cap versus floating cap. These differences persisted for a year (Bosso et al., 2009). Another practice that may influence extraction is the draining of a portion of the liquid prior to fermentation in order to increase the ratio of skin mass to juice and increase wine colour density. This practice is commonly referred to as saignee (Singleton, 1972, Harbertson et al., 2009). Singleton (1972) showed an increase of flavonoid and anthocyanin

content when 10% of the juice was removed in order to simulate small berry size. Harbertson et al. (2009) found the tannin concentration was increased in proportion to the amount of juice removed. Harbertson et al. (2009) also showed differences in anthocyanins at pressing, but the effect did not persist to 185 days.

The addition of cell wall-degrading enzymes during grape processing has been reported to have a positive impact on the colour and tannin structure of the wine (Guerrand and Gervais, 2002, Di Profio et al., 2011b). Commercial enzyme preparations may contain pectinases including polygalacturonase, pectin methyl esterase and pectin lyase, which act to improve maceration by hydrolysing cell wall polysaccharides (Romero-Cascales et al., 2008). Romero-Cascales et al. (2008) showed that enzyme addition increased the colour intensity and anthocyanin content on the third day of maceration, but the differences did not persist to the fifteenth day. Enzyme treatment also resulted in an increase in tannin content which did persist (Romero-Cascales et al., 2008). Pre-fermentation treatments with enzymes also resulted in greater levels of quercetin aglycones, as well as a higher degree of anthocyanin polymerization (Wightman et al., 1997). In contrast, Di Profio et al. (2011b) found that anthocyanin concentrations were lowered or unchanged with enzyme treatment.

Yeast strain may also play a role in wine phenolic composition and associated mouthfeel characters, though results vary with variety (Sacchi et al., 2005). Mazza et al. (1999) showed that total phenolics, flavanols, anthocyanins, tartaric esters and colour density in the final wines were not affected by yeast strain in Merlot or Cabernet Franc. However, a slow fermenting yeast resulted in lower colour density and phenolic content in Pinot Noir (Mazza et al., 1999). Colour may be decreased due to the ability of the yeast lees to absorb anthocyanins (Vasserot et al., 1997, Morata et al., 2003).

In addition to extraction from the grape, phenolic concentrations may change during the winemaking process as a result of chemical reactions that modify structures and change the intensity and stability of colour (Ribereau-Gayon and Glories, 1986, Zimman and Waterhouse, 2004, Fulcrand et al., 2006). Anthocyanins participate in phenolic interactions that aid in colour, stability, and the solubility of other phenolic compounds (Price et al., 1995b, Perez-Magarino and Gonzalez-San Jose, 2004, Fulcrand et al., 2006). Winemakers may also choose to decrease the phenolic content of wine through the use of fining agents. Protein fining agents such as gelatine, egg whites, casein or isinglass may be used to reduce wine astringency by precipitating tannins (Boulton et al., 1996, Margalit, 1996). Polyvinyl-polypyrrolidone (PVPP) may be used to reduce bitterness by adsorbing monomeric phenols (Boulton et al., 1996).

Further changes in wine chemistry occur during wine aging. Gomez-Plaza (2001) showed that wine colour and total pigments decreased after twelve months of wine storage. Storage yields a decrease in monomeric and an associated increase in polymeric anthocyanins (Heatherbell et al., 1997). Anthocyanin levels can decrease by up to 97% in just eight months (Ritchey and Waterhouse, 1999) while the polymeric pigments increase (Dallas and Laureano, 1994).

1.3.4 Influence of grape maturity on the extraction of phenolic compounds during winemaking

Grape maturity may have an effect on the phenolic composition in wine; however, very little has been published regarding the impact of maturity on phenolic extraction. Canals et al. (2005) showed that the extraction of anthocyanins from skins and proanthocyanidins from seeds and skins increased with

ripeness. Conversely, Fournand et al. (2006) saw no change in extraction associated with ripeness. Fournand et al. (2006) assert that tannin extraction is dependent on the size of the molecule, based on results showing the mDP was greater in the non-extracted fractions. This is in agreement with Downey et al. (2003a) who found increased yield of extension subunits with increased grape maturity when direct acid-catalysis of the grape seed residue was employed. Downey et al. (2003a) also showed mDP of proanthocyanidins in grape skin decreased with ripeness, though the quantities yielded by direct acid-catalysis remained constant with maturity.

1.3.4.1 Measurement of phenolic extractability

Romero-Cascales et al. (2005b) suggested that the extractability of phenolic compounds from grapes during winemaking can be estimated by macerating grapes for four hours at two pH levels (3.6 and 1.0) and determining the absorbance of the resulting extracts at 280 nm. The Extractability Index (EI) was calculated as follows:

Extractability index = $[(A_{pH1}-A_{pH3.6})/ A_{pH1}] X 100$

Extractability, under winemaking conditions, pH 3.6, is greatest when the extractability index is low (Romero-Cascales et al., 2005b). This analysis was based on the anthocyanin extractability reported by Glories et al. (Saint-Cricq de Gaulejac et al., 1998) with the pH modified to 3.6 as opposed to the original pH of 3.2. Romero-Cascales et al. (2005b) showed the extractability index value decreased with ripening which indicated that compounds extract more readily with maturity. However, Rolle et al. (2011) showed greater differences in chemical and mechanical properties between grape berry density classes than between harvest dates.

Extraction results may be affected by the polymerization of anthocyanins and proanthocyanidins (Bakker et al., 1986, Kennedy et al., 2001, Peyrot des Gachons and Kennedy, 2003) and interactions of proanthocyanidins with cell wall material (Bindon et al., 2010, Bindon and Kennedy, 2011). The sensorial consequences of differing extraction efficiencies may also be influenced by chemical interactions with other wine constituents. For example, Kennedy et al. (2001) found that with increased berry ripeness more uncharacterised material was extracted along with the proanthocyanidins. The material was characterised as pectin covalently associated with proanthocyanidins by electrospray ionization mass spectrometry (Kennedy et al., 2001). Taira et al. (1997) showed that increased pectin concentration reduced the astringency of soluble tannins in persimmons. Based on this, wines made from riper fruit may have a lower astringency due to greater concentrations of pectins.

Extraction results may also be affected by the analytical method (Herderich and Smith, 2005, Seddon and Downey, 2008, Harbertson and Downey, 2009, Fragoso et al., 2010). Grape tannins must be extracted from the berry in order to be analysed. Extraction may be affected by the solvent used and the amount of contact time. Downey and Hanlin (2010) compared the extraction of skin proanthocyanidins using ethanol and acetone mixtures of increasing concentrations. They showed that the class of compounds extracted was similar regardless of the solvent used; however, proanthocyanidin polymer number and length were greater in acetone extracts compared to ethanol (Downey and Hanlin, 2010). Downey and Hanlin (2010) showed extraction with 10-20% ethanol yielded average polymer lengths similar to those found in wine and suggested that only small proanthocyanidin polymers are extracted during winemaking. Extraction of grape proanthocyanidins with 70% acetone or 50% ethanol may overestimate the amount of proanthocyanidins that would be extracted into wine. Further studies are needed to determine the relationship between amount of total

tannins determined by current analytical methods and the amount that may be extracted into wine during vinification.

1.3.5 Use of water to assist fermentation

1.3.5.1 Use of water to assist fermentations (WAF) in industry

A desire for wines with strong fruit intensity and an absence of negative attributes, including green or vegetative aromas, has brought about a change in wine grape harvest criteria. While fruit sugar concentration previously served as the primary criterion for harvest, more recently, additional metrics including berry colour and flavour have been used to indicate grape harvest quality (Coombe and Iland, 2004, Winter et al., 2004, LeMoigne et al., 2008). It is generally believed that these fruit sensory attributes correlate with final wine characteristics and quality (LeMoigne et al., 2008).

The goal of production winemakers is to harvest the grapes when negative aromas have decreased, fruit aromas and flavours have peaked, and berry skin tannins have matured. Extended maturation is the practice of maintaining the fruit on the vine beyond traditional harvest maturities (ca 24°Brix) (Coombe and McCarthy, 1997). Extended maturation is commonly employed in California to achieve optimum berry flavour and mouthfeel development. However, extended maturation may also lead to undesirable field attributes such as berry shrivel and the associated yield losses. Similarly, hotter growing seasons brought on by changes in climate will yield riper fruit earlier in the season. Regardless of the cause, the high sugar fruit may yield fermentations that do not go completely dry due to the high alcohol or reduced wine quality due to excessive alcohol levels (Bisson, 1999).

In the United States, water may be added to the juice or must to facilitate fermentation, though the resulting density of the juice may not be lower than 22°Brix (Bureau of Alcohol Tobacco Firearms and Explosives, 1993). This practice is performed to mitigate the potential for a stuck fermentation or high alcohol wine due to harvesting grapes at elevated maturities. Water is added to the juice or must prior to fermentation to lower the sugar concentration at the start of fermentation. However, adding water may be seen as reducing the authenticity of the wine (ConeTech, 2012). Another option may be to use dealcoholized wine to lower the sugar concentration. Dealcoholized wine is said to retain the aromas, flavour, and body of the initial wine (TFC Wines & Spirits Inc., 2012). Therefore, the use of dealcoholized wine will minimize or eliminate the dilution of aromas and mouthfeel that may be associated with the use of water.

1.3.5.2 Impact of WAF on wine composition and sensory properties

Few studies have been performed to evaluate the impact of water addition on wine chemical attributes or sensory composition. Harbertson et al. (2009) assessed the effect of water additions, saignee and extended maceration on anthocyanin and tannin extraction from Merlot grapes and the associated sensory outcomes. This research showed that wines made with water additions had similar proportions of extracted skin and seed tannins whereas high alcohol wines had greater proportions of seed tannins (Harbertson et al., 2009). Increased seed tannin levels may make the wines too bitter and the high ethanol itself may increase the bitter perception (Fischer and Noble, 1994, Noble, 1994).

Low alcohol wines have also been studied, however, the wines used had the alcohol levels lowered after fermentation (Bui et al., 1986, Meillon et al., 2010). Bui et al. (1986) showed wines dealcoholized by reverse osmosis had decreased polyphenols and anthocyanin content, and lower colour intensity.

No sensory evaluation was conducted though the authors assert that the wines were not changed organoleptically (Bui et al., 1986). Consumer acceptance and preferences for low alcohol wines have also been studied. Meillon et al. (2010) showed Chardonnay wines with a 3% reduction in alcohol were statistically different from the control, but Sauvignon Blanc, Merlot, and Syrah with the same alcohol reduction were not. Wine professionals indicated the low alcohol Syrah wines (9.6% (v/v)) had a decrease in heat, mouthfeel, balance and sweetness compared to the original (12.7% (v/v)) (Meillon et al., 2010). The lack of data regarding water additions might reflect the reticence surrounding this practice.

High alcohol wines may also have some unfavourable sensory properties including the overbearing ethanol aromas and suppression of fruit characters. This may be due to changes in the extraction of key compounds from the grapes into the wine. Greater ethanol concentration increases the extraction of seed proanthocyanidins (Cheynier et al., 2006), and anthocyanins and proanthocyanidins from skin; however the increased ethanol also decreases colour stability by affecting copigmentation (Canals et al., 2005). Generally, winemakers prefer the mouthfeel associated with skin tannins rather than seed tannins and, therefore choose processing techniques to enhance skin extraction.

1.4 Proposed Research

Grape maturity is considered a factor in wine quality (Ough and Singleton, 1968, DuPlessis and VanRooyen, 1982). Extended maturation of the grapes is employed to achieve optimum berry flavour development and phenolic maturity for the desired wine style. Anecdotal evidence suggests that wines produced from grapes of greater maturity have greater colour and amounts of phenolic compounds, despite little change in grape phenolic concentration. While it has been suggested that

this increase in colour and phenolics might be due to an increase in extraction efficiency, this has not been thoroughly evaluated as most studies focus on early fruit development or from veraison to commercial harvest. The present study was conducted to determine the effects of extended maturation on grape phenolic concentrations and the extraction of those compounds into wine.

The aim of this research is to determine the driver for increased colour and phenolics in wines made from grapes harvested beyond historic or traditional maturity levels. To investigate this, berry phenolic composition and concentration will be measured throughout maturity of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes, along with the composition and concentration of colour and phenolics in the wines produced from these grapes. These attributes will be compared to wine sensory data to determine correlations between wine and grape chemistry and wine sensory. Ideally, the information obtained can be used to modify harvest and winemaking practices to achieve the desired wine style targets.

Another aim of this research is to determine how pre-fermentation sugar adjustment with water or dealcoholized wines impacts the phenolic concentration of wines. Wines will be made from Chardonnay and Zinfandel grapes harvested at high sugar levels. The sugar concentrations will be lowered with water or dealcoholized wine added pre-fermentation, and compared to wines made with no sugar adjustment. The phenolic concentration of these wines will be assessed and correlated to sensory data. This information can be used to determine how high total soluble solids fruit might be treated in order to avoid stuck or sluggish fermentations and to attain desired wine styles.

2 Fruit maturity influences the concentration, but not the extraction, of berry polyphenol compounds into Cabernet Sauvignon (*Vitis vinifera* L.) wines

Anecdotal evidence suggests that wines produced from grapes of greater maturity have greater colour and amounts of phenolic compounds. The study presented in Chapter 2 was conducted to determine the effects of extended maturation on grape phenolic concentrations and the extraction of those compounds into wine. Cabernet Sauvignon grapes were harvested weekly following historical maturity (23.5°B) and wines were produced. Grape and wine phenolic concentrations were measured to determine whether extraction of these compounds increases with grape maturity.

Chapter 2 was written in Australian English as the intent is to submit the manuscript embodied in this chapter to the Australian Journal of Grape and Wine Research for publication. The figures and tables have been inserted into the text for the convenience of the reviewer.

The references for this chapter have been incorporated into a single consolidated reference list that may be found at the rear of the thesis.

Fruit maturity influences the concentration, but not the extraction, of berry polyphenol compounds into Cabernet Sauvignon (*Vitis vinifera* L.) wines

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2.1 Abstract

Background and Aims: Grape composition is a key parameter determining wine quality. Historically, wine grapes were harvested based on total soluble solids and titratable acidity; however, additional metrics including berry flavour and tannin maturity are now commonly used to determine berry ripeness and optimum harvest date. These parameters are employed to ensure that optimum aroma and mouthfeel characteristics in the fruit are achieved in order to produce the desired wine style and flavour. The purpose of this study was to compare the concentration of individual phenolic compounds in berries as well as the resulting wine chemistry from grapes harvested over a period of extended maturation.

Methods and Results: Cabernet Sauvignon grapes from a commercial vineyard in Lodi, California were harvested weekly for eight consecutive weeks after the historical maturity level (23.5°Brix) had been reached. Grape and wine phenolic concentrations were analysed by reversed phase HPLC.

Conclusion: Malvidin-3-glucoside and polymeric tannin concentrations increased with fruit maturity in both grapes and wine; however, the proportion of each phenolic compound extracted did not increase with grape maturity.

Significance of the Study: Enhanced knowledge regarding the impact of grape ripeness on wine phenolic concentration can be used to attain desired wine styles.

Keywords: Cabernet Sauvignon, extraction, flavonoids, phenolics, ripening

2.2 Introduction

Phenolic compounds are an important class of compounds in grapes and wines. Phenols are the third most abundant constituent in grapes and wine, following carbohydrates (or the alcohol produced from them) and organic acids (Singleton, 1980). Phenols contribute to the colour and mouthfeel of the grapes and wines (Coombe and Iland, 2004) and may provide health benefits in the form of antioxidants (Prajitna et al., 2007).

Phenolic compounds are cyclic benzene compounds having one or more hydroxyl groups associated directly with the ring structure (Jackson, 2000). While they contain alcohol groups, they do not display the properties of an alcohol. Grape phenolics are primarily composed of two groups of compounds – nonflavonoids and flavonoids. Nonflavonoids include the hydroxycinnamic acids, hydroxybenzoic acids and their derivatives, and are found in both grapes and wine. Hydroxycinnamic acids are found in grape pulp and juice and are the primary phenols in white wines not aged in oak. Hydroxybenzoic acid derivatives are mainly products of the degradation of epicatechin gallate which is found in grape seeds, and appear with wine aging (Singleton et al., 1966). Though previously considered to contribute little to wine taste (Verette et al., 1988), nonflavonoids have recently been shown to contribute to the perceived astringency of wines (Hufnagel and Hofmann, 2008). Flavonoids are a class of compounds that have a C6-C3-C6 backbone. Flavonoids account for the majority of phenols in grapes (Boulton et al., 1996, Kennedy et al., 2006b) and red wines (Downey et al., 2006). Flavonoids represent approximately 95% or 5.35 g/kg GAE (gallic acid equivalent) and 80-90% or 1,000-2,000 mg/L GAE of grape and red wine phenols respectively (Margalit, 2004). This class of compounds includes flavanols, flavonols, and anthocyanins. They are found at high levels in the seeds and skins of red wine grapes and must be extracted into the resulting wine. However, the

concentration of phenols in wine will be less than that of the grapes due to incomplete extraction, polymerization, and binding to grape cell wall material or yeast cells (Singleton, 1980).

Flavanols are an important sub-class of flavonoids, contributing to the sensations of bitterness and astringency in red wines (Robichaud and Noble, 1990, Noble, 1994, Kennedy et al., 2006b). The most prevalent compounds in grapes and wines are catechin and epicatechin, with gallocatechin and epicatechin gallate present at lower levels (Boulton et al., 1996). Flavanols may exist free, as dimers including dimers B1, B2, B3, and B4, or as oligomers and polymers – called proanthocyanidins (or condensed tannins) (Cheynier et al., 2006, Downey et al., 2006). Flavonols are another subclass of the flavonoids. Flavonols are found in the grape skins, almost always in the glycosylated form. Quercetin-3- glucoside and quercetin-3-glucuronide are the most common flavonols present in grape skins though myricetin, kaempferol, and isorhamnetin are also present (Cheynier and Rigaud, 1986, Price et al., 1995a). Price et al. (1995a) reported quercetin glycoside concentrations ranged from 0.02 – 0.12 mg/g fresh weight from grape skin extracts and from 0.5 – 35.2 g/L in wine made from shaded and exposed Pinot Noir, respectively. Flavonols (e.g. quercetin glycosides) absorb UV radiation thereby providing some protection from UV damage (Price et al., 1995b). Flavonols are colourless, but may serve as anthocyanin copigments (Asen et al., 1972, Kennedy et al., 2002, Downey et al., 2003a, Downey et al., 2004). Anthocyanins are found in the skin of red and black grape varieties, and provide the colour to red grapes and young red wine. Malvidin-3-monoglucoside is the most prominent anthocyanin in *V. vinifera* (Rankine et al., 1958, Akiyoshi et al., 1963, Winkler et al., 1974).

Grape phenolic composition and concentration change over the course of berry development. Hydroxycinnamates (Ong and Nagel, 1978, Romeyer et al., 1983), monomeric flavanols (Lee and Jaworski, 1989, Kennedy et al., 2000a, Kennedy et al., 2002, Pastor del Rio and Kennedy, 2006), and

seed and skin tannins (Downey et al., 2003a, Herderich and Smith, 2005) form early in berry development and then decline with ripening. Conversely, the concentrations of dimer and trimer flavanols (Katalinic et al., 1997, Perez-Magarino and Gonzalez-San Jose, 2004, Downey et al., 2006), and the length of their polymeric forms, (the mean degree of polymerization mDP), increase with ripening (Kennedy et al., 2002, Fournand et al., 2006, Pastor del Rio and Kennedy, 2006). Fruit anthocyanin content also increases during ripening (Keller and Hrazdina, 1998, Matthews and Anderson, 1998, Burns et al., 2001, Kennedy et al., 2002, Ristic and Iland, 2005, Fournand et al., 2006). However, the anthocyanin content of grapes has been reported to decrease as they reach harvest or if they remain on the vine following extended maturation (Winkler et al., 1974, Keller and Hrazdina, 1998, Matthews and Anderson, 1998, Ryan and Revilla, 2003, Fournand et al., 2006). The anthocyanin composition changes with ripening. Keller and Hrazdina (1998) found that in *Vitis vinifera* variety Cabernet Sauvignon, although malvidin-, peonidin-, and cyanidin-3-glucosides were prevalent at veraison, by harvest the most common anthocyanins were malvidin and delphinidin-3-glucosides. Ryan and Revilla (2003) showed the relative amount of malvidin-3-glucoside increased with ripening, while delphinidin- and petunidin-3-glucoside amounts decreased with ripening.

Historically, the harvest date of wine grapes was determined on basic juice composition, including sugar, acid, and pH. However, more recently, additional metrics including berry colour and flavour have been used to indicate grape harvest quality (Winter et al., 2004) and partly as a consequence of this, the average total soluble solids at harvest has increased (Figure 2.1). The goal is to ensure the negative aromas have decreased, that positive fruit aromas have developed, and that the tannins have matured to achieve the desired wine style.

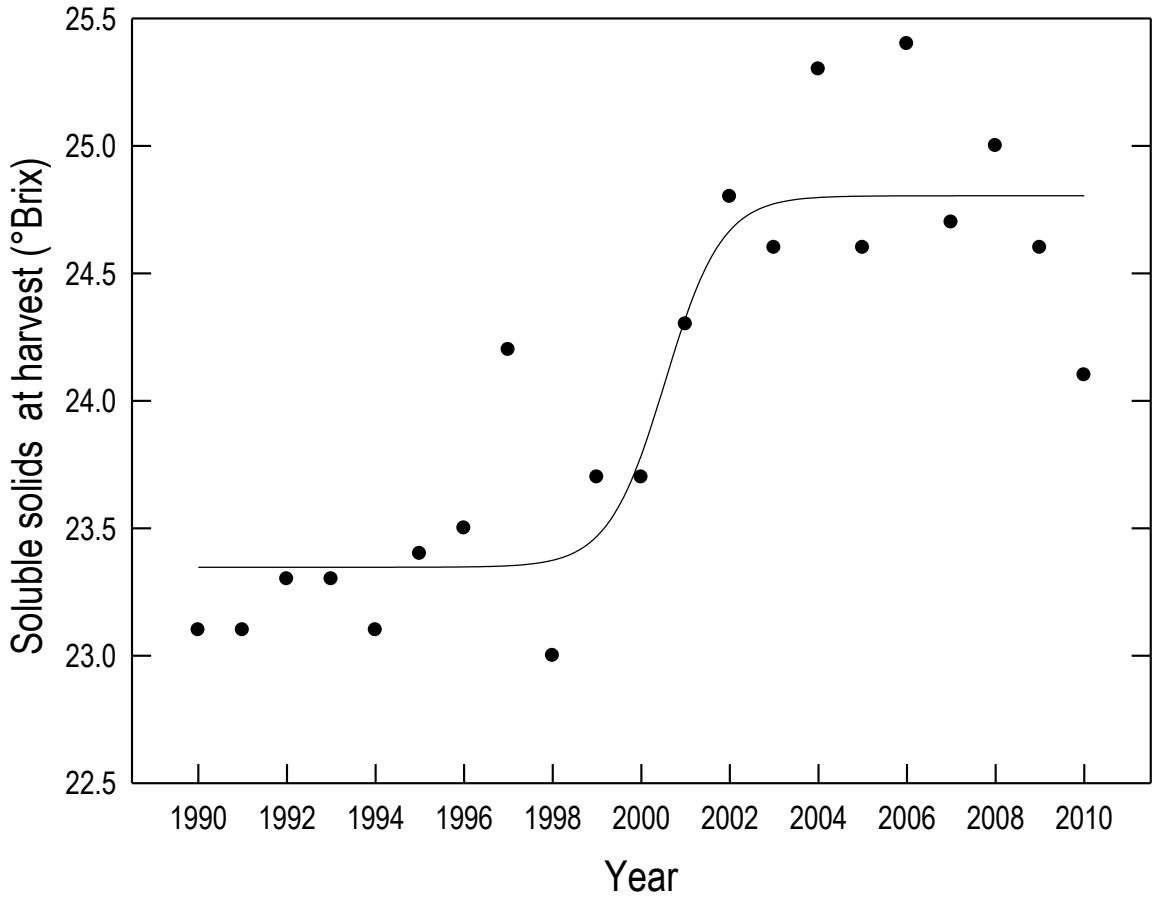

Figure 2.1 Average total soluble solids at harvest for California Cabernet Sauvignon grapes (Data from California Grape Crush Report, United States Department of National Agricultural Statistics Service, 2011)

The practice of maintaining the fruit on the vine beyond traditional harvest maturities (ca 24°Brix) is called extended maturation (Coombe and McCarthy, 1997). This technique is employed to achieve optimum berry flavour development for the desired wine style. Desirable flavours may include a decrease in vegetal aromas (LeMoigne et al., 2008) as well as a simplified volatile compound composition (Kalua and Boss, 2010). However, extended maturation may also lead to undesirable viticultural effects such as berry shrivel and the associated yield losses (Mendez-Costabel et al., 2012). Little research has been done to determine the effects of extended maturation on grape phenolic composition. Recently, Holt et al. (2010) reported on the total phenolics, total tannins, and

total anthocyanins in Cabernet Sauvignon during late stages of ripening. In five samples taken between 22° and 37°Brix, it was observed that the total phenolic and total tannin concentrations peaked at ~30°Brix while the total anthocyanin concentration peaked at ~27°Brix. Given the different sugar levels at which these parameters peak, these factors cannot be used as an indicator of optimal phenolic maturity (Holt et al., 2010). A few studies have assessed the impact of ripeness on phenolic extraction from grapes into wine. Canals et al. (2005) showed that extraction of anthocyanins and proanthocyanidins was improved with riper Tempranillo grapes. However, the last harvest date was less than six weeks post-veraison and sugar content did not exceed 18°Brix (ca 192 g/L) (Canals et al., 2005). In contrast, Fournand et al. (2006) showed no changes in extraction of skin anthocyanins or proanthocyanidins as sugar content of Shiraz grapes increased from 162.6-275.0 g/L (16.4-26.8°Brix).

The aim of the present work was to investigate the concentration of individual phenolic compounds of Cabernet Sauvignon berries harvested from veraison to veraison-plus fifteen weeks, and to compare the effect of harvest date on the concentration of individual phenolic compounds, the extraction of these compounds from grape into wines, and the resulting wine chemistry. The relationship between wine phenolic chemistry and wine sensory is presented in a companion paper (Chapter 3).

2.3 Materials and Methods

2.3.1 Vineyard

The experiment was conducted in a commercial vineyard located in the northern portion of the San Joaquin Valley, northeast of the town of Lodi, CA (38°10'48.21"N, 121°13'39.34"W) during the 2008 and 2009 growing seasons. The vines were planted in 1995 on a sandy loam soil with an approximate

rooting depth of 1.6 m. Plant materials consisted of *Vitis vinifera* L. cv. Cabernet Sauvignon (FPS clone 8) grafted on Teleki 5C rootstock (*V. berlandieri x V. riparia*). Vine rows were oriented east-west, spaced 1.5 m (between vines) x 3.3 m (between rows) and trained to a quadrilateral cordon, horizontally divided system. The trellis system consisted of two cordon wires, separated by 0.9 m and supported by a cross-arm located 1.35 m above ground, and two foliage support wires attached to a 1.1 m wide cross arm and placed 1.55 m above ground. The vines were spur pruned during dormancy to 24, 2-bud spurs per plant. At approximately 20 cm of shoot growth, vines were shoot thinned to retain two shoots per spur. Basal leaf removal in the fruiting zone was performed immediately following fruit set in both years. Vine irrigation requirements were estimated using daily reference evapotranspiration values (ETo) from weather stations located near the vineyard (less than one kilometre). Estimations of daily water requirements were obtained using the equation ETc = Kc x ETo (Williams et al., 1994), where Kc is the seasonal crop coefficient (Grimes and Williams, 1990). Irrigation amounts were calculated on a weekly basis to replenish the ETc value estimated for the previous week. The calculated irrigation requirement (L/vine/week) was applied in equal amounts over a four or five-day period each week. Nitrogen (20 kg/ha) and potassium (50 kg/ha) were applied to the vineyard following fruit set in both years.

2.3.2 Vineyard experimental design

The experiment was designed as a randomized complete block design with each harvest date (treatment) replicated four times using seven-vine plots. A total of 15 sample/harvest dates or treatments were included in the experiment. A different set of vines were either bunch sampled (post-veraison and prior to 21°Brix) or bunch sampled and harvested (at or following 21°Brix) each week. Sampling dates for 2008 and 2009 were July 21 through October 27 and July 20 through

October 26, respectively. All fruit used for fruit analysis and winemaking was collected from the middle five vines in each plot.

2.3.3 Sample collection and basic analysis

Fruit collection for compositional analyses commenced when soluble solids levels reach 18°Brix in both seasons. Randomly selected 20-bunch samples were taken weekly from their corresponding harvest date (treatment) vines and analysed for soluble solids (°Brix), pH, malic acid and potassium using Fourier Transform Infrared Spectroscopy (FTIR) (WineScan FT-120, FOSS North America, Eden Prairie, MN). Berry weights were also collected. Once fruit reached 21°Brix, in addition to compositional analyses, fruit was also harvested for winemaking purposes. All bunches were removed from the vines and total yield and bunch number recorded. Approximately 50 kg of fruit was harvested from each replicate plot for winemaking purposes. Field replicates were processed separately. All samples were held overnight at 2°C for processing the following morning.

2.3.4 Chemical analysis

2.3.4.1 Sample preparation

Berry bunches were destemmed via a destemmer crusher (manufactured by Wilkey Sheet Metal, Inc., Turlock, CA). The destemmed fraction was divided for further processing. Berries were analysed for total soluble solids by refractometer (Atago U.S.A., Inc, Bellvue, WA) and pH, titratable acidity (TA), reducing sugar (RS), ammonia, amino nitrogen, malic acid, volatile acidity (VA) by Fourier Transform Infrared Spectroscopy (FTIR) (WineScan FT-120, FOSS North America, Eden Prairie, MN). Berry polyphenols and colour analysis was conducted on berry homogenate extracted in the manner described by Iland et al. (2000). The homogenate was extracted in 50% (v/v) ethanol pH adjusted to

2.0 with 12M HCl at a solid:liquid ratio of 1:10. Samples were shaken for 1 hour at room temperature to keep the homogenate and solvent mixed. The sample was centrifuged for 15 minutes at 3,210 x g (Beckman Allegra 6 benchtop centrifuge) and the supernatant decanted for analysis.

2.3.4.2 Polyphenol analysis by HPLC

Standards. Linearity studies were generated for Gallic acid, Catechin, Caffeic acid, Quercetrin, Quercetin, and Malvidin-3-O-glucoside chloride at concentrations of 1, 5, 10, 25, 50, and 100 mg/L (ppm). Gallic acid Monohydrate, (+)-Catechin, (-)-Epicatechin, Caffeic acid, Quercetrin, and Quercetin were from Sigma Chemical (St. Louis, MO). Malvidin-3-O-glucoside chloride was from Indofine Chemical Company, Inc. (Hillsborough, NJ).

The supernatant obtained following extraction of the berry homogenate as described above was analysed by reversed phase HPLC coupled to a diode array detector using the method described by Waterhouse et al. (1999). The column was an Agilent Zorbax Eclipse XDB-C18 Rapid Resolution HT 4.6 X 50 mm, 1.8 µm column protected by an Agilent Zorbax Eclipse XDB-C18 analytical guard column 4.6 x 12.5 mm, 5 µm. The mobile phase was a gradient of 0.3% (v/v) phosphoric acid solution (mobile phase A) and 0.2% (v/v) phosphoric acid in acetonitrile (mobile phase B) at a flow rate of 1.0 ml/min. The elution conditions were as follows: 5% mobile phase B at time 0; 0-10 min 5-19% mobile phase B; 10.25-12.5 min hold at constant 33% mobile phase B; 12.5-13.5 min 33-95% mobile phase B; and 13.5-14.5 min 95-5% mobile phase B.

Eluting peaks were monitored at 230, 280, 320, 360, and 520 nm. Compounds eluting from the HPLC were identified and quantification was based on a comparison to authentic standards (except caftaric

acid, quercetin glycosides, and polymeric tannins). Chromatograms were integrated using Agilent ChemStation software. The compounds were monitored at the following wavelengths: polymeric tannins – 230 nm; catechin, epicatechin, and B2 dimers – 280 nm; caftaric acid – 320 nm; quercetin glycosides – 360 nm; and malvidin-3-glucoside – 520 nm. Compounds were quantified using the relative response to calibration compounds as follows: polymeric tannins, catechin, and B2 dimer to catechin; epicatechin to epicatechin; caftaric acid to caffeic acid; quercetin glycoside to quercetin; and malvidin-3-glucoside to malvidin-3-glucoside (Figure 2.2).

Peaks were identified based on comparison with authenticated standards. A library search was used to confirm peak identity. Since several compounds co-eluted with other compounds, manual integrations were needed. For example, quercetin glycoside elutes as the middle peak of a triplet of peaks and anthocyanins co-elute with the polymeric tannins at 280 nm. However, the polymeric tannins did not have absorption at 530 nm. The anthocyanin absorbance at 530 nm was about the same as their absorbance at 230 nm. The polymeric tannins were detected at 230 nm with the reference set at 530 nm in order to eliminate the interference from co-eluting anthocyanins.

2.3.5 Winemaking

Samples for wine lots commenced when the sugar concentration reached 21°Brix, and continued each week through the final week of October, to 27.3 and 26.0°Brix, for 2008 and 2009 respectively. Harvest dates are indicated as the week post-veraison. In 2008, the first wine lots were harvested on 25 August, or in the 6th week post-veraison while in 2009 the first wine lots were collected on 31 August, or in the 7th week post-veraison. The final berry samples were collected in the 15th week post-veraison in both years.

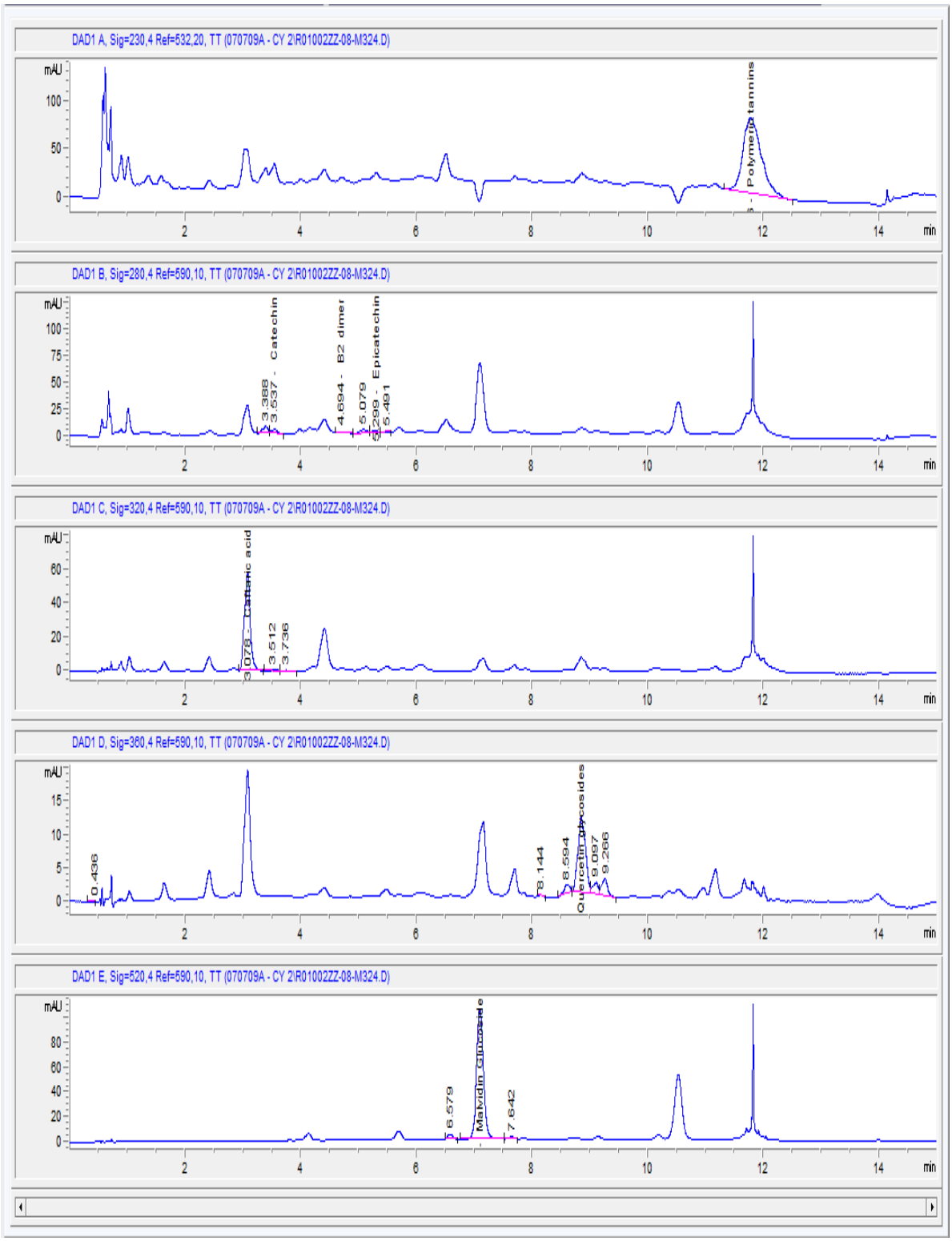

Figure 2.2 Representative HPLC chromatogram including identified peaks

The grapes from each harvest date were held overnight at 2°C and processed the following morning. Field replicates were kept separate for winemaking. The berries were destemmed/crushed using a Magitec model A15DC (Paarl, South Africa) directly into the fermentation tank. 60 ppm sulphur dioxide was added to the must immediately after crushing. In order to maintain similar alcohol levels, must sugar levels were adjusted to 24°Brix prior to fermentation for all wine lots. Samples with a starting sugar concentration less than 24°Brix were supplemented with 1:1 glucose:fructose mixture to reach 24°Brix. Samples with a high sugar level were diluted to 24°Brix using process water. The goal was to attain final wine alcohols of 14.0 ± 0.5% (v/v). This approach was utilized in an effort to minimize the influence of alcohol concentration on both the sensory perception of the wine (Fischer and Noble, 1994) and the extraction of phenolic compounds into the wine (Canals et al., 2005). The addition of water to the must may have diluted concentrations of phenolics in the must and resulting wine. To test these potential effects, wines were made with and without water addition in week 15 of each vintage.

Analysis of variance of the wine phenolic concentrations showed few significant differences in the wines made with and without water addition despite maximum dilutions of 8.95 and 7.39% (v/v) for 2008 and 2009 respectively (data not shown). Specifically, 2008 caftaric acid concentrations were lower for the wines with water addition than for those without. Week 15 was selected for these comparisons as the soluble solids at this time-point required the greatest dilution, and, therefore, it can be assumed that the impact of smaller dilutions would be negligible.

Diammonium phosphate (DAP) was added to achieve a must Yeast Assimilable Nitrogen (YAN) of 300 ppm and tartaric acid was added to bring the titratable acidity (TA) to 6 g/L. The must was inoculated with rehydrated N96 yeast (Anchor Yeast, Industria, South Africa) at a rate of 0.24 g/L.

Sixty-litre fermentations were conducted indoors at ambient conditions (approximately 21°C). Cap management was via submerged cap with one punchdown cycle per 24-hour period. Fermentations were monitored daily immediately following the punch down for temperature, Brix, and taste samples. The wines were pressed at 0°Brix using a Diemme AR 1.3 membrane press (Emilia-Romagna, Italy) to a maximum pressure of 1.8 bar. Fermentations were pressed after 7±1 days fermentation with the exception of 2009 week 9 wine, which was pressed after 5 days fermentation. Free run and press fractions were reconsolidated into a jacketed vessel maintained at ambient temperature. Daily sampling for reducing sugar (RS) and temperature continued until the wine was dry (<2 g/L).

Dry wines were cold settled for 3 days at 2°C and then racked into 10-gallon pressure rated cans. Wines were stored at 2°C under nitrogen until rough filtration. Rough filtration was through 1.0 µm nominal diatomaceous earth (DE) pads on a Filtrox plate and frame unit. The free sulphur dioxide was adjusted to 30 ppm, TA was adjusted to 5.5-5.8 g/L, and copper sulphate was used to remove sulphides prior to bottling. Malolactic fermentation was not performed. Wines were sterile filtered through Meissner 0.8 and 0.45 µm cartridge filters en route to bottling, using a GAI model 1006 modified for small lots. The filler bowl and bottles were purged with nitrogen gas. Wines were bottled into 750 ml bottles and sealed with screw caps. Packaged wines were stored at 13°C.

2.3.6 Wine analysis

2.3.6.1 Wine chemistry

Wines were analysed approximately 8 months after bottling and within 1 month of the completion of the wine sensory evaluation. Wine analysis included pH, titratable acidity, alcohol, reducing sugar (RS), lactic acid, malic acid, and volatile acidity (VA) by FTIR (WineScan FT-120, FOSS North America, Eden Prairie, MN). Wine colour metrics were determined as Absorbance at 420 nm and 520 nm by spectrophotometer (Lambda 35, Perkin Elmer. Waltham, MA. 1 mm path length). Hue was calculated as Abs420/Abs520 and intensity was calculated as Abs420+Abs520.

2.3.6.2 Polyphenol analysis by HPLC

Wines were filtered and loaded into HPLC vials for polyphenol analysis. Samples were analysed by the same method as described in the grape section.

2.3.7 Statistics

Extractability was calculated as the percent of a compound in wine relative to the concentration in the grapes. The wine concentrations were determined in mg/L and converted to mg/kg for comparison with the grape concentrations. The conversion of units was based on the average density of the wines in this trial, which was 0.993 g/ml. Extractability was expressed as a percentage (%) of the grape compounds extracted into wine.

Analysis of variance (ANOVA) was performed using PASW version 18 software (SPSS, Chicago, IL). The LSD test was used to separate the means ($p<0.05$).

2.4 Results

2.4.1 Berry development

Bunch samples commenced in mid-July (veraison) in both seasons and were collected weekly for 15 consecutive weeks. Sample dates were July 21 through October 27 in 2008 and July 20 through October 26 in 2009. Commercial harvest occurred in week 13 in both years. The commercial harvest date was determined by production winemakers based on total soluble solids, berry flavour, and tannin maturity.

Average maximum daily temperatures by month in 2008 were slightly greater than in 2009 during the first half of the year, while the average minimum temperatures were greater in the first part of 2009 (data not shown). Degree days by month were greater in the early part of berry development in 2008 compared to 2009 (Figure 2.3). The 2009 season was cooler, and also had a rain event of 125 mm on October 13. This date coincided with the thirteenth week of sampling.

Total soluble solids increased more rapidly in the early part of 2008 compared to 2009, but by week 10 total soluble solids were greater in 2009 than in 2008 (Table 2.1). In 2008, total soluble solids increased to a maximum of 27.3°Brix at the final harvest date. The 2009 total soluble solids were impacted by rain in week 13, resulting in lower total soluble solids for week 14 (24.8°Brix), then increasing to 26.0°Brix in week 15.

Berry weights varied little during the sampling period in each year (weeks 8-15) and between the two seasons. This is consistent with results reported by Pastor Del Rio and Kennedy (2006). Berry weights varied more over time in 2009 than in 2008.

Organic acid levels, titratable acidity (TA) and pH, showed similar patterns in both 2008 and 2009. However, TA values were significantly higher in 2009 compared to 2008. The pattern of malic acid decline was similar for both seasons, but the 2009 levels were greater at veraison. Grape nitrogen levels were significantly greater in 2008 than in 2009. This includes results for ammonia, amino nitrogen by OPA, and the calculated Yeast Assimilable Nitrogen (YAN) (Table 2.1).

2.4.2 Wine metrics

Wines from weeks 8-15 post-veraison were assessed for phenolic extractability. These wines were made from grapes harvested after historical commercial harvest dates (23.5°Brix). Fermentation temperatures decreased slightly with the progression of the season (Table 2.2). The musts were pressed once they reached 0°Brix. The timing for pressing ranged from 6-8 days (Table 2.2). This variability can be attributed to differences in the fermentation temperatures. All fermentations went to dryness with wines having less than or equal to 2.0 g/L reducing sugar at bottling. Wine alcohol levels were more consistent in 2009 than 2008 (Table 2.2). In 2008, the alcohol level in week 15 (14.8% (v/v)) was greater than the desired alcohol levels (14 ± 0.5% (v/v)). Wine TA and pH were similar throughout the sampling period, while wine malic acid levels declined (Table 2.1).

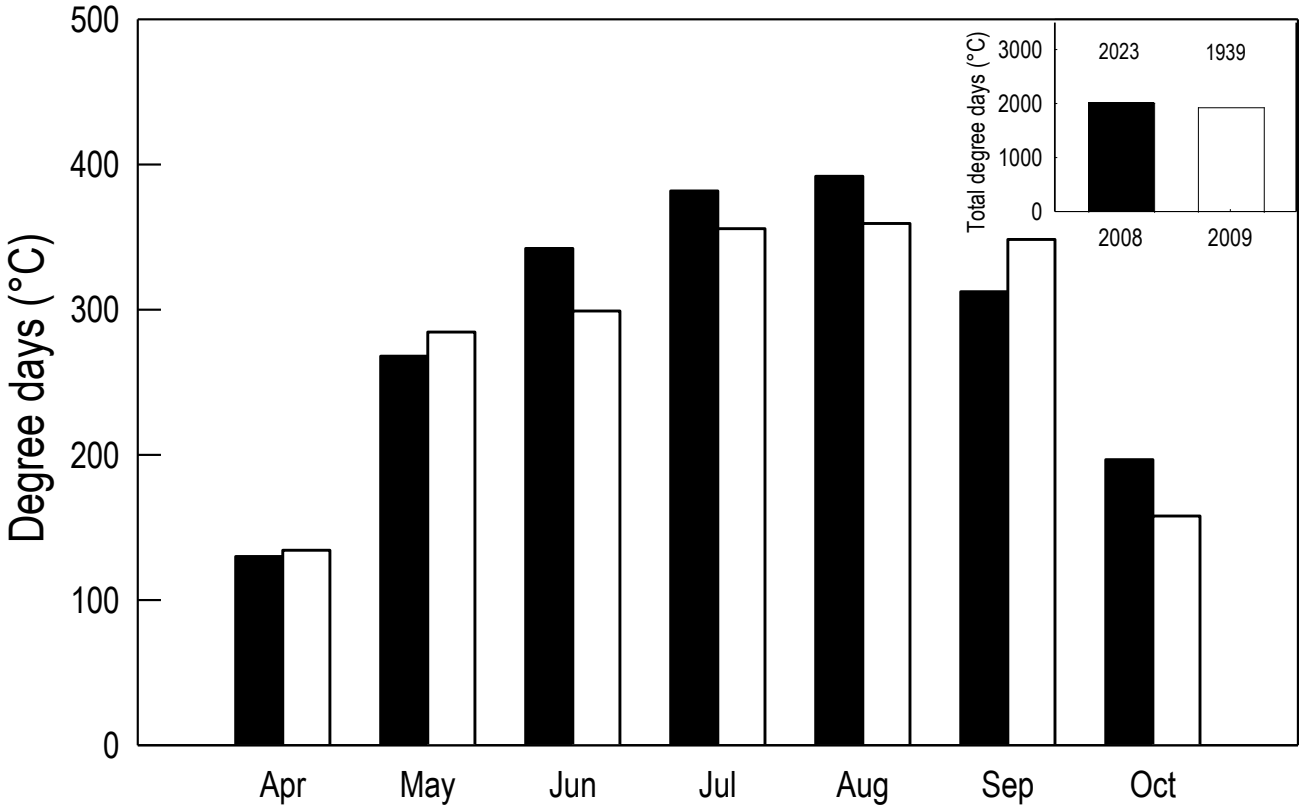

Figure 2.3 Degree days by month in Lodi, CA, in 2008 and 2009

Table 2.1 Harvest date and some compositional parameters for Cabernet Sauvignon grapes in 2008 and 2009.

Vintage	Weeks after veraison	Harvest Date	Soluble solids (°Brix)	Potassium (mg/L)	Malic acid (mg/L)	YAN (mg/L)	pH	Titratable acidity (g/L)	Berry weight (g)
2008	8	8-Sep	24.3 ± 0.1	1143.3 ± 10.9	2535 ± 107	213.0 ± 6.3	3.32 ± 0.01	5.3 ± 0.2	0.997 ± 0.01
	9	15-Sep	24.0 ± 0.2	1138.8 ± 43.2	2287 ± 48	251.0 ± 6.4	3.41 ± 0.03	5.0 ± 0.2	1.010 ± 0.01
	10	22-Sep	23.6 ± 0.3	1186.3 ± 20.6	1923 ± 145	239.8 ± 9.4	3.44 ± 0.01	4.2 ± 0.2	0.997 ± 0.01
	11	29-Sep	25.0 ± 0.1	1326.0 ± 36.8	1585 ± 51	223.5 ± 4.4	3.49 ± 0.03	4.3 ± 0.1	1.004 ± 0
	12	6-Oct	24.6 ± 0.1	1276.0 ± 15.4	1302 ± 81	222.8 ± 4.1	3.59 ± 0.02	3.7 ± 0.1	1.017 ± 0.01
	13	13-Oct	25.9 ± 0.1	1325.5 ± 49.0	1231 ± 72	201.0 ± 2.3	3.57 ± 0.02	3.8 ± 0.1	1.010 ± 0.01
	14	20-Oct	26.2 ± 0.2	1426.3 ± 46.2	1296 ± 66	202.3 ± 2.9	3.64 ± 0.01	3.5 ± 0.1	1.015 ± 0
	15	27-Oct	27.3 ± 0.1	1477.5 ± 43.1	1342 ± 90	206.0 ± 3.8	3.76 ± 0.02	3.2 ± 0.1	1.006 ± 0
2009	8	7-Sep	22.7 ± 0.2	1256.0 ± 44.0	2620 ± 173	206.5 ± 7.5	3.33 ± 0.02	6.3 ± 0.1	1.018 ± 0.01
	9	14-Sep	23.5 ± 0.2	1139.0 ± 52.8	1639 ± 65	156.5 ± 5.0	3.39 ± 0.02	5.3 ± 0.1	1.008 ± 0.01
	10	21-Sep	24.5 ± 0.1	1426.5 ± 44.1	1661 ± 157	155.3 ± 12.6	3.48 ± 0.02	5.7 ± 0.1	1.008 ± 0.01
	11	28-Sep	26.0 ± 0.5	1328.3 ± 15.1	1301 ± 170	128.8 ± 6.4	3.54 ± 0.02	4.6 ± 0.1	1.010 ± 0.01
	12	5-Oct	25.6 ± 0.2	1375.0 ± 21.3	1239 ± 148	126.3 ± 5.9	3.56 ± 0.03	4.2 ± 0.2	1.025 ± 0.01
	13	12-Oct	25.4 ± 0.1	1305.8 ± 33.3	1255 ± 64	142.8 ± 6.1	3.66 ± 0.02	3.6 ± 0.1	1.039 ± 0.01
	14	19-Oct	24.8 ± 0.4	1338.5 ± 50.0	1167 ± 68	111.3 ± 8.1	3.66 ± 0.04	3.7 ± 0.1	0.998 ± 0.01
	15	26-Oct	26.0 ± 0.2	1504.0 ± 40.4	1459 ± 45	114.3 ± 2.9	3.75 ± 0.04	3.9 ± 0.1	1.021 ± 0.01

Mean and standard error (n=4). YAN - yeast assimilable nitrogen

Table 2.2 Initial must °Brix, water additions by volume, sugar additions by weight, time to press and final alcohol levels for Cabernet Sauvignon wines produced in 2008 and 2009.

Vintage	Weeks after veraison	Soluble solids (°Brix)	Water addition (% v/v)	Sugar addition (g/L)	Final alcohol (% v/v)	Time to press (days)	Mean fermentation temperature (°C)	Maximum fermentation temperature (°C)
2008	8	25.3 ± 1.4	2.8 ± 0.6	0	13.9 ± 0.1	6.0 ± 0.0		25.1 ± 0.0
	9	23.9 ± 0.3	1.3 ± 0.6	0	13.6 ± 0.1	6.5 ± 0.3	22.4 ± 0.1	24.3 ± 0.1
	10	24.2 ± 0.2	0.7 ± 0.3	0	13.5 ± 0.1	6.0 ± 0.0	23.1 ± 0.0	24.3 ± 0.1
	11	24.3 ± 0.2	1.8 ± 0.8	0	13.9 ± 0.1	6.5 ± 0.3	22.4 ± 0.1	24.3 ± 0.4
	12	24.0 ± 0.1	1.4 ± 0.3	0	14.0 ± 0.1	7.0 ± 0.0	21.1 ± 0.1	23.9 ± 0.2
	13	25.5 ± 0.1	5.7 ± 1.1	0	14.1 ± 0.2	7.3 ± 0.3	21.1 ± 0.2	23.0 ± 0.2
	14	25.8 ± 0.2	6.3 ± 0.5	0	14.2 ± 0.1	7.5 ± 0.3	21.1 ± 0.1	22.7 ± 0.4
	15	26.9 ± 0.3	9.0 ± 1.2	0	14.8 ± 0.1	7.3 ± 0.3	20.6 ± 0.1	22.8 ± 0.1
2009	8	22.5 ± 0.2	0	10.1 ± 1.2	14.0 ± 0.1	6.0 ± 0.0	23.0 ± 0.1	25.3 ± 0.3
	9	23.0 ± 0.2	0	7.7 ± 1.2	14.0 ± 0.0	5.0 ± 0.0	22.3 ± 0.1	25.5 ± 0.2
	10	23.7 ± 0.2	0	1.3 ± 0.8	14.1 ± 0.1	6.0 ± 0.0	22.6 ± 0.0	24.6 ± 0.3
	11	25.1 ± 0.2	5.6 ± 0.8	0	14.4 ± 0.1	7.3 ± 0.3	21.4 ± 0.0	23.1 ± 0.0
	12	25.6 ± 0.3	6.8 ± 0.9	0	14.1 ± 0.1	6.3 ± 0.3	22.1 ± 0.1	24.2 ± 0.3
	13	25.8 ± 0.2	7.1 ± 0.5	0	14.1 ± 0.0	6.0 ± 0.0	21.0 ± 0.3	22.1 ± 0.1
	14	24.8 ± 0.3	3.0 ± 1.2	0	14.1 ± 0.1	6.0 ± 0.0	21.5 ± 0.0	22.7 ± 0.2
	15	26.1 ± 0.2	7.4 ± 0.5	0	14.0 ± 0.1	7.0 ± 0.0	21.7 ± 0.1	23.8 ± 0.5

Mean and standard error (n=4).

2.4.3 Polyphenolic compounds

2.4.3.1 Flavonoids

2.4.3.1.1 Anthocyanins

Malvidin-3-glucoside was tracked as this compound accounts for nearly three-quarters of the anthocyanins in Cabernet Sauvignon wine (Wulf and Nagel, 1978). Grape malvidin-3-glucose concentrations increased with fruit maturity, however, the rate of increase slowed as the grapes ripened in both years (Figure 2.4). This was consistent with data reported by Kennedy et al. (2002). Wine malvidin 3-glucoside concentrations also increased throughout the 2008 season. The malvidin 3-glucoside concentrations for 2009 wines were more variable and the trend less consistent. In 2008, malvidin-3-glucose extraction was stable over time. Malvidin 3-glucoside extraction was generally greater throughout most of ripening in 2009, and then trailed off, reaching levels similar to 2008 by the end of the season (Figure 2.4).

2.4.3.1.2 Flavonols

Grape quercetin glycoside concentrations did not differ significantly among years (Figure 2.5). However, the 2009 data trended downward with fruit maturity while 2008 concentrations were constant with maturity. Kennedy et al. (2002) found that flavonols reached maximum concentration two weeks before harvest and then declined. Price et al. (1995b) also reported a similar decrease in wine flavonols following periods of elevated temperatures. The downward trend reported here has

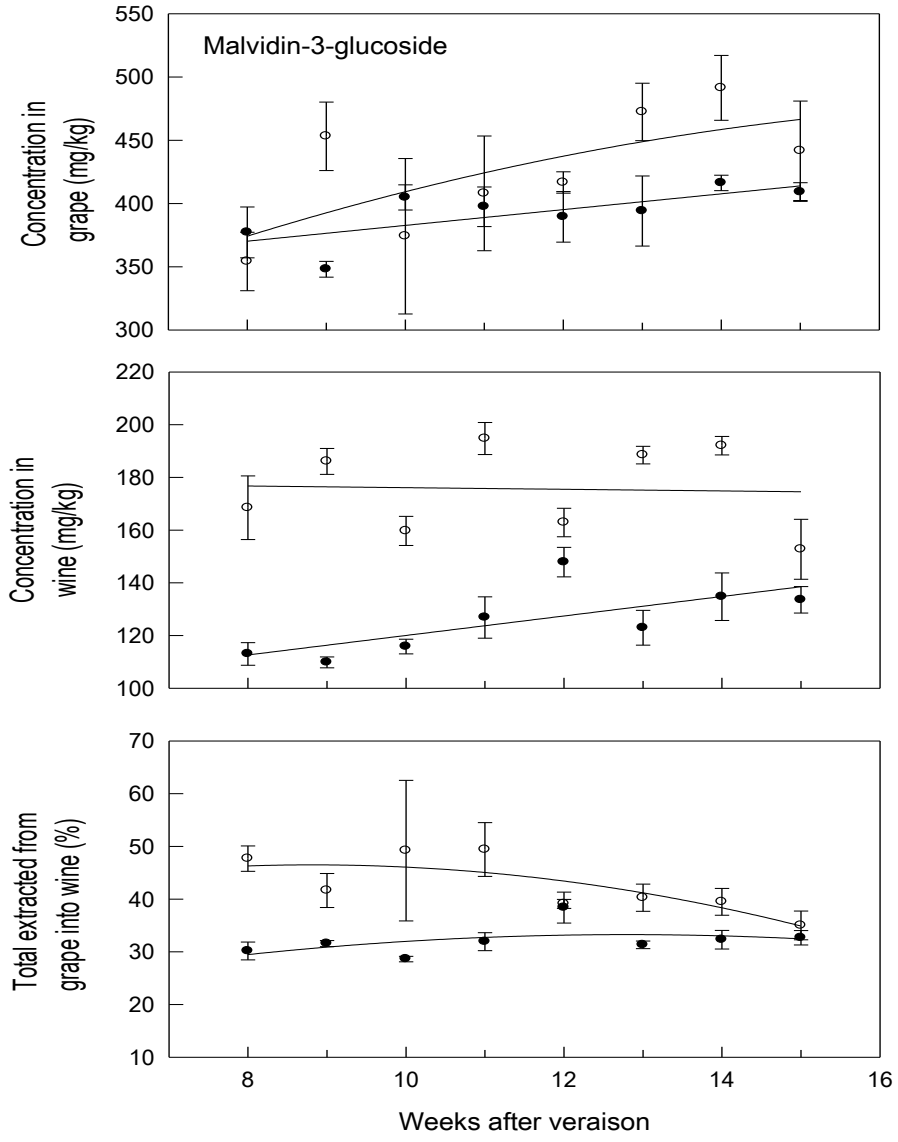

Figure 2.4 Influence of grape maturity on malvidin-3-glucoside concentration in grape (upper graph), concentration in wine (middle graph), and total grape malvidin-3-glucoside extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (°). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=316.3+357.3(1-e^{-0.021x})$ ($R^2 = 0.569$) for 2008 and $f=-300.677+123.8x-4.163x^2$ ($R^2 = 0.730$) for 2009. Wine concentration data were fitted to the following regression equations $f=67.72+3.362(1-e^{-0.00001x})$ ($R^2 = 0.648$) for 2008 and $f=25.544+27.540x-1.212x^2$ ($R^2 = 0.233$) for 2009. Extraction data were fitted to the following regression equations $f=-7.056+6.488x-0.259x^2$ ($R^2 = 0.552$) for 2008 and $f=77.35-3.916x+0.079x^2$ ($R^2 = 0.687$) for 2009.

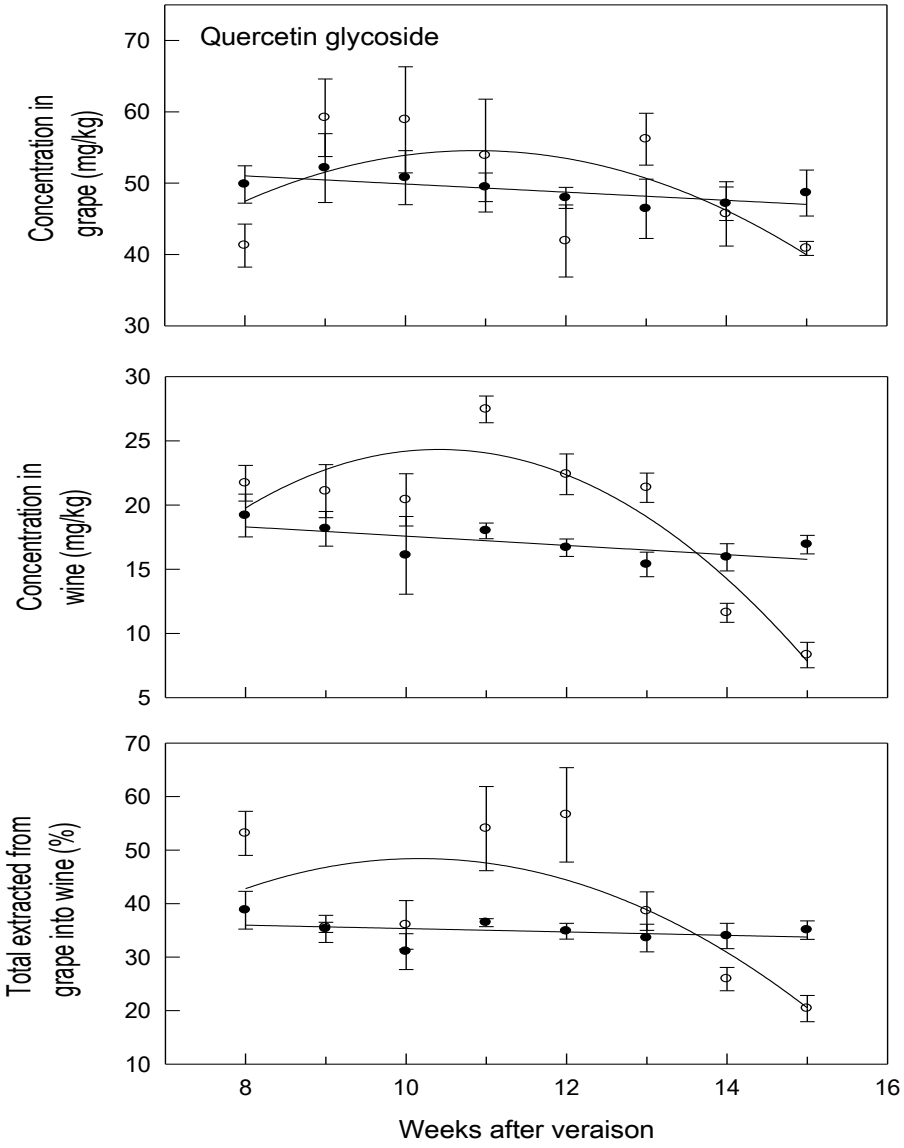

Figure 2.5 Influence of grape maturity on quercetin glycoside concentration in grape (upper graph), concentration in wine (middle graph), and total grape quercetin glycoside extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=48.39+0.573x-0.043x^2$ ($R^2 = 0.410$) for 2008 and $f=27.415+6.016x-0.336x^2$ ($R^2 = 0.274$) for 2009. Wine concentration data were fitted to the following regression equations $f=20.33-0.377x+0.006x^2$ ($R^2 = 0.289$) for 2008 and $f=-2.604+6.444x-0.376x^2$ ($R^2 = 0.695$) for 2009. Extraction data were fitted to the following regression equations $f=43.93-1.536x+0.061x^2$ ($R^2 = 0.098$) for 2008 and $f=-21.821+15.228x-0.82x^2$ ($R^2 = 0.511$) for 2009.

been reproduced consistently in grapes grown in the central valley of California over several vintages (Personal Communication, Mike Cleary, E.& J. Gallo Winery). Wine quercetin glycoside values remained constant with grape maturity in 2008. However, the trend was not as clear in 2009. Quercetin glycoside concentrations declined in the later stages of ripening in 2009. Quercetin glycoside extraction was consistent at 35% regardless of sampling week in 2008. In 2009, quercetin glycoside extraction in later stages of ripening (20%) was significantly lower than for grapes harvested earlier (53%).

2.4.3.1.3 Flavanol monomers

The concentrations of grape catechin and epicatechin (mg/kg) declined over time in both years, though the rate of decline was slower in 2009 (Figures 2.6 and 2.7). Grape catechin concentrations declined to similar levels and then remained constant in both years. Epicatechin concentrations declined along with catechin, however, epicatechin concentrations were approximately 50% lower than catechin. Wine catechin and epicatechin concentrations declined with fruit ripening in 2008. In 2009, wine catechin levels declined, while the wine epicatechin levels remained relatively constant throughout ripening (Figures 2.6 and 2.7). Wine catechin concentrations were generally similar for both years. Wine epicatechin concentrations were significantly greater in 2009 compared to 2008, similar to the grape data.

For both seasons, catechin extraction trended upward with berry maturity, and then reached a plateau. However, the percent of catechin extracted was greater in 2008 than 2009. In 2008, catechin extraction was similar in early and late ripening. Epicatechin extraction trends were opposite in 2008

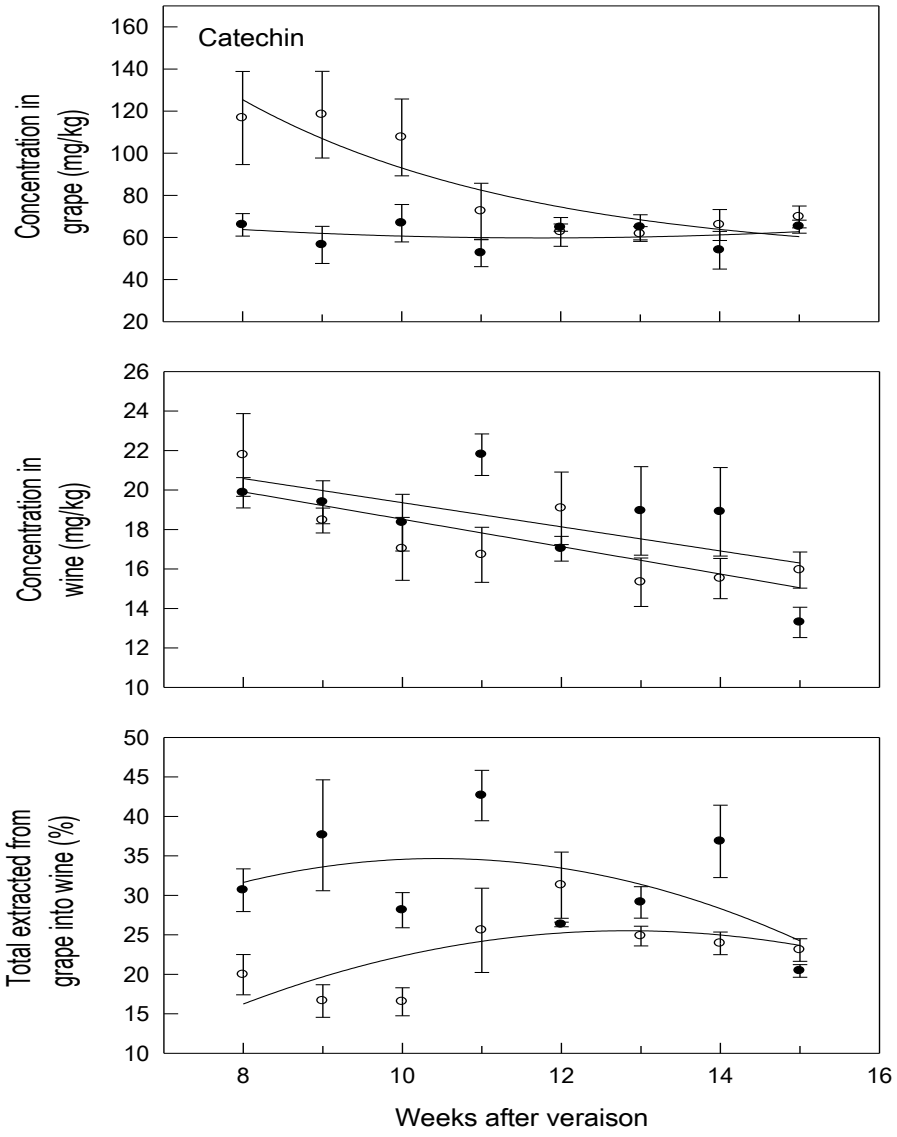

Figure 2.6 Influence of grape maturity on catechin concentration in grape (upper graph), concentration in wine (middle graph), and total grape catechin extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=60.64+495815944e^{-2.313x}$ ($R^2 = 0.893$) for 2008 and $f=45.336+522.255e^{-0.239x}$ ($R^2 = 0.890$) for 2009. Wine concentration data were fitted to the following regression equations $f= 28.47-0.828x-0.002x^2$ ($R^2 = 0.552$) for 2008 and $f=-21.962+46.002e^{-0.014x}$ ($R^2 = 0.521$) for 2009. Extraction data were fitted to the following regression equations $f=-42.79+14.35x-0.662x^2$ ($R^2 = 0.332$) for 2008 and $f=-33.184+9.050x-0.350x^2$ ($R^2 = 0.600$) for 2009.

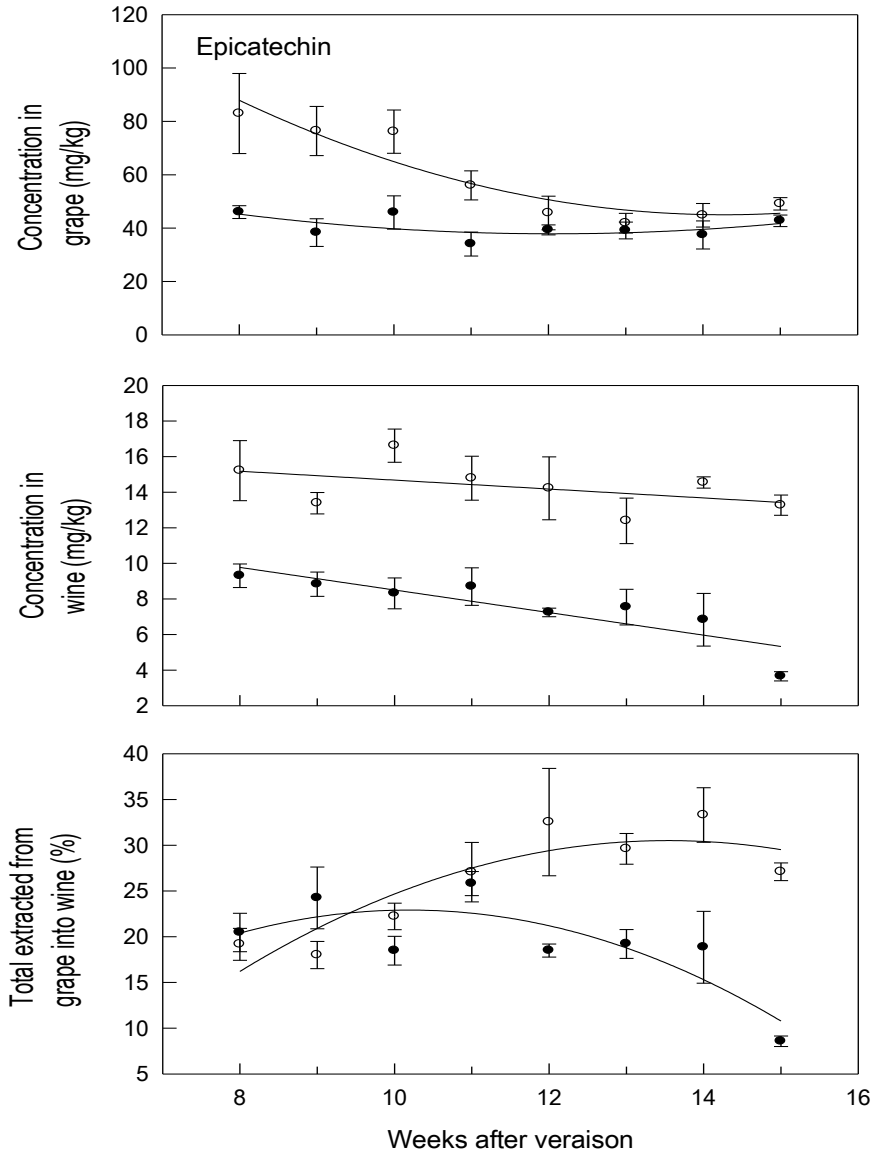

Figure 2.7 Influence of grape maturity on epicatechin concentration in grape (upper graph), concentration in wine (middle graph), and total grape epicatechin extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=39.008+2882e^{-0.784x}$ ($R^2 = 0.578$) for 2008 and $f=50.133+128407.5e^{-0.989x}$ ($R^2 = 0.945$) for 2009. Wine concentration data were fitted to the following regression equations $f=14.23-0.356e^{0.018x}$ ($R^2 = 0.825$) for 2008 and $f=-6.557+1.597e^{0.078x}$ ($R^2 = 0.205$) for 2009. Extraction data were fitted to the following regression equations $f=-3.629+5.937x-0.328x^2$ ($R^2 = 0.641$) for 2008 and $f=-66.629+14.611x-0.548x^2$ ($R^2 = 0.905$) for 2009.

and 2009. In 2008, the extraction diminished with ripening while in 2009 the extraction increased with grape maturity.

2.4.3.1.4 Dimers

Grape B2 dimer concentration levels and patterns were different in both years (Figure 2.8). In 2008, the B2 dimer concentration initially decreased, then increased with fruit maturity. In 2009, B2 dimer concentrations declined over time. B2 dimer concentrations were significantly greater in 2009 versus 2008 until week 12 post-veraison, but reached similar levels in weeks 13-15. Consistent with previous literature, the B2 dimer concentrations were lower than the flavanol monomer concentrations at all time points in both seasons (Katalinic and Males, 1997). Wine B2 dimer concentrations decreased slightly with maturity in both years, though the range of the data was very small. B2 dimer concentrations in 2009 were significantly greater than 2008 values. The B2 dimer extraction showed opposing trends in 2008 and 2009. In 2008, values declined over time whereas in 2009 the extraction into wine increased with fruit maturity. The 2008 B2 dimer extraction declined rapidly during the later stages of ripening. Though the extraction trends are opposing, the range of extraction was relatively small (8-19%).

2.4.3.1.5 Proanthocyanidins

As with the B2 dimers, grape polymeric tannin concentration levels and patterns differed by year (Figure 2.9). Grape polymeric tannin concentrations increased over time in 2008. Week to week variation in 2009 made the trend less clear; however, the concentrations were relatively constant with fruit ripening. The 2009 data is consistent with Hanlin and Downey (2009) who reported that Shiraz and Cabernet Sauvignon skin proanthocyanidin concentrations remained relatively constant after

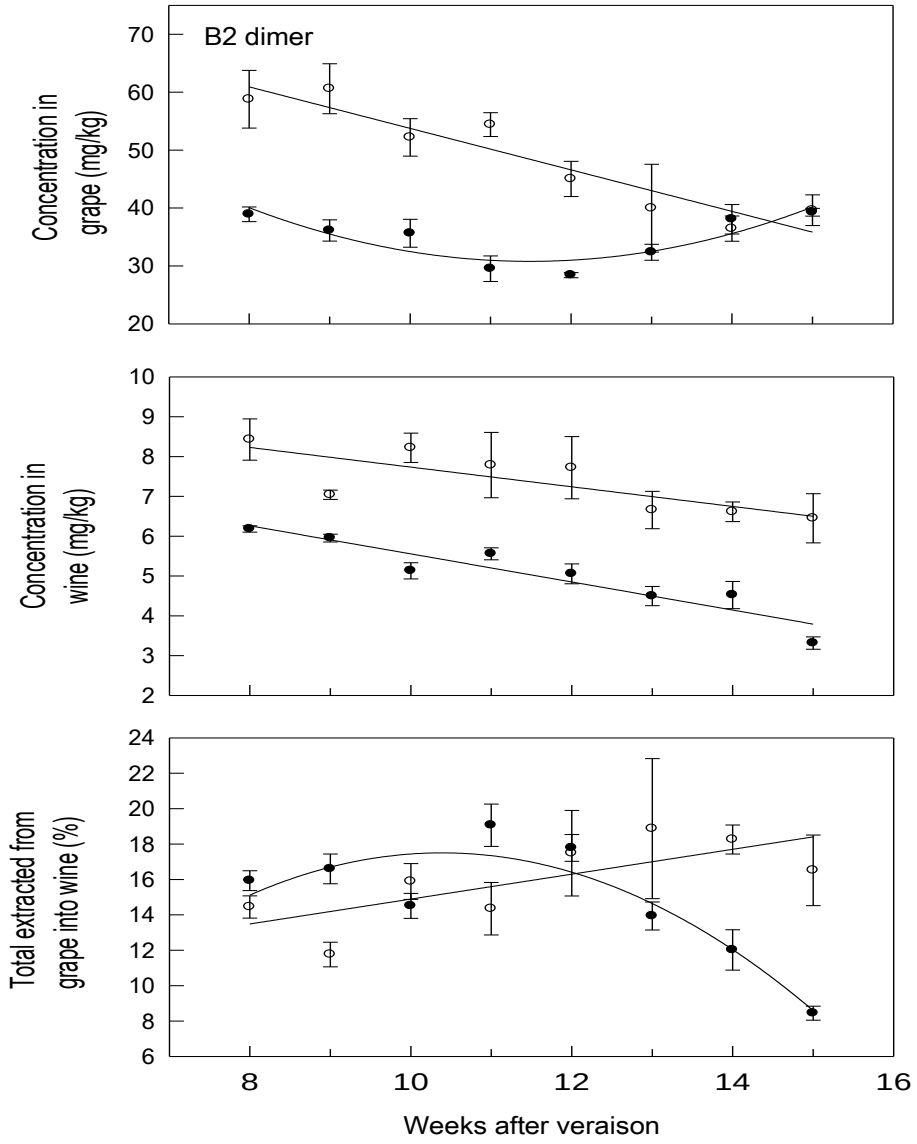

Figure 2.8 Influence of grape maturity on B2 dimer concentration in grape (upper graph), concentration in wine (middle graph), and total grape B2 dimer extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=123-16.08x+0.703x^2$ ($R^2 = 0.860$) for 2008 and $f=60.534+1.260x-0.197x^2$ ($R^2 = 0.857$) for 2009. Wine concentration data were fitted to the following regression equations $f=9.991-0.466x+0.003x^2$ ($R^2 = 0.906$) for 2008 and $f=4.701+0.726x-0.042x^2$ ($R^2 = 0.623$) for 2009. Extraction data were fitted to the following regression equations $f=-11.3+5.912x-0.304x^2$ ($R^2 = 0.765$) for 2008 and $f=4.313+1.445x-0.036x^2$ ($R^2 = 0.597$) for 2009.

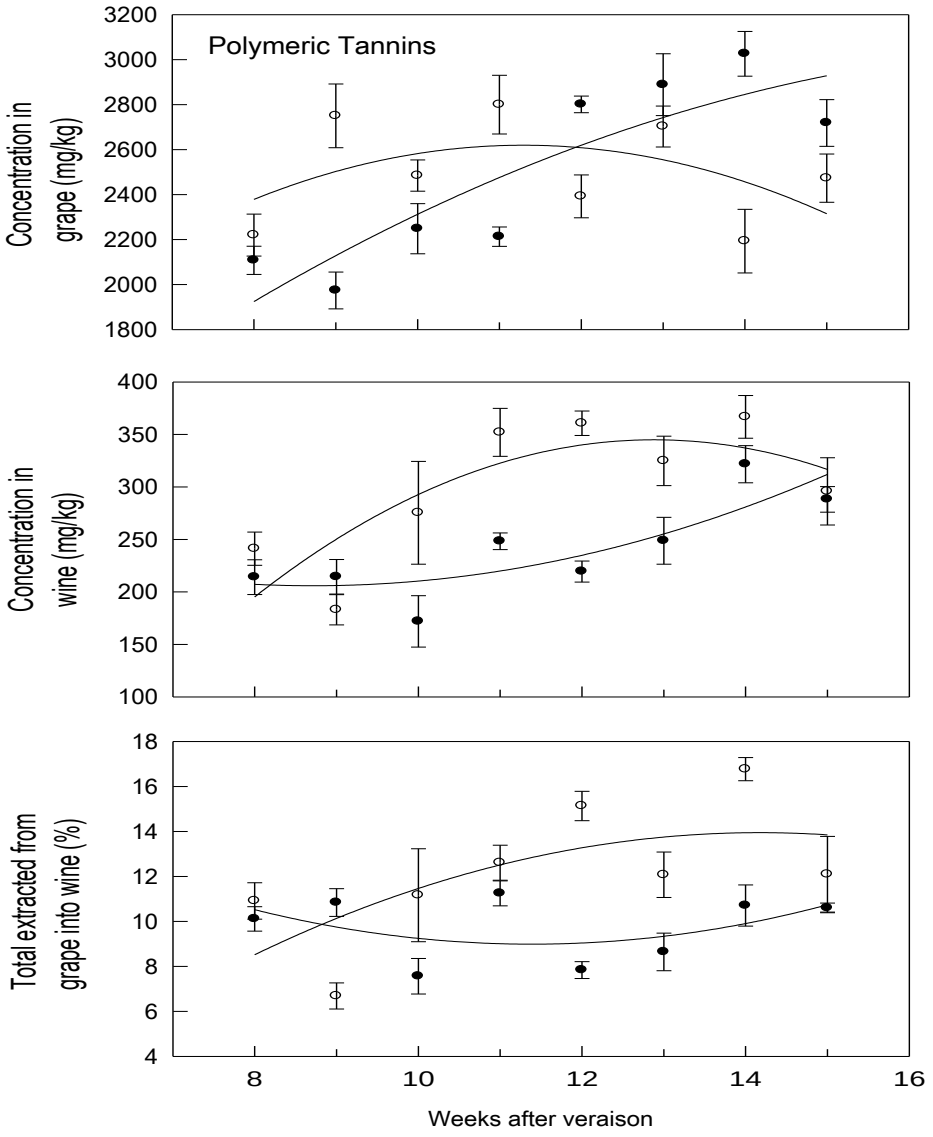

Figure 2.9 Influence of grape maturity on polymeric tannin concentration in grape (upper graph), concentration in wine (middle graph), and total grape polymeric tannin extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=155.2+275.3x-5.954x^2$ ($R^2 = 0.825$) for 2008 and $f=1333.922+238.315x-11.326x^2$ ($R^2 = 0.122$) for 2009. Wine concentration data were fitted to the following regression equations $f=242.1-18.27x+1.506x^2$ ($R^2 = 0.721$) for 2008 and $f=-338+100.7x-3.775x^2$ ($R^2 = 0.660$) for 2009. Extraction data were fitted to the following regression equations $f=17.9-1.611x+0.074x^2$ ($R^2 = 0.118$) for 2008 and $f=-7.410+2.803x-0.091x^2$ ($R^2 = 0.527$) for 2009.

veraison and 3 weeks post-veraison respectively. Polymeric tannin extraction in 2008 was relatively constant but extraction increased in 2009.

2.4.3.2 Non-flavonoids

Grape caftaric acid concentrations declined with maturity in both years (Figure 2.10), with each weekly concentration significantly greater in 2009 (40-21 mg/kg) than 2008 (37-16 mg/kg) ($p<0.05$). Wine caftaric acid concentrations also declined with fruit ripening in both years. Wine caftaric acid concentrations did not significantly differ between 2008 and 2009. Interestingly, caftaric acid concentrations were consistently greater in the wine compared to the grapes.

The low grape caftaric acid values yielded high extraction percentages, which were significantly greater in 2008 compared to 2009. In 2008, the harvest weeks 8-13 had the greatest percentage of caftaric acid extraction (453±117%), while in 2009 the extraction was relatively constant with a slight increase toward the later sampling data (169- 270%) (Figure 2.10).

2.4.4 Wine colour metrics

2.4.4.1 Spectrophotometer metrics

Wine absorbance at 420 nm, 520 nm, and intensity values increased over maturity in 2008 (Figure 2.11). The 2009 colour metrics increased initially, and then subsequently declined in the later stages of ripening. Hue remained relatively constant across all time points (data not shown). The spectrophotometric measurement trends paralleled the wine malvidin-3-glucoside concentrations.

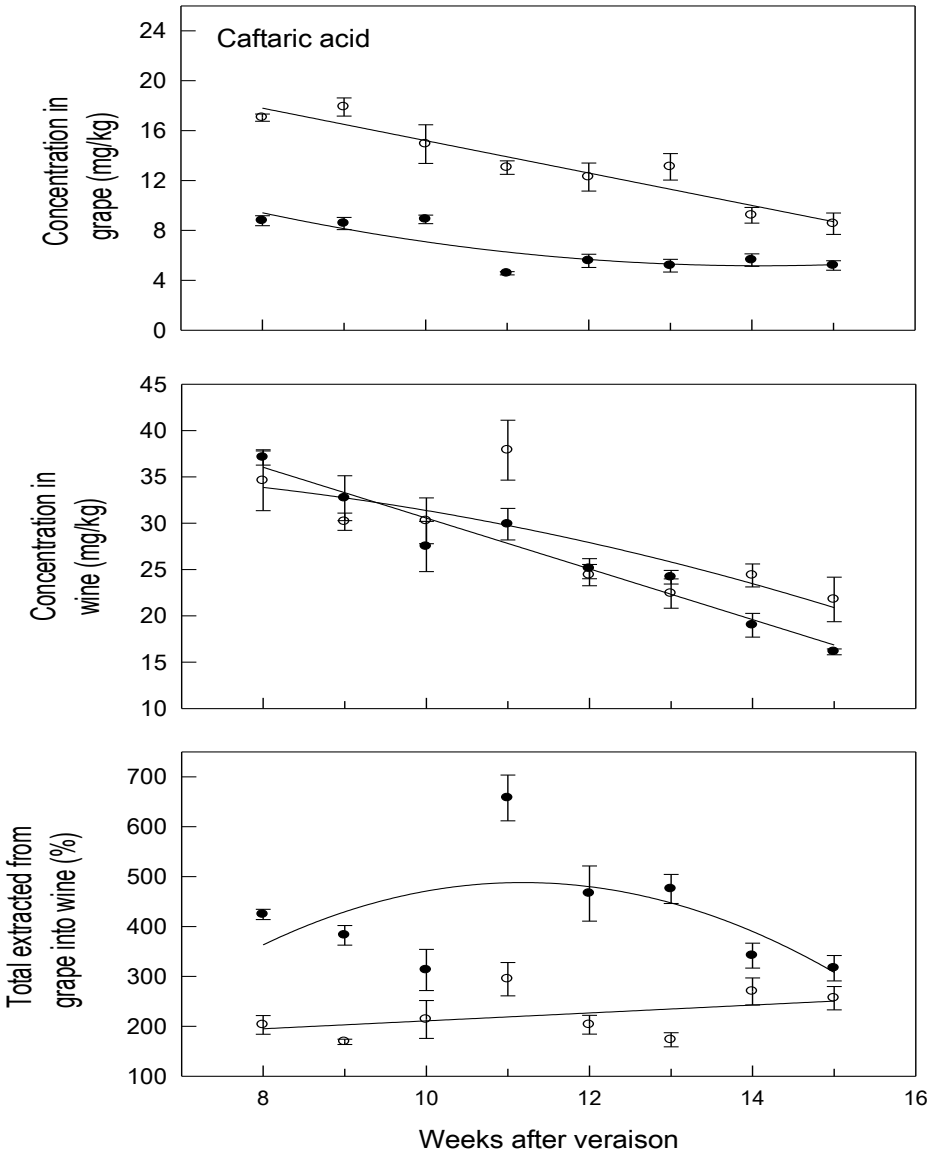

Figure 2.10 Influence of grape maturity on caftaric acid concentration in grape (upper graph), concentration in wine (middle graph), and total grape caftaric acid extracted into wine (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. Grape concentration data were fitted to the following regression equations $f=4.907+196.5e^{-0.458x}$ ($R^2 = 0.868$) for 2008 and $f=-26.855+59.046e^{-0.034x}$ ($R^2 = 0.934$) for 2009. Wine concentration data were fitted the following regression equations $f=49.07-1.181x-0.066x^2$ ($R^2 = 0.955$) for 2008 and $f=-15.872+75.018e^{-0.046x}$ ($R^2 = 0.706$) for 2009. Extraction data were fitted to the following regression equations $f=-997.60+266.30x-11.94x^2$ ($R^2 = 0.414$) for 2008 and $f=-190.880+-2.183x+0.419x^2$ ($R^2 = 0.199$) for 2009.

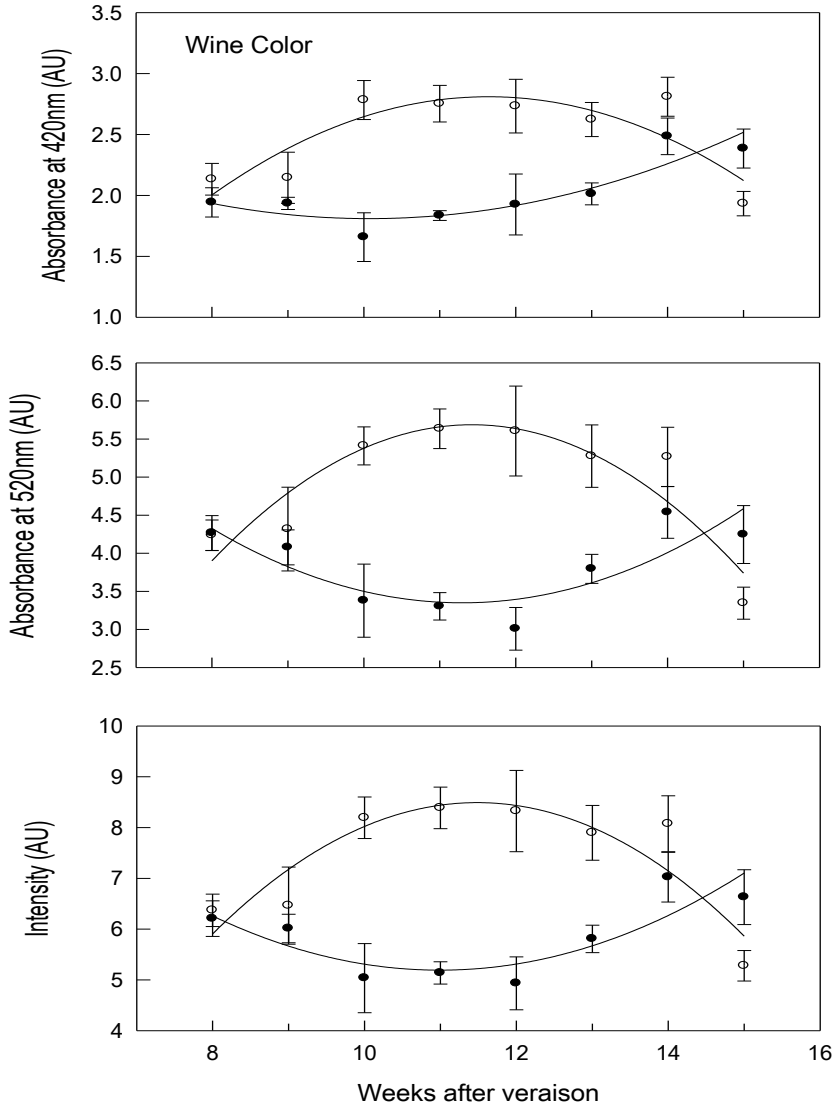

Figure 2.11 Influence of grape maturity on wine color measured as Absorbance at 420 nm (upper graph), Absorbance at 520 nm (middle graph), and Intensity (lower graph) of Cabernet Sauvignon grapes in 2008 (•) and 2009 (o). Each data point represents the mean of 4 replicates. All color data were fitted to quadratic regressions. Absorbance at 420nm data were fitted to the following regression equations $f=2.196-0.150x+0.011x^2$ ($R^2 = 0.718$) for 2008 and $f=-3.526+1.101x-0.048x^2$ ($R^2 = 0.727$) for 2009. Absorbance at 520nm data were fitted to the following regression equations $f=5.978-0.527x-0.028x^2$ ($R^2 = 0.240$) for 2008 and $f=-10.039+2.7734x-0.1239x^2$ ($R^2 = 0.809$) for 2009. Intensity data were fitted to the following regression equations were $f=8.173-0.676x-0.039x^2$ ($R^2 = 0.400$) for 2008 and $f=-13.594+3.87x-0.171x^2$ ($R^2 = 0.787$) for 2009.

2.5 Discussion

It is known that sample preparation impacts the extraction of phenolics (Singleton et al., 1985). Phenolic extraction is dependent on temperature, contact time and the solvent used (Sun et al., 1999, Koyama et al., 2007, Downey and Hanlin, 2010). In this study, whole berry homogenate was extracted with 50% (v/v) ethanol pH adjusted to 2.0 with HCl, with the aim of achieving maximum extraction of grape phenolics (Downey and Hanlin, 2010). This allows the comparison of total grape phenolic concentrations to the amounts extracted into the wine. Homogenization of the grape allows disruption of the skin and seed thereby allowing the hydro-alcoholic solution to contact and extract compounds from all locations within the grape berry. This technique was also used by Holt et al. (2008b, 2010). Mattivi et al. (2009) used lower ethanol, (12.88% (v/v)) for 5 days, and used whole skins and seeds to mimic the winemaking conditions. Other studies have separated the skin and seed material and used acetone or higher ethanol concentrations for extraction (Kennedy et al., 2000a, Kennedy et al., 2001, Harbertson et al., 2002, Kennedy et al., 2002, Downey et al., 2003a, Hanlin and Downey, 2009). Downey and Hanlin (2010) showed skin proanthocyanidin extraction to be similar in 50% ethanol and 70% acetone solutions. Fragoso et al. (2010) showed similar anthocyanin extraction when three different extraction methods were used (ITV, Glories at pH 1.0 and AWRI). However, all of these methods are likely to extract more phenolic compounds than would occur in wine due to the lower alcohol content of wine.

Grape phenolic results are frequently presented as weight per berry (Downey et al., 2003a) or moles per seed (Kennedy et al., 2000a) while wine phenolics are reported as weight per volume of wine (Katalinic et al., 1997). In the current study, grape and wine data were presented as concentrations (mg/kg) rather than as content. Concentrations were selected for ease of application to commercial winemaking as well as to be able to compare grapes and wines. The expression of data as total

content was ruled out due to volume losses in winemaking and sampling that may have impacted volume measurements and, therefore, the accuracy of the wine total content and calculations of extraction.

The extractability data for this study in wine were calculated as percent of total grape phenolic content. This differs from the extractability index proposed by Saint-Cricq de Gaulejac et al. (1998), which considered all available phenolics relative to those extractable at wine pH. Similarly, Sun et al. (2001) compared the phenolic content of the solid components of grapes before and after fermentation. In the current study, we calculate the amount of each compound extracted into the wine rather than the amount remaining in the grape solids. This expression was used in order to be more relevant to commercial winemaking.

The low grape caftaric acid concentrations found in this trial may be an artefact of the sample preparation. Singleton et al. (1984) found that juice that was not protected from enzymic oxidation yielded a caftaric reaction product (CRP), later termed grape reaction product (GRP) (Singleton et al., 1985). Grape samples from the current trial had peaks consistent with GRP (data not shown). The presence of GRP in the grape sample suggests that oxidation may have occurred during the homogenization process. The relatively high wine caftaric acid concentrations are contrary to data reported in previous literature. Singleton et al. (1985) reported a significant loss of caftaric acid during crushing with little subsequent loss during fermentation, while Nagel and Wulf (1979) showed a decrease in caftaric acid with fermentation. In the present experiments, the grapes for analysis were destemmed/crushed, homogenised in a grinder, and extracted for 1 hour on a shaker. This process was likely very oxidative. In contrast, the small-scale fermentors were closed, and the punch downs were gentle, so low amounts of oxygen were introduced into the system. The wines in the current

study were sampled approximately seven months after bottling in order to link the chemistry data to wine sensory data (Chapter 3). This delay in analysis may have resulted in lower wine polyphenol concentrations due to polymerization. For example, Nagel and Wulf (1979) showed anthocyanin concentrations decline quickly in the 8 months after fermentation. Differences in wine composition themselves may have impacted the polymerization and solubility of certain compounds including flavonols and anthocyanins (Price et al., 1995b). Despite these potential issues, the extraction trends would be the same given that all the grape samples and winemaking were processed similarly in this study.

In the current study, the sugar content of the must was adjusted to 24°Brix prior to fermentation in order to remove possible effects of the ethanol content on the extraction of phenolic compounds. Canals et al. (2005) showed that the extraction of total anthocyanins and total phenolics from grapes to wine increased with increased ethanol concentration. Hernandez-Jimenez et al. (2012) showed that ethanol was not necessary to extract seed proanthocyanidins, but the rate of extraction increased with ethanol present. In previous studies assessing the impact of grape maturity on wine composition, the must sugar levels were not adjusted. Wines made with grapes harvested at higher sugar levels would have greater alcohol concentrations and, therefore, the extraction would be greater (Canals et al., 2005). Increases in extractability attributed to grape maturity may have been due to solvent effects rather than changes in the grapes.

2.5.1 Extraction of phenolic compounds from grapes into wine

Many factors affect the extraction of compounds into wine, including the location and chemical structure of each compound found in the berry. Location includes the specific berry tissue where the

compounds are found, the berry pulp, skin, or seed, as well as the cellular location within the tissue (Sun et al., 1999) (Figure 2.12). The chemical structure of each compound determines its solubility and the potential to interact with other compounds (Koyama et al., 2007). For example, water-soluble compounds such as anthocyanins are extracted earlier during the fermentation than the flavanols (Gonzalez-Manzano et al., 2004, Canals et al., 2005). Although ethanol is not necessary for extraction of flavanols, the ethanol concentration increases the rate of extraction (Hernandez-Jimenez et al., 2012). Based on this, different compounds are extracted at different rates during the winemaking process. Winemaking practices, including cold soak and extended maceration, can also modify the extraction of compounds.

2.5.1.1 Location

The percent extraction may also be affected by the constituent's location within the grape berry. Hydroxycinnamates primarily in the pulp of the berry extract readily into the juice. Compounds in the skin, such as anthocyanins and flavonols, extract more rapidly than compounds in the seeds such as the flavanol monomers. Sun et al. (1999) studied the extraction of flavanol monomers and polymers from different grape tissues including stems. They found that catechins and proanthocyanidins are more readily extracted from skins and stems than seeds. Sun et al. (1999) also found that almost all of the stem catechins and proanthocyanidins are extracted during winemaking and can therefore impact the content of these compounds in the wine. Koyama et al. (2007) showed that phenolics in the grape skin extract faster than those found in seeds and Gomez-Plaza (2001) showed an increase in seed based compounds with longer maceration times. Given that a velvety, less grippy mouthfeel is generally preferred for red wines (Winter et al., 2004, Hufnagel and Hofmann, 2008), knowledge of the extraction kinetics of different compounds during fermentation in conjunction with ripeness parameters will allow processing decisions to achieve the desired wine styles.

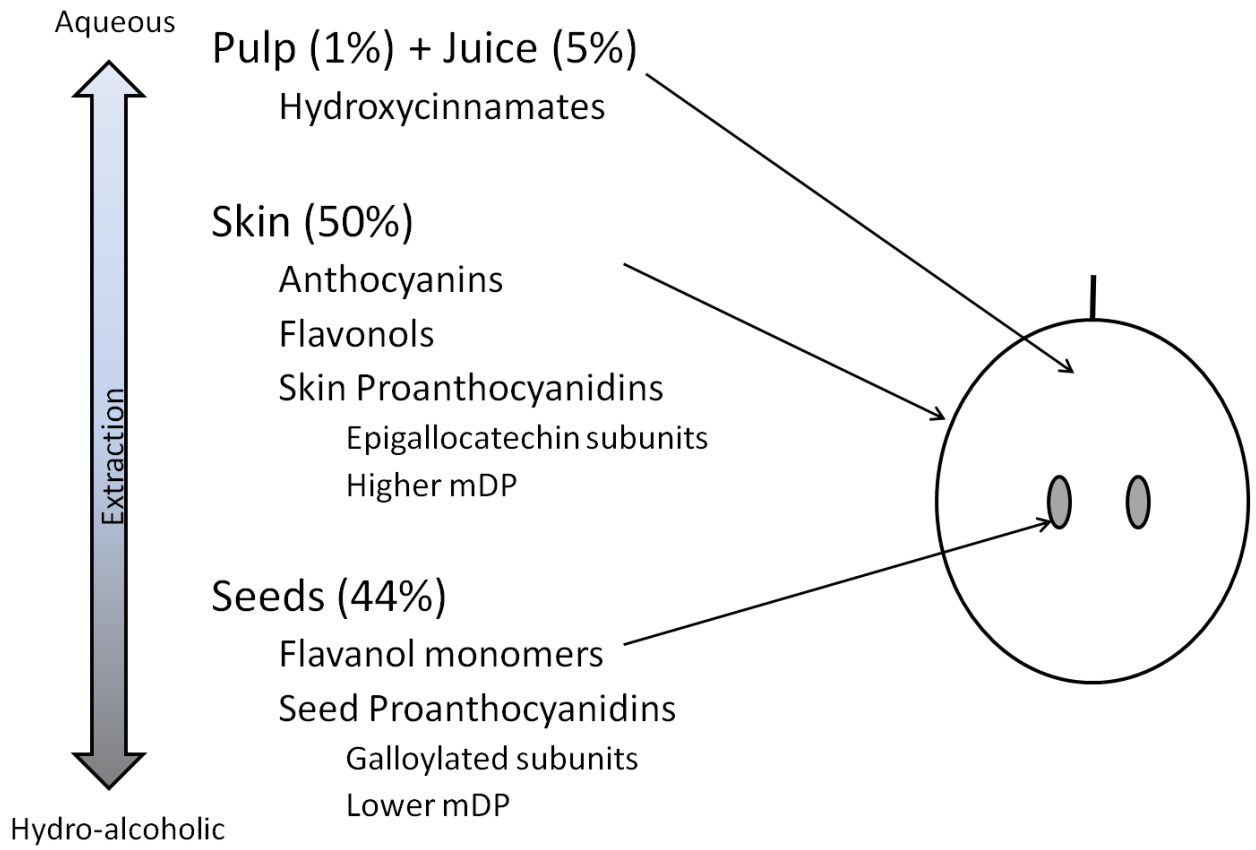

Figure 2.12 Location and extractability of grape phenolic compounds.

In this trial, constituent location in the berry appeared to correlate with the extraction of phenolic compounds. Skin-based constituents had greater extractability than seed-based constituents. Wine malvidin-3-glucoside concentrations increased with grape ripening; however, the increase was due to an increase in the grape concentration as opposed to an increase in malvidin-3-glucoside extractability (Figure 2.4).

Previous studies have shown a decline in the concentration of grape seed flavanol monomers and polymers with maturity, though different mechanisms for the declines have been proposed (Katalinic and Males, 1997, Saint-Cricq de Gaulejac et al., 1997, Kennedy et al., 2000a, Kennedy et al., 2000b, Ristic and Iland, 2005). Ristic and Iland (2005) reported that differences in the seed colour scores are inversely correlated to seed tannin extractability and suggested that seed dormancy may result in the impermeability of the seed coat. This is in agreement with Kennedy et al. (2000a) who proposed seed polyphenol oxidation resulted in decreased extractability. Saint-Cricq de Gaulejac et al. (1997) reported a decrease in small procyanidins (<3 subunits) with grape maturity and suggested the extraction is impacted by polymerization of these compounds and associated decrease in solubility. Pastor del Rio and Kennedy (2006) showed a decrease in wine proanthocyanidins despite an increase in Pinot Noir grape proanthocyanidins with ripeness. This is consistent with the results of the current study (Figure 2.9). Bindon and Kennedy (2011) found little or no difference in the percent of proanthocyanidins bound by cell wall material from early season to later ripeness dates in Cabernet Sauvignon. This is in agreement with the small differences in polymeric tannin extraction shown over the period of ripeness in the current study. In the current study, polymeric tannin extraction was very low (<17%) (Figure 2.9), though similar to extraction from Shiraz grapes (~25%) as reported by Bindon et al. (2010). The low extraction rates could be due to the formation of associations with

polysaccharides or proteins (Pastor del Rio and Kennedy, 2006, Bindon et al., 2010, Hanlin et al., 2010, Bindon and Kennedy, 2011). The trends in extraction in the current study are consistent with previous data (Pastor del Rio and Kennedy, 2006, Bindon and Kennedy, 2011).

2.5.1.2 Chemical structure

Chemical structure may also impact extraction of compounds into wine. Some compounds are more soluble in water while others require ethanol for extraction. For example, anthocyanins are soluble in water whereas seed tannins require some ethanol for extraction (Canals et al., 2005). Thus, skin contact time and the timing of the contact can impact the outcome. Short maceration times may give maximum colour, but lower total phenolic concentration. All wines in this study had maceration times ranging from 7-8 days (data not shown). Larger tannin molecules likely extract more slowly than smaller molecules (Fournand et al., 2006). Fournand et al. (2006) and Koyama et al. (2007) showed that skin proanthocyanidins with a lower average degree of polymerization were extracted more rapidly than those with a high average degree of polymerization from Shiraz and Cabernet Sauvignon grapes respectively. The extraction of catechin and epicatechin both exceeded that of B2 dimers and polymeric tannins in the current study. This is consistent with previous literature (Fournand et al., 2006, Koyama et al., 2007).

Phenolic extraction may also be affected by other factors. Polymerization may also result in decreased solubility of compounds, which results in lower concentration of these compounds in the wine. Alternatively, phenolics may interact with other phenolics or compounds including cell wall material and polysaccharides (Pinelo et al., 2006, Vicens et al., 2009, Bindon et al., 2010, Bindon and Kennedy, 2011). Vasserot (1997) reported less than 50% of colour and tannin is extracted into wine

while the rest remains adhered to the yeast lees. In the present study, the extraction of both catechin and epicatechin were much lower in week 15 of 2008 than for other harvest dates. This may indicate a decrease in extraction due to polymerization or an increased interaction with other cellular components. While interactions with cellular materials may result in lower extraction, copigmentation associations often result in greater extraction of pigment from skins into wine (Boulton, 2001). The difference in quercetin glycoside extractability between the years may be due to differences in flavonol-anthocyanin interactions that would increase the solubility of the flavonols (Price et al., 1995b).

2.5.1.3 Vintage effects

Influence of grape maturity on the extraction of polyphenolic compounds showed trends, though the patterns varied by compound and vintage. For example, catechin extraction increased with maturity in both years, while epicatechin, B2 dimer and polymeric tannins extraction decreased with maturity in 2008 but increased with maturity in 2009. The decline in extraction of seed phenolics in 2008 is consistent with previous data (Czochanska et al., 1979, Romeyer et al., 1986). The increases in extraction in 2009 may be due to the lower air temperature, and the associated slower ripening and/or the rain event in 2009, which may have lowered the concentration of compounds in the fruit. The differences in polymeric tannin extraction in 2008 versus 2009 may be due to compositional changes rather than concentrations (Kennedy et al., 2000a, Fournand et al., 2006). Not only was 2008 slightly warmer than 2009, but the crop load was also lower. This may also yield differences in the ripening patterns and subsequent extraction of compounds into wine. As reviewed by Downey et al. (2006) and Schultz and Stoll (2010), grape cultural practices and environmental stressors influence the concentration of phenolic compounds available in the grape and, therefore, the wine.

2.5.2 Influence of extended maturation

In this study, extended fruit maturation did not result in the extraction of disproportionately increased concentrations of polyphenolic compounds from grape into wine. Trends in the 2008 grape and wine phenolic data suggest that concentrations in wine are directly related to the grape concentrations. Unfortunately, the 2009 data do not reveal such a clear picture (Figures 2.4-2.10). Grape caftaric acid, catechin, epicatechin, and B2 dimer concentrations decline with ripening, and this was reflected in their concentrations in the wine. The decreases in grape caftaric acid, catechin, and epicatechin concentrations are consistent with previously published results that showed these compounds increased early in berry development and then declined with ripening (Singleton et al., 1986, Kennedy et al., 2000a, Kennedy et al., 2002, Pastor del Rio and Kennedy, 2006). However, the decline in B2 dimer concentration with fruit maturity was contrary to Romeyer et al. (1986), Perez-Magarino et al. (2004), and Katalinic (1997), who reported an increase of B2 dimer with seed maturation. Romeyer (1986) suggested that the increase of B2 dimer concentration along with the associated decrease in flavanol monomers supports the notion that the polymers are being synthesized from the monomers.

Consistent with previous reports, grape malvidin-3-glucoside concentrations increased with ripening (Keller and Hrazdina, 1998, Matthews and Anderson, 1998, Kennedy et al., 2002, Fournand et al., 2006) and the wine concentrations trended similarly. Despite the late maturity dates in the current study, the malvidin-3-glucoside concentrations did not decrease in overripe berries as has been previously reported (Ryan and Revilla, 2003, Fournand et al., 2006). In 2009, the final maturity sample contained less malvidin-3-glucoside than fruit from previous weeks. This is in agreement with the wine colour metrics. This decline may be due to the rain event two weeks earlier and subsequent breakdown of the berry skin. The notion that the decline might be due to dilution does not seem likely given that the percent of water added was similar to that in week 13, yet no decline was observed on

that date. Further work to determine the cause of the week 15 decline in anthocyanins and colour could be of interest as colour is indicative of wine quality (Somers and Evans, 1974).

2.5.3 Industry relevance

Historically Cabernet Sauvignon grapes in California were harvested at 23-23.5°Brix (Figure 2.1). However, the current total soluble solid levels at harvest are now closer to 25°Brix (Figure 2.1), requiring approximately two additional weeks of ripening. This change has come about due to changes in wine style preference and the development of berry sensory analysis techniques that utilise a series of attributes to gauge ripeness as opposed to sugar or acid content (Winter et al., 2004). One goal is a low level of the herbaceous characters associated with methoxypyrazines. Winemakers are also looking for “soft” or mature tannins to produce wines with less harsh astringency. This may be due to changing consumer patterns toward drinking wines immediately upon purchase rather than aging the wines. Softer, more mature tannins are less astringent and therefore more appealing to consumers.

The data reported here do not support the notion of increased extractability of phenolic compounds with grape maturity. However, the wine phenolic concentrations themselves might be more commercially relevant than extractability. Grapes harvested at greater ripeness provided higher concentrations of malvidin-3-glucoside and polymeric tannins and a slight decrease in the caftaric acid and flavanol monomers and oligomers compared to grapes harvested earlier in the season. Despite the increase in malvidin-3-glucoside concentration, wine colour was not significantly greater when measured as absorbance at 420 or 520 nm. The increase in polymeric tannin concentrations might reduce the ‘green tannins’ and perceived astringency associated with riper fruit. Changes in

wine sensory attributes associated with harvest date are described and discussed in Chapter 3. Kassara and Kennedy (2011) reported that proanthocyanidin composition might be more important than the concentration. However, proanthocyanidin composition data was not collected in the present work. The decrease in flavanols may result in a decrease in bitterness; however, the concentrations are far below the threshold values reported by Hufnagel and Hofmann (2008) and should therefore have little impact on the mouthfeel of the wine.

Grape harvest date is one tool that can be used to achieve targeted wine style, but the effects of winemaking practices as reviewed by Sacchi et al. (2005) might also be considered. Skin phenolics are generally preferred over seed phenolics for improved tannin quality (Cheynier et al., 1999). Various techniques have been shown to increase anthocyanin extraction including: pre-fermentation juice runoff (Singleton, 1972), elevated fermentation temperatures (Reynolds et al., 2001), thermovinification (with no additional phenolic extraction) (Auw et al., 1996), and extended maceration (Auw et al., 1996, Zimman et al., 2002). Vinification at higher temperature increases high molecular weight proanthocyanidins, but not the low molecular weight proanthocyanidins (Zimman et al., 2002) and thermovinification enhances anthocyanins without additional phenolic extraction (Auw et al., 1996). Higher vinification temperature causes some of the same desired increase in colour and riper tannins as extended hang time of grapes. Employing vinification techniques might be a better strategy to attain desired wine styles due to risk of weather with extended hang time.

Based on the compounds assessed in this study, extended fruit maturation has little impact on the concentration of phenolic compounds in wine. However, the wines made with grapes of different

ripeness can be differentiated based on sensory attributes as shown in a related study (Chapter 3). It appears that the individual phenolic compounds measured in the current study do not provide a clear marker for grape maturity. Further work is needed to determine the identity of the compounds responsible for the differences in mouthfeel associated with extended fruit maturation found in this study.

2.6 Conclusions

The results of this study indicate that extended maturation, or maintaining fruit on the vine beyond 24°Brix, does not increase the extractability of phenolic compounds in the associated wines. However, wines from riper grapes have greater concentrations of anthocyanins and polymeric tannins compared to those made from grapes harvested at earlier maturities. In a related paper (Chapter 3), it is shown that the wines can be differentiated by grape maturity based on sensory attributes. The phenolic compounds measured in this study are not adequate to explain the differences reported by the sensory panel. Further work is needed to elucidate chemical metrics that change with extended maturity which can be linked to the sensory differences.

2.7 Acknowledgements

The authors wish to thank the following staff of E. & J. Gallo Winery: Mike Cleary and Phil Chou for their help with chemistry and data interpretations, Robert Sui for his assistance with the HPLC and integration of the chromatograms, David Vasquez for vineyard preparation and grape sampling, and Susana Rodriguez-Vasquez, Steven Kukesh, Garrett Cosenza, David Santino, and Mike Owens for assistance with winemaking.

3 Impact of fruit maturity on polyphenol compounds in Cabernet Sauvignon (*Vitis vinifera* L.) wine and correlations to sensory perception

The extraction of phenolic compounds was studied based on anecdotal evidence that wine mouthfeel improves with grape maturity. In chapter 2, grape and wine phenolic concentrations and the extraction of these compounds from grape to wine were determined. In chapter 3, the wines produced from the grapes harvested at weekly intervals are assessed by descriptive analysis. The goal was to determine correlations between phenolic compounds and wine mouthfeel.

Chapter 3 was written in Australian English as the intent is to submit the manuscript embodied in this chapter to the Australian Journal of Grape and Wine Research for publication. The figures and tables have been inserted into the text for the convenience of the reviewer.

The references for this chapter have been incorporated into a single consolidated reference list that may be found at the rear of the thesis.

Impact of fruit maturity on polyphenol compounds in Cabernet Sauvignon (*Vitis vinifera* L.) wine and correlations to sensory perception

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3.1 Abstract

Background and Aims: Wine grape harvest is traditionally determined based on total soluble solids, acidity, fruit aroma and mouthfeel characteristics with the goal of achieving desired wine style and flavour. The purpose of this study was to determine the impact of grape harvest date on the sensory perception of the resulting wine and how wine sensory profiles correlate with wine chemistry.

Methods and Results: Cabernet Sauvignon grapes were harvested weekly for eight consecutive weeks in 2008 and 2009 after the historical maturity level (23.5°Brix) had been reached. Wine phenolic compound concentrations were determined and sensory descriptive analysis performed. Wines made with riper grapes have higher ratings of mouthfeel, colour, and dark fruit attributes. Wine colour measured by absorbance at 420 and 520 nm was strongly correlated with colour intensity ratings, though malvidin-3-glucoside concentrations were not. Wine polymeric tannin and alcohol concentrations were strongly correlated with mouthfeel ratings in 2008; however, few strong correlations were found in 2009.

Conclusion: The phenolic compound concentrations measured in this study did not fully explain the impact of harvest date on wine mouthfeel properties. Additional chemical metrics may be needed to explain the increase in wine mouthfeel observed in this study.

Significance of the Study: Enhanced knowledge regarding the impact of grape ripeness on wine phenolic concentrations and how that affects wine sensory attributes.

Key words: descriptive analysis**,** mouthfeel, phenolics, ripening

3.2 Introduction

Wine phenolic compounds are responsible for several important wine sensory factors including colour, bitterness and astringency (Noble, 1994, Gawel, 1998). Bitterness is a taste and is perceived through the taste receptors on the tongue, soft palate and upper digestive tract, while astringency is a tactile sensation defined by dryness, puckering, and roughness in the oral cavity perceived by the trigeminal sense (Peleg et al., 1999). Reactions involving phenolic compounds also contribute to the changes in wine during aging, including colour shifts and decreased astringency (Somers, 1971, Timberlake and Bridle, 1976, Cheynier et al., 2006, McRae et al., 2010). These attributes play an important role in wine quality and consumer acceptance and preference of wines.

Flavonoids are extracted from the grape seed and skins during vinification and are the primary phenolic compounds in wine (Singleton and Noble, 1976). Flavanol monomers and polymers contribute to wine bitterness and astringency (Rossi and Singleton, 1966a, Robichaud and Noble, 1990) and anthocyanins, such as malvidin-3-glucoside, provide colour to red grapes and wine. Non-flavonoids are also present in wine; however, hydroxycinnamates do not contribute to bitterness at white wine concentrations (Verette et al., 1988).

The molecular size of flavanols affects their relative bitterness and astringency. Monomers are more bitter than astringent, while large molecular weight derivatives are more astringent than bitter (Robichaud and Noble, 1990, Brossaud et al., 2001). Peleg et al. (1999) showed that intensity and persistence of bitterness decreased from monomer to trimer, yet Lea et al. (1978) found decreasing bitterness with polymers longer than four subunits. Vidal et al. (2003a) compared proanthocyanidins with greater degrees of polymerization and found no differences in bitterness perception. Other

components may also contribute to or enhance the bitter perception of wine. Ethanol increases the intensity and duration of bitterness (Noble, 1994) and was itself shown to be bitter (Fischer and Noble, 1994). A 3% increase in ethanol concentration increased the bitterness intensity more than addition of 1,400 mg/L catechin (Noble, 1994).

Astringent wines are commonly described as 'tannic' (Gawel, 1998). Tannins have been defined as phenolic compounds, with molecular weights between 500 and 3000, which can precipitate alkaloids, gelatine and other proteins (White, 1957). However, monomeric flavanols e.g. catechin do not crosslink collagen (White, 1957) or precipitate gelatine (Singleton and Trousdale, 1992), yet still elicit astringent sensations (Robichaud and Noble, 1990, Thorngate, 1995). Lee and Lawless (1991) found that dryness and roughing correlated with astringency. Studies to determine the mechanism for astringency are focused on two theories: the precipitation of salivary proteins, and interactions of procyanidin or protein-procyanidin complexes with oral epithelium (Green, 1993, Payne et al., 2009). Payne et al. (2009) found the binding of procyanidins to oral epithelial cells to be dose-, temperature-, and pH-dependent based on half-tongue tests. Astringency thresholds are decreased at lower pH (3.5 vs. 7.0) and ethanol decreases the perception of astringency, likely due to increases in viscosity (Payne et al., 2009). However, astringency intensity and duration increases with repeated ingestion (Guinard et al., 1986).

High degrees of polymerization are associated with astringency, drying and coarseness (Francis et al., 2001). Peleg et al. (1999) showed maximum astringency is greater in dimers and trimers compared to monomers and Robichaud and Noble (1990) showed an increase in astringency relative to bitterness with increasing polymer length. However, Rossi and Singleton (1966a) found a lower amount of highly polymerized tannins was needed to elicit a change in perceived astringency in dry

white wine than with less polymerized tannins. The increase in perceived astringency associated with increased polymer length may be due to hydrogen bonding between phenolic hydroxyl groups and the carbonyl groups of peptide linkages (Peleg et al., 1999). Seed proanthocyanidins have a mean degree of polymerization (mDP) around 10, while skin proanthocyanidins have an mDP around 30 (Prieur et al., 1994, Souquet et al., 1996, Hanlin et al., 2011).

Changes in the proportions of different size molecules may enhance or disrupt the balance of a wine. Young red wines containing a greater proportion of oligomers containing four or less subunits are more bitter and astringent and are termed “hard” (Noble, 1994). Lea and Arnold (1978) suggested that the ratio of bitterness to astringency can be used to define ‘hard’ versus ‘soft’ tannins. Flavanol monomers were more bitter than dimers (Peleg et al., 1999). The ability of proanthocyanidins to elicit astringency increases with degree of polymerization (Lea and Arnold, 1978). However, astringency declines with wine age. This may be due to increased intramolecular hydrophobic interactions causing a compact tannin conformation, thereby leaving fewer binding sites available (McRae et al., 2010).

Phenolic compound conformation can also impact the perception of bitterness and astringency. Despite being stereoisomers, epicatechin has a greater maximum intensity and longer persistence of bitterness and astringency than catechin (Noble, 1994, Thorngate, 1995, Peleg et al., 1999). This is of interest as seed proanthocyanidins have a greater percentage of epicatechin than catechin (Noble, 1994). Epicatechins serve as proanthocyanidin extension subunits, while catechin serves as the terminal unit (Prieur et al., 1994, Souquet et al., 1996). Epicatechin gallate subunits are also present in seeds and represent greater than 50% of the total seed flavanol content (Prieur et al., 1994), while skin proanthocyanidins contain less than 2% epicatechin gallate (Souquet et al., 1996). Thus, seed

tannins have a greater percent galloylation (28.4%) than skin tannins (3.8%) (Brossaud et al., 2001). Vidal et al. (2003a) showed the percent galloylation of proanthocyanidins impacts perception of astringency. Degalloylated seed fractions were rated lower in dry, chalky, and coarse grain attributes compared to fractions with the same degree of polymerization with higher percent galloylation (Vidal et al., 2003a).

Bond position can also impact perceived bitterness and astringency. Peleg et al. (1999) found catechin-catechin dimers with a 4-6 linkage were more bitter than either catechin-catechin or catechin-epicatechin with 4-8 linkage. However, the 4-8 catechin-catechin dimer was the least astringent of the three dimers.

Anthocyanins provide red wine with its colour which is perceived to be an indication of wine quality (Somers and Evans, 1974). Morrot et al. (2001) showed positive correlations between red wine colour descriptors and measured colour parameters. This can be important when assessing wine sensory attributes. Colour has been shown to bias odour identification and affect liking of the odours (Zellner et al., 1991). Hue, brightness and saturation of colour may all play a role in the intensity ratings of odours (Zellner and Kautz, 1990). Morrot et al. (2001) showed that red wine descriptors were used when white wine was artificially coloured red. Wine colour may be altered due to pH, free SO_2 level, and the age of the wine (Boulton et al., 1996). Colour stability corresponds to the degree of polymerization between phenolics and anthocyanins (Auw et al., 1996). The tannin-anthocyanin complexes are less sensitive to decolourization by either an increase in pH or the addition of bisulfite (Ribereau-Gayon and Glories, 1986). Anthocyanins themselves do not contribute to mouthfeel, but may have a contribution when they are polymerized with tannins (Waters, 1997). Incorporation of

anthocyanins into the tannin structure reduces the tannin hydrophobicity and, therefore, the perceived astringency (Kennedy et al., 2006a).

Sensorially, tannin quality improves with grape maturity. This may be due to a decrease in low molecular weight tannins and the associated decrease in 'harsh' tannins. This change in sensory characteristics may also be attributed to an increase in skin tannins versus seed tannins thereby decreasing the bitterness (Kennedy et al., 2002). Ristic et al. (2002) found higher quality wines had greater amounts of anthocyanins and skin phenolics, and a lower amount of total flavanols and seed procyanidins.

Although several studies have assessed the impact of grape maturity on the extraction of phenolic compounds (Canals et al., 2005, Fournand et al., 2006) or wine composition (Kennedy et al., 2000a, Kennedy et al., 2001, Kennedy et al., 2002, Perez-Magarino and Gonzalez-San Jose, 2006), these studies have not assessed the impact of grape maturity on wine sensory properties. Cabernet Sauvignon is the top selling red variety in the United States (Cuellar et al., 2010). The aim of this work is to investigate the relationship between phenolic concentrations and the sensory attributes of wines made from Cabernet Sauvignon grapes harvested at different maturities.

3.3 Materials and Methods

A condensed version of experimental methods is provided here. The full details of the materials and methods are provided in Chapter 2.

3.3.1 Vineyard

The experiment was conducted during the 2008 and 2009 growing seasons in a commercial vineyard located in the northern portion of the San Joaquin Valley, northeast of the town of Lodi, California (38°10'48.21"N, 121°13'39.34"W). Plant materials consisted of *Vitis vinifera* L. cv. Cabernet Sauvignon (FPS clone 8) grafted on Teleki 5C rootstock (*V. berlandieri x V. riparia*). The vines were planted in 1995 on a sandy loam soil. Vine rows were oriented east-west and trained to a quadrilateral cordon, horizontally divided system. The vines were spur pruned during dormancy to 24, 2-bud spurs per plant. Basal leaf removal in the fruiting zone was performed immediately following fruit set in both years. Vine irrigation requirements were estimated using daily reference evapotranspiration values (ETo) from weather stations located near the vineyard (less than one kilometre). Irrigation amounts were calculated on a weekly basis to replenish the ETc value estimated for the previous week. The calculated irrigation requirement (L/vine/week) was applied in equal amounts over a four or five-day period each week. Nitrogen (20 kg/ha) and potassium (50 kg/ha) were applied to the vineyard following fruit set in both years.

3.3.2 Vineyard experimental design

The experiment was designed as a randomized complete block design with each harvest date (treatment) replicated four times using seven vine plots. A total of 8 harvest dates or treatments were included in the experiment. A different set of vines were bunch sampled and harvested each week (at or following 21°Brix). Sampling dates for 2008 and 2009 were September 1 through October 27 and August 31 through October 26, respectively. All fruit used for fruit compositional analysis and winemaking was collected from the middle five vines in each plot.

3.3.3 Sample collection and basic analysis

Fruit collection for winemaking commenced when soluble solids reached historical commercial harvest levels (23.5°Brix) and continued each week through the final week of October, to Total Soluble Solids (TSS) values of 27.3 and 26.0°Brix for 2008 and 2009, respectively. Harvest dates are indicated as the week post-veraison. The wine lots were produced for post-veraison weeks 8-15 in both years.

Approximately 50 kg of fruit was harvested from each replicate for winemaking purposes. Field replicates were processed separately. All samples were held overnight at 2°C for processing the following morning.

3.3.4 Winemaking

The berries were detemmed/crushed using a Magitec model A15DC (Paarl, South Africa) destemmer/crusher directly into 80 L stainless steel fermentation tanks with adjustable height lids. Sixty ppm sulphur dioxide was added to the must immediately after crushing. In order to maintain similar alcohol levels, must sugar levels were adjusted to 24°Brix prior to fermentation for all wine lots. Samples with a starting sugar concentration less than 24°Brix were supplemented with 1:1 glucose:fructose mixture to reach 24°Brix. Samples with a high sugar level were diluted to 24°Brix using process water. The goal was to attain final wine alcohols of 14.0 ± 0.5% (v/v). This approach was utilized in an effort to minimize the influence of alcohol concentration on both the sensory perception of the wine (Fischer and Noble, 1994) and the extraction of phenolic compounds into the wine (Canals et al., 2005). Diammonium phosphate (DAP) was added to achieve a must Yeast Assimilable Nitrogen (YAN) of 300 ppm and tartaric acid was added to bring the titratable acidity (TA)

to 6 g/L. The must was inoculated with N96 yeast rehydrated per packet instructions (Anchor Yeast, Industria, South Africa) at a rate of 0.24 g/L.

Sixty-litre fermentations were conducted indoors at ambient conditions (approximately 21°C). Cap management was via submerged cap with one punchdown cycle per 24-hour period. Fermentations were monitored daily immediately following the punchdown for temperature, Brix, and flavour samples. The wines were pressed at 0°Brix using a Diemme AR 1.3 (Emilia-Romagna, Italy) membrane press. Free run and press fractions were reconsolidated into 40 L jacketed stainless steel vessel maintained at ambient temperature. Daily sampling for reducing sugar (RS) by FTIR and temperature continued until the wine was dry (<2 g/L).

Dry wines were cold settled for 3 days at 2°C and then racked into 40 L pressure rated cans. Wines were stored at 2°C under nitrogen until rough filtration. Rough filtration was through 1.0 µm nominal diatomaceous earth (DE) pads on a Filtrox plate and frame unit. The free sulphur dioxide was adjusted to 30 ppm, TA was adjusted to 5.5-5.8 g/L, and copper sulphate was used to remove sulphides prior to bottling. Malolactic fermentation was not performed. Wines were sterile filtered through Meissner 0.8 and 0.45 µm cartridge filters en route to bottling, using a GAI model 1006 modified for small lots. The filler bowl and bottles were purged with nitrogen gas. Wines were bottled into 750 ml bottles and sealed with screw caps. Packaged wines were stored at 13°C.

3.3.5 Wine analysis

3.3.5.1 Wine chemistry

Wines were analysed approximately 8 months after bottling and within 1 month of the completion of the wine sensory evaluation. Wine analysis included pH, titratable acidity, alcohol, reducing sugar (RS), lactic acid, malic acid, and volatile acidity (VA) by Fourier Transform Infrared Spectroscopy (FTIR) (WineScan FT-120, FOSS North America, Eden Prairie, MN). Wine colour metrics were determined as Absorbance (Abs) at 420 nm and 520 nm by spectrophotometer (Lambda 35, Perkin Elmer. Waltham, MA). Hue was calculated as Abs420/Abs520 and intensity was calculated as Abs420+Abs520.

3.3.5.2 Polyphenol analysis by HPLC

Calibration curves were generated for gallic acid, catechin, caffeic acid, quercetrin, quercetin, and malvidin-3, 5-diglucoside chloride at concentrations of 1, 5, 10, 25, 50, and 100 mg/L (ppm). Gallic acid monohydrate, (+)-catechin, (-)-epicatechin, caffeic acid, quercetrin, quercetin were from Sigma Chemical (St. Louis, MO). Malvidin-3, 5-diglucoside chloride was from Indofine Chemical Company, Inc. (Hillsborough, NJ).

Wines were filtered and loaded into HPLC vials for polyphenol analysis by reversed phase HPLC coupled to a diode array detector using the method as described by Waterhouse et al. (1999). The column was an Agilent Zorbax Eclipse XDB-C18 Rapid Resolution HT 4.6 X 50 mm, 1.8 µm column protected by an Agilent Zorbax Eclipse XDB-C18 analytical guard column 4.6 x 12.5 mm, 5 µm. The mobile phase was a gradient of 0.3% (v/v) phosphoric acid solution (mobile phase A) and 0.2% (v/v) phosphoric acid in acetonitrile (mobile phase B) at a flow rate of 1.0 ml/min. The elution conditions were as follows: 5% mobile phase B at time 0; 0-10 min 5-19% mobile phase B; 10.25-12.5 min hold

at constant 33% mobile phase B; 12.5-13.5 min 33-95% mobile phase B; and 13.5-14.5 min 95-5% mobile phase B.

Compounds eluting from the HPLC were identified and quantification was based on a comparison to authentic standards (except caftaric acid, quercetin glycosides, and polymeric tannins). Chromatograms were integrated using Agilent ChemStation software. The compounds were monitored at the following wavelengths: polymeric tannins – 230 nm; catechin, epicatechin, and B2 dimers – 280 nm; caftaric acid – 320 nm; quercetin glycosides – 360 nm; and malvidin-3-glucoside – 520 nm.

Since several compounds co-eluted with other compounds, manual integrations were needed. For example, polymeric tannins have co-eluting shoulder peaks that must be skimmed. Quercetin glycoside elutes as the middle peak of a triplet of peaks. A library search was used to confirm peak identity.

3.3.6 Wine sensory

3.3.6.1 Descriptive analysis

The wines produced in 2008 were evaluated from April through June in 2009. The panel was composed of 11 University of Adelaide staff and students from the Viticulture and Oenology program (4 females and 7 males). The wines produced in 2009 were evaluated April through July 2010. The panel comprised 10 University of Adelaide students and staff from the Viticulture and Oenology program and non-university personnel (7 females and 3 males).

Some panellists had previous DA panel experience; however, all were trained in 2-hour sessions held over 12 weeks. During these sessions, panellists were tasked with describing the wines and asked to come to consensus on the list of attributes and definitions to distinguish the wines. In order to determine attributes that defined the wines, the 2009 panellists saw each of the 44 wines from 2008 and the 2010 panellists saw the 40 wines from 2009 on at least one occasion during this process. Mouthfeel standards, represented by different fabric swatches, were available to the panellists during each session. Aroma reference standards and colour swatches were available to panellists during each session and were modified based on their feedback to produce a final set of standards for the formal evaluation sessions. Unoaked Cabernet Sauvignon wine made in the University of Adelaide teaching winery was used as the base wine for the aroma reference samples.

The wines were presented in clear, INAO (ISO standard), 215 ml tasting glasses covered with a petri dish. Each sample (30 ml) was identified by a 3-digit random code. The panel agreed upon descriptive terms including one colour, eight aroma, five flavour, two taste, four mouthfeel, and one aftertaste attribute for the 2008 wines and three colour, nine aroma, six flavour, four taste, four mouth feel, and one aftertaste attribute for the 2009 wines. The selected attributes, their definitions, and order of assessment for 2008 and 2009 wines are presented in Table 3.1 and Table 3.2, respectively. Panellists practiced scoring the wines on intensity of each attribute using an unstructured 15 cm line scale. The scale was anchored using indented end points at 10% and 90% of the scale representing “low” and “high” intensity, respectively. Raspberry cordial diluted in water 1:40 and 1:5 was used as the low and high intensity standards, respectively. Prior to formal evaluation of the wines, the panel was familiarized with the sensory booths and computer interface. The panel’s performance was evaluated by having each panellist assess a subsample of the wines in triplicate. The data was

analysed for panellist by sample interactions using PanelCheck (Nofima Mat and DTU – Informatics and Mathematical Modelling, Norway) and SENPAQ software (version 4.3 Qi Statistics, UK). Final assessment of the samples commenced when no significant interactions were found.

Prior to the formal assessment of the wines, the panellists were informed of the formal sample evaluation process. The formal evaluation was conducted under fluorescent light in individual booths within a temperature controlled sensory lab. During each formal evaluation session, each panellist was presented with 20-22 wines. Wines were presented as 30 ml samples in clear INAO (ISO standard) 215 ml tasting glasses covered with a petri dish. Each sample was identified by a 3-digit random number code. Wines were presented to the panellists in a randomized order, balanced for carry over effects. Distilled water and unsalted crackers were provided to each panellist as palate cleansers. Panellists were forced to rest for one minute between each sample and to take a 5-minute break after each of 5 wines. During this 5-minute period the panellists were asked to re-familiarize themselves with the reference samples. The reference and intensity standards were available to the panellists outside the booths throughout each formal assessment period. The 2008 wines were assessed in triplicate while the 2009 wines were assessed in duplicate over the course of the formal evaluations. The sessions for each set of replicates were held on separate days to avoid panellist fatigue.

Table 2.1 2008 Descriptive analysis attributes and definitions.

Attribute	Definition
Colour	
Colour Intensity	transparent to opaque
Aroma	intensity from low to high*
Dark Fruit	any combination of blackberry, blackcurrant, dark plum, dark cherry (1 black cherry, 2 blackberries, 2 black currants; all frozen and mashed)
Red Fruit	any combination of strawberry, raspberry, red cherry, red plum (1/2 strawberry, 2 raspberries, 1cm^3 red plum, 2 red currants; all frozen and mashed)
Green – Plant	any combination of plant, cut grass, mint, menthol, capsicum, asparagus (1cm^3 frozen capsicum, pinch mixed herbs (Masterfoods, NSW) chopped tomato leaves, grass leaves)
Green – Unripe fruit	green/unripe banana (1 cm length of green banana)
Pepper	either white or black (1 white +1 black peppercorn, broken)
Spice	spice combination (1/4 tsp. mixed spice, McKenzies Pty Ltd., Altona, Vic)
Earthiness	dirt and mulch (1 tsp dirt taken from Waite campus, SA)
Chocolate/Coffee	chocolate, coffee, mocha (1/2 tsp Nescafe Mocha powder, Nestle Australia Ltd. Rhodes, NSW)
Flavour	intensity from low to high (as per aroma definitions)
Fruit	any combination of dark and red fruit
Green	any combination of green characters including plant and unripe fruit
Pepper	either white or black
Spice	spice combination
Chocolate/Coffee	chocolate, coffee, mocha
Taste	
Acid	low to high acidity
Bitter	low to high bitter
Mouthfeel	
Tannin	silky to rough (touch standards satin material and coarse grade sandpaper)
Astringency	low drying to high drying
Body	light to full bodied
Alcohol	no heat to hot
Aftertaste	
Length - Flavour	length of time flavour/taste remains after expectoration where short is defined as <5 seconds, medium lasts about 10 seconds, and long is defined as >20 seconds

All standards were prepared in black glasses and presented in 40ml of an unoaked, 2008 South Australian Cabernet Sauvignon wine produced by the University of Adelaide research winery unless otherwise indicated. *Low and high intensity were anchored by 1:5 and 1:40 raspberry cordial in water, respectively. (Raspberry flavored cordial, Woolworth's Home Brand, Bella Vista, NSW).

Table 3.2 2009 Descriptive analysis attributes and definitions.

Attribute	**Definition**
Colour	
Hue	deep magenta to perylene violet (colour patches provided, acrylic paint Liquitex Artist Materials, Piscataway, N.J., U.S.A.)
Rim	pink to purple
Depth	transparent to opaque
Aroma	intensity from low to high*
Dark Fruit	any combination of black cherry, blueberry, blackberry, dark berry, mulberry (1 black cherry, 2 blackberries, 2 black currants; all frozen and mashed)
Red Fruit	any combination of strawberry, plum, raspberry, red currants, red cherry, plum fruit (1/2 strawberry, 2 raspberries, 1cm^3 red plum, 2 red currants; all frozen and mashed)
Confectionary	lolly, bubblegum, musk, cordial, estery, artificial fruit (1cm^3 pink 'Hubba Bubba' bubble gum broken into bits (Wrigley Company Pty.Ltd, Asquith, NSW), 1mm strawberries and cream, (Nestle Australia Ltd. Rhodes, NSW))
Developed Fruit	dried fruit, jammy, liqueured fruit, concentrated fruit (40 ml 2004 Bastian West Wines, Grenache Grenock, Barossa Valley)
Fresh Green	capsicum, green olives, tomato bush/leaf, herbaceous, stalky, grassy (1 cm3 frozen capsicum, pinch mixed herbs (Masterfoods, NSW), chopped, frozen tomato leaves, grass leaves)
Medicinal Green	eucalyptus, disinfectant, medicinal, Dencorub, Vicks (3 drops of a 0.001% solution of eucalyptus oil (Bosisto's Eucalyptus Oil, Felton Grimwade & Bosistos Pty Ltd., Oakleigh, South Vic), a pinch of Vicks Vaporub (Proctor & Gamble Australia Pty Ltd., McQuarie Park, NSW))
Floral	rose, violet (1 drop rose oil, Sunspirit Aromatherapy, Balling, NSW)
Chocolate/Coffee/Mocha	chocolate, coffee, mocha (1/2 tsp Nescafe Mocha powder, Nestle Australia Ltd. Rhodes, NSW)
Spice	black pepper, cinnamon, spicy, licorice/fennel/aniseed (pinch mixed spice, McKenzies Pty Ltd., Altona, Vic)
Flavour	intensity from low to high (as per aroma definitions)
Dark Fruit	any combination of black cherry, blueberry, blackberry, dark berry, mulberry
Red Fruit	any combination of strawberry, plum, raspberry, red currants, red cherry, plum fruit
Confectionary	lolly, bubblegum, musk, cordial, estery, artificial fruit
Developed Fruit	any combination of dried fruit, jammy, liqueured fruit, concentrated fruit
Green	any green character perceived on the palate
Spice	black pepper, fennel, licorice, aniseed, cloves, cinnamon
Taste	
Acid	low to high acidity
Bitter	low to high bitter
Mouthfeel	
Alcohol	low to high warmth on the palate
Tannin quality	silky to coarse (touch standards provided)
Body	light to full bodied
Astringency	not drying to drying
Aftertaste	
Length	length of time flavour/taste remains after expectoration. Anchored by 0 and > 20 seconds, with graduations at 2 seconds and 10 seconds.

All standards were presented in 40ml of a 2L cask of Shiraz wine unless otherwise indicated.

*Low and high intensity were anchored by 1:5 and 1:40 raspberry cordial in water, respectively. (Raspberry flavored cordial, Woolworth's Home Brand, Bella Vista, NSW)

3.3.7 Statistics

For the DA, a mixed model two-way ANOVA with assessors as random and samples as fixed factor effects was used, with Fishers LSD (least significant difference) post-hoc test where $p<0.1$ was considered significant using SENPAQ version 4.3 (Qi Statistics, UK). Pearson correlation tests, Principal Component Analysis (PCA), and Partial Least Squares Regressions (PLSR) were performed using XLStat 2011 (Addinsoft SARL, France). Pearson correlation tests were used to determine correlations between the sensory attribute and chemical metric means by harvest week (95% significance level). The inputs for PCA and PLSR were the mean of the four replicates for each harvest week.

3.4 Results

Wines made from grapes harvested at different maturities were dissimilar in both sensory attributes and chemical composition (Chapter 2).

3.4.1 Principal component analysis

3.4.1.1 Vintage differences

Principal component analysis (PCA) of the combined sensory data illustrated clear differences between the two vintages (data not shown), therefore, subsequent analysis was performed separately for each year.

3.4.1.2 Sensory attributes

To further assess the impact of grape harvest date on the resulting wine, PCA was conducted on all significant sensory attributes ($p<0.1$) for each year with the phenolic and colour data included as supplementary data. The attributes selected to define the wines were different for each vintage (Table

3.1 and 3.2). Significant differences between the means ($p<0.1$) were found for 15 and 12 attributes for 2008 and 2009, respectively. Sensory attributes that did not significantly differentiate the wines were excluded from the analysis. The PCA for 2008 attributes accounted for 82.48% of variation with the first 2 principal components (Figure 3.1). PC1 (62.81%) separated the harvest dates based on mouthfeel attributes including body, alcohol burn, tannin quality, length of aftertaste, astringency, and bitterness as well as dark fruit aroma, fruit, spice and chocolate/coffee flavour. PC2 (19.67%) divided the wines on red fruit aroma opposed to green and earth aromas.

The supplementary colour and phenolic data correlated well with the sensory ratings in 2008 (Figure 3.1, Table 3.3). Colour analyses (absorbance at 420 and 520 nm) had strong positive correlations with colour intensity ratings, though not with malvidin-3-glucoside concentration. Flavanol monomer concentrations displayed a moderate positive correlation with green aroma and flavour and were negatively correlated with dark fruit flavours in 2008. Polymeric tannin concentrations had strong positive correlations with all mouthfeel attributes, aftertaste length, and bitter taste, as well as colour in 2008.

The 2009 PCA accounted for 63.53% of the variation on the first two principal components (Figure 3.2). PC1 (33.43%) separated the data by colour ratings while PC2 (30.10%) divided the harvest weeks based on the developed fruit aroma and flavour opposed to red fruit aroma. PC3 (13.42%) separated the weeks based on the mouthfeel attributes astringency and tannin quality.

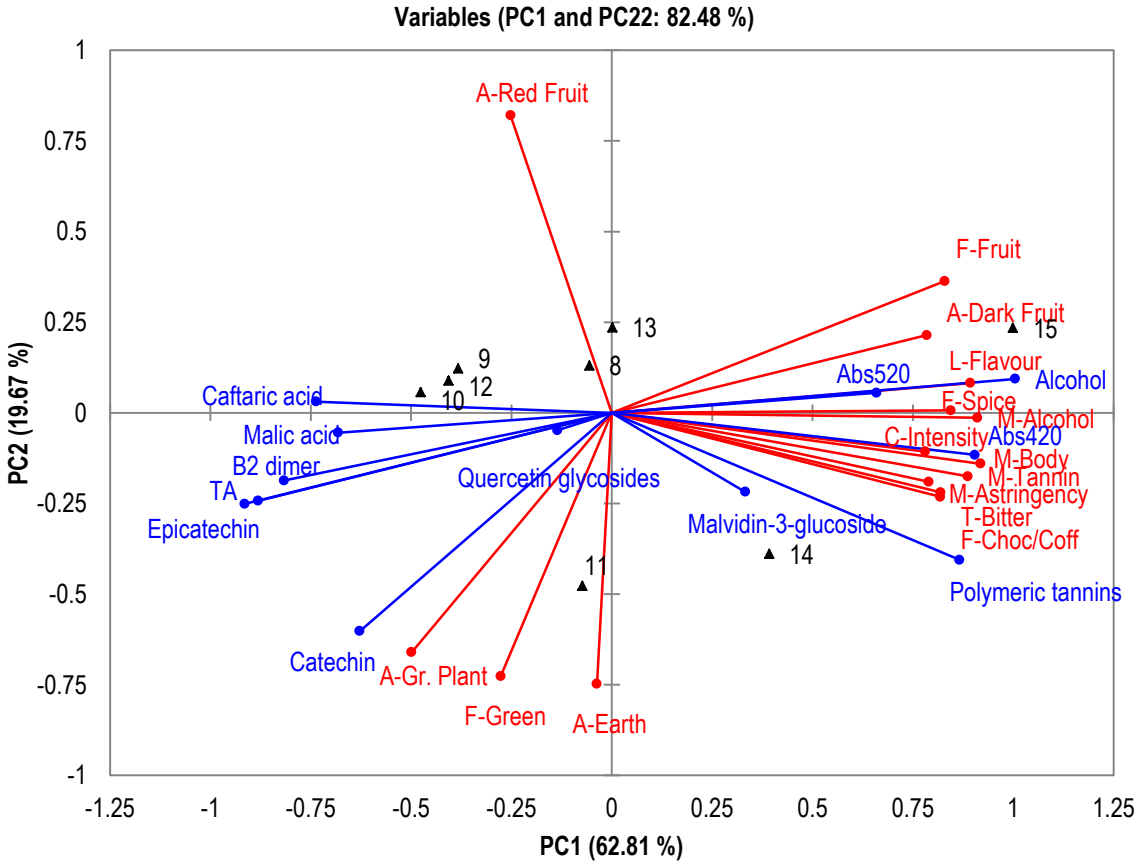

Figure 3.1 Principal Component Analysis of 2008 significant sensory attributes ($p<0.1$). Data represents the mean score for the 4 replicates from each week. Chemical metrics (indicated in blue) were treated as supplementary variables. A-aroma attribute; F-flavour attribute; C-colour attribute; M-mouthfeel attribute; and L-length.

Table 3.3 Pearson (n-1) correlation matrix for 2008 sensory and chemistry data. Values in bold are significantly different from 0 ($p<0.05$).

	Alcohol	Malic acid	TA	Abs420	Abs520	Caftaric acid	Catechin	Epicatechin	B2 dimer	Polymeric tannins	Quercetin glycoside	Malvidin-3-glucoside	C-Intensity	T-Acid	T-Bitter	M-Tannin	M-Astringency	M-Body	M-Alcohol	L-Flavour
Alcohol	**1.00**																			
Malic acid	**-0.76**	**1.00**																		
TA	**-0.81**	**0.79**	**1.00**																	
Abs420	**0.83**	-0.62	**-0.73**	**1.00**																
Abs520	0.43	-0.03	-0.46	**0.72**	**1.00**															
Caftaric acid	**-0.74**	**0.92**	**0.86**	-0.71	-0.17	**1.00**														
Catechin	-0.67	0.63	**0.81**	-0.48	-0.16	0.69	**1.00**													
Epicatechin	**-0.88**	**0.86**	**0.94**	**-0.71**	-0.26	**0.89**	**0.89**	**1.00**												
B2 dimer	**-0.81**	**0.91**	**0.93**	-0.65	-0.19	**0.96**	**0.78**	**0.95**	**1.00**											
Polymeric tannins	**0.80**	-0.67	-0.62	**0.93**	0.57	-0.69	-0.25	-0.61	-0.61	**1.00**										
Quercetin glycoside	-0.19	0.66	0.45	-0.24	0.13	**0.73**	0.32	0.43	0.66	-0.27	**1.00**									
Malvidin-3-glucoside	0.55	-0.68	-0.31	0.43	-0.29	-0.66	-0.44	-0.58	-0.55	0.49	-0.42	**1.00**								
C-Intensity	0.66	-0.45	**-0.74**	**0.83**	**0.84**	-0.56	-0.28	-0.55	-0.58	**0.80**	-0.23	-0.03	**1.00**							
T-Acid	**0.92**	-0.49	-0.60	0.65	0.38	-0.45	-0.52	-0.70	-0.57	0.64	0.15	0.42	0.51	**1.00**						
T-Bitter	**0.84**	-0.42	-0.62	**0.75**	0.48	-0.55	-0.46	-0.68	-0.57	**0.73**	0.04	0.46	0.62	**0.86**	**1.00**					
M-Tannin	**0.92**	-0.60	-0.69	**0.81**	0.52	-0.58	-0.42	**-0.72**	-0.63	**0.85**	0.03	0.40	**0.73**	**0.92**	**0.89**	**1.00**				
M-Astringency	**0.87**	-0.48	-0.54	**0.80**	0.49	-0.46	-0.39	-0.63	-0.47	**0.81**	0.20	0.48	0.58	**0.90**	**0.87**	**0.95**	**1.00**			
M-Body	**0.89**	-0.60	**-0.80**	**0.86**	0.66	-0.66	-0.44	**-0.74**	-0.70	**0.87**	-0.13	0.27	**0.89**	**0.82**	**0.86**	**0.95**	**0.84**	**1.00**		
M-Alcohol	**0.94**	-0.59	**-0.80**	**0.82**	0.56	-0.64	-0.62	**-0.82**	-0.70	**0.77**	0.01	0.39	**0.72**	**0.92**	**0.92**	**0.96**	**0.91**	**0.94**	**1.00**	
L-Flavour	**0.87**	-0.69	**-0.95**	**0.86**	0.65	**-0.79**	-0.67	**-0.87**	**-0.85**	**0.77**	-0.32	0.26	**0.88**	**0.71**	**0.77**	**0.83**	0.69	**0.93**	**0.90**	**1.00**

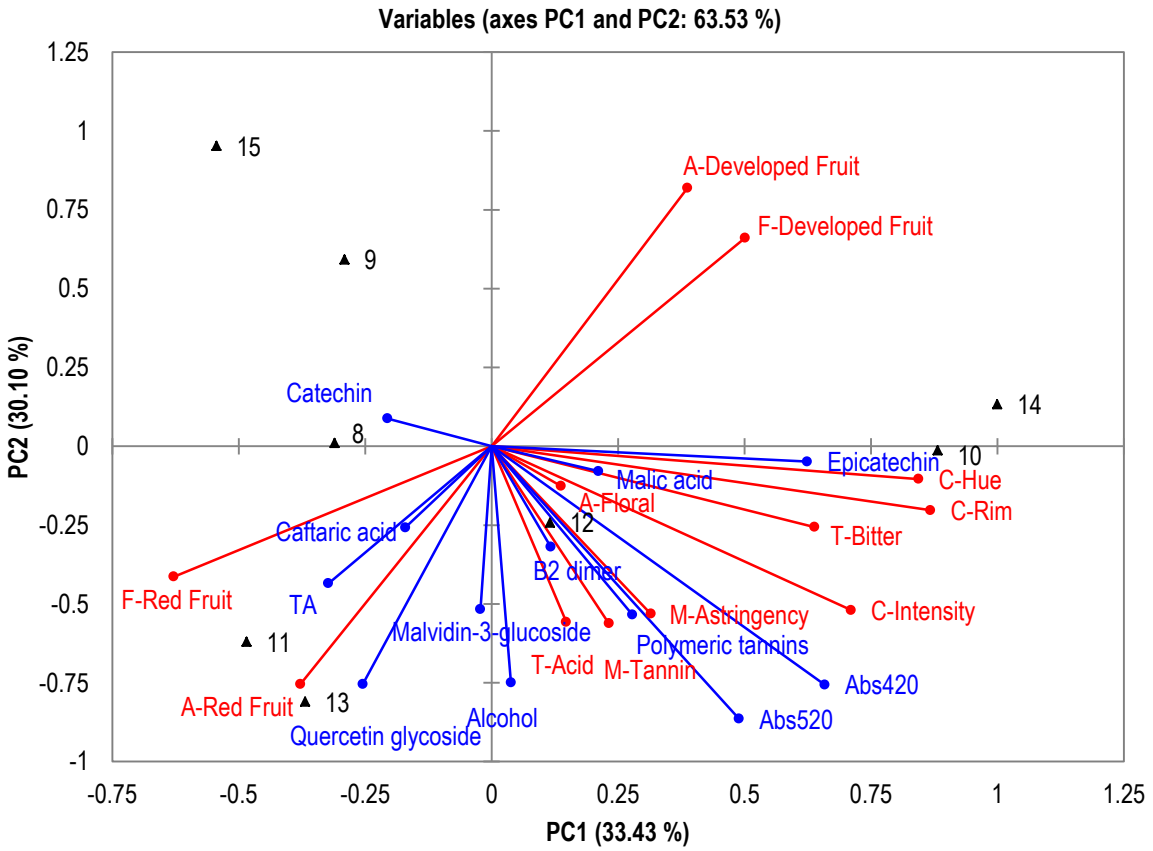

Figure 3.2a. Principal Component Analysis of 2009 significant sensory attributes ($p<0.1$). Data represents the mean score for the 4 replicates from each week. PC1 and PC2. Chemical metrics (indicated in blue) were treated as supplementary variables. A-aroma attribute; F-flavour attribute; C-colour attribute; and M-mouthfeel attribute.

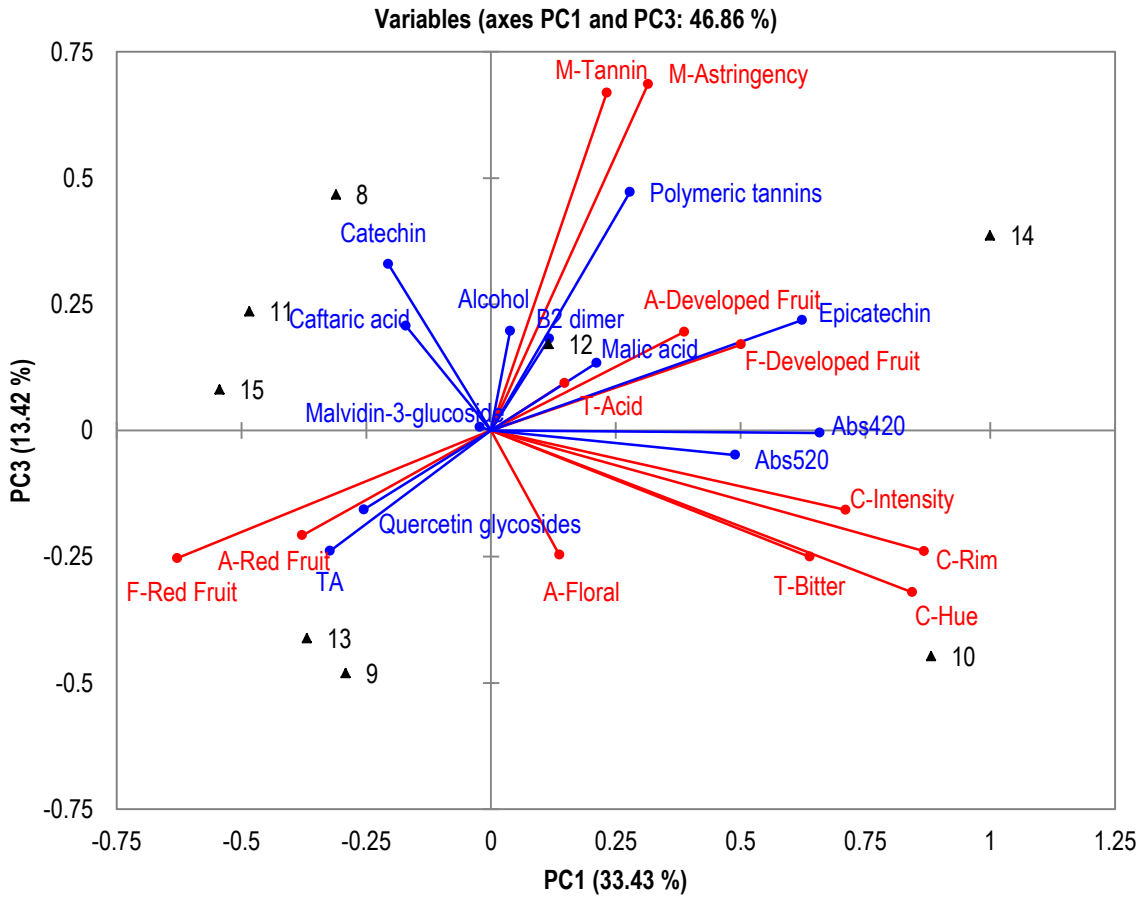

Figure 3.2b. Principal Component Analysis of 2009 significant sensory attributes ($p<0.1$). Data represents the mean score for the 4 replicates from each week. PC1 and PC3. Chemical metrics (indicated in blue) were treated as supplementary variables. A-aroma attribute; F-flavour attribute; C-colour attribute; and M-mouthfeel attribute.

The supplementary colour and phenolic data correlations were not as strong in 2009 compared to 2008 (Figure 3.2, Table 3.4). As in 2008, colour analyses (absorbance at 420 and 520 nm) had strong positive correlations with colour intensity ratings, though not with malvidin-3-glucoside concentration. Flavanol concentrations had few significant correlations in 2009. In 2009, polymeric tannins were positively correlated with the mouthfeel attributes tannin quality and astringency; however, these were the only mouthfeel attributes with significant differences ($p<0.1$).

3.4.1.3 Mouthfeel and wine phenolic concentrations

In order to determine the correlations between sensory ratings and phenolic composition, PCA was performed using only the sensory attributes that describe mouthfeel, taste, aftertaste, or colour. The TA (g/L), alcohol (% v/v), phenolic concentrations (mg/L), and absorbance measurements were included as supplementary data. A correlation matrix (Pearson (n-1)) was calculated to quantify the relationships as indicated by the PCA vectors (Table 3.3 and 3.4).

PCA of the 2008 descriptive analysis attributes accounted for 95.08% of the variation in the first two dimensions with 85.56% and 9.52% explained by PC1 and PC2, respectively (Figure 3.3). PC1 separated the harvest weeks by alcohol, tannin quality, and bitterness. PC2 further separated the wines based on colour intensity, acid, and astringency. The vectors for the supplementary data indicated strong correlations between mouthfeel alcohol perception and the wine alcohol concentration (% v/v) ($r=0.944$) (Table 3.3). Body, tannin quality, astringency, and colour were

Table 3.4 Pearson (n-1) correlation matrix for 2009 sensory and chemistry data. Values in bold are significantly different from 0 (p<0.05).

	Alcohol	Malic acid	TA	Abs420	Abs520	Caftaric acid	Catechin	Epicatechin	B2 dimer	Polymeric tannins	Quercetin glycoside	Malvidin-3-glucoside	C-Intensity	T-Acid	T-Bitter	M-Tannin	M-Astringency	M-Body	M-Alcohol	L-Flavour
Alcohol	**1.00**																			
Malic acid	-0.12	**1.00**																		
Titratable acidity	0.18	0.54	**1.00**																	
Abs420	**0.72**	-0.06	-0.03	**1.00**																
Abs520	**0.76**	-0.03	0.19	**0.96**	**1.00**															
Caftaric acid	0.43	0.67	0.71	0.06	0.19	**1.00**														
Catechin	-0.30	0.52	0.53	-0.34	-0.18	0.49	**1.00**													
Epicatechin	0.15	0.70	0.10	0.36	0.31	0.56	0.33	**1.00**												
B2 dimer	0.15	**0.72**	0.52	0.18	0.31	**0.73**	**0.72**	**0.78**	**1.00**											
Polymeric tannins	0.61	-0.44	-0.53	0.68	0.58	-0.28	-0.48	-0.02	-0.19	**1.00**										
Quercetin glycoside	0.56	0.28	**0.80**	0.37	0.59	0.68	0.40	0.22	0.65	-0.06	**1.00**									
Malvidin-3-glucoside	0.63	-0.06	0.44	0.39	0.44	0.27	-0.29	-0.26	-0.24	0.18	0.35	**1.00**								
C-Intensity	0.34	0.04	0.07	**0.85**	**0.82**	-0.13	-0.20	0.27	0.11	0.39	0.23	0.36	**1.00**							
T-Acid	0.18	**0.73**	0.59	0.34	0.46	0.56	0.57	0.62	**0.88**	-0.05	0.67	-0.05	0.36	**1.00**						
T-Bitter	0.52	-0.34	-0.34	**0.82**	0.70	-0.24	-0.68	0.18	-0.23	0.55	-0.03	0.30	**0.71**	-0.17	**1.00**					
M-Tannin	0.64	0.10	-0.16	0.62	0.57	0.18	-0.07	0.31	0.24	**0.82**	0.16	0.25	0.35	0.37	0.24	**1.00**				
M-Astringency	0.55	0.05	-0.13	0.64	0.60	0.10	0.05	0.28	0.24	**0.79**	0.17	0.26	0.47	0.38	0.25	**0.96**	**1.00**			
M-Body	0.63	-0.39	-0.28	**0.87**	**0.83**	-0.24	-0.37	0.07	0.01	**0.88**	0.23	0.18	0.67	0.19	0.68	0.68	0.70	**1.00**		
M-Alcohol	-0.24	0.30	-0.08	0.32	0.31	-0.06	0.44	0.60	0.61	0.06	0.12	-0.59	0.46	0.60	0.10	0.15	0.27	0.32	**1.00**	
L-Flavour	-0.38	-0.63	**-0.81**	-0.21	-0.34	-0.68	-0.39	-0.21	-0.47	0.12	-0.64	-0.62	-0.25	-0.66	0.23	-0.36	-0.36	0.04	0.10	**1.00**

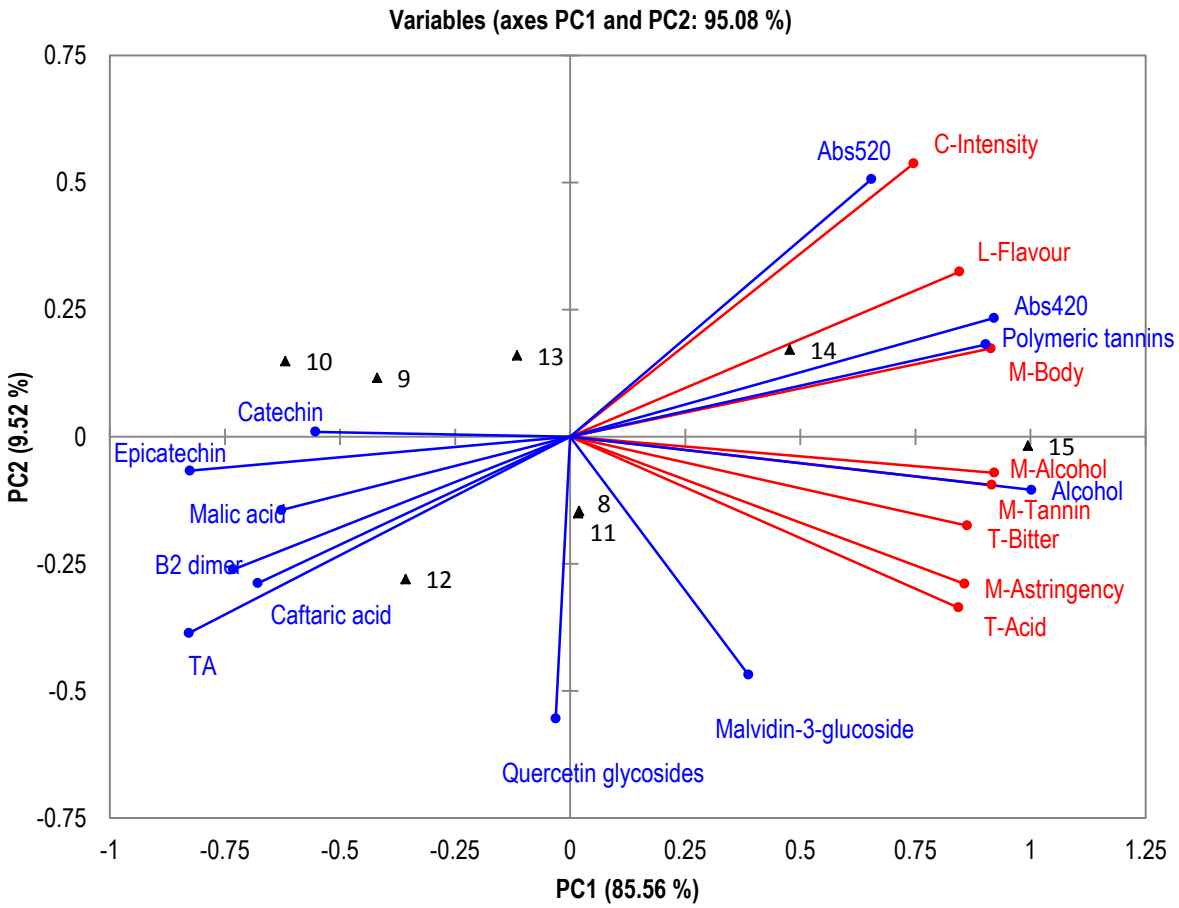

Figure 3.3 Principal Component Analysis of 2008 sensory taste and mouthfeel attributes ($p<0.1$). Data represents the mean score for the 4 replicates from each week. Chemical metrics (indicated in blue) were treated as supplementary variables. A-aroma attribute; F-flavour attribute; C-colour attribute; M-mouthfeel attribute; and L-length of perception after expectoration.

strongly correlated with polymeric tannins (r=0.871, 0.853, 0.810, and 0.803 respectively). Colour intensity scores were closely related with the wine colour measurements (AU) (Abs420 r=0.829 and Abs520 r=0.843). However, colour intensity and malvidin-3-glucoside concentration appeared unrelated given the vectors are located approximately perpendicular to one another. Bitterness and astringency were closely correlated to one another (r=0.836), but were negatively related to the concentrations of flavanol monomers and dimer (catechin, epicatechin and B2 dimer). Acidity scores were negatively correlated to wine TA and malic acid concentration. None of the sensory attributes were significantly correlated with wine malvidin-3-glucoside and quercetin glucoside concentrations.

The first two dimensions in the PCA of the 2009 DA scores explained 70.11% of variation. PC1 (46.49%) separated the harvest weeks based on astringency, body, tannin quality, and colour intensity (Figure 3.4). PC2 (23.62%) separated the weeks based on acid and bitter taste, and aftertaste length. The 2009 data had few significant correlations between sensory attributes and chemical data. The mouthfeel attributes tannin quality, body, and astringency were positively correlated with polymeric tannin concentrations (r=0.817, 0.881, and 0.786 respectively) (Table 3.4). Colour intensity was strongly correlated to the colour measurements (AU) (Abs 420nm r=0.854; Abs 520 nm r=0.820), but not malvidin-3-glucoside concentration (r=0.355). Acidity showed a stronger correlation with malic acid concentration (r=0.731) than with TA (r=0.589). Bitter taste was negatively correlated to the concentrations of flavanol monomers and dimers.

In order to determine the relationship between wine chemistry and sensory attributes, colour, taste and mouthfeel attribute ratings (y variables) were modelled based on chemical metrics (x variables) by partial least squares regression (PLSR). In 2008, three components were required to explain the average sensory attribute ratings with $Q^2cum = 0.713$. The 2008 model was driven by polymeric

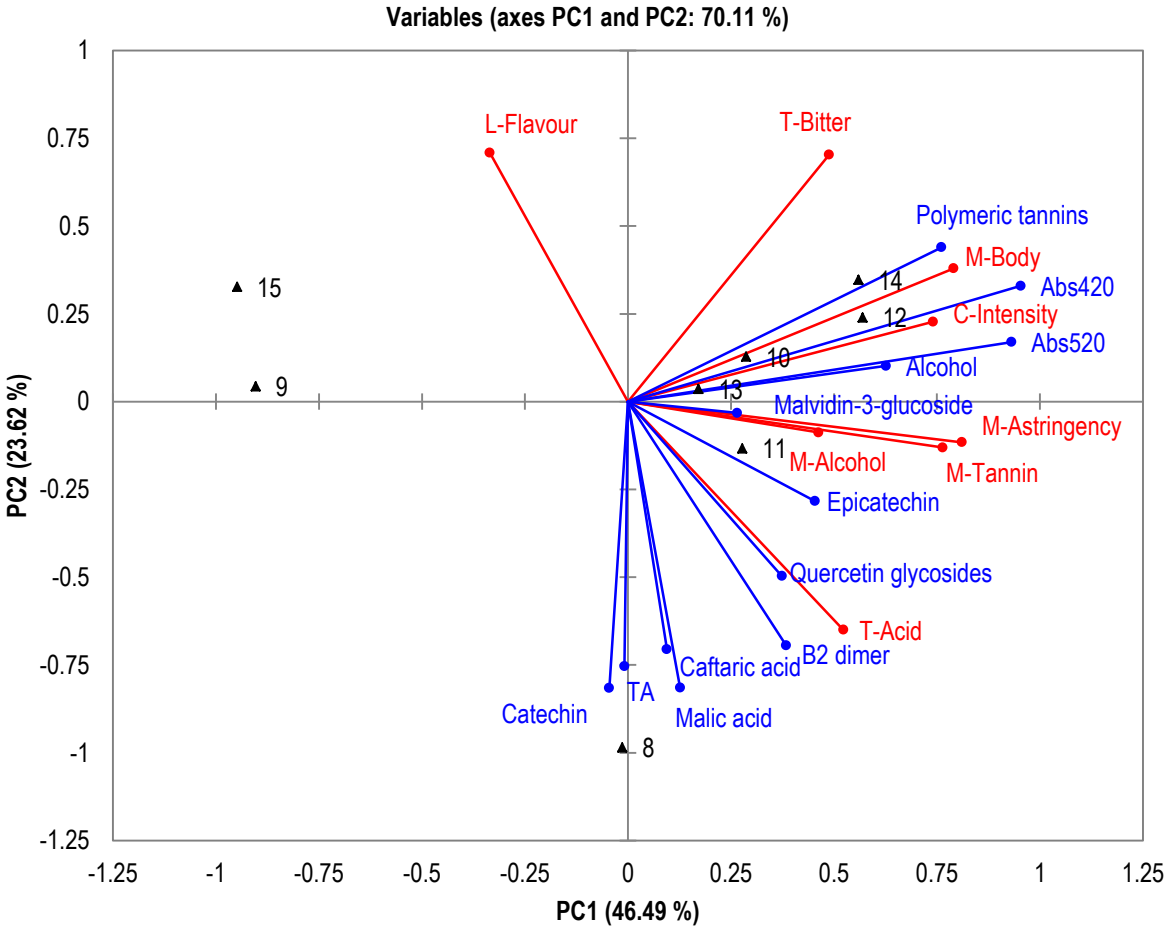

Figure 3.4 Principal Component Analysis of 2009 sensory taste and mouthfeel attributes attributes ($p<0.1$). Data represents the mean score for the 4 replicates from each week. Chemical metrics (indicated in blue) were treated as supplementary variables. A-aroma attribute; F-flavour attribute; C-colour attribute; M-mouthfeel attribute; and L-length of perception after expectoration.

tannin, alcohol, and quercetin glycoside concentrations (Figure 3.5). The alcohol and polymeric tannin concentration contributions to the model were consistent with the Pearson correlations; however, quercetin glycoside showed no significant Pearson correlations with any of the sensory attributes (Table 3.3). Malvidin-3-glucoside concentrations were a large negative contributor to the colour intensity model (data not shown) despite the low correlation scores and vector position in the PCA (Table 3.3, Figure 3.3). The PLSR model for 2009 sensory attributes rating was not as strong with Q^2cum = 0.122 with three components. The PLSR regression coefficients were lower in 2009 than 2008 (Figure 3.6). Polymeric tannin concentration was the strongest contributor to the 2009 PLSR sensory model. This was consistent with the Pearson coefficients, which showed strong correlations between polymeric tannin and the mouthfeel attributes tannin quality, astringency, and body.

3.5 Discussion

Extended maturation of grapes is utilized to achieve optimum berry flavour and phenolic maturity in order to produce wines of that meet specific wine style targets. The aim of this study was to determine the chemical compounds responsible for increased wine colour and mouthfeel in wines made from grapes harvested beyond historic or traditional maturity levels. Wine sensory data was compared with wine colour metrics and phenolic compound concentrations to determine correlations between wine chemistry and wine sensory. Wines made from grapes harvested at different maturities had significantly different sensory attributes. In general, wines made with riper grapes had higher ratings of mouthfeel, colour, and dark fruit attributes.

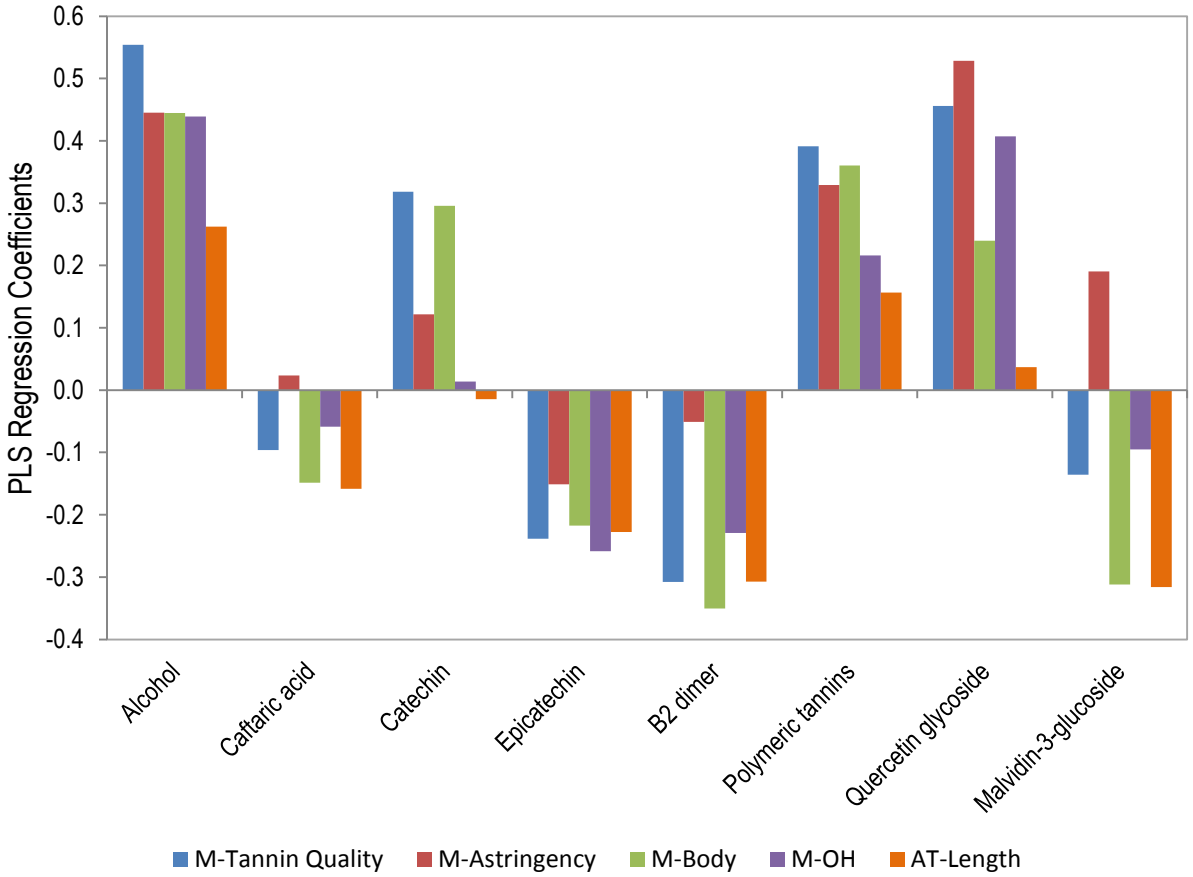

Figure 3.5 Partial least squares regression standardized coefficients of 2008 mouthfeel attributes modeled on composition variables (n=8). Q^2cum= 0.713.

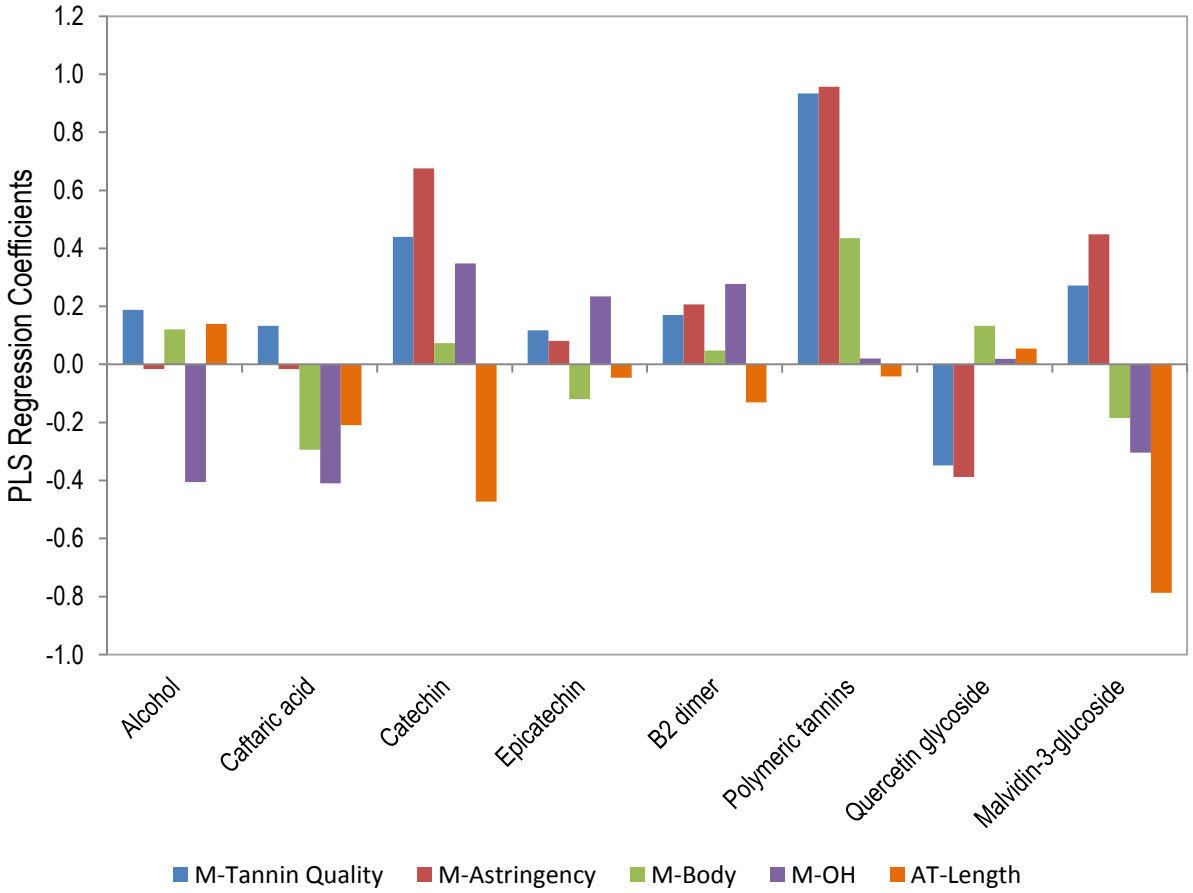

Figure 3.6 Partial least squares regression standardized coefficients of 2009 mouthfeel attributes modeled on composition variables (n=8). Q^2cum= 0.122.

Grapes harvested beyond historic or traditional maturity levels have high TSS levels that result in high alcohol in the wine. In the current study, must TSS was adjusted to 24°Brix to achieve consistent wine alcohol concentrations. This was done to ensure extraction of the phenolic compounds was due to grape phenolic composition as opposed to greater extraction in the presence of increased alcohol in the medium (Downey and Hanlin, 2010, Hernandez-Jimenez et al., 2012). The consistent wine alcohol levels also diminished the impact of alcohol on the sensory perception of the wine (Fischer and Noble, 1994).

3.5.1 Flavonoids

3.5.1.1 Anthocyanins

In the current study, malvidin-3-glucoside was measured as this is the most prominent anthocyanin in *V. vinifera* (Winkler et al., 1974). Malvidin-3-glucoside concentrations increased throughout the 2008 season, but remained constant in 2009 (Section 2.4.3.1.1). The 2009 concentration data trend was influenced by the low malvidin-3-glucoside concentration in week 15, which was likely due to the rain event in week 13. Malvidin-3-glucoside concentration was not closely related to the panel's colour intensity ratings or the colour as measured as absorbance at 420 or 520 nm. This may also be due to the content and concentration of individual anthocyanin forms. Roggero et al. (1986) showed that the percent of cyanidin and delphinidin in Syrah grapes declined and the percent of malvidin increased following veraison. However, the ripest grapes were sampled only 7 weeks after veraison, or earlier than the start of the current study. The concentration of individual anthocyanins was not determined in the current study; however, malvidin-3-glucoside was the dominant peak at 520 nm accounting for approximately 50% of the anthocyanins at all sample points in both years (data not shown). Wine colour is also affected by anthocyanin reactions in the wine including copigmentation, which enhances the colour of young red wines (Boulton, 2001).

Anthocyanins do not contribute much to wine taste (Brossaud et al., 2001) or to mouthfeel, but may have a contribution when they are polymerized with tannins (Waters, 1997). Malvidin-3-glucoside was not closely related to any of the mouthfeel attributes (Figure 3.3 and 3.4). This is in agreement with previous literature. Landon et al. (2008) showed that anthocyanin concentrations do not have a strong relationship with tannins, bitterness or astringency. Vidal et al. (2004) showed that addition of 0.5 g/L anthocyanin fractions does not modify bitterness or perception of astringency in a model wine solution.

3.5.1.2 Flavonols

Quercetin glycoside was weakly correlated with all sensory attributes. In 2008 the quercetin glycoside concentrations remained constant with grape ripeness (Section 2.4.3.1.2). However, the quercetin glycoside coefficients for bitter, tannin, astringency and alcohol indicated that quercetin glycoside was a contributor in the 2008 PLSR model predicting sensory from chemistry (Figure 3.5). This may be due to the low threshold of astringency for quercetin glycoside (1.0 mg/L) (Hufnagel and Hofmann, 2008). Slight changes in concentration would therefore have a considerable effect on mouthfeel. Hufnagel and Hofmann (2008) described quercetin glycoside as providing a 'velvety astringency'. It is possible that this velvety astringency was neither perceived nor identified as readily as a drying or puckering astringency.

3.5.1.3 Flavanols

Flavanols contribute to the sensations of bitterness and astringency (Robichaud and Noble, 1990, Noble, 1994, Brossaud et al., 2001, Kennedy et al., 2006b). Hufnagel and Hofmann (2008) reported catechin and epicatechin as being both bitter and astringent and reported the bitter threshold for these

compounds to lie between 270-290 mg/L in water. In the current study, wine flavanol monomer and dimer concentrations decreased with grape maturity (Section 2.4.3.1.3). However, bitterness ratings changed little with grape maturity. This is contrary to Arnold and Noble (1978) who showed a correlation between bitter scores and flavanol monomer concentrations; however, the levels used (25, 80, and 135 mg/L GAE) were much greater than in the current study. The flavanol monomer concentrations in the current study were well below the threshold values reported by Hufnagel and Hofmann (2008), and the sensory scores showed no perceptible change in bitterness.

Epicatechin has a greater maximum intensity and longer persistence of bitterness and astringency than catechin (Peleg et al., 1999). In 2008, both catechin and epicatechin levels in the wine were negatively correlated with bitterness (Table 3.3). In 2009, catechin was negatively correlated, but epicatechin was positively correlated (Table 3.4). The negative correlations are likely due to declines in wine catechin concentrations with ripeness in both 2008 and 2009 and a decline in epicatechin concentration in 2008. However, the epicatechin concentration decreased over a smaller range in 2009. Despite the statistical significance associated with the changes in flavanol monomer concentrations, at the concentrations recorded there would be at most only negligible differences in astringency and bitterness perception in the wines. The increase in bitterness may have been due to compounds other than flavanol monomers.

3.5.1.4 Dimers

Increased flavanol oligomers size decreases the intensity and persistence of bitterness (Peleg et al., 1999); however, proanthocyanidin degree of polymerization did not affect bitterness perception

(Vidal et al., 2003a). Hufnagel and Hofmann (2008) reported B2 dimer having bitter and astringent thresholds of 280 mg/L and 110 mg/L, respectively. These thresholds are similar to those of catechin. Yet, Peleg et al. (1999) showed maximum astringency was greater in dimers and trimers compared to monomers when evaluated in a 1% aqueous ethanol solution. In the current study PCAs, the B2 dimer concentrations were closely related to those of the monomers catechin and epicatechin (Figures 3.3 and 3.4). Wine B2 dimer concentrations declined with ripeness in both seasons, but the concentrations were low and the range over which they declined was minimal.

3.5.1.5 Proanthocyanidins

Proanthocyanidins contribute to wine mouthfeel and colour stability (Vidal et al., 2002, Downey et al., 2004). Hufnagel and Hofmann (2008) described polymeric tannins (proanthocyanidins) as having a 'puckering astringency' above the threshold of 22 mg/L. In the current study, wine polymeric tannin concentrations increased with grape ripeness (Section 2.4.3.1.5). In 2008, polymeric tannin concentrations had strong positive correlations with tannin quality, astringency and body, alcohol, and aftertaste length as well as colour intensity (Figure 3.3; Table 3.3). In 2009, similar patterns were observed, but the correlations with colour intensity were weaker (Figure 3.4; Table 3.4). The sensory descriptors themselves were also correlated. This is consistent with Kennedy et al. (2006a) who also showed a correlation between tannin concentration and perceived astringency.

Phenolic compound size and conformation can impact the perception of bitterness and astringency, though the balance of attributes may also be important. Ristic et al. (2002) found higher quality wines had greater amounts of anthocyanins and skin phenolics, and a lower amount of total flavanols and seed procyanidins. This may be attributed to the decrease in bitterness associated with an increase in

skin tannins versus seed tannins (Kennedy et al., 2002, Kennedy, 2007). In the current study, polymeric tannin concentrations increased while monomer and dimer concentrations decreased with grape ripeness. The astringency and tannin quality rating increased with grape ripeness with the exception of 2009 week 15, indicating that the wines became more drying and had more prominent tannins when made from riper grapes. The increase in tannins and astringency is associated with increased red wine quality (Gawel et al., 2001a). This supports the idea that extended maturation of the grape provided improved mouthfeel attributes. Differences in proanthocyanidin composition may also impact wine mouthfeel (Kassara and Kennedy, 2011). Evaluation of proanthocyanidin composition would be of interest to examine in future work.

Wine tannins change with aging (Fulcrand et al., 2006). Improved tannin quality over time may be due to a decrease in low molecular weight tannins and the associated decrease in 'harsh' tannins. Polymerization may also impact perception as large proanthocyanidins are more stable and do not react with salivary proteins, thereby decreasing the astringency (Vidal et al., 2004). In the current study, descriptive analysis of the wines was conducted only eight months after bottling. Polymerization may be minimal in these relatively young wines and the astringency might change with wine aging. In addition, few red wines are consumed this close to production.

3.5.2 Non-flavonoids

Hufnagel and Hofmann (2008) reported that caftaric acid added to water provided 'puckering astringency', as with polymeric tannins. In the current study, the caftaric acid concentrations were approximately twice the threshold (22 mg/L) (Hufnagel and Hofmann, 2008). Caftaric acid concentrations decreased with increasing grape ripeness as seen with the flavanol monomers and

dimers, therefore, caftaric acid is positively correlated to these compounds. However, caftaric acid was negatively correlated with astringency and the other mouthfeel attributes. The correlations are greater in the 2008 data compared to 2009. Caftaric acid did not appear to provide astringency as was previously reported (Hufnagel and Hofmann, 2008). Hufnagel and Hofmann (2008) assessed the individual phenolic fractions in water. The impact of caftaric acid on astringency in the current study may have been masked due to low concentrations of caftaric acid in the wine relative to the concentration of polymeric tannins or due to interaction of other compounds such as ethanol, organic acids, and polysaccharides in the wine.

3.5.3 Other chemical metrics

Despite a lack of correlation between wine colour ratings and malvidin-3-glucoside concentration, perceived wine colour intensity was closely correlated with the absorbance at 420 and 520 nm. As reviewed by Delwiche (2004), colour can influence the perception of food and beverages. Wine flavour descriptors can be influenced by wine colour. Morrot et al. (2001) found that white wine coloured red was described with red wine attributes. Colour can also impact the perception of odour intensities (Zellner and Kautz, 1990). In the current study, colour intensity scores increased with grape maturity. In 2008, colour intensity had significant, positive correlations with the mouthfeel attributes of tannin quality, astringency, body, alcohol, and aftertaste. This could be due to the visual impact of increased wine colour with later grape harvest dates. In 2009, the wine colour declined at the final harvest date and the correlations to mouthfeel attributes were not significant. The wine colour decline at week 15 may have been due to the rain at week 13 diluting phenolic compounds. This was consistent with the chemical results as shown in chapter 2. Further studies using black glasses or under red light for the sensory analysis would reduce potential biases based on wine colour; however, this was not done in the current study as colour was also a parameter of interest.

3.5.4 Correlations between sensory metrics

The wines for each year were evaluated approximately eight months after bottling. The panellists available to participate in the descriptive analysis were different for each year. Each panel came to consensus on the attribute terms, standards, and intensity scales that differentiated the wines for that year (Tables 3.1 and 3.2). Although several attribute terms were common to both 2008 and 2009, the attribute definitions and scaling used differed by year. Some of the differences between 2008 and 2009 may be due to the inclusion of fewer significant sensory attributes in 2009. However, the chemistry data for 2009 also had weaker trends than in 2008 (Chapter 2).

Phenolic compounds provide colour, astringency and bitterness to wines. The phenolic compounds measured in the current study do not explain the changes in sensory properties seen with increasing grape ripeness. The perception of bitterness and astringency may be altered also by ethanol (Fischer and Noble, 1994), the acidity of the wine (Fischer and Noble, 1994, Peleg et al., 1998), viscosity (Smith et al., 1996), polysaccharides (Vidal et al., 2003b, Carvalho et al., 2006), and anthocyanins (Vidal et al., 2003b, Llaudy et al., 2004). Further studies are needed to determine the chemical interactions between polyphenols and these compounds as well as the impact of these interactions on perception of wine mouthfeel attributes.

3.6 Conclusions

The results of this study indicate that sensory analysis can discriminate wines made from grapes harvested at weekly intervals after reaching the historical commercial maturity level. Sensory attributes describing colour and astringency may be predicted using chemical metrics in some years, but vintage effects may be stronger than changes in the chemical concentrations of these

compounds. The phenolic compounds measured in this study did not fully explain the impact of harvest date on wine mouthfeel properties. Additional chemical metrics may need to be added to explain the interaction effects and synergies in wine observed in this study.

3.7 Acknowledgements

The authors wish to thank Susana Rodriguez-Vasquez, Steven Kukesh, Garrett Cosenza, David Santino, and Mike Owens for assistance with winemaking, and the DA panellists for evaluating the wines.

4 Water addition to facilitate fermentation: Impacts on the concentration of polyphenolic compounds and sensory properties of wines

The wines assessed in Chapters 2 and 3 were produced following a must TSS adjustment to 24°Brix. This was done in order to standardize the alcohol levels in all wines in order to rule out the effects of alcohol on extraction or sensory perception. The study discussed in Chapter 4 was performed to determine the impact of materials added to dilute must TSS. Chardonnay and Zinfandel grapes harvested at high TSS were used as this required the highest dilution rates and would be expected to have the greatest impact. Cabernet Sauvignon was not used as high TSS fruit was not available in 2010. The must sugar concentrations were lowered with water or dealcoholized wine added pre-fermentation, and compared to wines made with no must sugar adjustment. The phenolic concentration of these wines was assessed and correlated to sensory data.

Chapter 4 was written in American English as the intent is to submit the manuscript embodied in this chapter to the American Journal of Enology and Viticulture. The figures and tables have been inserted into the text for the convenience of the reviewer.

The references for this chapter have been incorporated into a single consolidated reference list that may be found at the rear of the thesis.

Water addition to facilitate fermentation: Impacts on the concentration of polyphenolic compounds and sensory properties of wines

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4.1 Abstract

In many cases wine grapes accumulate high levels of sugar (>24°Brix) before reaching the optimum flavor development required for harvest. This may cause issues with the completion of primary fermentation, as well as resulting in wines that are higher in alcohol than desired. Under these conditions, winemakers in the U.S.A. may choose to decrease the final alcohol content of the wine by adding water to the must and diluting the initial must sugar concentration. Since water may dilute the concentration of wine aroma and mouthfeel compounds, another option is to use dealcoholized wine to reduce initial must sugar content. The purpose of this study was to determine the impact of these practices on wine composition and sensory characteristics. Chardonnay and Zinfandel grapes harvested at 24.9 and 26.3°Brix, respectively, were crushed and evenly divided into the following treatments prior to primary fermentation: no addition (Con), diluted with process water (WA), or diluted with dealcoholized wine (DAL). For Zinfandel, an additional treatment diluted with water following saignee (or juice run off) of equal volume (SA) was included. The dilution volumes were calculated to attain final wine alcohol concentrations of 14.0 ± 0.5% (v/v) or 15.0 ± 0.5% (v/v) for Chardonnay and Zinfandel, respectively. Wines were assessed for phenolic and aroma compound concentrations. Water addition lowered color intensity and phenolic compound concentrations, though saignee prior to water addition minimized the effect. Dealcoholized wine addition yielded fewer differences in phenolic compound concentrations from the control compared to water addition. Aroma compound concentrations were lowered by all treatments in Chardonnay, but few differences existed between the Zinfandel wines. Despite few disparities in wine chemistry, the treatments were discriminated sensorially. The results suggest that pre-fermentation adjustment with dealcoholized wine is preferable to addition of water.

Key words: dealcoholized wine, phenolics, aroma compounds, wine sensory

4.2 Introduction

A desire for wines with strong fruit intensity and an absence of negative attributes, including green or vegetative aromas, has brought about a change in wine grape harvest criteria. It is generally believed that fruit sensory attributes correlate with final wine characteristics and quality (Coombe and Iland, 2004). While fruit sugar concentration and total acidity have historically served as the primary criteria for harvest, more recently additional metrics including berry color and flavor have been used to indicate grape maturity and quality (Winter et al., 2004, LeMoigne et al., 2008).

The goal of production winemakers is to harvest the grapes when negative aromas have decreased, fruit aromas and flavors have peaked, and berry skin tannins have matured. Extended maturation is the practice of maintaining the fruit on the vine beyond traditional harvest maturity (ca 24°Brix) (Coombe and McCarthy, 1997). Extended maturation is commonly employed in California to achieve optimum berry flavor and mouthfeel development. However, extended maturation may also lead to undesirable field attributes such as berry shrivel and the associated yield losses (Mendez-Costabel et al., 2012). Furthermore, high sugar levels in the fruit may also result in fermentations that do not go completely dry due to alcohol inhibition of the yeast (Bisson, 1999).

In the United States, water may be added to the juice or must to facilitate fermentation; however, the resulting density of the juice may not be lower than 22°Brix (U.S. 27CFR Part 24 Bureau of Alcohol Tobacco Firearms and Explosives, 1993). This practice is performed to mitigate the potential for incomplete or stuck fermentations due to alcohol induced inhibition of yeast performance or viability resulting from excessive alcohol in the wine (Bisson, 1999). Excessive residual sugar levels may also

result from yeast inhibition. Water is added to the juice or must prior to the addition of yeast in order to lower the sugar concentration at the start of fermentation.

Ingredient quality is important to food and beverage quality. Most food product manufacturers have strict ingredient quality standards to ensure consistency of the product (Strategic Diagnostics Inc., 2012); however, water from municipal sources is generally considered acceptable without further monitoring (Reynolds, 2002). The brewing and fruit juice industries have water quality standards in place (AquaFit4Use, 2010), but the wine industry does not. Further work to determine the effects of water quality on wine sensory properties is therefore needed.

In the application of these techniques to red winemaking, to avoid the dilution of compounds associated with added water, winemakers may prefer to perform a pre-fermentation juice run off (or saignee) before adding the water (Gawel et al., 2001b). Saignee is generally used to increase the ratio of skins to juice to emulate small berry size. However, saignee followed by replacement of an equivalent volume of water can be employed to maintain the equivalent skin to juice ratio.

Few studies have evaluated the impact of water addition on wine chemical attributes or sensory composition. Harbertson et al. (2009) assessed the effect of water addition and saignee on Merlot anthocyanin, iron reactive phenolics, and tannin extraction as well as wine sensory. The initial TSS level (28°Brix) was decreased in all treatments, including the control (water added to adjust to 24.3°Brix). The high ethanol treatment was most similar to unaltered must, with a 4.5% volume increase due to the water addition. Two levels of saignee were evaluated (16 and 32% (v/v)). The low saignee treatment had an equivalent volume of water added back to the must, whereas the high

saignee had approximately half the volume replaced in order to achieve a TSS level consistent with the other treatments in the study. The low saignee treatment was repeated in conjunction with a 20-day extended maceration. Harbertson et al. (2009) found that wines produced from the low and high saignee treatments had greater anthocyanin concentrations than the wines from control or high ethanol treatments, which had similar concentrations. Low saignee with extended maceration treatment wine had the lowest anthocyanin concentration. Wine polymeric pigments were measured as large polymeric pigments (LPP), and small polymeric pigments (SPP). Large polymeric pigments are those that precipitate with bovine serum albumen, while small polymeric pigments do not. Total polymeric pigments (TPP) was calculated as the sum of these measurements (Harbertson et al., 2003, Harbertson et al., 2009). All polymeric pigment concentrations trended similarly for the treatments in decreasing order as follows: high saignee, high ethanol, low saignee, and control. The low saignee with extended maceration treatment had low small polymeric pigment concentration, though equivalent to the control; however, this treatment yielded the highest concentrations of large and total polymeric pigments. Sensory analysis showed that the perceived alcohol separated the high ethanol wines from the other treatments. The low saignee with extended maceration was noted for the drying and dynamic characters. The control and high and low saignee treatments had the highest ratings for fresh fruit and smoothness.

Wine alcohol levels may be altered by adjusting the juice or must, or the wine itself. Technologies for alcohol removal from wine have been reviewed by Pickering (2000). Alcohol removal may be performed on a portion of the wine and then blended back in order to minimize the percent of wine that goes through processing. Another option is to use dealcoholized wine to lower the juice or must sugar concentration. It is believed that the use of dealcoholized wine will minimize or eliminate the dilution of aroma and mouthfeel compounds that may be associated with the use of water.

High alcohol wines may also have some unfavorable sensory properties. This may be due to changes in the extraction of key compounds from the grapes into the wine. Greater ethanol concentrations increase the extraction of seed proanthocyanidins (Cheynier et al., 2006), anthocyanins, and proanthocyanins; and also decrease color stability by affecting copigmentation (Canals et al., 2005). Generally, winemakers prefer the mouthfeel associated with skin tannins rather than seed tannins and therefore choose processing techniques to enhance skin extraction and minimize compounds extracted from seeds (Kennedy, 2007). Harbertson et al. (2009) showed that wines made with water additions had similar proportions of extracted skin and seed tannins, whereas high alcohol wines had greater proportions of seed tannins. Increased seed tannin levels may make the wines too bitter and the high ethanol itself may increase the bitter perception (Fischer and Noble, 1994, Noble, 1994).

Bui et al. (1986) showed that wines dealcoholized by reverse osmosis exhibited decreased concentrations of polyphenols and anthocyanins, as well as lower color intensity. The authors assert that the wines were not organoleptically changed, though no sensory evaluation was conducted (Bui et al., 1986). Consumer acceptance and preferences for low alcohol wines have also been studied. Meillon et al. (2010) showed a negative consumer perception for Chardonnay wines with a 4.5% reduction in alcohol compared to the control, but no differences in liking for Sauvignon Blanc, Merlot, or Syrah with the same alcohol reduction. Low alcohol Syrah wines (9.6% (v/v)) had less heat, mouthfeel, balance and sweetness than the original (12.7% (v/v)) (Meillon et al., 2010).

The aim of the current study was to investigate the effects of water or dealcoholized wine added to the juice or must to facilitate fermentation and the impact on concentrations of phenolic, color, and aroma compounds and sensory properties of the resulting wines.

4.3 Materials and Methods

4.3.1 Vineyard

The commercial vineyards used in this study were located in Lodi, California. The Chardonnay (FPS Clone 4) and Zinfandel (FPS Clone 7) vines were bilateral cordon trained and trellised to the California sprawl system. Both varieties were planted in 1997 on 1103P rootstock, and grown using standard cultural practices for the variety and region. Grapes were harvested at high sugar concentrations to explore extreme cases of dilution-based sugar adjustment (Table 4.1 and Table 4.2). Chardonnay was harvested on October 5, 2010 and Zinfandel on October 13, 2010. Grapes were hand harvested from two adjacent vine rows into 13 kg tubs. The bins were randomly assigned into sets of 4 bins containing approximately 50 kg of grape clusters. The sets of 4 bins were then processed differently for the two varietals. For Zinfandel, the sets of 4 bins were randomly assigned to the treatments and replicates described below, whereas the Chardonnay replication arose only for winemaking treatments.

4.3.2 Data collection

At harvest, randomly selected 20-cluster samples were taken from each variety and analyzed for Total Soluble Solids (TSS, °Brix), pH, and malic acid using Fourier Transform Infrared Spectroscopy (FTIR) (WineScan FT-120, FOSS North America, Eden Prairie, MN). Grape polyphenolics were extracted using methods described by Iland et al. (2000) and measured by reversed-phase high

performance liquid chromatography (HPLC). All samples were held overnight at 2°C for processing the following morning.

4.3.3 Winemaking

4.3.3.1 Chardonnay

Chardonnay grapes were harvested on October 5, 2010 at a TSS of 24.9°Brix (Table 4.3). Grape clusters were detemmed/crushed using a Magitec model A15 (Paarl, South Africa) crusher-destemmer into a temporary holding vessel. Sulfur dioxide (40 ppm) was added immediately after crushing and the must was pressed using a Diemme AR1.3 (Emilia-Romagna, Italy) membrane press to a maximum pressure of 1.8 bar. The pressed juice was cold settled in a single large tank over night at 4°C, and then racked off into a second tank to remove the gross lees. Tartaric acid was added to bring the titratable acidity (TA) to 6 g/L. The juice was homogenized and distributed to individual forty-liter tanks for fermentation. The juice lots were then left with high sugar (Con) or were diluted with either process water (WA) or dealcoholized Chardonnay wine (DAL) based on treatment in order to attain final wine alcohol concentrations of 14.0 ± 0.5% (v/v) (Table 4.7). The dealcoholized wine was a commercially produced Chardonnay wine that had the alcohol concentration reduced by reverse osmosis (Table 4.3). The juice was inoculated with rehydrated N96 yeast (Anchor Yeast, Industria, South Africa) at a rate of 0.12 g/L.

Forty-liter fermentations were conducted in jacketed vessels at 18.3 ± 2°C. Fermentations were monitored daily for temperature, Brix, and flavor. After the wine reached 0°Brix, sampling for reducing sugar (RS) and temperature continued daily until the wine was dry (<2 g/L RS).

Table 4.1 Compositional parameters for Chardonnay juice before and after additions.

		Treatments - after additions		
	Initial juice*	**Control**	**WA**	**DAL**
Volume added (%)	-	0.0 c	18.7 b	24.0 a
Soluble solids (°Brix)	24.9	25.6 a	22.1 b	21.5 c
RS (g/L)	267.0	272.0 a	233.7 b	223.0 c
pH	3.55	3.45 a	3.45 a	3.41 b
TA (g/L)	5.30	5.83 a	4.93 b	5.93 a
YAN (mg/L)	311.0	313.3 a	256.3 b	262.0 b

Numbers within the same row followed by different letters are different at $p \leq 0.05$ using LSD (n=3).
*The initial juice values are for the homogenized juice. No replicates existed at this processing point. WA, water added to must treatment; DAL, dealcoholized wine added to must treatment.

Table 4.2 Compositional parameters for Zinfandel must before and after additions.

	Control	WA	SA	DAL
Must before additions				
Soluble solids (°Brix)	26.5a	26.7a	25.9b	26.0b
RS (g/L)	286.3ab	289.0a	280.0b	279.7b
pH	3.93a	3.93a	3.92a	3.94a
TA (g/L)	4.77a	4.63a	4.70a	4.83a
YAN (mg/L)	311.3a	295.7a	284.7a	318.0a
Must after additions				
Volume removed (%)	0.0b	0.0b	13.2a	0.0b
Volume added (%)	0.0d	17.4b	13.2c	19.9a
Volume change (%)	0.0c	17.4b	0.0c	19.9a
Soluble solids (°Brix)	28.3a	23.8b	23.6bc	23.1c
RS (g/L)	298.7a	247.7c	269.3b	227.7d
pH	3.55a	3.48ab	3.52a	3.43b
TA (g/L)	6.80ab	6.47b	6.50b	7.27a
YAN (mg/L)	367.3a	285.3b	307.7b	277.3b
Alcohol (% v/v)	0.23b	0.37b	0.30b	1.73a

Numbers within the same row followed by different letters are different at $p \leq 0.05$ using LSD (n=3). WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment.

Table 4.3 Compositional parameters for Chardonnay and Zinfandel dealcoholized wine.

	Dealcoholized wine	
	Chardonnay	Zinfandel
Alcohol (% v/v)	5.11	6.47
pH	3.35	3.58
RS (g/L)	4.3	0.9
TA (g/L)	6	6.2
VA (g/L)	0.3	0.66
Malvidin-3-glucoside (mg/L)	0	6
Quercetin glycoside (mg/L)	2	7
Gallic acid (mg/L)	3	46
Caftaric acid (mg/L)	9	17
Catechin (mg/L)	3	14
Epicatechin (mg/L)	3	11
Polymeric tannins (mg/L)	22	420

Dry wines were cold settled for 2 days at 2°C and then racked into 40-liter pressure-rated cans and topped with nitrogen gas. Wines were stored at 2°C under nitrogen until filtration. Rough filtration was through 1.0 µm nominal diatomaceous earth (DE) pads on a Filtrox plate and frame unit. The free sulfur dioxide was adjusted to 30 ppm and copper sulfate was used to remove sulfides prior to bottling. Wines were sterile filtered through Meissner 0.8 and 0.45 µm cartridge filters en route to GAI model 1006 filler and capper modified for small lots. The filler bowl and bottles were purged with nitrogen gas. Wines were bottled into 750 ml bottles and sealed with screw caps. Packaged wines were stored at 13°C.

4.3.3.2 Zinfandel

Zinfandel grapes were harvested on October 13, 2010 at a TSS of 26.5°Brix (Table 4.2). The grapes were held overnight at 2°C and processed the following morning. Each 50 kg lot of Zinfandel grape clusters was destemmed/crushed using a Magitec model A15 (Paarl, South Africa) destemmer/crusher directly into an eighty-liter fermentation tank. Sixty ppm sulfur dioxide was added to each lot immediately after crushing. Zinfandel must lots were held at ambient temperature and sampled every 4 hours for the first 48 hours after crushing to determine the time required to get a representative sugar sample. After 31 hours, the must was adjusted to create the treatments. The treatments were as follows: unadjusted must with high sugar (Con); diluted with process water (WA); diluted with process water following saignee (or juice run off) of equal volume (SA); or diluted with dealcoholized wine (DAL). The dealcoholized wine was a commercially produced Zinfandel wine that had the alcohol concentration reduced by reverse osmosis (Table 4.3). The must dilution volumes were calculated to attain final wine alcohol concentrations of 15.0 ± 0.5% (v/v) (Table 4.8). The water and dealcoholized wines were added to the must for those treatments. For the saignee treatment, a portion of juice was drawn off from the must prior to the water addition. The volume drawn off was

equal to the volume added. Tartaric acid was added to each 50 kg treatment replicate to lower the pH to 3.6. The must was inoculated with rehydrated N96 yeast (Anchor Yeast, Industria, South Africa) at a rate of 0.18 g/L.

Sixty-liter fermentations were conducted indoors at ambient conditions (approximately 21°C). Cap management was via submerged cap with one punchdown cycle per 24-hour period. Fermentations were monitored daily immediately following the punchdown for temperature, °Brix, and flavor. The wines were pressed at 0°Brix using a Diemme AR 1.3 (Emilia-Romagna, Italy) membrane press to a maximum pressure of 1.8 bar. Free run and press fractions were reconsolidated into a 40-liter jacketed vessel maintained at ambient temperature. Daily sampling for RS and temperature continued until the wine was dry (<2 g/L RS). Dry wines were cold settled, filtered, and packaged in the manner previously described for Chardonnay wines.

4.3.4 Wine analysis

4.3.4.1 Wine chemistry

Wines were analyzed approximately eight months after bottling and within one month of the completion of the wine sensory evaluation. Wine analysis included pH, titratable acidity (TA), alcohol, reducing sugar (RS), lactic acid, malic acid, and volatile acidity (VA) by FTIR (WineScan FT-120, FOSS North America, Eden Prairie, MN). Wine color was determined as absorbance at 420 nm and 520 nm by spectrophotometer (Lambda 35, Perkin Elmer, Waltham, MA. 1 mm path length), and tristimulus colorimetry as Hunter color (L, a, and b) (Ultrascan Pro Spectrophotometer, Hunter Labs).

4.3.4.2 Polyphenol analysis by HPLC

Standards. Linearity studies were generated for gallic acid, catechin, caffeic acid, quercetrin, quercetin, and malvidin-3, 5-diglucoside chloride at concentrations of 1, 5, 10, 25, 50, and 100 mg/L (ppm). Gallic acid monohydrate, (+)-catechin, (-)-epicatechin, caffeic acid, quercetin, and quercetrin were from Sigma Chemical (St. Louis, MO). Malvidin-3-O-glucoside chloride was from Indofine Chemical Company, Inc. (Hillsborough, NJ).

Wines were filtered prior to analysis by reversed phase HPLC coupled to a diode array detector using the method described by Waterhouse et al. (1999). The column was an Agilent Zorbax Eclipse XDB-C18 Rapid Resolution HT 4.6 X 50 mm, 1.8 µm protected by an Agilent Zorbax Eclipse XDB-C18 analytical guard column 4.6 x 12.5 mm, 5 µm. The mobile phase was a gradient of 0.3% (v/v) phosphoric acid solution (mobile phase A) and 0.2% (v/v) phosphoric acid in acetonitrile (mobile phase B) at a flow rate of 1.0 ml/min. The elution conditions were as follows: 5% mobile phase B at time 0; 0-10 min 5-19% mobile phase B; 10.25-12.5 min hold at constant 33% mobile phase B; 12.5-13.5 min 33-95% mobile phase B; and 13.5-14.5 min 95-5% mobile phase B.

Eluting peaks were monitored at 230, 280, 320, 360, and 520 nm. Compounds eluting from the HPLC were identified and quantification based on a comparison to authentic standards (except caftaric acid, quercetin glycosides, and polymeric tannins). Chromatograms were integrated using Agilent ChemStation software. The compounds were monitored at the following wavelengths: polymeric tannins – 230 nm; catechin, epicatechin, and B2 dimers – 280 nm; caftaric acid – 320 nm; quercetin glycosides – 360 nm; and malvidin-3-glucoside – 520 nm. Compounds were quantified using the relative response to calibration compounds as follows: polymeric tannins, catechin and B2 dimer to

catechin; epicatechin to epicatechin; caftaric acid to caffeic acid; quercetin glycoside to quercetin; and malvidin-3-glucoside to malvidin glucoside.

Peaks were identified based on comparison with authenticated standards. A library search was used to confirm peak identity. Since several compounds co-eluted with other compounds, manual integrations were needed. For example, quercetin glycoside elutes as the middle peak of a triplet of peaks and anthocyanins co-elute with the polymeric tannins at 280 nm. However, the polymeric tannins did not have absorption at 530 nm. The anthocyanin absorbance at 530 nm was about the same as their absorbance at 230 nm. The polymeric tannins were detected at 230 nm with the reference set at 530 nm in order to eliminate the interference from co-eluting anthocyanins.

4.3.4.3 Aroma analysis by GC-MS

The concentrations of 25 volatile wine aroma compounds were determined by headspace solid phase micro-extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) using the method described by Siebert (2005). The profile included fatty acids, alcohols, acetates, and ethyl esters.

A Hewlett Packard 5975 mass selective (MS) detector with HP 6890 chromatograph and CTC Combi-Pal auto-sampler was used for analysis. The GC column was a DB-5MS+DG 30 m X 0.250 mm I.D., 0.5 µm film column from J&W Scientific (Santa Clara, CA). The SPME fiber assembly was Divinylbenzene/Carboxen on polydimethylsiloxane coating from Supelco (Part no. #57298-U Sigma-Aldrich, St. Louis, MO). The starting oven temperature was 40°C for 5 minutes, then increased to 170°C at a rate of 3°C min, then increased to 275°C at 30°C/min.

Calibration was performed using ethyl isobutyrate, ethyl butanoate, ethyl-2-methyl butanoate, ethyl isovalerate, 2-methyl butyl acetate, 1-octen-3ol, ethyl hexanoate, 2,6-nonandienal, ethyl octanoate, nerol, geraniol, ethyl dihyrocinnamate, ethyl cinnamates, and β-ionone from Aldrich (St. Louis, MO); linalool, 2-phenylethyl acetate, α-ionone, cis-rose ocide 1 and cis-rose oxide 2 from Fluka (St. Louis, MO); isoamyl acetate from Mallickrodt (St. Louis, MO); β-damascenone from Advanced Biotech; and 2-phenylethanol from Sigma-Aldrich (St. Louis, MO) (Table 4.4).

4.3.5 Wine sensory

4.3.5.1 Descriptive analysis

Descriptive analysis (DA) was performed separately for each variety. The panel comprised 11 University of Adelaide students and staff from the Viticulture and Oenology program and non-university personnel (6 females and 5 males). Panelists were 22-63 years old. The Chardonnay and Zinfandel wines were evaluated from April through May 2011 and May through August 2011, respectively.

Some panelists had previous DA experience; however, all were trained for the present task in 2-hour sessions held over 6 weeks per variety. During these sessions, panelists were tasked with describing the wines and asked to come to consensus on the list of attributes and definitions to distinguish the wines. In order to determine attributes that defined the wines, the panelists saw each of the wines on at least one occasion during this process. Mouthfeel standards, represented by different fabric swatches, were available to the panelists during each session. Aroma reference standards and color swatches were available to panelists during each session and were modified based on their feedback

Table 4.4 Wine aroma by GC-MS. Calibration validation results.

Wine aroma method	Elution time (min)	Concentration in standard solution (ppb)	Recovery of injected standards (%)	Standard Deviation of % Recovery
ethyl isobutyrate	9.709	100	99.8	0.22
ethyl butanoate	12.042	100	101.3	0.24
ethyl-2-methyl butanoate	14.902	100	105	1.31
ethyl isovalerate	15.182	100	106	0.91
1-hexanol	16.110	2,000	103.5	0.42
isoamyl acetate	16.516	600	113.2	0.17
2-methylbutyl acetate	16.621	100	111.7	2.61
1-octen-3-ol	22.554	20	103.7	13.21
ethyl hexanoate	23.613	300	109.7	0.4
linalool	29.280	100	71.9	5.4
2-phenyl ethanol	30.040	10,000	101.5	3.14
ethyl octanoate	34.235	300	111.2	0.34
2-phenylethyl acetate	37.065	100	83.9	1.06
ethyl dihydrocinnamate	41.500	20	76.9	11.66
β-damascenone	43.009	20	92.3	0.38
ethyl decanoate	43.613	20	125.6	0.39
β-ionone	47.350	20	88.5	4.04

n=3

to produce an appropriate set of final standards for the formal evaluation sessions. The reference standards were made using a commercial un-oaked Chardonnay and a commercial Shiraz as the base wine for the Chardonnay and Zinfandel aroma reference samples, respectively. Shiraz wine was used for the Zinfandel standards due to unavailability of un-oaked Zinfandel wine.

The wines were presented in clear, INAO (ISO standard), 215 ml tasting glasses covered with a petri dish. Each sample (30 ml) was identified by a 3-digit random code. The panel descriptive terms agreed upon included two color, eight aroma, six flavor, two taste, four mouth feel, and two aftertaste attributes for Chardonnay wines; and two color, seven aroma, seven flavor, two taste, three mouth feel, and two aftertaste attributes for Zinfandel wines. The selected attributes, their definitions, and order of panel assessment for Chardonnay and Zinfandel wines are presented in Tables 4.5 and 4.6, respectively. Panelists practiced scoring the wines on the intensity of each attribute using an unstructured 15 cm line scale. The scale was anchored using indented end points at 10% and 90% of the scale. Raspberry cordial diluted in water 1:70 and 1:10 was used as the low and high intensity standards, respectively. Prior to formal evaluation of the wines, the panel was familiarized with the sensory booths and computer interface. The panel's performance was evaluated by having each panelist assess a subsample of the wines in duplicate. The data were analyzed for panelist by sample interactions using PanelCheck (Nofima Mat and DTU – Informatics and Mathematical Modelling, Norway) and SENPAQ software (version 4.3 Qi Statistics, UK). Final assessment of the samples commenced when no significant interactions were found.

Table 4.5 Descriptive analysis panel attribute terms, definitions, and reference standards for Chardonnay wines.

Attribute	Description
Color	
Hue	watery/grey to yellow
Rim	yellow to green/grey
Aroma	anchored by no intensity to very intense
Tree fruit	apple, pear (1 cm^3 piece of each - Gala apple, Granny Smith apple, pear, all frozen and mashed)
Tropical fruit	pineapple, melon (2mm slice banana, 1 cm^3 piece of pineapple and rock melon, all frozen and mashed)
Stone fruit	peach, nectarine (1 cm^3 white peach, 1 cm^3 yellow peach, 2 X 1 cm^3 white nectarine, all frozen and mashed)
Citrus fruit	lemon, lime, grapefruit (2 pieces 2mm X 1cm lemon peel, frozen and thawed)
Confected fruit	banana lolly, estery (½ banana lolly, cut up (Nestle Australia Ltd. Rhodes, NSW))
Green	grassy (1/2 Tbsp fresh grass, chopped; 4cm of grape bunch rachis, 1cm lengths, cut longitudinally in half; 2 tomato leaf 'stars', broken)
Alcohol	alcohol perceived in aroma
Reduced	no or yes, scored as 0 and 15, respectively
Taste	
Acid	low to high acidity
Bitter	low to high bitter
Flavor	anchored by no intensity to very intense as for aroma
Tree fruit	apple, pear
Tropical fruit	pineapple, melon
Stone fruit	peach, nectarine
Citrus fruit	lemon, lime, grapefruit
Confected fruit	banana lolly, estery
Green	grassy
Mouthfeel	
Alcohol	low to high warmth on the palate
Body	light to full bodied
Aftertaste	
Length - Fruit	length of time fruit flavor/taste remains after expectoration. Anchored by 0, 10 and 20 seconds
Length -Other	length of time everything other than fruit flavor remains after expectoration, anchored by 0, 10 and 20 seconds

All standards were presented in 40ml of a 2L cask of 2010 Yalumba unwooded Chardonnay wine unless otherwise indicated.
*Low and high intensity were anchored by 1:10 and 1:70 raspberry cordial in water, respectively. (Raspberry flavored cordial, Woolworth's Home Brand, Bella Vista, NSW)

Table 4.6 Descriptive analysis panel attribute terms, definitions, and reference standards for Zinfandel wines.

Attribute	Description
Color	
Hue	pink to deep purple (3 color swatches provided)
Depth	transparent to opaque
Aroma	anchored by no intensity to very intense
Red fruit	raspberry, plum, red fruits (2 frozen raspberry, ½ frozen strawberry, 1 small piece red plum, 2 frozen red currants, all frozen and mashed)
Black fruit	blueberry, black currant, dark cherry (2 frozen blackberries, 1 frozen black cherry, 2 frozen black currants, all frozen and mashed, plus 3 slices black olives (Woolworth's HomeBrand, Bella Vista, NSW)
Jammy fruit	jammy dark fruit (1½ tsp. strawberry jam)
Confectionery	strawberry lolly, confectionery (4 mm strawberries and cream candy, chopped (Nestle Australia Ltd. Rhodes, NSW))
Green	green bean, green capsicum, stalky, leafy, menthol, medicinal, eucalypt (1 Tbsp fresh grass, chopped; 6 X 1 cm lengths grape bunch rachis, cut longitudinally in half, 1cm piece of bean and asparagus)
Spice	spice, pepper, licorice/anise seed (¼ tsp. mixed spice (McKenzies Pty Ltd., Altona, Vic) + 1 grind black peppercorn + crushed star anise)
Alcohol	alcohol perceived in aroma
Reduced	no or yes, scored as 0 and 15, respectively
Taste	
Acid	low to high acidity
Bitter	low to high bitter
Flavor	anchored by no intensity to very intense
Red fruit	raspberry, red fruits, strawberry
Black fruit	dark fruit, ripe/dark cherry, dark plum, blackberry, black olive
Jammy fruit	dark fruit jam
Confectionery	confectionery
Green	green bean, green capsicum, stalky, leafy, menthol, medicinal, eucalypt (1 Tbsp fresh grass, chopped; 6 X 1 cm lengths racchis, cut longitudinally in half, 1cm piece of bean and asparagus)
Spice	spice, pepper, licorice/anise seed (¼ tsp. mixed spice (McKenzies Pty Ltd., Altona, Vic) + 1 grind black peppercorn + crushed star anise)
Mocha/chocolate	mocha or chocolate (1/2 tsp mocha powder)
Mouthfeel	
Alcohol	from no to very hot on the palate
Tannin quality	silky/velvety to suede, coarser (touch standards provided)
Astringency	From not drying to very drying
Aftertaste	
Fruit	length of time flavor/taste remains after expectoration. Anchored by 0, 10 and 20 seconds
Non fruit	length of time everything other than fruit flavor remains after expectoration, anchored by 0, 10 and 20 seconds

All standards were presented in 40ml of a 2L cask of 2010 Yalumba Shiraz wine unless otherwise indicated.

*Low and high intensity were anchored by 1:10 and 1:70 raspberry cordial in water, respectively. (Raspberry flavored cordial, Woolworth's Home Brand, Bella Vista, NSW)

Prior to the formal of assessment of the wines, the panelists were informed of the formal sample evaluation process. The formal evaluation was conducted under fluorescent light in individual booths within a temperature controlled sensory lab. During each formal evaluation session, each panelist was presented with 20-22 wines. Wines were presented as 30 ml samples in clear INAO (ISO standard) 215 ml tasting glasses covered with a petri dish. Each sample was identified by a 3-digit random number code.

Wines were presented to the panelists in a randomized order, balanced for carry over effects (McFie and Bratchell, 1989). Distilled water and unsalted crackers were provided to each panelist as palate cleansers. Panelists were forced to rest for one minute between each sample and to take a 5-minute break after each bracket of 5 wines. During this 5-minute period the panelists were asked to re-familiarize themselves with the reference samples. The reference and intensity standards were available to the panelists outside the booths throughout each formal assessment period. The wines were assessed in duplicate over the course of the formal evaluations.

For the Chardonnay DA, the control was presented in two manners: as bottled (Con); and following dilution with dealcoholized Chardonnay wine to 14 ± 0.5% (v/v) alcohol post-bottling (PB). The dealcoholized Chardonnay was the same wine as added for the DAL treatment. This approach was utilized in an effort to minimize the influence of alcohol concentration on the sensory perception of the wine (Fischer and Noble, 1994). Dealcoholized wine was used to lower the alcohol concentration rather than water as post-fermentation addition of dealcoholized wine was consistent with commercial winemaking practices (ConeTech, 2012). This approach was not used for the Zinfandel DA due to off-

notes in the dealcoholized Zinfandel wine during panel training sessions. The panelists therefore only assessed the Zinfandel control wines as bottled during the formal evaluation sessions.

4.3.6 Statistics

Analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post-hoc test was used for means separation ($p<0.05$ for chemical metrics; $p<0.1$ for sensory scores). Principal component analysis (PCA) and Multiple factor analysis (MFA) were performed using XLStat version 2011.2.02 (Addinsoft SARL, France).

4.4 Results

4.4.1 Chardonnay

4.4.1.1 Must chemistry

The Chardonnay grapes were harvested at 24.9°Brix. The TSS measurements after pressing were slightly higher (25.6°Brix) (Table 4.1). The experimental treatments required that the juice was diluted with water or dealcoholized wine to determine the impact of these additions, and therefore the sugar measurements of the treatments as °Brix and RS were lower than the control (Table 4.1). The dealcoholized Chardonnay wine used for the dilutions contained 5.11% (v/v) alcohol and 4.3 g/L RS (Table 4.3), so the quantity of dealcoholized wine added to the juice was greater than that of the water. The WA treatments had lower TA and Yeast Assimilable Nitrogen (YAN) compared to the control. The DAL treatments were lower in pH and YAN compared to the control.

4.4.1.2 Wine chemistry

4.4.1.2.1 Basic chemistry

The target wine alcohol concentrations were attained (14 ± 0.5% (v/v)) (Table 4.7). The Con wine alcohol level was significantly greater than for the treatments, as intended. The WA treatment had lower pH, malic acid, and VA than the control. The DAL treatment had lower RS, pH, and VA than the control. The PB wine was not significantly different from the control, with the exception of alcohol concentration, which was comparable to the other treatments.

4.4.1.2.2 Wine color

The color of the treatments was not significantly different from the control; however, the Hunter values (L,a,b) were different between the WA and both the DAL and the PB treatments (Table 4.7). The Hunter color values for the dealcoholized wine were a=-1.09; b=6.02; L=95.2 compared to a=-1.14; b=5.73; L=95.4 for the base wine. The change in Hunter values indicated that the dealcoholized wine is more red than green; more yellow than blue; and more opaque than transparent.

4.4.1.2.3 Wine phenolics

Concentrations of phenolic compounds were low in all wines (Table 4.7). Catechin, epicatechin, and gallic acid concentrations were significantly lower in the WA wines compared to the controls. Catechin and gallic acid were also lower in the DAL wine compared to the control. The catechin and gallic acid levels for the DAL wine were intermediate to the Con and WA wines. Catechin, epicatechin, and gallic acid concentrations for the PB treatment were significantly greater than the control.

Table 4.7 Compositional parameters for Chardonnay wines.

	Control	WA	DAL	PB
Dilution (% v/v)	0.0c	18.7b	24.0a	19.5b
Alcohol (% v/v)	15.8a	13.8b	14.0b	13.6b
RS (g/L)	1.67ab	1.40ab	0.87b	2.03a
pH	3.34a	3.15c	3.27b	3.33a
TA (g/L)	6.20ab	6.43a	6.33ab	6.13b
Lactic acid (mg/L)	150.0a	150.0a	147.7a	170.0a
Malic acid (mg/L)	1755.0a	1524.7b	1771.7a	1720.0a
VA (g/L)	0.25a	0.19b	0.15b	0.29a
Abs420 (AU)	0.062ab	0.051b	0.067a	0.071a
Abs520 (AU)	0.013ab	0.011b	0.015a	0.016a
Hue	4.770a	4.787a	4.640a	4.553a
Intensity	0.074ab	0.062b	0.082a	0.087a
Hunter a	-0.577ab	-0.493a	-0.600b	-0.617b
Hunter b	4.233ab	3.597b	4.617a	4.800a
Hunter L	99.067ab	99.233a	98.933b	98.867b
Quercetin glycoside (mg/L)	0.31b	0.41ab	0.34ab	0.49a
Catechin (mg/L)	7.41a	5.69c	6.61b	6.69b
Epicatechin (mg/L)	2.10a	1.80b	1.85ab	1.82b
B2 dimer (mg/L)	0.58a	0.56a	0.56a	0.58a
Polymeric tannins (mg/L)	2.04a	1.93a	2.48a	1.83a
Caftaric acid (mg/L)	15.55ab	16.00ab	17.64a	14.01b
Caffeic acid (mg/L)	1.81a	1.58a	1.88a	2.05a
Gallic acid (mg/L)	1.19a	0.72c	0.90b	0.83bc

Numbers within the same row followed by different letters are different at $p \leq 0.05$ using LSD (n=3). WA, water added to must treatment; DAL, dealcoholized wine added to must treatment; PB, control wine with post-bottling addition of dealcoholized wine treatment.

4.4.1.2.4 Wine aroma compounds

Concentrations of aroma compounds varied by treatment (Table 4.8). The WA wines were significantly lower in 1-hexanol, 1-octen-3-ol, ethyl butanoate, ethyl dihydrocinnamate, and isoamyl acetate concentrations, and had a higher concentration of ethyl isobutyrate, compared to the control. Concentration of 1-octen-3-ol was significantly lower in the DAL wines than the control and this was the only significant difference in aroma compound concentrations between these treatments. PB aroma concentrations were similar to the DAL treatments as is expected given the same dealcoholized Chardonnay wine was used in both treatments. However, the PB wines had lower concentrations of ethyl butanoate and ethyl octanoate than DAL wines.

4.4.1.3 Wine sensory

Wine sensory ratings were assessed by ANOVA. Of the 22 sensory attributes assessed by the DA panel (Table 4.5), 12 were significantly different ($p<0.1$) between the treatments. Principal component analysis (PCA) was performed on these 12 attributes only (Figure 4.1). The PCA for Chardonnay accounted for 61.38% of the variation on the first two principal components. PC1 (33.33%) separated the wines by color and stone fruit aroma and flavor as opposed to citrus flavor, while PC2 (28.05%) separated the wines mainly by alcohol sensation and aroma. The Con wines were separated from the treatments along PC2, based on alcohol perception, length of perception after expectoration, tropical fruit flavor, body, and green aromas. The WA wines were negatively loaded on PC1 and were perceived as more citrus flavored. The DAL wines were located close to the center of the PCA, indicating that they were more moderate in all attributes. The PB treatments were positively loaded on PC1, and were closely related to higher color ratings and stone fruit aroma and flavor.

Table 4.8 Aroma compounds of Chardonnay wines subject to three pre-fermentation and one post-fermentation treatments.

		(µg/L)			
Compound	**Perception Threshold (µg/L)**	**Control**	**WA**	**DAL**	**PB**
1-hexanol	8,000[a]	778.00a	644.00b	803.00a	704.67ab
1-octen-3-ol	1[c]	8.70a	7.13b	0.50c	0.50c
2-methylbutyl acetate	30[b]	302.33a	263.00a	368.33a	312.00a
2-phenyl ethanol	14,000[b]	42416.00a	32509.00a	37819.00a	37595.33a
2-phenylethyl acetate	250[a]	1015.00ab	775.67b	994.67ab	1175.67a
β-damascenone	0.05[a]	10.07a	7.97a	9.13a	8.67a
β-ionone	0.09[b]	0.23a	0.13a	0.13a	0.17a
ethyl butanoate	20[a]	976.00a	810.67b	975.33a	841.00b
ethyl decanoate	200[b]	422.33a	414.00a	425.67a	380.67a
ethyl dihydrocinnamate	1.6[b]	0.87a	0.32b	0.57ab	0.13b
ethyl hexanoate	14[b]	1939.10ab	2248.27a	2073.33ab	1827.80b
ethyl isobutyrate	15[b]	60.00b	77.67a	51.67b	56.00b
ethyl isovalerate	3[b]	25.60a	29.10a	25.37a	22.27a
ethyl octanoate	5[b]	2685.00a	2672.03a	2605.07a	2146.07b
ethyl-2-methyl butanoate	18[b]	9.00a	11.00a	9.67a	7.67a
isoamyl acetate	30[a]	7394.67a	5219.67b	6964.33ab	6077.67ab
linalool	25[b]	13.23b	15.53ab	15.40ab	17.97a

Numbers within the same row followed by different letters are different at $p \leq 0.05$ using LSD (n=3). WA, water added to must treatment; DAL, dealcoholized wine added to must treatment; PB, control wine with post-bottling addition of dealcoholized wine treatment. Perception thresholds are as listed in 10% ethanol ([a]) (Guth, 1997), synthetic wine at 11% alcohol ([b]) (Ferreira et al., 2000), or in water ([c])(Buttery et al., 1988).

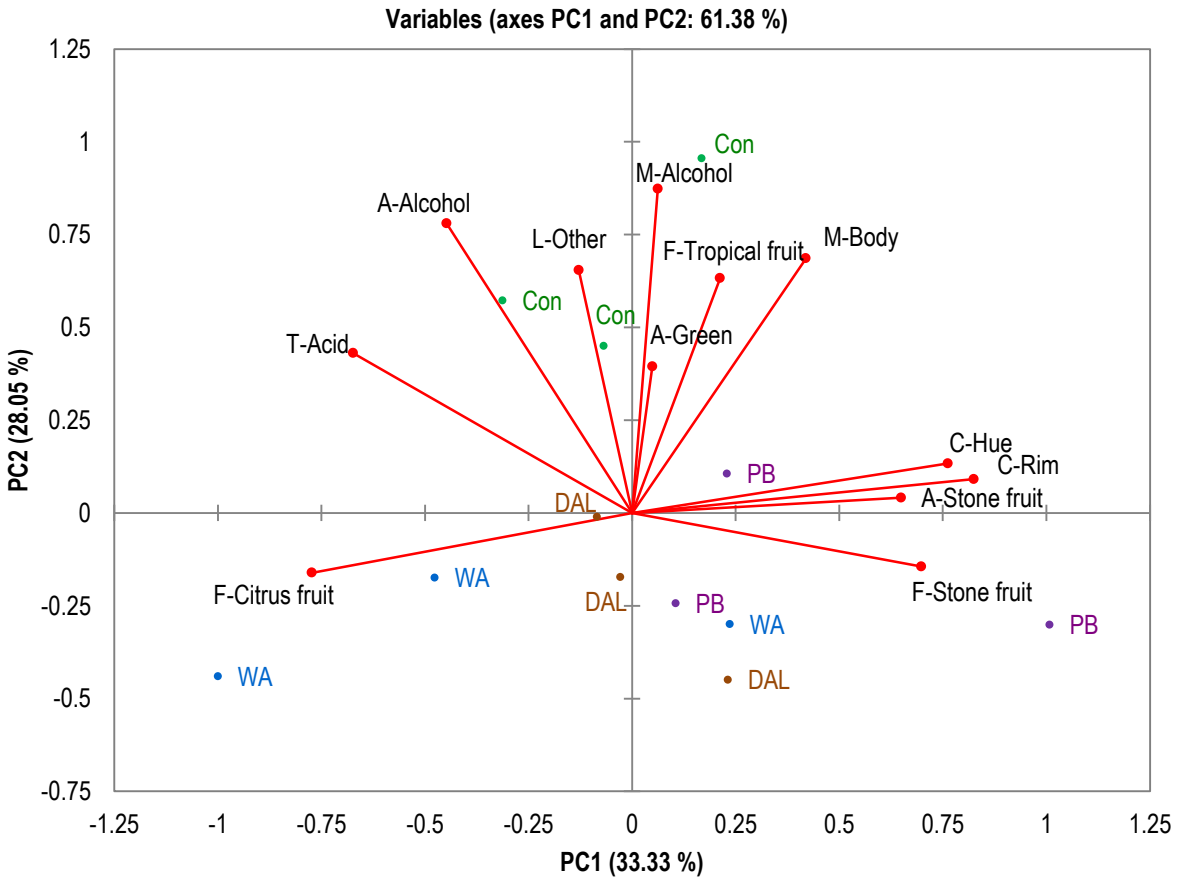

Figure 4.1 Principle component analysis of significant Chardonnay wine sensory attributes (p<0.1). Con, control; WA, water added to must treatment; DAL, dealcoholized wine added to must treatment; PB, control wine with post-bottling addition of dealcoholized wine treatment. A-aroma attribute; T-taste attribute; F-flavor attribute; C-color attribute; and L-length of perception after expectoration.

Multiple factor analysis (MFA) was performed using all significant attributes. The four tables included in the MFA were: chemistry and color, phenolic compounds, aroma compounds, and sensory attributes (Figure 4.2). The MFA for Chardonnay explained 57.45% of the variation in the first two factors. Factor 1 (32.80%) separated the treatments based on gallic acid and catechin. Factor 2 (24.65%) separated the wines based on acid and 1-octen-3-ol opposed by absorbance at 420 and 520 nm. Factor 3 explained an additional 12.61% of the variation (data not shown). Factor 3 separated the wines based on caftaric acid, ethyl hexanoate, and ethyl octanoate. The Con wines were positively loaded on both factor 1 and 2. The Con wines were closely associated with all alcohol metrics, chemical and sensory. The WA wines were loaded negatively for factor 1 and positively for factor 2. The WA wines were defined by citrus, ethyl isobutyrate, and ethyl hexanoate, and had low color. The DAL treatments were central to the MFA on factors 1 and 2, but were separated by factor 3, which is associated with caftaric acid. The PB wines were separated on factor 2. These wines had greater color by absorbance at 420 and 520 nm and color as perceived by the DA panel and were also low in acid.

4.4.2 Zinfandel

4.4.2.1 Must chemistry

Zinfandel grapes were crushed in 50 kg lots. The mean must sugar concentrations for the Con and WA treatments were greater than those of the SA and DAL treatments (Table 4.2). The sugar content of the Zinfandel must was monitored at four-hour intervals from the time of crushing to 48 hours post-crushing. The sugar concentration increased for the first 24 hours (Figure 4.3 upper graph). The treatments were initiated 31 hours after crushing. Following the addition, sugar concentrations again increased for eight hours (Figure 4.3 lower graph).

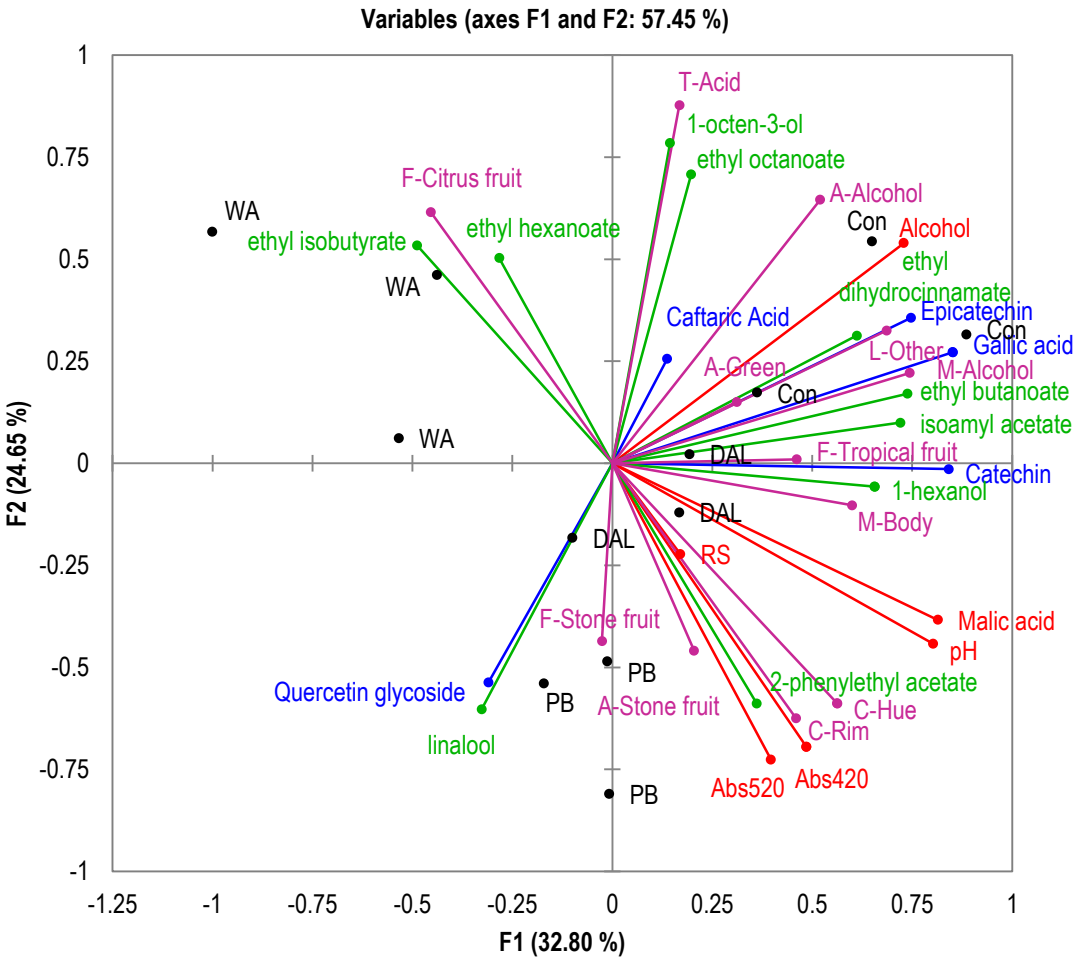

Figure 4.2 Multiple factor analysis of significant Chardonnay wine sensory attributes ($p<0.1$) and chemistry ($p<0.05$). Con, control; WA, water added to must treatment; DAL, dealcoholized wine added to must treatment; PB, control wine with post-bottling addition of dealcoholized wine treatment. A-aroma attribute; T-taste attribute; F-flavor attribute; C-color attribute; and L-length of perception after expectoration.

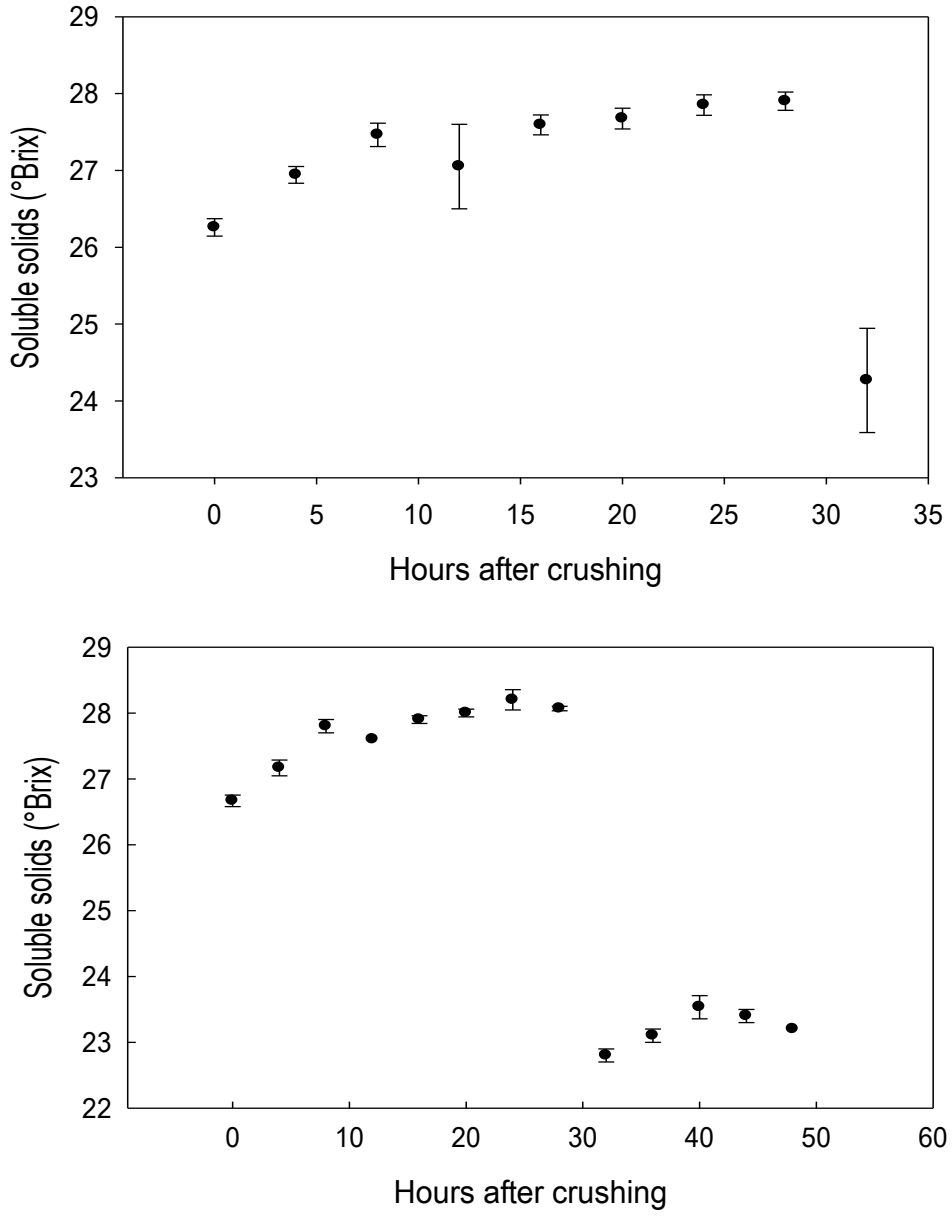

Figure 4.3 Soluble solids for Zinfandel must after crushing. Must treatments were created at 31 hours and inoculation of the must at 32 hours after crushing. Upper graph: All replicates through the creation of treatments (n=12). Lower graph: Water added treatment (WA) (n=3).

To establish the treatments, the must was diluted with water (WA) or dealcoholized (DAL) Zinfandel wine. As intended, the sugar measurements as °Brix and reducing sugar (RS) were lower for all treatments than the control (Table 4.2). The dealcoholized Zinfandel wine used for the dilutions contained 6.4% (v/v) alcohol (Table 4.3) so the must alcohol concentrations were greater in the DAL treatment (Table 4.2). The DAL treatment also had greater TA and lower pH compared to the control. The DAL, WA and SA treatments all had lower YAN than the control.

4.4.2.2 Wine chemistry

4.4.2.2.1 Wine basic chemistry metrics

The target wine alcohol concentrations were attained for all treatments (15 ± 0.5% (v/v)) (Table 4.9). The Con wine alcohol was greater than for the treatments, as intended. The WA and SA treatments were similar. Both treatments had significantly lower TA and VA than the control. The DAL treatment had significantly greater lactic acid than the Con or WA treatments. This is due to the dealcoholized Zinfandel wine having been through malolactic fermentation prior to being dealcoholized.

4.4.2.2.2 Wine color

WA treatment wine color was different from the Con wine (Table 4.9). The absorbance values at 420 and 520 nm were lower than the control while the Hunter values (L, a, b) were all greater for the WA treatment than the control. SA and DAL wine colors were not significantly different from the control.

4.4.2.2.3 Wine phenolics

Wine phenolic concentrations varied due to treatment (Table 4.9). WA had significantly lower concentrations of quercetin glycoside, catechin, epicatechin, polymeric tannins, and gallic acid. SA had a lower polymeric tannin concentration compared to the control, but significantly higher when compared to the WA treatment. DAL phenolic concentrations were not significantly different from the control.

4.4.2.2.4 Wine aroma compounds

The concentrations of aroma compounds were not greatly impacted by treatment (Table 4.10). The WA had higher concentrations of 2-phenethyl acetate and ethyl hexanoate, and lower concentrations of ethyl isobutyrate compared to the control. The SA and DAL treatments had higher concentrations of 2-phenethyl acetate than the control.

4.4.2.3 Wine sensory

PCA was performed only on the 13 sensory attributes that were found to be significantly different among the treatments ($p<0.1$) (Figure 4.4). The 10 attributes that were not significantly different were excluded (Table 4.6). The first two principal components accounted for 66.66% of the variation in the Zinfandel wines. PC1 separated the wines by red fruit aroma and flavor and confectionery aroma as opposed to perceived color and alcohol and PC2 separated the wines by black fruit aromas and color. The Con wines were negatively loaded on both PC1 and PC2. Two of the replicates were negatively loaded on PC1. This indicated higher color and alcohol perception. The third Con wine was loaded negatively on PC2, or opposite the descriptors for black fruit. It should be noted that the first two Con

Table 4.9 Compositional parameters for Zinfandel wines.

	Control	WA	SA	DAL
Dilution (% v/v)	0.0d	17.4b	13.2c	19.9a
Alcohol (% v/v)	16.70a	14.77b	15.13b	15.21b
RS (g/L)	0.17a	0.09a	0.09a	0.11a
pH	3.75a	3.67b	3.71ab	3.72a
TA (g/L)	6.10a	5.40b	5.43b	5.80ab
Lactic acid (mg/L)	68.3b	56.3b	69.3b	380.7a
Malic acid (mg/L)	1760.0a	1701.3a	1706.7a	1600.0a
VA (g/L)	0.03a	0.02b	0.02b	0.03ab
Abs420 (AU)	3.917a	2.717b	3.170ab	3.767a
Abs520 (AU)	6.723a	4.850b	5.727ab	6.750a
Hue	0.592a	0.560a	0.553a	0.558a
Intensity	10.650a	7.567b	8.897ab	10.533a
Hunter a	45.297b	51.090a	47.907ab	45.073b
Hunter b	23.380b	31.030a	26.730ab	22.910b
Hunter L	13.733b	19.100a	15.800ab	13.367b
Malvidin 3-glucoside (mg/L)	173.87a	173.40a	188.17a	173.83a
Quercetin glycoside (mg/L)	11.00ab	8.18c	9.96b	12.15a
Catechin (mg/L)	34.31a	22.47b	25.24ab	24.47ab
Epicatechin (mg/L)	21.65a	14.62b	18.23ab	17.21ab
B2 dimer (mg/L)	4.00a	3.12a	3.93a	3.63a
Polymeric tannins (mg/L)	313.37a	179.63c	234.21b	292.54a
Caftaric acid (mg/L)	35.61a	35.14a	38.82a	44.16a
Caffeic acid (mg/L)	3.19a	2.44a	2.16a	3.11a
Gallic acid (mg/L)	26.72a	19.19b	23.32ab	27.16a

Numbers within the same row followed by different letters are significantly different at $p \leq 0.05$ using LSD (n=3). WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment.

Table 4.10 Aroma compounds of Zinfandel wines subject to four pre-fermentation treatments.

		(µg/L)			
Compound	**Perception Threshold (µg/L)**	**Control**	**WA**	**SA**	**DAL**
1-hexanol	8,000[a]	1526.50a	1247.67a	1300.00a	1292.33a
1-octen-3-ol	1[c]	13.95a	14.63a	13.73a	13.03a
2-methylbutyl acetate	30[b]	200.50a	207.33a	252.33a	217.33a
2-phenyl ethanol	14,000[b]	58104.00a	52251.33a	49029.00a	40368.67a
2-phenylethyl acetate	250[a]	105.00c	301.67a	237.67ab	214.33b
β-damascenone	0.05[a]	9.55a	14.10a	8.70a	8.13a
β-ionone	0.09[b]	0.25a	0.43a	0.37a	0.40a
ethyl butanoate	20[a]	327.00a	369.67a	349.67a	344.67a
ethyl decanoate	200[b]	98.00a	136.33a	129.67a	145.67a
ethyl dihydrocinnamate	1.6[b]	0.65ab	0.73a	0.60b	0.60b
ethyl hexanoate	14[b]	604.35b	829.57a	662.30ab	573.53b
ethyl isobutyrate	15[b]	48.00a	32.33b	37.67ab	41.00ab
ethyl isovalerate	3[b]	17.25a	16.63a	17.73a	17.53a
ethyl octanoate	5[b]	606.40ab	756.00a	624.53ab	549.37b
ethyl-2-methyl butanoate	18[b]	8.00a	7.33a	7.67a	6.67a
isoamyl acetate	30[a]	1991.00a	2558.00a	2655.00a	2260.00a
linalool	25[b]	6.25a	42.40a	7.60a	8.07a

Numbers within the same row followed by different letters are different at $p \leq 0.05$ using LSD (n=3). WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment. Perception thresholds are as listed in 10% ethanol ([a]) (Guth, 1997), synthetic wine at 11% alcohol ([b]) (Ferreira et al., 2000), or in water ([c])(Buttery et al., 1988).

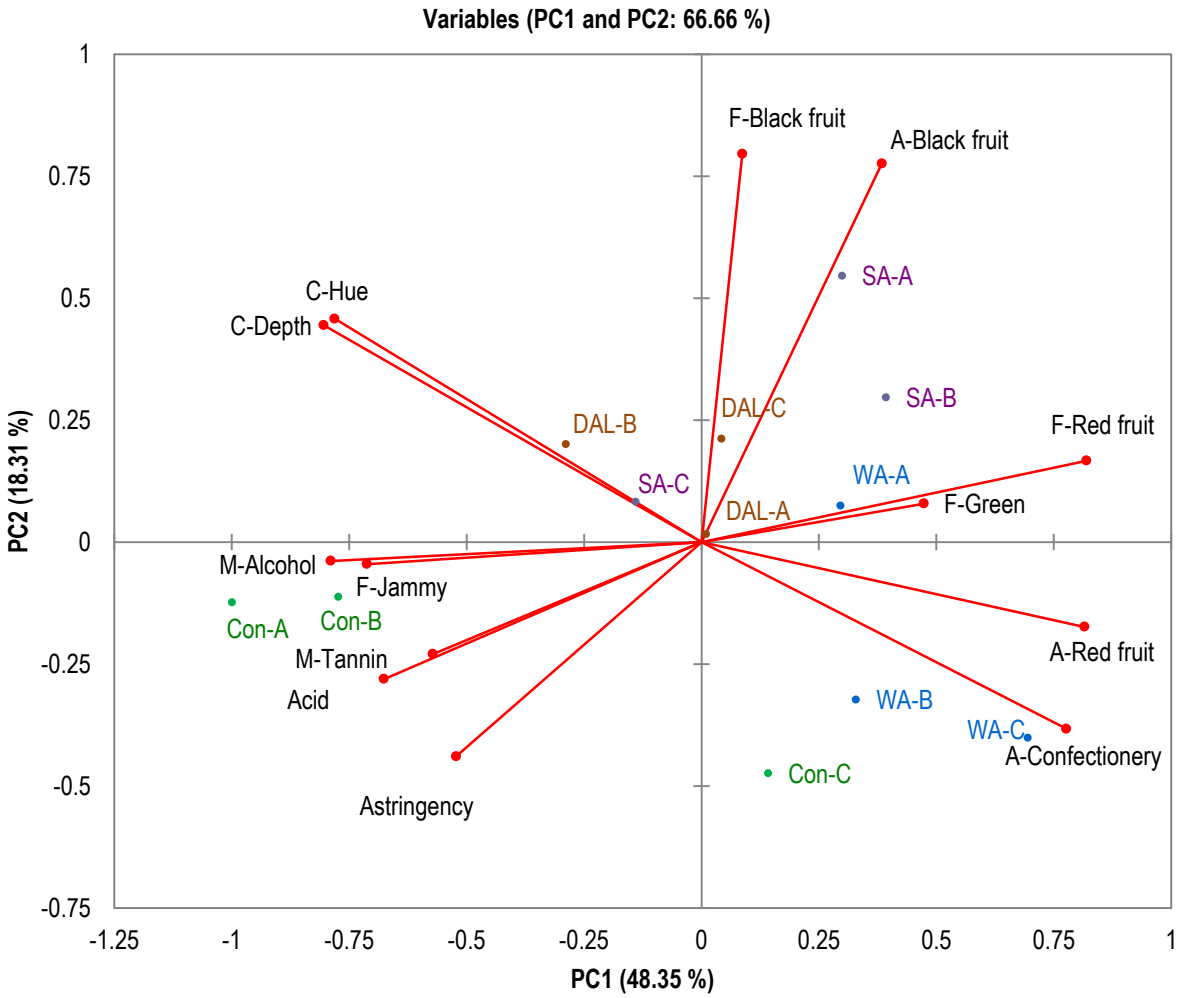

Figure 4.4 Principle component analysis of significant Zinfandel wine sensory attributes ($p<0.1$). Con, control; WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment. A-aroma attribute; T-taste attribute; F-flavor attribute; C-color attribute; and L-length of perception after expectoration.

replicates were noted as being ‘reduced’ (or sulfidic) by the DA panel. The WA treatments were defined by red fruit and confectionery aromas, while the SA wines were aligned with black fruit aroma and flavor. The DAL wines were close to the center of the plot. These wines were not highly scored for any particular attribute that defined the model.

Multiple factor analysis (MFA) was performed using all significant attributes. The 4 tables included in the MFA were: chemistry and color, phenolic compounds, aroma compounds, and sensory attributes (Figures 4.5 a and b). Con replicate A was not included in the MFA due to missing aroma data. The MFA for Zinfandel wines explained 66.05% of the variation in the first two factors. Factor 1 (50.66%) separated the treatments based on gallic acid, quercetin glycoside, and polymeric tannins opposed by ethyl hexanoate, ethyl octanoate, and 2-phenethyl acetate. Factor 2 (15.38%) separated the wines based on catechin, ethyl isobutyrate, and red fruit aroma. Factor 3 explained an additional 13.04% of the variation. Factor 3 separated the wines based on acid opposed to red fruit flavor and black fruit aroma and flavor. The two Con wine replicates did not cluster together. Con C was loaded positively along factor 2, and was closely related to catechin concentrations. Con B loaded positively for factor 1, but negatively for factors 2 and 3. This indicates higher polymeric tannin and gallic acid concentrations, lower catechin concentration and less red fruit, and increased acidity. The WA wines were loaded negatively for factor 1 and relatively central for factors 2 and 3. The WA wines were correlated to ethyl hexanoate, ethyl octanoate, and 2- phenethyl acetate concentrations. The SA wines were central on factor 1 and 2 and were positively loaded on factor 3. This suggests the SA wines do not have high values for factor 1 or 2 attributes, but have higher red and black fruit flavors. The DAL wines were positively loaded along factor 1, central to the MFA on factor 2, and slightly

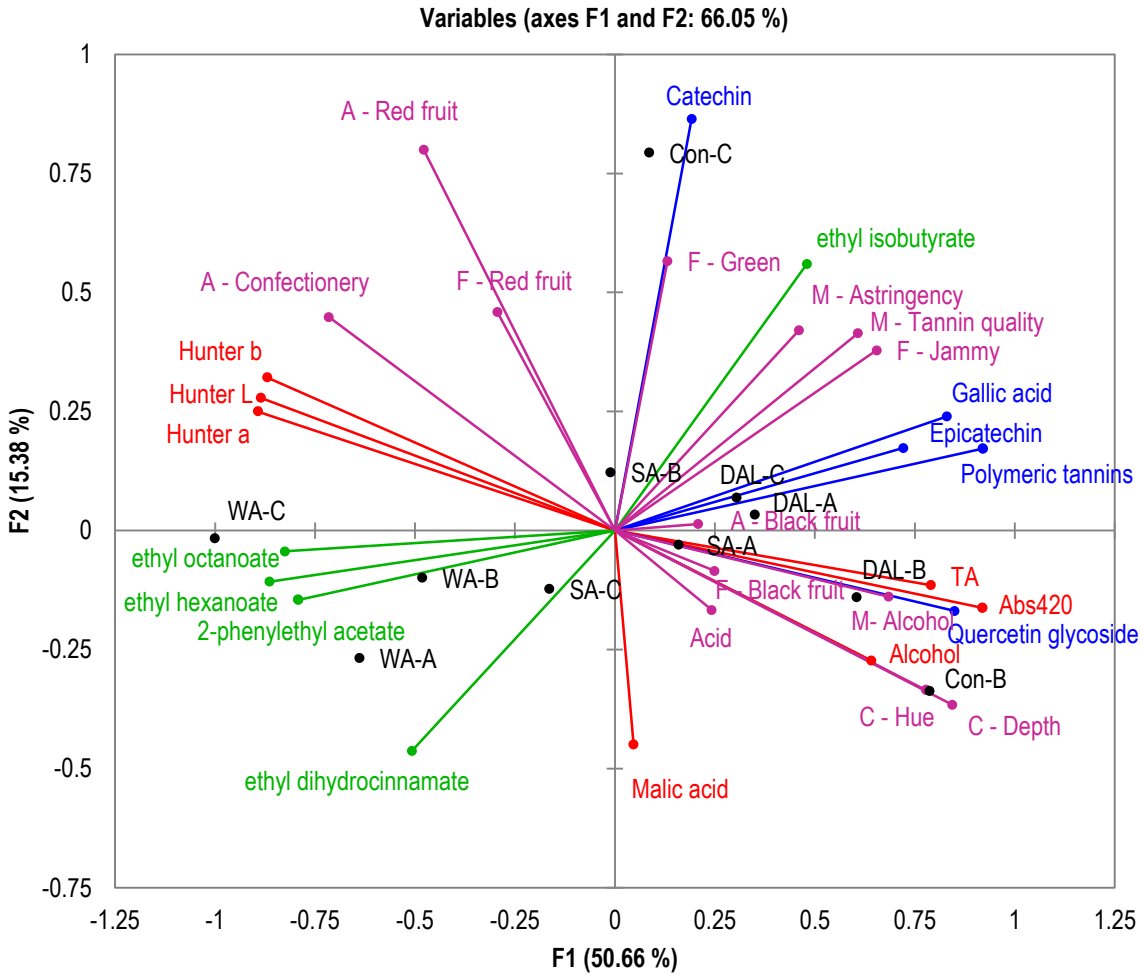

Figure 4.5a Multiple factor analysis of significant Zinfandel wine sensory attributes ($p<0.1$) and chemistry ($p<0.05$). Con, control; WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment. A-aroma attribute; T-taste attribute; F-flavor attribute; C-color attribute; and L-length of perception after expectoration.

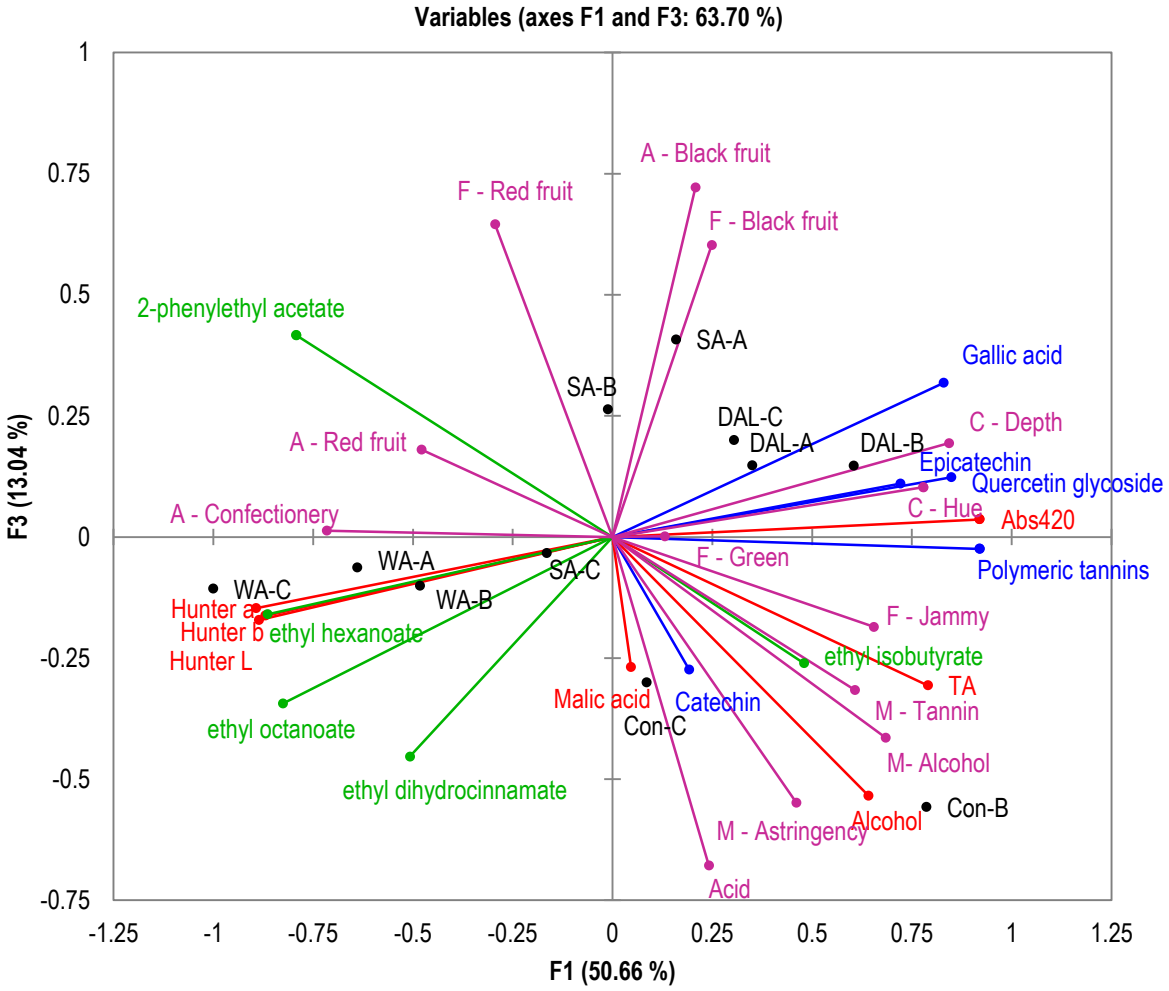

Figure 4.5b Multiple factor analysis of significant Zinfandel wine sensory attributes ($p<0.1$) and chemistry ($p<0.05$). Con, control; WA, water added to must treatment; SA, saignee prior to water addition treatment; DAL, dealcoholized wine added to must treatment. A-aroma attribute; T-taste attribute; F-flavor attribute; C-color attribute; and L-length of perception after expectoration.

positively loaded along factor 3. This indicates slightly higher polymeric tannin, quercetin glycoside, and gallic acid concentrations and higher red and black fruit flavors.

4.5 Discussion

Fermentation of high sugar juice or must may result in stuck or sluggish fermentations (Bisson, 1999). Even if the fermentations reach dryness, the resulting wines may be higher in alcohol than desired for the target wine style. To avoid either of these outcomes, juice or must TSS may be diluted with water, dealcoholized wine, or low alcohol wine produced from unripe fruit (Kontoudakis et al., 2011); or juice may undergo concentration and fractionation (Pickering, 2000). Alternatively, the resulting high alcohol wine, or a portion thereof, can be dealcoholized. The method used to lower the final wine alcohol content may alter the final wine and reduce wine quality.

Ingredient quality is considered an important aspect of food and beverage quality. The brewing and fruit juice industries have water quality standards in place (AquaFit4Use, 2010), but the wine industry does not. Dealcoholized wine used as an ingredient may also impact the final wine quality. Dealcoholized wine quality may be affected by production techniques and the level of alcohol removed (Pickering, 2000). Pickering (2000) noted that dealcoholized wine may have flavor imbalances, lack of body, cooked characters, and enhanced bitterness and astringency. Aroma compounds are also lost in the dealcoholizing process (Pickering, 2000). Wines dealcoholized by reverse osmosis had lower polyphenol and anthocyanin concentrations (Bui et al., 1986).

The current study showed that the material used to lower must sugar concentrations may yield wines with different chemical composition and sensory characters. In order to produce wines of a desired wine style, processing steps and ingredient additions must be considered.

4.5.1 Chardonnay

The TA of the WA treatment juice was significantly lower than the control. The Chardonnay juice acid adjustment occurred prior to the addition of water. The TA was diluted by the water. Interestingly, the pH was similar to the control, presumably because the extent of dilution was insufficient to significantly alter hydronium ion concentration. The pre-fermentation additions decreased the juice YAN for the WA and DAL treatments compared to the control (Table 4.1). These decreases also could be attributed to dilution.

The WA treatment wines had lower pH than the other treatments despite similar TA. This suggests the buffering capacity of the WA was different from the other treatments. Chardonnay wine VA was significantly lower in both pre-fermentation treatments compared to the control (Table 4.7). These decreases could be attributed to dilution. It should be noted that the VA values of all treatments were very low and therefore sensory impact would be minimal. Malic acid was lower in the WA versus the control. This could be attributed to dilution.

The control wine Hunter color values were intermediate to the treatments, though not significantly different from the treatments (Table 4.7). The WA wines were significantly more transparent than DAL wines. This is not surprising as the dealcoholized Chardonnay wine used for the addition was visibly browned compared to the base wine. Addition of dealcoholized wine either before or after

fermentation increased the opacity of the wine and increased the yellow hue, though the difference was significant only when compared to WA. Based on PCA, the DA panel distinguished the PB, and to some extent the DAL, wines from the other treatments by color attributes being more yellow with greener rim (Figure 4.1). The change in color associated with material used for must TSS adjustment could also serve to tailor the wine color to a meet a particular wine style.

Additional attributes also separated the wines by PCA. The Con wines were rated higher in alcohol aroma and perception of alcohol, which agreed with the chemically measured, significantly greater alcohol concentrations compared to the other treatments. Wine body was correlated with the perception of alcohol. This is consistent with previous literature that showed that increases in ethanol concentration increased wine viscosity (Runnebaum et al., 2011).

Body and aftertaste length were the only mouthfeel attributes rated that were significantly different among the treatments. Given that skin and seed contact time is minimized in Chardonnay wine production, it is not surprising that the Chardonnay wine phenolic concentrations were low. Caftaric acid was the only compound measured with a concentration above reported thresholds (5 mg/L) (Hufnagel and Hofmann, 2008) (Table 4.7); however, astringency of the treatments was not significantly different.

Dealcoholized wine for dilution was proposed in order to minimize the dilution effect of constituents other than sugar/alcohol caused by the addition of water. Lower 1-octen-3-ol concentration in the DAL was the only significant difference from the control (Table 4.8). This is most likely a positive change given that 1-octen-3-ol is associated with a moss or mushroom-like aroma. In contrast, the water

addition resulted in significant differences involving several other compounds as well. Using MFA, correlations between aroma compound concentrations and sensory attribute scores emerged (Figure 4.2). For example, isoamyl acetate, a compound associated with banana, was closely related to tropical fruit flavor, which was defined by the panel as banana, pineapple, and rock melon (or cantaloupe). 2-phenylethyl acetate, described as honey, spice, or floral, was closely related to stone fruit aroma and flavor. Ethyl isobutyrate, associated with fruit aromas, and ethyl hexanoate, associated with apple aromas, were both closely related to citrus fruit attribute defined by the panel. The WA treatments were loaded with the citrus flavor and away from the stone and tropical fruits, whereas the PB and, to a lesser extent, the DAL wines were loaded closer to the stone fruit attributes. The PB treatments were loaded near linalool, a compound associated with citrus and lavender aromas. This is consistent with the chemical metrics as the PB treatment had significantly a higher linalool concentration compared to the other treatments.

4.5.2 Zinfandel

Zinfandel must was not homogenized, so individual lots had some natural variation even prior to must adjustment (Table 4.2). Con and WA had higher sugar concentrations than the SA or DAL treatments. The sugar sampling showed an increase in sugars (°Brix) for 24 hours before reaching a plateau (Figure 4.3 upper graph). This increase is consistent with reports in the earlier literature (Ough and Nagaoka, 1984, Jones and Ough, 1985) and may be attributed to the lack of a representative sample; however, this is unlikely as the must was stirred prior to each sample point. Instead, it may be that the sugars diffuse from grape solids over time. This is supported by the second increase in sugar concentration, which followed the water addition (Figure 4.3 lower graph). The second rise in sugar suggests that the change in solute shifts the equilibrium such that more sugar is extracted from the grape and into solution. Eight hours after the water addition, the sugar concentration reached a peak.

The concentration may have continued to increase, but the fermentation had initiated at this point. It takes approximately 24 hours after crushing to reach equilibrium between the sugar concentrations in the liquid and solid portions of the must. This needs to be considered when making sugar adjustment calculations or the resulting alcohols will be greater than the target.

After must adjustment, YAN was greater for the control than for any of the treatments (Table 4.2). Despite this, diammonium phosphate (DAP) was not added as all treatments had sufficient YAN (>250 mg/L). Tartaric acid was added to each of the lots in order to attain a pre-fermentation must pH of 3.6. The DAL pH was significantly lower than the control or SA and the DAL TA was significantly higher than the WA or SA. Calculations were based on standard grape must and the premise that 1 g/L of tartaric acid will decrease the pH of the must by 0.1 units. The additions were calculated separately for each lot. This may have resulted in some variation. Alternatively, the resulting pH and TA values may be due to differences in the buffering capacity of the adjusted must.

Wine color varied by treatment (Table 4.9). The WA wines were significantly lower in absorbance at 420 and 520 nm and significantly higher for Hunter color (a, b, L) compared to the Con and DAL wines. The SA wines were intermediate to those groups but not significantly different from either. By tristimulus colorimetry, WA wines were significantly more transparent, red and yellow than DAL or Con wines. This may be due to dilution. The DA panel was also able to distinguish the WA wines as more transparent and more pink than deep purple.

Wine SO_2 concentrations can impact wine color as SO_2 slows browning of the wine (Bakker et al., 1998). Moreover, young red wines, in which monomeric anthocyanins are still abundant, will be

bleached by the formation of bisulfite:anthocyanin adducts. The lower free SO_2 levels of the WA wines and the lower Abs520 nm values suggest that this bleaching reaction may have occurred in these wines. Future investigations should include a more comprehensive analysis of the proportions of the SO_2-bleachable and total pigments present in each treatment wine to examine this possibility. Wine pH values were similar, so color differences cannot be attributed to pH shifts (Glories, 1984). The wines were assessed eight months post-bottling. Bakker et al. (1986) showed that young red wines contained 20-35% polymeric pigments and this increases over time. Thus color differences may in part also be due to polymerization rather than pH or SO_2 effects. The copigmentation in the WA treatment may be decreased compared to the other wines due to dilution of the pigment concentration (Boulton, 2001). This would explain the lower value for blue tones (Hunter b) in the WA wines. The wines were analyzed at an age where copigmentation would be relevant to the wine color.

Concentrations of phenolic compounds could not be ascribed to volume changes due to the treatments, and concentrations of malvidin-3-glucoside, caffeic acid, and caftaric acid were not significantly different among the treatments (Table 4.9). Previous authors have reported similar anthocyanin concentrations in control and treatment wines when wine was produced from the fruit removed during cluster thinning, and later added to must in order to lower the initial must sugar concentration for Merlot, Cabernet Sauvignon, and Bobal (Kontoudakis et al., 2011). Wine produced from low maturity fruit removed during cluster thinning was lower in alcohol due to its early harvest date, and the addition of this wine prior to fermentation was therefore similar to the DAL treatment in the current study. Harbertson et al. (2009) showed that anthocyanin concentration in the control wines (18.7% water added) did not differ from the high ethanol (4.5% water added) wines. These treatments are comparable respectively to the WA and Con treatment in the current study. However, the anthocyanin concentrations in the current study are approximately half the values reported by

Harbertson et al. (2009). Contrary to the results of the current study, Harbertson et al. (2009) reported significant anthocyanin concentration increases in the high saignee and low saignee wines (32.7 and 18.1% volume removed, respectively) compared to the control, when sampled after 185 days. The difference in findings may be due to the greater volume changes used by Harbertson et al. (2009). Anthocyanin copigmentation shifts the equilibrium of free anthocyanins thereby enabling greater retention of pigment and a higher wine anthocyanin concentration (Boulton, 2001). Volume changes and relative skin to liquid ratios may also shift the free anthocyanin concentrations. Fermentation parameters may also contribute to the relatively low anthocyanin extraction. Harbertson et al. (2009) conducted commercial scale fermentations whereas the current study was performed at 50 kg scale. Schmid et al. (2009) showed greater temperature gradients in large scale fermentors compared to small scale fermentors. Elevated temperatures increased the extraction of anthocyanins and the formation of polymerized anthocyanins (Gao et al., 1997). Cap management technology may also yield differences in anthocyanin extraction. Mechanical punch downs and pump overs have been shown to increase phenolic extraction compared to manual punch downs (Fischer et al., 2000). Lower fermentation temperatures and manual punch downs associated with the small lot fermentations may explain the lower anthocyanin concentrations in the present study compared to those of Harbertson et al. (2009). Alternatively, the lower anthocyanin concentrations may be due to varietal differences between Zinfandel and Merlot.

The WA treatment had significantly lower quercetin glycoside, polymeric tannin, catechin, epicatechin and gallic acid (Table 4.9). All of these concentrations were lower than could be explained by simple dilution of the compounds. DAL treatment phenolic concentrations were not significantly different from the control. Previous literature has shown no difference in polymeric tannin concentration when must sugars are diluted with wine from unripe grapes (Kontoudakis et al., 2011). This supports the rationale

for using DAL or low-alcohol wine in place of water. The SA treatment phenolic concentrations were intermediate to those of the WA and DAL treatments. The polymeric tannin concentrations for SA and WA were lower than would be explained by dilution alone. This is contrary to Harbertson et al. (2009) who stated that tannins increased in proportion to the volume of juice removed; however, the tannin concentrations were not significantly different between treatments. The low polymeric tannin concentrations recorded in the current study may be due to relatively low proanthocyanidin extraction resulting from the low fermentation temperatures (24°C) (Zimman et al., 2002). The difference might also be attributed to the fermentation scale as extraction of proanthocyanidins has also been shown to be slower in microscale compared to commercial fermentations (Sampaio et al., 2007). However, the fermentation parameters in the current study were consistent for all treatments, and therefore results could be compared among treatments. In the current study, DAL eliminated the dilution of phenolic compounds that occurs with water additions. Use of saignee prior to water addition mitigated the dilution of phenolics associated with water addition.

In order to achieve equivalent final alcohol concentrations in all wines, it was necessary to use differing addition volumes, and hence the treatments have different dilution factors (Table 4.2). The base ingredients may impact the result, but differences could also be due to solvent effects and changes in the extraction of compounds from different grape tissues. Caftaric acid and gallic acid, which are located in grape pulp, exhibited few differences between the treatments. Since these compounds are readily extracted during crushing, it might be expected that these concentrations would be diminished due to dilution. The seed-based compounds catechin and epicatechin were lower in all treatments compared to the control; however, B2 dimer concentrations were not different. Gallic acid concentrations, which may be attributed to the hydrolysis of epicatechin gallate (Singleton et al., 1966), were also significantly lower in the WA treatment compared to the Con, but the DAL and

SA treatments did not have a dilution effect and the concentrations were not significantly different from the control. Polymeric tannins, which may be from seeds or skins, were at lower concentrations than the control in all treatments, though the DAL treatment was not significantly different. Skin-based compounds did not have consistent trends. Malvidin-3-glucoside concentrations were not significantly different among the treatments, whereas quercetin glycoside concentrations were lowest in the WA and greatest in the DAL treatment.

The phenolic concentrations and associated attributes were closely related as shown by MFA (Figure 4.5). Polymeric tannin and gallic acid concentrations and astringency and tannin quality were positively loaded on factor 1. Polymeric tannin concentrations were more than eight times greater than the threshold (22 µg/L), and gallic acid concentrations were approximately one-half the threshold value (50 µg/L) (Hufnagel and Hofmann, 2008) (Table 4.9). This suggests that perceived astringency differences might be based on the polymeric tannin concentrations as opposed to gallic acid. Malvidin-3-glucoside was not included in the MFA as there were no significant differences between treatments. Interestingly the concentration of quercetin glycoside, another skin-based compound, was closely related to color attribute scores and chemistry.

Only five aroma compounds were significantly different among the treatments and, therefore, included in the MFA (Figure 4.5). Confectionary aroma was positively correlated with 2-phenethyl acetate, ethyl hexanoate, and ethyl octanoate. 2-phenethyl acetate has been described as honey, spice, rose, or lilac; ethyl hexanoate as apple, fruity, strawberry, or anise; and ethyl octanoate as pear, sweet soap, pineapple, and floral. These sweet fruit descriptors may be perceived as confectionary. In the current study, the concentrations of ethyl octanoate, ethyl hexanoate, and ethyl isobutyrate for all treatments

were well above the thresholds found in synthetic wines (5, 14, and 15 mg/L, respectively) (Ferreira et al., 2000); whereas 2-phenethyl acetate was above the threshold (250 mg/L) only in the WA wines (Table 4.10). The WA wines had the highest concentrations of ethyl octanoate, ethyl hexanoate, and 2-phenethyl acetate across all treatments. The high concentrations of these compounds may separate the WA wines along factor 1 of the MFA. The increase in aroma compounds with WA addition may be due to fewer solids in the must. Girard et al. (1997) reported increased ester, alcohol, and total volatile concentrations in wines made from low insoluble solids Pinot Noir must, however these treatments also involved must heating and the effect of solids versus temperature was not resolved. Gawel et al. (2001b) reported an increase in aroma and flavor intensities of Syrah wines following 20% juice run-off, compared to control wines and wines made with 10% run-off. Importantly, this effect was specific to fruit from one block within a vineyard and was not observed in a comparable trial using fruit from another block in the same vineyard. It should be noted that the juice run off in Gawel et al. (2001b) was not followed by a water addition as in the current study.

Two of the Con replicates (A and B) were considered reduced by the DA panel; therefore Con C was the only wine that could be properly assessed for fruity attributes. The Con wines presented to the panel also had higher alcohol concentrations than the other treatment wines and this can impact the perception of viscosity and bitterness (Fischer and Noble, 1994, Pickering et al., 1998). Yu and Pickering (2008) showed that consumers could detect ethanol concentration differences as low as 1% (v/v). In the current study, the control alcohol concentrations were 1.4 – 2.0% (v/v) greater than the treatment wines. Ideally, the DA panelists would have assessed both the Con wines and an additional treatment of the control wines with dealcoholized Zinfandel added post-bottling as was done for the Chardonnay wines (PB). However, the dealcoholized Zinfandel wine was oxidized, so this treatment was eliminated prior to the formal sensory evaluation.

Sensorially, the WA treatment wines lacked mouthfeel and color compared to the control. This is consistent with the low concentrations of polymeric tannins and quercetin glycosides and low color metrics in these wines. SA wines had improved fruit characteristics, but the mouthfeel ratings were still lower than the controls. In contrast, the DAL wines had sensory ratings equivalent to those of the controls. Therefore, the use of dealcoholized wine to lower red must sugar concentrations could be used to reduce final wine alcohol without reducing the mouthfeel characters in the wine.

The raw materials used in winemaking produce a different final wine. The data from this study showed that wine color, aroma, and mouthfeel were affected by the use of water to lower must soluble solids (°Brix). The use of dealcoholized wine instead of water resulted in fewer differences from the control, so the use of this material could be used to attain a different, lower alcohol product. The choice of raw materials could be used to target certain wine styles.

4.6 Conclusions

The results of this study indicate that the choice of material used for pre-fermentation must sugar adjustment can significantly alter the wine style composition. The use of water reduced the color intensity of the wines for both varieties, while fewer differences from the control were evident when dealcoholized wine was used in place of water. The results also indicate that dealcoholized wine of the same variety can be used to avoid the dilution of phenolic compound concentrations associated with water use. Saignee performed prior to water addition yielded concentrations that were intermediate to the water and dealcoholized wine addition. In Chardonnay, aroma compound concentrations trended similarly with the water and dealcoholized wine treatments when compared to

the control. In Zinfandel, few aroma compound concentrations were significantly different from the control, though patterns varied by treatment. Despite few chemical differences, treatments could be separated by sensory analysis. The choice of must sugar adjustment technique may be a useful tool for making wines to meet different consumer preferences. Dealcoholized wine can be used pre-fermentation to reduce final wine alcohol without diminishing the color or the mouthfeel qualities. Alternatively, water can be used to create a lighter style of wine. This provides winemakers with additional decision points for creating the desired wine.

4.7 Acknowledgements

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5 Conclusions

5.1 Introduction

Phenolic compounds provide several important wine sensory factors including colour, bitterness and astringency (Noble, 1994, Gawel, 1998). These attributes play an important role in consumer acceptance and preference of wines.

Grape maturity is considered a factor determining wine quality (Ough and Singleton, 1968, DuPlessis and VanRooyen, 1982); however, very little has been published regarding the impact of fruit maturity on the extraction of phenolic compounds during winemaking. Canals et al. (2005) and Fournand et al. (2006) are among the few who have studied extraction of phenolic compounds from grapes into wine at different stages of fruit ripeness. However, these studies assessed total solvent extractability in hydro-alcoholic solutions as opposed to extraction during vinification. Canals et al. (2005) showed improved extraction of anthocyanins and proanthocyanidins as the maturity of Tempranillo grapes increased. In contrast, Fournand et al. (2006) showed no changes in extraction of skin anthocyanins or proanthocyanidins due to increased fruit maturity.

During the past 15 years, in common with what has been seen in many of the grape-growing regions of the world, the total soluble solids content of wine grapes at harvest has increased in California. This extended maturation period is employed to achieve optimum berry flavour development and phenolic maturity for the desired wine style. However, most research regarding the impact of grape maturity on resulting wine quality has been performed on grapes harvested near historical commercial harvest maturities (i.e. 23.5°Brix). For example, the ripest fruit used by Canals et al. (2005) was harvested at soluble solids levels less than 18°Brix. Few studies have evaluated the effect of advanced maturity on wine composition. Holt et al. (2010) reported on the total phenolics, total tannins, and total

anthocyanins in Cabernet Sauvignon grapes during the latter stages of ripening, but extraction of these compounds into wine was not assessed.

Extended fruit maturation and warmer growing seasons brought on by climate change will produce riper fruit. The resulting high sugar fruit content may yield fermentations that do not go completely dry due to the high alcohol (Bisson, 1999). High alcohol wines may also have some unfavourable sensory properties. This may be due to changes in the extraction of key compounds from the grapes into the wine, though ethanol itself increases the perception of bitterness (Fischer and Noble, 1994).

In the United States, water may be added to the juice or must to mitigate the potential for a stuck fermentation or high alcohol wine due to harvesting grapes at elevated maturities (U.S. 27CFR Part 24, Bureau of Alcohol Tobacco Firearms and Explosives, 1993). However, few studies have been performed to evaluate the impact of water addition on wine chemical attributes or sensory composition. Harbertson et al. (2009) assessed the effect of water additions, saignee and extended maceration on anthocyanin and tannin extraction and the associated sensory outcomes. Wines made with water additions had similar proportions of extracted skin and seed tannins whereas high alcohol wines have greater proportions of seed tannins (Harbertson et al., 2009). This is consistent with Canals et al. (2005) who showed increased extraction of anthocyanins and seed procyanidins with greater ethanol concentrations. Given that winemakers generally prefer the mouthfeel associated with skin tannins rather than seed tannins (Kennedy, 2007), high ethanol wines may fail to meet stylistic targets.

The first goal of the research described in this thesis was to assess the impact of extended maturation of *Vitis vinifera* L. cv. Cabernet Sauvignon on grape phenolic compound concentrations, the extraction of these compounds into wine, and the associated sensory impact. Grapes were harvested weekly for eight consecutive weeks after the historical maturity level (23.5°Brix) had been reached. This corresponds to week 8 through week 15 post-veraison.

A second goal of this research was to determine how pre-fermentation sugar adjustment, with water or dealcoholized wines, impacted the phenolic concentration of wines. Wines were made from Chardonnay and Zinfandel grapes harvested at high sugar levels. Must sugar concentrations were lowered by adding water or dealcoholized wine added pre-fermentation, and compared to wines made with no sugar adjustment. Wine phenolic concentrations were assessed and correlated to sensory data. Ideally, the information obtained from these experiments can be used to modify harvest and winemaking practices to avoid stuck or sluggish fermentations and achieve the desired wine style targets.

5.2 Summary of research outcomes

5.2.1 Phenolic extraction

In the current study, the hypothesis that the extractability of phenolic compounds increases with grape maturity was not supported. Riper grapes contained higher concentrations of some phenolic compounds, and hence these concentrations themselves might be more commercially relevant than their extractability. Grape caftaric acid, catechin, epicatechin, and B2 dimer concentrations declined, while malvidin-3-glucoside and polymeric tannin concentrations increased with ripening. These changes in grape composition with maturity are consistent with previous literature (Singleton et al.,

1986, Kennedy et al., 2000a, Kennedy et al., 2002, Fournand et al., 2006). Increased anthocyanin and polymeric tannin concentrations are associated with wine colour, tannin structure and body, and smaller flavanols with bitterness, therefore, later grape harvest date does impact the quality of the resulting wine.

These changes in grape phenolic concentrations with grape maturity were reflected in their concentrations in the wine in 2008, but not in 2009. Previous reports of comparable experiments have also yielded mixed results. Gonzalez-Neves et al. (2004) showed high correlations between grape and wine phenols and anthocyanins, yet Harbertson et al. (2002) found the total tannins per berry did not correlate with wine tannins. These conflicting outcomes may be due to extraction, polymerization or chemical reactions occurring during fermentation and aging. Analytical techniques including extraction solvent and time may also influence reported phenolic concentrations, thereby affecting comparisons between studies (Downey and Hanlin, 2010).

In this study, constituent location in the berry appeared to correlate with the extraction of phenolic compounds. Skin-based constituents had greater extractability than seed-based constituents. Wine malvidin-3-glucoside concentrations increase with grape ripening; however, the increase was due to an increase in the grape concentration as opposed to an increase in malvidin-3-glucoside extractability.

Smaller molecules may extract more rapidly than large molecules found in the same tissue. Catechin and epicatechin extraction both exceeded that of B2 dimers and polymeric tannins. This is consistent with Fournand et al. (2006), who showed that skin proanthocyanidins with a lower average degree of

polymerization were extracted more rapidly than those with a high average degree of polymerization from Shiraz grapes. However, in the current study, the extraction of both catechin and epicatechin was much lower in week 15 after veraison of 2008 than for other harvest dates. This may indicate a decrease in extraction due to polymerization or an increased interaction with other cellular components. Polymerization may decrease solubility and result in lower concentrations of these compounds in the wine. Alternatively, phenolics may interact with other phenolics or compounds including cell wall material, proteins, and polysaccharides as shown by Bindon et al. (2010) and Bindon and Kennedy (2011).

The influence of grape maturity on the extraction of polyphenolic compounds showed trends, though the patterns varied by compound and vintage. Catechin extraction increased with grape maturity in both years; however, epicatechin, B2 dimer and polymeric tannins extraction decreased with maturity in 2008, but increased with maturity in 2009. The decline in seed phenolic extraction with maturity shown in 2008 is consistent with previous data (Czochanska et al., 1979, Romeyer et al., 1986). Climate and viticultural practices may have impacted the outcomes of the current study. In 2009, the maximum temperatures were lower than in 2008 and the fruit ripened more slowly. The 2009 crop load was lower than in 2008. Also, it rained in the 13th week after veraison in 2009. The rain may have resulted in a dilution of fruit phenolic concentrations. These differences may have impacted the extraction of compounds from grapes into wines. Alternatively, differences in extraction in 2008 versus 2009 may be due to polymeric tannin compositional changes as opposed to concentration differences, as shown by Kennedy et al. (2000a) and Fournand et al. (2006) in Shiraz grape seeds and Cabernet Sauvignon grape skins, respectively. However, proanthocyanidin composition was not measured in the current study, so this cannot be determined. Evaluation of the proanthocyanidin composition would be of interest to examine in the future.

5.2.2 Sensory correlations with chemistry

While wine chemistry is important commercially, the ability to make wines to meet desired wine style preferences is paramount. Malvidin-3-glucoside was not closely related to any of the mouthfeel attributes. This is in agreement with Vidal et al. (2004) who showed that addition of anthocyanin fractions did not modify bitterness or perception of astringency. Quercetin glycoside also had weak correlations with all sensory attributes. This may be due to the similarity of wine quercetin glycoside concentrations observed for all 2008 wines regardless of harvest dates. The threshold of astringency for quercetin glycoside is low (1.0 mg/L) (Hufnagel and Hofmann, 2008), so slight changes in concentration might be expected to have an effect on mouthfeel. However, the PCA analysis was based on the sensory results and the chemical parameters were supplementary. The sensory attributes that defined the first few principle components were not strongly correlated with quercetin glycoside.

Wine flavanol monomer and dimer concentrations decreased with grape maturity, though bitterness ratings were constant. The decrease in monomer and dimer concentrations is consistent with results reported by Downey et al. (2003a). No perceptible change to bitterness ratings was evident as the concentrations of these compounds were well below their reported sensory thresholds (Hufnagel and Hofmann, 2008).

In 2008, polymeric tannin concentrations had strong positive correlations with tannin quality, astringency, body, alcohol, and aftertaste length as well as colour intensity. This is in agreement with Kennedy et al. (2006a) who showed a correlation between tannin concentration and perceived astringency.

Ristic et al. (2002) found higher quality wines had greater amounts of anthocyanins and skin phenolics, and a lower amount of total flavanols and seed procyanidins. In the current study the monomer and dimer concentrations declined, and the polymeric tannin concentrations increased with grape ripeness. This is consistent with the practice of utilizing extended maturation in order to achieve phenolic maturity and therefore higher quality wines. However, the phenolic compounds measured in the current study did not fully explain the sensory differences in wines made from grapes harvested at different maturities. Further work examining phenolic compounds and the interactions of compounds with polysaccharide, ethanol, and organic acid concentrations is needed to determine what contributes to the sensory differences associated with extended maturation.

5.2.3 Pre-fermentation adjustment of high TSS must

In the maturity component of this study (Chapters 2 and 3), must sugar concentrations were adjusted to 24°Brix prior to fermentation in order to remove possible effects of the ethanol content on the extraction of phenolic compounds. Wines made with grapes harvested at higher sugar levels would have higher alcohol concentrations and, therefore, the extraction might be greater (Canals et al., 2005), or the rate of seed proanthocyanidin extraction may have been increased (Hernandez-Jimenez et al., 2012). The must sugar levels were not adjusted in previous studies assessing the impact of grape maturity on wine composition; therefore increases in extractability attributed to grape maturity may have been due to solvent effects rather than changes in grape composition. The consistent starting sugar concentrations in the current study also resulted in consistent fermentation times and, therefore, skin contact time for all wines. However, the use of water additions with the riper fruit treatments may have diluted the wine phenolic concentrations. The work in Chapter 4 was conducted to test the impacts of water addition.

High sugar Chardonnay and Zinfandel musts were used to assess the impact of pre-fermentation water additions. Dealcoholized wine was also used to test the impact of the ingredient used for dilution. The use of water reduced the colour intensity of both Chardonnay and Zinfandel wines. Fewer differences from the control were evident when dealcoholized wine was used in place of water. The results also indicated that dealcoholized wine of the same variety can be used to avoid the dilution of phenolic compound concentrations associated with water use. Saignee performed prior to water addition yielded concentrations that were intermediate to the water and dealcoholized wine addition.

In Chardonnay, the wine colour was altered based on the diluent material used. Water resulted in a more transparent wine, while dealcoholized wine addition yielded a darker wine. The high alcohol control wines had greater body ratings. This is consistent with previous literature, which showed that increases in ethanol concentration increased wine viscosity (Runnebaum et al., 2011). As might be expected, the phenolic compound concentrations were low for all Chardonnay wines. Caftaric acid was the only compound measured with a concentration above reported thresholds (5 mg/L) (Hufnagel and Hofmann, 2008); however, astringency of the treatments was not significantly different.

Aroma compound concentrations yielded more differences between treatments. The water addition resulted in significantly lower 1-hexanol, 1-octen-3-ol, ethyl butanoate, ethyl dihydrocinnamate, and isoamyl acetate concentrations, and greater ethyl isobutyrate, compared to the control. When dealcoholized Chardonnay wine was used, only the 1-octen-3-ol concentration was lower than the control. The differences in wine aroma associated with the material used for dilution could be used to

target a particular wine style or consumer segment. Dealcoholized Chardonnay can be added to lower the final wine alcohol concentration, whereas water can be used to enhance the apple or citrus notes by decreasing the green, floral and banana character of the wine.

As with the Chardonnay, the colour of Zinfandel wines could be differentiated by treatment, with the water treatment being most transparent. Wine phenolic compound concentrations could not be ascribed to volume changes due to the treatments. Concentrations of malvidin-3-glucoside, caffeic acid, and caftaric acid did not differ among the treatments. This is consistent with Harbertson et al. (2009) who showed that anthocyanin concentration in the control wines did not differ from the high ethanol, or no sugar adjustment, wines. Water addition yielded lower quercetin glycoside, catechin, epicatechin, polymeric tannins, and gallic acid concentrations, whereas the use of dealcoholized wine for pre-fermentation dilution resulted in no differences from the control for these compounds. Polymeric tannin and gallic acid concentrations were both positively correlated to astringency; however, polymeric tannin concentrations were more than eight times greater than their astringency threshold (22 µg/L), while gallic acid concentrations were approximately one-half the threshold value (50 µg/L) (Hufnagel and Hofmann, 2008). This suggests that perceived astringency differences might be based on the polymeric tannin concentrations as opposed to gallic acid. The use of saignee in conjunction with water addition yields wine colour and phenolic concentrations intermediate to that of the water or dealcoholized wine addition. Aroma compound concentrations were not impacted by treatment.

The use of dealcoholized wine to reduce high sugar in must yielded wines that were similar to those of the undiluted controls. This is consistent with Kontoudakis et al. (2011) although the source of low alcohol wine was different. If dealcoholized wine is not available, or its use not reasonable from a

financial standpoint, saignee prior to water addition can moderate the dilution effect. However, legal constraints within winemaking regions and the countries to which they export wines must be considered.

Some of the techniques employed in the current study may have confounded the results or comparison of the results to other studies. Previous studies have assessed grape phenolic content using strong extraction solvents (e.g. acetone and ethanol) and separate samples of grape skins and seeds to determine the total amount of phenolics present in the grapes (Kennedy et al., 2000b, Kennedy et al., 2001, Harbertson et al., 2002, Downey et al., 2003a). Grape berry homogenate has been extracted in a model wine-like solution to determine grape phenolic extractability (Canals et al., 2005, Fournand et al., 2006). In the current study, grape berry homogenate extracted with 50% ethanol was used to determine grape phenolic concentration and wine was produced to test the extraction of grape phenolics into wine. The aim was to quantify the total phenolics available compared to the amount extracted during winemaking.

In the current study, wines were produced from 50 kg fruit lots. These small-scale fermentations were used for process control and replication. Anecdotally, small-scale winemaking extraction does not reflect that of commercial winemaking. The extraction in small scale is expected to be less than in large scale due to lower temperatures. This assumption is based on the larger surface area to volume ratio and the associated dissipation of heat (Boulton et al., 1996). Recently Schmid et al. (2009) determined the temperature gradients in large (3,450 L) and small scale fermentors (50 L) and found similar temperature gradients despite scale. This is consistent with Schmid et al. (2007) who showed few compositional differences between wines made at 20 kg, 50 kg and 300 kg scale.

The intent of the current study was to assess extraction of grape phenolic compounds into wine and any differences brought about due to addition of water to reduce high must sugar concentrations. The winemaking process used did not include malolactic fermentation or oak contact as would be typical in commercial winemaking. The winemaking employed was chosen to minimize process variability, rather than to make a product to appeal to the consumer.

5.3 Future research

Grape varieties differ in their phenolic compositions. Knowledge of these differences is helpful for aiming for a particular wine style, though this may be variable due to region or weather patterns. The differences between 2008 and 2009 made it difficult to draw general conclusions regarding the extraction of grape phenolic compounds into wine. Weather cannot be predicted or controlled, so additional years of data may only add complexity to the data set.

The measured chemistry did not explain the sensory differences in the wines. Further method development to characterize specific proanthocyanidins along with sensory trials would be necessary to determine how proanthocyanidin conformation impacts wine sensory. This could be a step to establishing links between wine chemistry and sensory; however, phenolics do not appear to be the right metrics.

Polysaccharides and cell wall interactions have also been investigated (Bindon et al., 2010, Bindon and Kennedy, 2011). More work in this area may elicit some further understanding of mouthfeel.

However, the chemical interactions among various compound classes must also be considered. The impact of these interactions on sensory perception of wine mouthfeel attributes needs to be addressed.

5.4 Conclusions

The concentrations of individual phenolic compounds in wine varied based on grape maturity, with some increasing while others decreased. Importantly, the wines made with grapes of different ripeness could be differentiated on the basis of their sensory attributes. Ideally, the chemical metrics of these wines could predict their sensory changes. It appears however that the individual phenolic compounds measured in the current study did not provide a clear marker for predicting wine sensory properties. Extended fruit maturation had little impact on the extraction of grape phenolic compounds into wine based on the compounds assessed in this study.

Where legally permitted, high sugar must concentrations may be reduced; however, the material used for pre-fermentation must sugar adjustment impacts the resulting wine. Dealcoholized wine of the same variety can be used to avoid the dilution of colour intensity and phenolic compound concentrations associated with water use. Saignee performed prior to water addition can partially mitigate the effects of the water addition. Treatments can be differentiated by sensory analysis even when differences in chemical concentrations are small.

6 Bibliography

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