Literature review

Use of Exogenous Tannins during Post-Fermentation Extended Maceration to Enhance Colour Stability and Potential Longevity of Pinot Noir

1.1 Introduction

Pinot noir is an important grape variety for Australia, with an estimated 35,790 tonnes harvested in 2011 (WFA Vintage report, 2011). It is capable of producing high quality Table wines, but it can be a difficult challenge for viticulturists and oenologists (Smart 1992). Although it can produce wines of great potential during a good vintage, Pinot noir is also known to produce disappointingly light, short-lived wines, which affects quality consistency (Parley, et al. 2001). It is low in phenols and anthocyanin content as compared to most common cultivars, which makes extraction during winemaking and colour stability important considerations for Pinot noir (Gao, et al. 1997).

Since colour is probably the first recognizable aspect of wine quality for red wine, winemakers employ various techniques like cold soak, thermovinification and extended maceration to optimise colour extraction and maintain pigment stability (Sacchi, et al. 2005). Cold soak treatment involves holding the must at low temperatures, usually around 10 to 15°C for several days before primary fermentation (des Gachons and Kennedy 2003)). Thermovinification involves heating the skins, usually to a temperature around 60°C for a short time, extracting pigments into the juice before being pressed and cooled down for fermentation ((Somers and Evans 1974)). These methods usually involve introduction of a winemaking step, sometimes paired with additions of favourable exogenous compounds. Post-fermentation extended maceration is one such method and it involves leaving the wine in contact with pomace, which consists of skins, seeds and grape solids for a period of time beyond the completion of primary alcoholic fermentation to achieve increased phenolic extraction (Peynaud 1984).

Commercial oenological tannins or exogenous tannins have been used increasingly over the past few decades due to their antioxidant activity (Hagerman, et al. 1998) and their ability to stabilise the colour of red wine (Bautista-Ortin, et al. 2005). This review focuses on the use of these two processes, namely post-fermentation maceration for extracting colour from grapes and using exogenous tannins in conjunction with this method to stabilise the colour and protect against oxidation.

1.2 Grape and Wine Phenolics

A wine's phenolic composition depends on the grape variety, vintage and winemaking conditions under which it is made (Bautista-Ortin, et al. 2005). Pinot noir, as a grape is known to have lower phenolic content compared to most cultivars and depending on the vintage, this content can either increase or decrease (Smart 1992). A good vintage would be classified by viticulturalists as one where the weather, water absorption and viticultural conditions are at their desirable levels, leading to a higher phenolic content. Although these can be adjusted to a certain extent, they are not in complete control of the viticulturalist. Thus, during a bad vintage, winemakers have to rely on using various winemaking techniques to modify the wine's phenolic composition and hence improve its quality.

In grapes, the phenolic compounds are mainly found in seed and skin tissues (Cheynier 2005)

During ripening, the phenolic composition of the various parts of the grapes changes as the phenolics located in seeds migrate from the vacuolar liquid to the thick walls of the epidermis where they are available for extraction during winemaking (Joscelyne

2009). After the grape has ripened, most of the anthocyanins are restricted to the grape skins and the tannins are contained in the seeds and the skins of grapes (Haslam 1998). The extraction and stabilization of these phenolics during vinification is important for determining final wine quality (Boulton, et al.).

1.2.1 Monomeric Flavonoids and Non-Flavonoids

Common polyphenols found in red grapes and young red wine can be divided into two main classes (Figure 1.1), the flavonoids (which include the coloured anthocyanins, extracted from the skin and colourless flavonols, mainly derived from seeds) and the non-flavonoids (which are largely lost during vinification and thus, are of little relevance to this study).

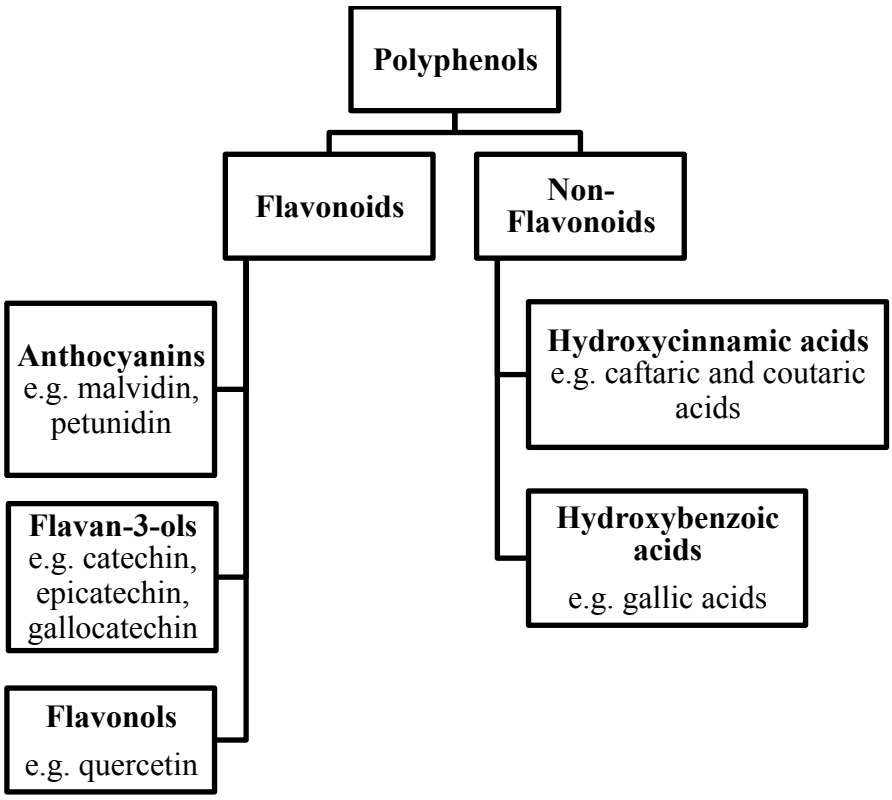


Figure 1.1: Polyphenols found in red wine (adapted from Birse, 2007).

Flavonoids, which include flavan-3-ols, anthocyanins and flavonols, share a basic C_6-C_3-C_6 structure, consisting of A and B aromatic rings joined by a three carbon chain closed by oxygen to form a heterocyclic ring, illustrated in Figure 1.2

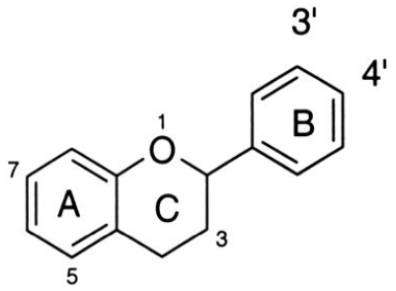
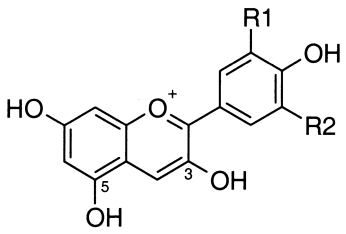


Figure 1.2: Flavonoid C_6-C_3-C_6 structure (Waterhouse 2002).

For understanding the chemistry behind the colour of red wine, flavonoids are of particular importance as they are responsible for most colour and ageing characteristics. Anthocyanins are naturally occurring, highly reactive class of water-soluble vacuolar pigments that transmit light in the red to blue range of the visible spectrum (Villiers, et al. 2004). Sulfur dioxide bleaching and pH are the major influences on the colour of anthocyanins and their colour ranges from red at low pH to blue at higher pH (Eiro and Heinonen 2002).

In *Vitis vinifera* grapes, the commonly found anthocyanidins, which lack a glycoside, are malvidin, delphinidin, petunidin, peonidin and cyanidin and these aglycones are linked to a glucose molecule at the 3-position of the C-ring (Cheynier, et al. 2006). The structures of these five anthocyaninidins are summarised in Figure 1.3.



R1, R2
H, OH; cyanidin
H, OCH_3; peonidin
OH, OH; delphinidin
OH, OCH_3; petunidin
OCH_3, OCH_3; malvidin

Figure 1.3: Structures of anthocyanin 3-glucosides commonly found in *Vitis vinifera* red grapes and wines (from Cheynier, Duenas-Paton et al., 2006).

In addition, the sugar molecule maybe acylated with acetic, *p*-coumaric or caffeic acid in some varieties to give 10 main forms of anthocyanins (Cheynier, et al. 2006). Pinot noir grapes contain all the above-mentioned anthocyanin glucosides but lack the acylated forms (Mazza and Miniati 1993), with malvidin-3-glucoside being the most prevalent form at 80% of all anthocyanins.

Red wine contains numerous combinations of anthocyanins, anthocyanins co-pigmented with other phenolic wine compounds (Gomez-Cordoves 2004), and anthocyanin derived pigments which may contribute to wine colour and thus need to be extracted from the pomace into the wine. These pigments can be monomeric, oligomeric or polymeric in nature and are more colour stable than free anthocyanins to the effects of change in pH (Bakker and Timberlake 1997). Thus, winemakers seek to modify free anthocyanins into their polymeric forms to make the wine more colour stable.

1.2.2 Proanthocyanidins and Tannins

Proanthocyanidins, also known as oligomeric procyanidins, leucoanthocyanins or condensed tannins, are polymeric chains of catechins, of molecular weights from 500 to 3000 g/mol that can cross-link proteins (Herderich and Smith 2005). Tannins are polymeric, high molecular weight structures consisting of multiple flavan-3-ol (or flavan-e, 4-diol) monomers (from 2 to more than 60 monomers) linked by C_4-C_8 or less commonly by C_4-C_6 bonds as illustrated in Figure 1.4 and 1.5 (Dennis 2010).

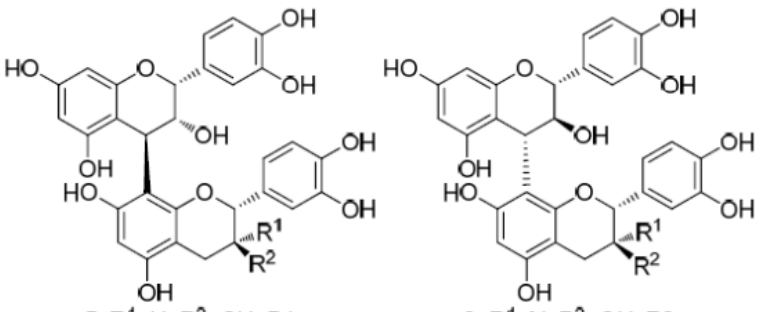


Figure 1.4: C_4-C_8 proanthocyanidin dimers B1-B4 (Dennis 2010).

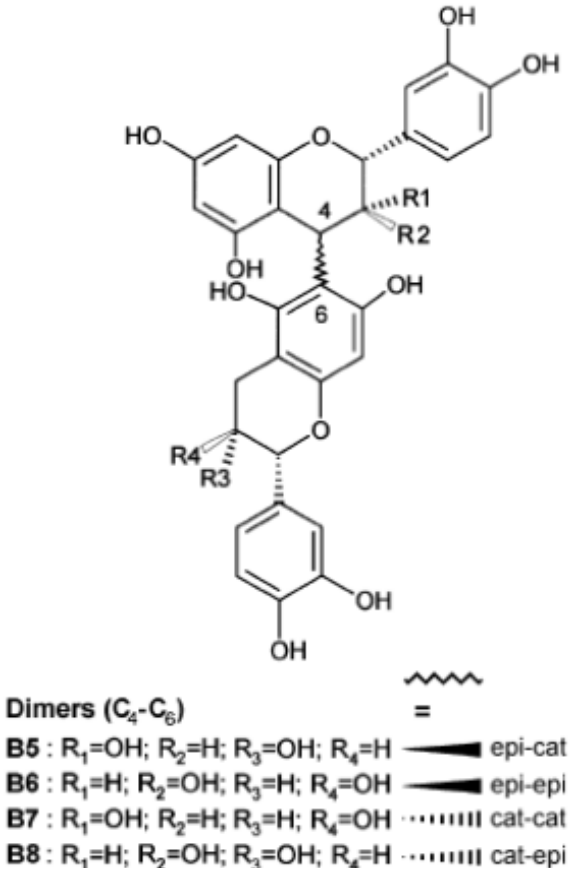


Figure 1.5: C_4-C_6 proanthocyanidin dimers B5-B8 (Mateus, et al. 2001)

Low pH catalyses the polymerisation of these compounds, sometimes complemented by cross-linking with acetaldehyde (Joscelyne 2009), and they are more stable than their monomeric constituents (Herderich and Smith 2005). They occur naturally in grape skins and seeds (Fougere-Rifot, et al. 1993), (Amrani Joutei, et al. 1994)), however, total tannin is significantly higher in seeds than the skins (Souquet, et al. 1996). Skin tannins also have a higher degree of polymerisation than seed tannins and were found to have a mean degree of polymerisation (mDP) of approximately from 27 to 42 monomeric units, while the seeds were found to have an mDP of approximately from 6 to 9 in Pinot noir (del Rio and Kennedy 2006). Though tannins are colourless, they have reactivity towards electron deficient sites and form red coloured pigmented polymers by acting as co-polymers with anthocyanin molecules, thereby contributing significantly to the colour of the wine (Cheynier, et al. 2006). These anthocyanin derived pigments can vary in colour from orange to blue, depending on composition (Cheynier, et al. 2006)and are only found in small concentrations in Pinot noir (Harbertson, et al. 2002), indicating they are mostly formed during vinification. These pigmented polymers contribute 70% to 90% to red wine colour (Schwarz, et al. 2003) and are more stable to bisulfite bleaching (Cheynier, et al. 2006) and effects of pH on colour expression (Gawel 1998). Moreover, it has also been found that these polymeric pigments contribute about 50% and 90% to the colour of one-year old and 10-year old wines respectively suggesting that polymeric wine pigments become the major source of colour with storage time (Somers 1971).

As red wine ages, the pigments increase in molecular weight due to polymerisation between anthocyanins and proanthocyanidins (Saucier, et al. 2004). Saucier *et al.,* (2004) also found that polymerisation between anthocyanins and tannins also enhances wine colour and protects it from oxidation. Polymerisation reactions are dependent on anthocyanidin composition and the ratio of proanthocyanidin and anthocyanin (Timberlake and Bridle 1967).

1.2.3 Exogenous Tannins

Enological tannins can be classified in to two groups, namely hydrolysable tannins, which originate from oak, and chestnut; and condensed tannins, which mostly originate from grapes. Tannin derived from quebracho is condensed tannin, which is also used as exogenous tannin. Hydrolysable tannins are polyhydric alcohols (typically D-glucose) and depending on if their hydroxyl groups have been esterified by gallic or hexahydroxydiphenic (HHDP) acid, are classified as gallotannins or ellagitannins respectively (Hagerman and Butler 1991). Grape tannins are usually considered superior to other tannins in oenological practice because their chemical structure is similar to those of red wine proanthocyanidins (Vivas, et al. 2004). Grape-derived tannins or condensed tannins are polymerised flavanol units consisting of catechin, epicatechin, epigallocatechin and epicatechin gallate (Prieur, et al. 1994).

Exogenous tannins are commercially added to wine to assist the precipitation of proteins, help increase aging potential, stabilise wine colour and limit the activity of laccase (Zoecklein 1991). The tannins used commercially are mostly ellagic tannins, proanthocyanidic tannins or catechins or a blend. To cover three main types of exogenous tannins used in the wine industry, the oenotannins used in this experiment

are ‘Quertannin’ which is derived from oak heartwood and is composed of ellagic tannins, ‘VR Supra’ which is composed of ellagic and proanthocyanidic tannins and ‘Tannin SR’ which is a combination of catechin tannins reinforced with grape seed tannins. Condensed tannins contribute to the astringency, browning and turbidity of wine (Ricardo-da-Silva, et al. 1993); (Robichaud and Noble 1990). The use of these tannins however, has been based mostly on the producing company’s claims for the above-mentioned effects and anecdotal evidence and has not all been scientifically researched. It is also important to understand the influence of the timing of addition and type of tannin employed as they are crucial to achieve the desired effects and deviations in these can have undesirable effects such as decrease in colour density (Kovac, et al. 1992,Kovac, et al. 1995).

1.3 Maceration in Red Winemaking

Winemakers employ a variety of techniques to extract phenolics from the skins and seeds of grapes. This primarily takes place by diffusion during the alcoholic fermentation of grape must. The leaching of phenolics, from grapes, into the must is defined as maceration and it is primarily used to increase the extraction of phenolic components into the wine (Amrani Joutei and Glories 1995); (Robinson and Harding 2006)). In red winemaking, maceration can be practiced before, during or after alcoholic fermentation. Pre-fermentation maceration, also referred to as ‘cold soak’, as it is carried out at a temperature lower than fermentation temperature, increases anthocyanin extraction, which improves wine colour intensity (Nemanic, et al. 2002), (Parenti, et al. 2004)). To encourage further extraction during ‘cold soak’, practices such as agitation of the must ((Ough and Amerine 1961); (des Gachons and Kennedy 2003)), cap management techniques such as plunging or pump-overs (Boulton, et al.),

and addition of pectolytic enzymes (Romero-Cascales, et al. 2005)are implemented. The application of these practices is influenced by many factors such as the volume of must, the quality of fruit and the equipment available. Moreover, each method implemented has a different effect on both, the rate of extraction and the type of phenolics extracted (Zoecklein 1991). The Pinot noir wines made with cold soak for the study conducted by Price, (Price, et al. 1995), however, were lower in anthocyanins (300 mg/L as compared to 500 mg/L during most vintages), colour intensity and flavanols and thus data from this study cannot be extrapolated to normal levels. Also, the study conducted by (Fischer 1997) as quoted in (Sacchi, et al. 2005), reported the rate of colour extraction measured as absorbance at 520 nm to be slower for Pinot noir at lower temperatures, which makes cold soak disadvantageous as time is often a constraint during vintage.

Increase in the length of contact time between pomace is said to increase phenolic extraction (Singleton and Trousdale 1992). Increase in phenolic extraction is also known to occur with increase in temperature or percentage of alcohol in the must (Gonzalez-Manzano, et al. 2004). An increase in temperature is said to increase the permeability of hypodermal cells in the skins and seeds of grapes, while certain phenolic compounds, particularly those from the seeds, become soluble at different ethanol concentrations (Gonzalez-Manzano, et al. 2004); (Sacchi, et al. 2005)). During alcoholic fermentation, polymeric seed proanthocyanidins are not extracted (Sun, et al. 1999) and this is probably due to the existence of linkages too strong to be broken by weak solvents present in the fermenting must (Amrani Joutei, et al. 1994). Since Pinot noir does not show increase in extraction of monomeric anthocyanins during pre-fermentation extended maceration (Parley, et al. 2001), the possibilities for

a more favourable outcome, considering the above factors may lay with post-fermentation extended maceration.

1.4 Post-fermentation Extended Maceration

1.4.1 Practices/Chemical implications

Many winemakers extend the opportunity to extract phenolic compounds from pomace beyond the period of alcoholic fermentation as this has been shown to increase the concentrations of gallic acid, catechin, epicatechin, and polymeric phenols ((Auw, et al. 1996); (Nemanic, et al. 2002); (Sipiora and Granda 1998). Wines produced in this way from Pinot noir have been found to have a high concentration of (+)-catechin and (-)-epicatechin and a higher (+)-catechin to (-)-epicatechin ratio than the control while having lower concentrations of myricetin and quercetin (Goldberg *et al.,* 2000). It is possible to extract from 15% to 30 % of the grape phenolics into wine (Ribereau-Gayon 1974). Longer maceration times have shown to increase the concentrations of these compounds (Sipiora and Granda 1998)along with increase in colour density (Scudamore-Smith, et al. 1990).

Wine colour density immediately after pressing was greater in wines made with post-fermentation extended maceration (Gomez-Plaza, et al. 2001). However, Scudamore-Smith, Hooper et al*.,* (1990) found that these differences were insignificant after 400 days post fermentation suggesting the need to retain the increase in colour density. Wine colour hue - the proportion of brown colour relative to red colour (Somers and Evans 1974) was also lower in post fermentation extended maceration wines up to 12 months after processing (Auw, et al. 1996); (Gomez-Plaza, et al. 2001)) possibly due

to earlier and increased production of stable pigmented polymers with absorbance maxima more towards 420 nm.

Anthocyanins self-associate when in high concentrations and form vertical stacks, protecting the anthocyanidins from hydrating, which results in an increase in colour (Hoshino 1992) leading to the perceived increase in colour intensity. Mazza and Brouillard (Mazza and Brouillard 1990) however found that these anthocyanin stacks persist only until the ethanol content of the wine reaches a level that interferes with the hydrogen bonding of water and thus this colour intensity decreases after alcoholic fermentation. Somers and Evans (1979) found out that the extent of this colour loss is dependent on the particular juice or wine, pH, anthocyanin content and alcohol level.

During and after alcoholic fermentation, there are increases in the concentrations of alcohol, carbon dioxide and sulfur dioxide, which together with the heat generated during fermentation, increase the permeability of skin and seed cells and other membranes, which leads to increased phenolic extraction (Sacchi, Bisson et al., 2005). (Berg and Akiyoshi 1956), (Singleton and Draper 1964) and (Oszmianski, et al. 1986) also suggested that extraction of tannins increases during post-fermentation extended maceration. In Pinot noir, greater amounts of polymeric phenols were extracted with longer pomace contact (Kantz and Singleton 1991); (Singleton and Trousdale 1992)) and this is attributed to the formation of tannin-anthocyanin adducts which may be acetaldehyde bridged polymers (Thorngate Iii and Singleton 1994) involving polymeric phenols and anthocyanins (Dallas, et al. 1996). However, wines made from Pinot noir were not found to contain the tannin-anthocyanin adduct until a year of aging, possibly due to low presence of tannin (Thorngate Iii 1993).

Sacchi et al., (2005) also suggested that since anthocyanin levels have been found to drop so early in fermentation, increase in polymeric pigments could possibly be due to more increased extraction of tannins than of anthocyanins. Thus, presence of adequate amount of tannins early in fermentation could possibly be crucial to trap anthocyanins to generate more polymeric pigment in the wine.

1.5 Experimental Investigation

In an effort to make better Pinot noir, winemakers and use various techniques such as maceration and agitation to achieve a wine with increased colour stability and longevity ((Drouhin 1991); (Ewart and Gawel 1993)). Investigations by (Parley, et al. 2001), concluded that pre-fermentation extended maceration was more suitable for winemakers who wanted to increase the aromatic and flavour profile expressed by Pinot noir, whereas, post-fermentation extended maceration extracted more anthocyanins and tannins, which upon polymerisation, was believed to increase colour stability.

(Mazza and Ford 2005) conducted studies of the effect of one or three weeks of post-fermentation extended maceration on Pinot noir wines which were subsequently assessed for colour, phenolic composition and sensory analysis for bitterness and astringency. Anthocyanin, tannin and pigmented polymer concentrations up to 140 days after fruit processing were analysed by HPLC. Mazza and Ford, (2005) conducted an analysis of (+)-catechin and (-)-epicatechin showed that when compared to the control, the 1 week post fermentation extended maceration treatment had double the concentration and when compared to the 3 week post-fermentation extended maceration treatment, had four times the concentration of (+)-catechin and

(-)-epicatechin. Gel permeation Chromatography (GPC) was employed to characterise wine tannin size distribution between treatments but due the absence of standards only relative comparisons between treatment samples could be made. The replications of each treatment were discrete fermentations from the initial parcel of fruit, which may have introduced variables from must composition or fermentation dynamics.

(Neves, et al.) conducted a study that investigated the effects of addition of commercial grape seed tannins on chromatic characteristics, phenolic composition and antioxidant properties. Neves, Spranger et al., (2010) used GSE100 and GSE300, which were grape seed tannins and determined their phenolic composition. They also used two different maceration times, which corresponded respectively to wines rich and poor in polyphenols. The results, which were analysed for phenolic composition, chromatic characteristics and antioxidant activity respectively, showed that the addition of grape seed tannins increased the colour intensity only in the wines poor in polyphenols. Importantly, (Neves, Spranger et al., (2010) found that tannins added after alcoholic fermentation had a better effect on phenolic composition of red wine than tannins added before alcoholic fermentation. The scope of this study however, was limited to grape seed tannin and so cannot be extrapolated to hydrolysable tannins.

Mazza and Ford, (2005), in their review of previous studies into post fermentation extended maceration suggested that experiments conducted at a commercial scale frequently lacked scientific rigour. Specifically, the lack of standard addition rates and standard lengths of post-fermentation extended maceration has made it difficult to compare findings. Also, studies such as the ones conducted by Parley et al., (2001)

and (Goldsworthy 1993) showed that pre-fermentation extended maceration is not particularly effective for increasing colour intensity, and also the colour extracted needs to be stabilised before it precipitates due to increased size and hydrophobicity of polymeric pigments. This has led to interest in using exogenous tannins during post-fermentation extended maceration. No research has been published regarding the complete effect of exogenous tannins during post-fermentation extended maceration on increasing the colour stability and longevity of Pinot noir derived wines and this has led to potential gaps in our understanding.

1.6 Analytical Methods

1.6.1 Spectral Analysis

Red wine is a complex solution and although it is straightforward to measure its colour using spectral techniques, the composition of wine colour is rather difficult to determine due to the effects like polymerisation, which are reviewed in the preceding sections. Somers and Evans, (1979) developed a set of spectral measures on light absorbance at 420 and 520 nm as anthocyanins in wine have an absorbance maximum in the range 520-535 nm, and a minimum at about 420 nm. While newer methods of colour analysis, such as CIELab (Birse 2007) are being adopted by many wine researchers, the Somers methods are still used as they provide simple and reproducible results for the measurement of wine colour (Iland, et al. 2000). These methods will provide results that can be compared between treatments to conclude which tannin increases colour intensity.

1.6.2 Methyl Cellulose Precipitable Tannin (MCPT) Assay

Tannins can be quantified by a number of analytical methods (Herderich and Smith, 2005). Many of these however, require a lot of work, are expensive or are not suitable for a large number of samples (Mercurio, et al. 2007). The MCPT assay is a simple method to determine tannin concentration (Sarneckis, et al. 2006). It uses the tendency of tannin to bind and precipitate to polymers such as methyl cellulose (Mercurio, et al. 2007). In this assay, a control sample (with no methyl cellulose added) is compared to the unknown sample, in which the formation of an insoluble complex followed by precipitation causes a decrease in the absorbance of the wine at 280nm (Mercurio, et al. 2007). This allows for determination of the concentration of tannin in the wine.

1.6.3 High Performance Liquid Chromatography (HPLC) analysis

Reverse-phase HPLC technique is a quick and reliable method for quantifying anthocyanins, flavan-3-ols and simple proanthocyanidins (Mazza, et al. 1999); (Peng, et al. 2002). This method is mostly used to analyse simple, monomeric wine phenolics; Peng, Iland et al., (2002) showed that the pigmented polymer content of red wines may also be analysed by this method but only as an unidentified collection of molecules, represented by a 'hump' in the HPLC trace. (Kennedy and Jones 2001) found that the sub-unit composition and mean degree of polymerisation (mDP) of proanthocyanidins can be assessed through acid catalysed cleavage in the presence of an excess of a nucleophile such as phloroglucinol. Benzyl mercaptan can also be used as a reagent but phloroglucinol is preferred as it is odourless and there is more selectivity in the formation of 3, 4-t*rans* adducts from 2, 3-*trans* flavan-3-ol extension subunits (Hemingway, 1989). Electrophilic intermediates are released by the cleavage of proanthocyanidins which form analysable adducts with a nucleophilic reagent like phloroglucinol (Kennedy and Jones, 2001). RP-HPLC analysis gives information on

proanthocyanidin constituents as well as the ratio of terminal units to extension units, which allows the mDP to be calculated. The assay can also be used to differentiate the origin of berry-derived proanthocyanidins from exogenous tannins, due to difference in compositions and mean degrees of polymerisation ((Gawel 1998),(Kennedy and Jones 2001), (Kennedy and Peyrot des Gachons 2003).

A limitation of the phloroglucinol assay is its inability to provide information on size and weight distribution of proanthocyanidins in wine due to acid induced cleavage of the polymer and it cannot identify specific subunit sequences but only provide a mean proanthocyanidin size (Herderich and Smith, 2005). mDP may be then determined by analysing the isolated proanthocyanidins by the phloroglucinol assay (Kennedy and Taylor 2003). To calculate the apparent mDP, Kennedy and Jones, (2001) divided the sum of all subunits (flavan-3-monomer and phloroglucinol adduct, in moles) by the sum of all flavan-3-ol monomers (in moles).

1.7 Conclusion and Proposed Research

There is little scientific research and industry significant information indicating that use of exogenous tannins affects the phenolic composition and thus the colour stability of the wine. Further, there is limited research on the effects of post-fermentation extended maceration on the extraction of phenolics into the wine.

However, there is limited research investigating the effects of the use of exogenous tannins during post-fermentation extended maceration on colour stability. Furthermore, these results have not always been positive and have been also found to reduce wine colour if not applied correctly. There is also a lack of definitive research of the impact of these treatments on Pinot noir, which suggests further investigation of the consequences.

This study aims to compare the effects of the addition of three different exogenous tannins, ‘Quertannin’, ‘VR Supra’ and ‘Tannin SR’ during three weeks of post fermentation extended maceration to help identify their contribution towards the phenolic composition of the wine and hence, its colour stability. Along with the treatment wines, control wine, made with and without post-fermentation extended maceration will be included. The rate of addition of these tannins will be as recommended by the suppliers, as they show optimum results at those levels, so comparisons between them to observe which product increases colour stability the most would be valuable to winemakers who are concerned about economic costs and logistic pressures.

In this study, phenolic composition and concentration will be compared during various stages of treatment as well as between different treatments to identify distinct chemical markers for the outcome of adding the above mentioned tannins during post-fermentation extended maceration. Any significant differences and merits found between treatments may provide winemakers with information that could help to make purchase decisions between the range of tannins available, which in turn could help to optimise the effects of post-fermentation extended maceration on the wine.

2. Materials and methods

2.1 Introduction

Previous work by Scudamore-Smith et al. (1990), Auw et al. (1996), Mazza (2005) and Joscelyne (2009) showed the effects of post-fermentation extended maceration on colour expression. These studies however, did not examine the effect of the addition of different types of exogenous tannins during post-fermentation extended maceration. Sims and Morris (1986) showed that the addition of condensed tannins to wine increased the total phenol concentration of the wine. The effect of the addition of these tannins during post-fermentation extended maceration on colour expression was not studied and so a correlation between increase in phenolic content and colour stability was not established. Zoecklein (1991) also suggested that addition of tannins could help to stabilise colour compounds. However, the aforementioned properties of commercial exogenous tannins have not been scientifically researched for their effects on colour expression of Pinot noir when added during post-fermentation extended maceration. For this project, a single fruit source was used to produce the wines with appropriate replication to allow comparisons of colour parameters, measured by the methods described below.

2.2 Experimental Winemaking

2.2.1 Winemaking Protocol

Approximately 840kg of *Vitis vinifera* cv. Pinot noir from the Oakbank Estate Vineyard Adelaide Hills, harvested on 13th April 2010 was used to produce the wines for this study at the Hickinbotham Roseworthy Wine Science Laboratory (HRWSL, University of Adelaide, Waite campus). Total soluble solids (TSS) were expressed as

Baumé, pH and titrable acidity (TA) was analysed for a sample of fruit prior to crushing.

The total batch of fruit was destemmed and crushed using a Miller crusher/destemmer unit. After crushing (sulfur dioxide added in the form of PMS: 30 mg/L) and de-stemming into a 1 tonne Potter-type fermenter fitted with a brine cooling jacket fixed pump-over irrigator. The must was inoculated with inoculation with *Saccharomyces cerevisiae* yeast strain Maurivin PDM strain of wine yeast at the rate of 30g/hL. The yeast was rehydrated 1 in 10 parts water at 38°C using174 g of Dynastart (Laffort) according to manufacturer's instructions. A decrease in the Baumé of the must was noted 12 hours after inoculation, which indicated a rapid onset of fermentation.

During alcoholic fermentation, the wine was pumped over twice daily for 5 minutes in the morning and afternoon. At the onset of fermentation, the temperature was at 14 -15 °C which was very cold so the cooling was turned off. When the must reached 10.3 °Be the cooling in the winery failed, which caused the Baumé to drop to 7.4 °Be overnight. Since the cooling was not working for the next day as well, the Baumé further dropped to 2.7 °Be within 12 hours.

At 96 hours post-inoculation, when the must was fermented to 1°Be (determined by hydrometry) the ferment was divided into 15 × 47 kg replicates. This was achieved by collecting the liquid and solid portion separately in pre-weighed vessels. The liquid was drained from the fermenter via gravity for 1.5 hours. Seeds and skins (the solid portion) were collected and weighed separately. A solids to liquid ratio was determined for the original fermentation which allowed the reconstitution of 15 x 47 kg fermentations in 60 L plastic drums. Each replicate consisted of 2.1 kg of seeds; 11.6 kg of skins and 33.3 kg of wine and this was achieved by draining the liquid

from the fermenter and collecting the seeds with a strainer, weighing the components and dividing the liquid, seeds and skins into appropriate proportion. This was done to minimize differences between replicates due to the variable effects of primary fermentation, and to decrease the number of ferments that needed to be managed over the initial stages.

The 15 replicates were then randomly allocated to one of the five treatment groups. The five treatment groups included a control (without post-fermentation extended maceration), a control (with post- fermentation extended maceration) and three treatments with Laffort Quercetin Sweet tannin, VR Supra tannin and Tannin SR respectively, all of which were subjected to post fermentation extended maceration after the tannins were added at the rate of 100mg/L hydrated 1 in 5 parts of wine as per the manufacturer's instructions. The control without the post-fermentation extended maceration treatment was pressed the day after the ferment was divided, using a Diemme press (Lugo, Italy) which had a 12 minute pressing cycle, following overnight storage at 15 °C. Samples of the wine before and after pressing were collected and frozen. The control wines were racked off gross fermentation lees five days after pressing into 30 L plastic drums with lids, without ullage.

The treatments were then stored in a 20 °C temperature controlled room at the HRWSL for three weeks after being inoculated with Lalvin VP41 strain of lactic acid bacteria *Oenococcus oeni* for malolactic fermentation (MLF). The wines were stored in 60 L plastic containers with lids and the ullages were gassed with dry ice. During storage for post fermentation extended maceration, the wines were gently plunged at three-day intervals. The wines underwent three weeks of post-fermentation extended

maceration before being pressed and minimum ullage was maintained to prevent oxidation.

The treatment wines completed MLF 7 days after pressing and 100 mg/L SO_2 as PMS was added. At this point in time, every replicate was about 30 L in volume, with additional topping wine, which was the leftover control wine (approx. 5 L) for each treatment. After the final racking, 9 L (12 bottles) of each treatment was bottled under screwcap in 750 mL Bordeaux bottles.

2.2.2 Winemaking analysis

Winemaking analysis was conducted according to methods described in Iland et al., (2000). Sample pH was determined using a pH meter (Radiometer Analytical) TA was determined by titration with 0.1M NaOH. Total soluble solids through fermentation and EM were determined by refractometry (fruit samples), and hydrometry. The end of fermentation was determined by the rebelein method and MLF monitored by thin-layer chromatography. Alcohol content was measured by ebulliometry. A combined aspiration still was used to determine free and total SO_2 and volatile acidity levels.

2.3 Analytical Methods

2.3.1 Spectral Measures

Wine colour was measured by the Somers method (Iland et al., 2000) using a Multiskan Spectrum spectrophotometer (Thermo, Finland), with a computer running SkanIt Software 2.2. A Greiner UV Star 370 µL 96 well disposable plate was used, which has an optical window down to 200 nm. The spectrophotometer had an in-built

path correction function that normalised the pathlength of each well to 10 mm and corrected any variation in sample volume.

The reagents consisted of Buffer 1 (model wine, 0.5 w/v tartaric acid in 12% v/v ethanol adjust to pH 3.4 with 5M NaOH), 0.375 % w/v sodium metabisulfite, 0.1% v/v acetaldehyde and 1M HCl. First, the absorbance of the wine in its original state (with regard to wine pH and SO_2 concentration) was recorded at 420 nm (yellow/orange pigments) and 520 nm (red pigments), and from these values, the parameters wine colour density and wine colour hue were calculated. The second reading was taken after the addition of excess SO_2 allowing for the measurement of colour (A520) resulting from the SO_2-resistant pigments present in wine. Third, the original wine was treated with excess acetaldehyde, which permits the estimation of coloured anthocyanins at wine pH. Finally, the wine was diluted with 1M hydrochloric acid, lowering the pH and converting all anthocyanins and many other pigments into their coloured forms. The acidified solution was then monitored at A520 and A280 to give an indication of the concentration of total red pigments and total phenolics respectively.

The main modifications to the original method used in Mercurio et al., (2007), were the standardisation of the wine pH to pH 3.4 and the alcohol concentration to 12% v/v using a buffer solution prior to the initial analysis.

Measurements were made on the samples for all the wines before and after the steps of tannin addition, pressing, sulfur dioxide addition and bottling. Samples were centrifuged in 10 mL tubes for 15 minutes at 3,500 rpm prior to analysis.

Calculations for Modified Somers Colour Parameters (Iland, Ewart et al,. 2000)

Total phenolics (au)	$(A_{280HCl} \times 50) - 4$
Sulfur dioxide resistant pigments	$A_{520sulfite} \times 10$
Wine colour density (au)	$(A_{420buffer1} + A_{520buffer1}) \times 10$
Modified wine colour density (au)	$(A_{420acetal} + A_{520acetal}) \times 10$
Wine colour hue (no units)	$A_{420buffer1} / A_{520buffer1}$
Modified wine colour hue (no units)	$A_{520acetal} / A_{420acetal}$
Degree of red pigment colouration (%)	$\{[(A_{520buffer1} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$
Modified degree of red pigment colouration (%)	$\{[(A_{520acetal} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$
Total Anthocyanins (mg/L)	$20 \times [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]$

2.3.2 Analysis for the presence of laccase

Laccase is a strongly oxidative enzyme produced when the mould *Botrytis cinerea* infects grapes and is particularly active on anthocyanins (Iland, Ewart et al., 2000). A red wine, with residual activity can brown rapidly if exposed to air (Iland, Ewart et al., 2000). For this experiment, the presence of laccase was tested using a bench-top method as instructed by verbal communication from Dr Paul Grbin.

For this method, bottles for the replicates for the Control wines and the Quertannin wines were opened and their wine colour density was measured. These wines were then exposed to oxidation by leaving them in open beakers on the Table overnight. The wine colour density was measured on the second day and the measures were plotted on a graph to observe any differences. A significant difference in their wine colour densities would indicate the presence of laccase.

There is also a more accurate method to detect the presence of laccase called the syringaldazine method, which works on the rate at which the laccase oxidizes syringaldazine to a purple coloured oxidation product (Iland, Ewart et al., 2000). I was decided that if the initial bench-top method showed a significant difference, the more accurate method would then be used to confirm the presence of laccase.

2.3.3 Statistical Analysis

Data were analysed using Jump version 5.0, and statistical differences between treatments were determined using one-way Anova method of comparison with 95% confidence intervals, unless otherwise indicated.

3. Results

3.1 Initial must analysis

Table 3.1.1: Initial must analysis prior to inoculation.

Date	13/04/2011
TSS (°Baume)	11.8
pH	3.5
TA (g/L H_2T)	8.5
Free and total SO_2 (g/L)	0.0
Temperature (°C)	0.0
Condition of the fruit	Diseased (*B. Cinerea*) and damaged

The fruit was delivered from the Adelaide Hills at 0.0 °C. The Total Soluble Solids (TSS) were lower in value (Table 3.1.1) than the traditional harvest criteria of 13-14 °Be. pH and TA levels were within acceptable parameters. There was no free or total sulfur dioxide detected upon fruit receival so 80 mg/L of sulfur dioxide in the form of potassium metabisulfite was added to offer protection against oxidation. The fruit was infected with *Botrytis cinerea* and also a large percentage of berries were split which resulted in browning and loss of juice.

3.2 Fermentation kinetics

Temperature and total soluble solids were used to monitor fermentation. The total duration of the fermentation was about 96 hours, at which the TSS was recorded as 0 °Be, as observed in Figure 3.1. The duration of the fermentation was short compared to the traditional period of two weeks as the cooling at HRWSL stopped working on the third day, which resulted in an increase in temperature to 26°C, which resulted in rapid fermentation.

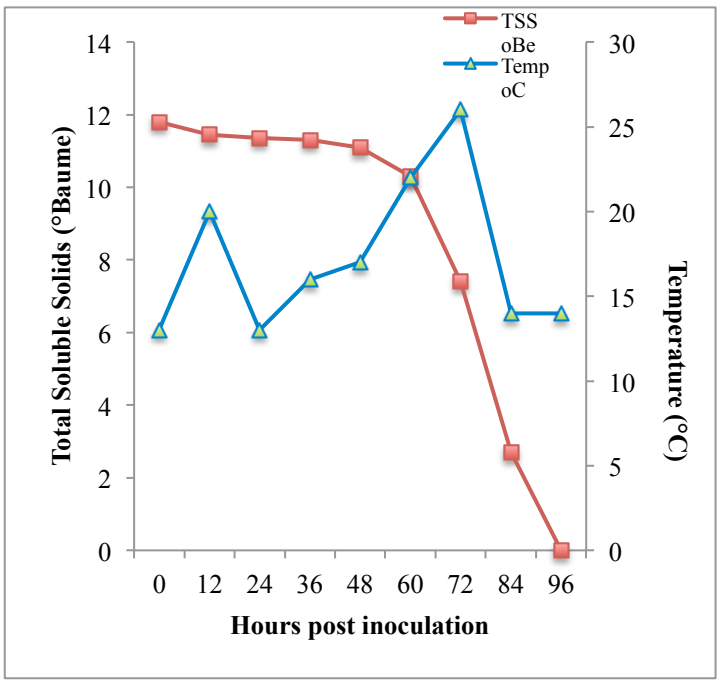


Figure 3.1 Fermentation curve of the initial batch up to 96 hours post inoculation.

3.3 Basic wine analysis

One day prior to bottling, the wines were analysed for the basic parameters of pH, TA, free SO_2, VA and percentage of alcohol (Table 3.1.2). Significant differences were found for pH, Free SO_2 and VA. The pH of all the wines was within the range considered stable for bottling (Table 3.1.2). Therefore no additions of tartaric acid were made prior to bottling. Contradictory to observations made by Mazza, (2005), post fermentation extended maceration did not result in an increase in pH. Table 3.1.2 shows that addition of exogenous tannins had no effect on the pH of the wines. The highest pH was exhibited by the VR Supra treatment, while the Quertannin treatment had the lowest pH. The Control EM treatment had the lowest value for free SO_2, while VR Supra had the highest value. There was no trend of a significant difference between the control wines and the tannin treatment wines. The Control EM wine also had the highest value for VA. The control wine had the lowest value followed by

Quertannin treatment wines. The treatments VR Supra and tannin SR also showed higher levels of VA. There were no significant differences between treatments for TA and percentage of alcohol.

Table 3.1.2: Winemaking analysis conducted prior to bottling.

Treatment	pH	TA (g/L H_2T)	Free SO_2 (mg/L)	VA (g/L)	Alcohol (%v/v)
Control	3.42 b	6.4 a	34.2 b	0.32 c	13.8 a
Control EM	3.41 bc	6.3 a	32.4 d	0.44 a	13.9 a
Quertannin	3.40 c	6.4 a	33.7 bc	0.35 c	13.8 a
VR Supra	3.44 a	6.3 a	34.9 a	0.41 b	13.8 a
Tannin SR	3.42 b	6.3 a	33.3 c	0.43 b	13.9 a

Mean values with the same column designated with a different letter differ significantly.

The wines were also analysed two months post bottling to observe if there was any difference in parameters between treatments and if these were consistent with the values obtained prior to bottling. The values of free SO_2 have dropped consistently over all the treatments (Table 3.1.3). The pH of the wines has increased for all treatments. The values for VA were highest for Control EM treatment, which is consistent with the results prior to bottling. The values for VA also had increased considerably 2 months post-bottling for the control EM treatment as compared to the others. The percentage of alcohol and TA show no significant difference between treatments two months post-bottling.

Table 3.1.3: Winemaking analysis conducted 2 months post-bottling.

Treatment	pH	TA (g/L H_2T)	Free SO_2 (mg/L)	VA (g/L)	Alcohol (%v/v)
Control	3.43 b	6.4 a	33.2 ab	0.33 c	13.8 a
Control EM	3.46 bc	6.2 a	30.6 c	0.48 a	13.9 a
Quertannin	3.42 c	6.4 a	31.9 a	0.35 c	13.8 a
VR Supra	3.43 a	6.3 a	31.8 a	0.42 b	13.8 a
Tannin SR	3.42 b	6.2 a	30.3 bc	0.41 b	13.9 a

Mean values with the same column designated with a different letter differ significantly.

3.4 Spectral analysis

3.4.1 Total phenolics

Total phenolics give a measure of the concentration of all the phenolic material present in the wine (Iland et al., 2000). The formula used to obtain these values is mentioned in the methods and materials section. The subtraction of the value of 4 allows for absorbance of non-phenolic material (Iland et al., 2000).

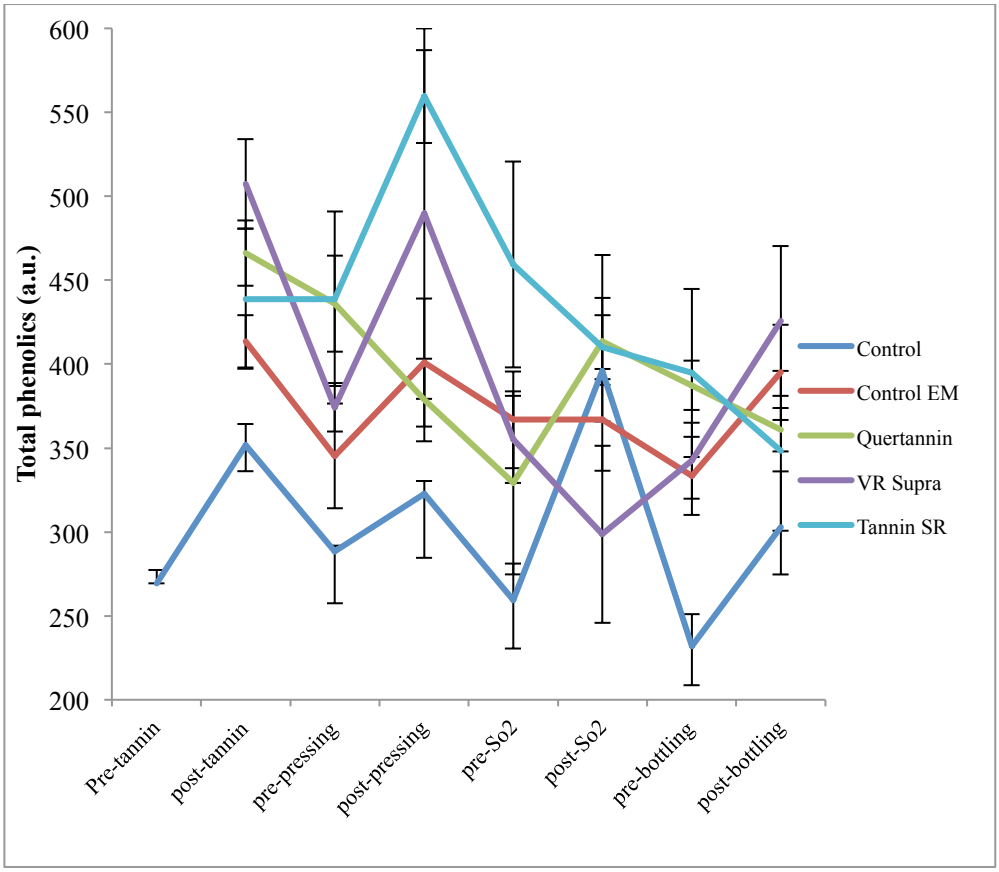


Figure 3.2: Spectrophotometric analysis of total phenolics in fermentation and wine samples expressed in a.u. Total phenolics were determined as (A_{280HCl} × 50) – 4. Error bars represent ± standard error (n=3) divided by the square root of n.

Figure 3.2 shows that total phenolics of all the treatments increased post tannin addition. Control EM wines also showed an increase in total phenolics when compared to the control wine. The highest increase in total phenolics post addition was observed for the VR Supra treatment. Pressing decreased the total phenolics for all the wines, particularly for VR Supra. Post pressing, there was a consistent increase in total phenolics for all the wines except quertannin, with Tannin SR showing the highest increase. SO_2 addition resulted in a sharp increase in the total phenolics for the control wine, whereas VR Supra and Tannin SR showed a steady decrease. Post bottling, we can observe, that tannin addition and post-fermentation extended maceration has resulted in a significant increase in total phenolics for VR Supra treatment (Figure 3.3).

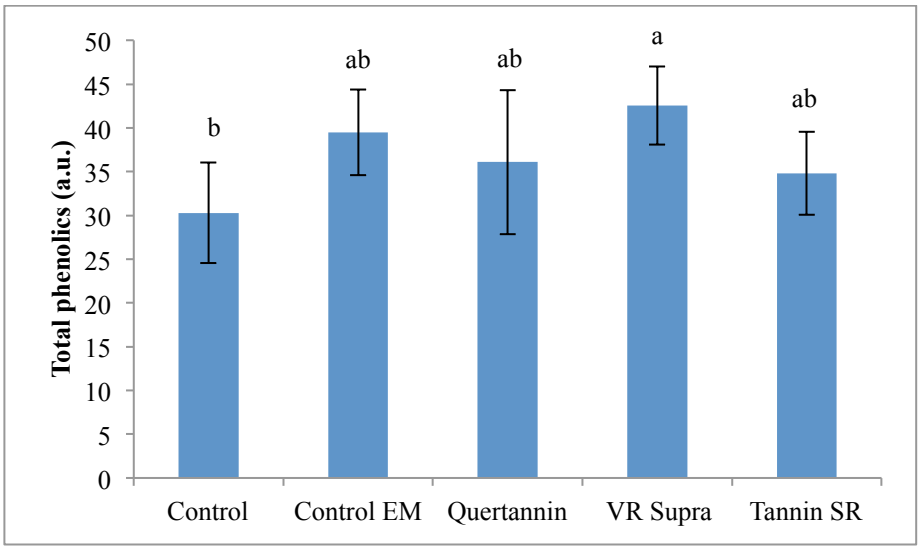


Figure 3.3: Spectrophotometric analysis of Total phenolics of treatments post-bottling. Total phenolics were determined as (A_{280HCl} × 50) – 4. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

3.4.2 Sulfur dioxide resistant pigments

Sulfur dioxide pigments give an estimate of the concentration of red coloured pigments that are resistant to decolouration (bleaching) on the addition of sulfur dioxide (Iland, Ewart et al. 2000). Figure 3.4 shows that the addition of tannin and post-fermentation extended maceration has significantly increased the amount of SO_2 resistant pigments in the wines. There was also a significant loss of SO_2 resistant pigments during post-fermentation extended maceration for the all the treatment wines except Tannin SR. The control wines also showed a considerable drop in SO_2 resistant pigments. The SO_2 resistant pigments were almost restored to their post-tannin levels after pressing. There was a steady drop in SO_2 resistant pigments post-pressing till the wines were bottled. Quertannin treatment wines showed a small increase in SO_2 resistant pigments post bottling. Figure 3.5 shows that there was no significant difference between the wines for SO_2 resistant pigments post-bottling.

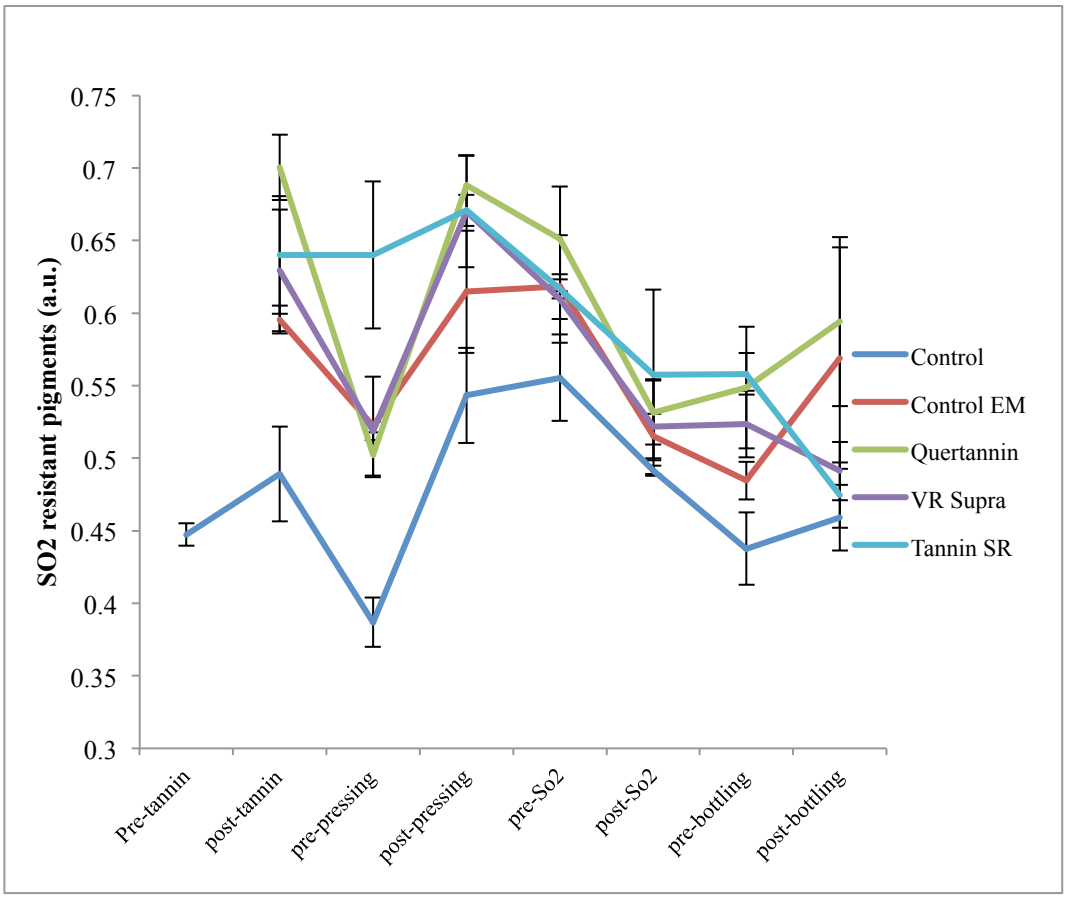


Figure 3.4: Spectrophotometric analysis of estimate of SO_2 resistant pigments in fermentation and wine samples expressed in a.u. SO_2 resistant pigments were determined as $A_{520sulfite}$ × 10. Error bars represent ± standard error (n=3) divided by the square root of n.

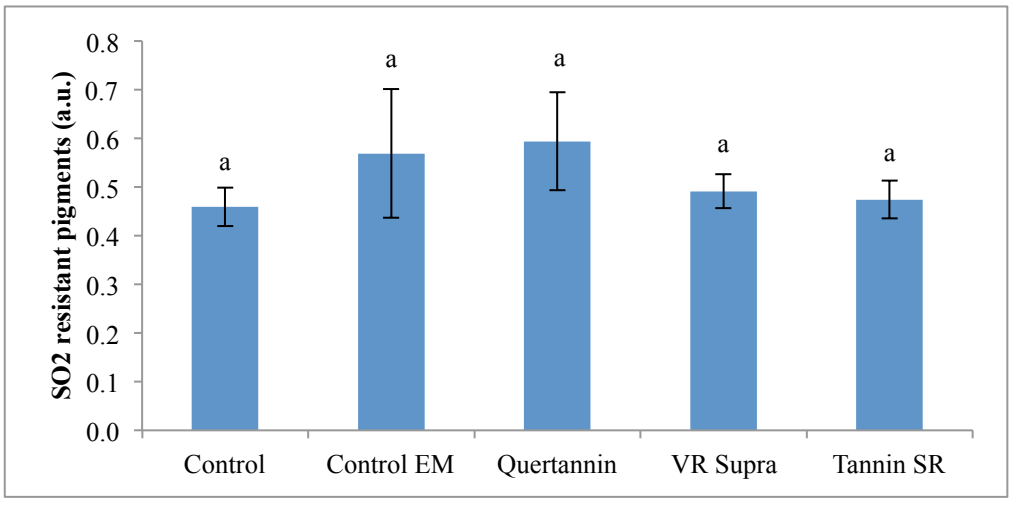


Figure 3.5: Spectrophotometric analysis of Estimate of SO_2 resistant pigments post-bottling. SO_2 resistant pigments were determined as $A_{520sulfite}$ × 10. Error bars

represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

3.4.3 Wine colour density

Wine colour density describes the intensity of the colour and is the sum of the values of absorbance for red coloured pigments and yellow/brown coloured pigments (Iland et al., 2000).

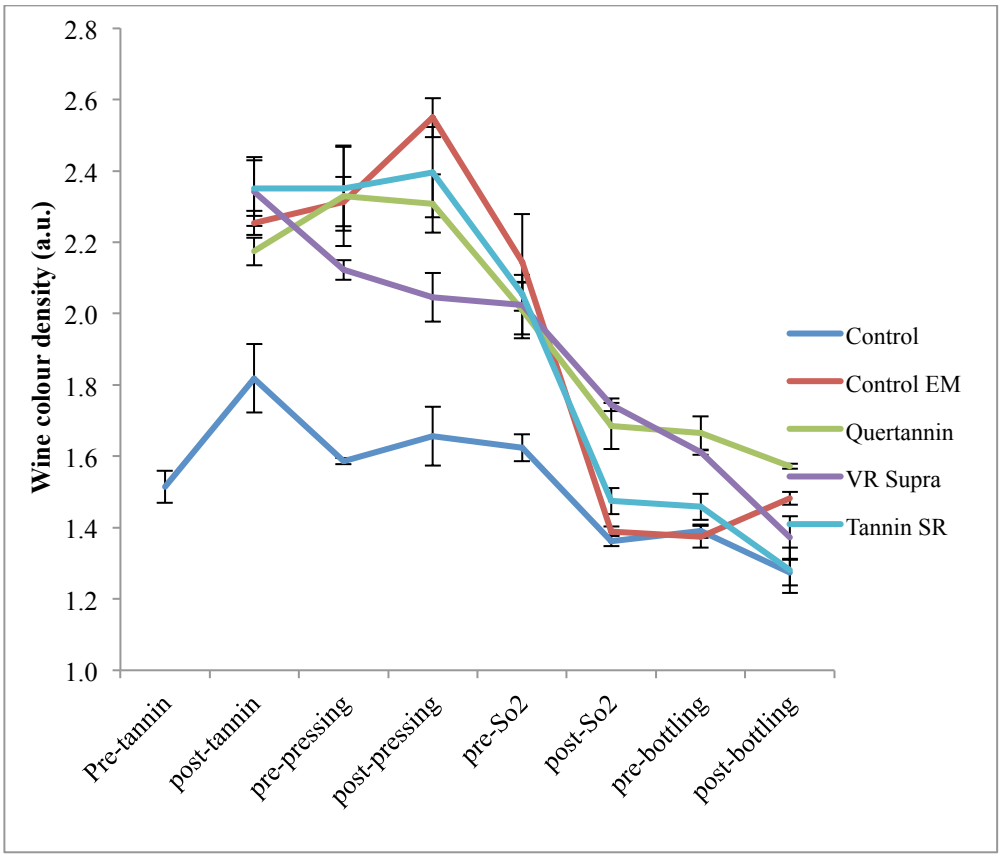


Figure 3.6: Spectrophotometric analysis of wine colour density in fermentation and wine samples expressed in a.u. Wine colour density was determined as ($A_{420buffer1}$ + $A_{520buffer1}$) × 10. Error bars represent ± standard error (n=3) divided by the square root of n.

Figure 3.6 illustrates that the wine colour density increased after the addition of exogenous tannins. The Control EM treatment also showed an initial increase in wine colour density. The wine colour density was observed to decrease for all the wines

post-pressing. There was a significant drop in wine colour density between pressing and addition of sulfur dioxide, particularly for the Control EM, Tannin SR and quertannin treatments. All the treatments showed a further decrease in wine colour density towards bottling except the Control EM treatment, which showed a slight increase.

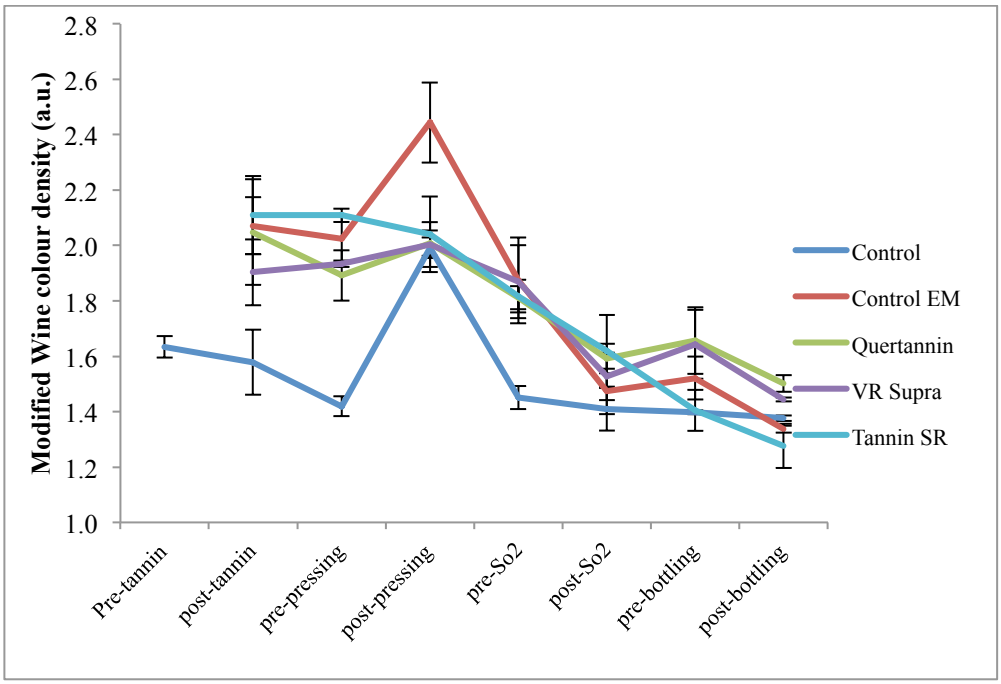


Figure 3.7: Spectrophotometric analysis of modified wine colour density in fermentation and wine samples expressed in a.u. Modified wine colour density was determined as ($A_{420acetal}$ + $A_{520acetal}$) × 10. Error bars represent ± standard error (n=3) divided by the square root of n.

The modified wine colour density was also measured for all the samples as comparing wines with different pH and sulfur dioxide values can often be confusing. Figure 3.7 shows that most of the treatments behaved quite similarly except for the control treatment which showed an increase in modified wine colour density from pre-pressing, where it was 1.41 a.u. to post-pressing stage, where it was 1.99 a.u.

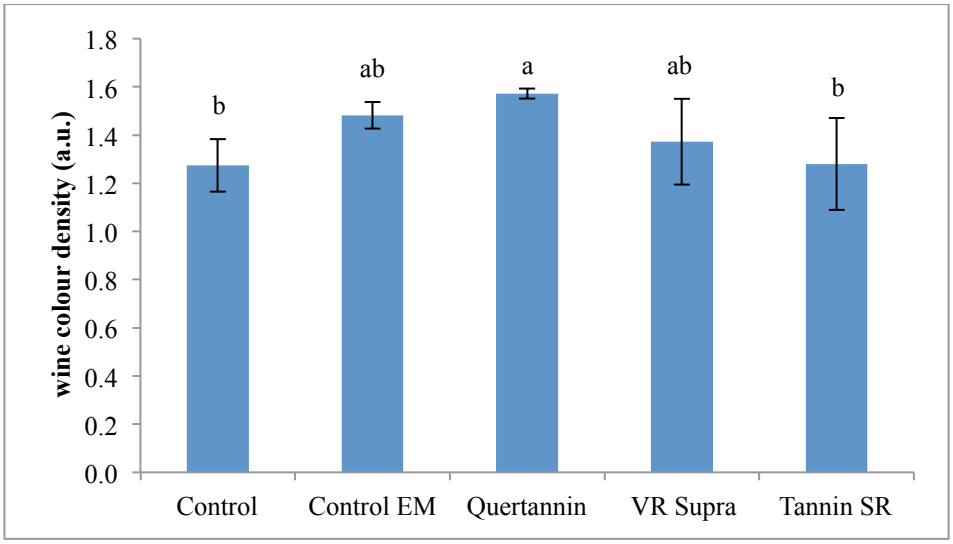


Figure 3.8: Spectrophotometric analysis of wine colour density post-bottling (a.u.). Wine colour density was determined as ($A_{420buffer1}$ + $A_{520buffer1}$) × 10. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

Figure 3.8 shows that Quertannin showed the highest value for wine colour density among the treatments. The Control EM also showed a slight increase in wine colour density when compared to the control wines. There was no significant increase in wine colour density for the Tannin SR treatment when compared to the control.

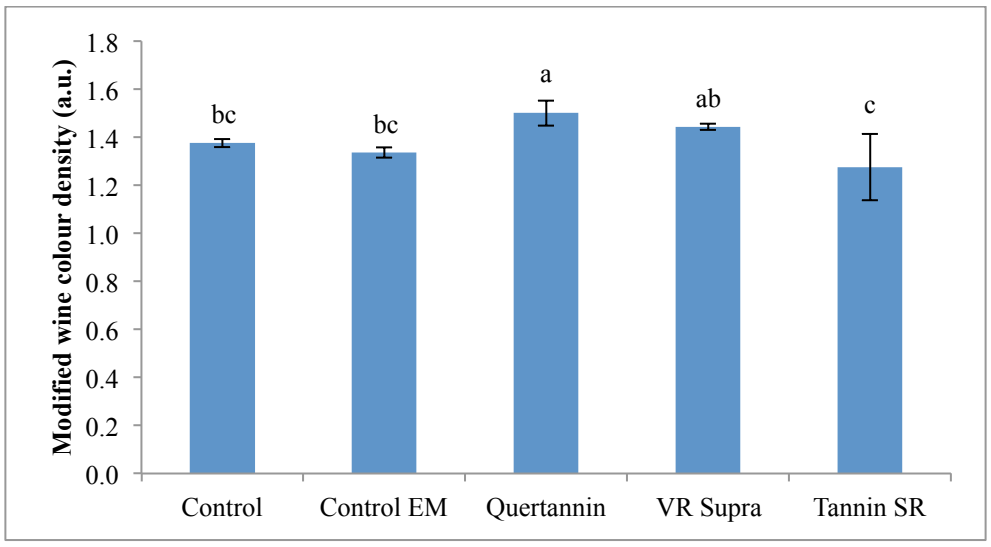


Figure 3.9: Spectrophotometric analysis of modified wine colour density post-bottling expressed as a.u. Error bars represent ± standard error (n=3). Modified wine colour density was determined as ($A_{420acetal}$ + $A_{520acetal}$) × 10. Significance of differences between treatments is indicated by different letters.

Figure 3.9 shows us that there was a significant difference in modified wine colour density between the treatments Quertannin and Tannin SR. The other treatments along with the controls did not show a significant difference.

3.4.4 Wine colour hue

At wine pH and sulfur dioxide levels, wine colour hue expresses the tint or shade of the colour rather than its intensity.

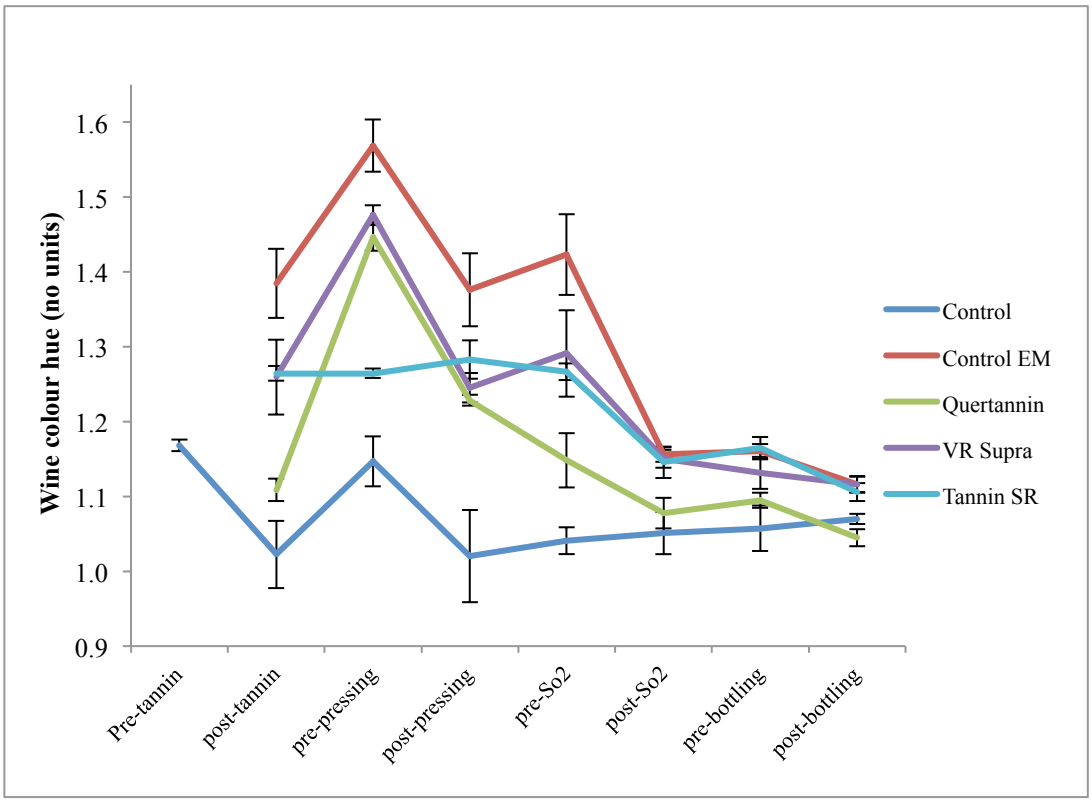


Figure 3.10: Spectrophotometric analysis of wine colour hue in fermentation and wine samples. Wine colour hue was determined as $A_{420buffer1}$ / $A_{520buffer1}$. Error bars represent ± standard error (n=3) divided by the square root of n.

As illustrated in Figure 3.10, wine colour hue increased for all the treatments post-tannin addition except for quertannin, where the wine colour hue decreased. The Control EM treatment had the highest value for wine colour hue post tannin addition and continued to remain highest until sulfur dioxide was added. The wine colour hue decreased for all the treatments post-bottling except for the control treatment, which

showed a steady increase in wine colour hue from the pressing stage to the bottling stage. We can also observe the steep decrease in wine colour hue after the pressing and the sulfur dioxide addition stages for all the treatments.

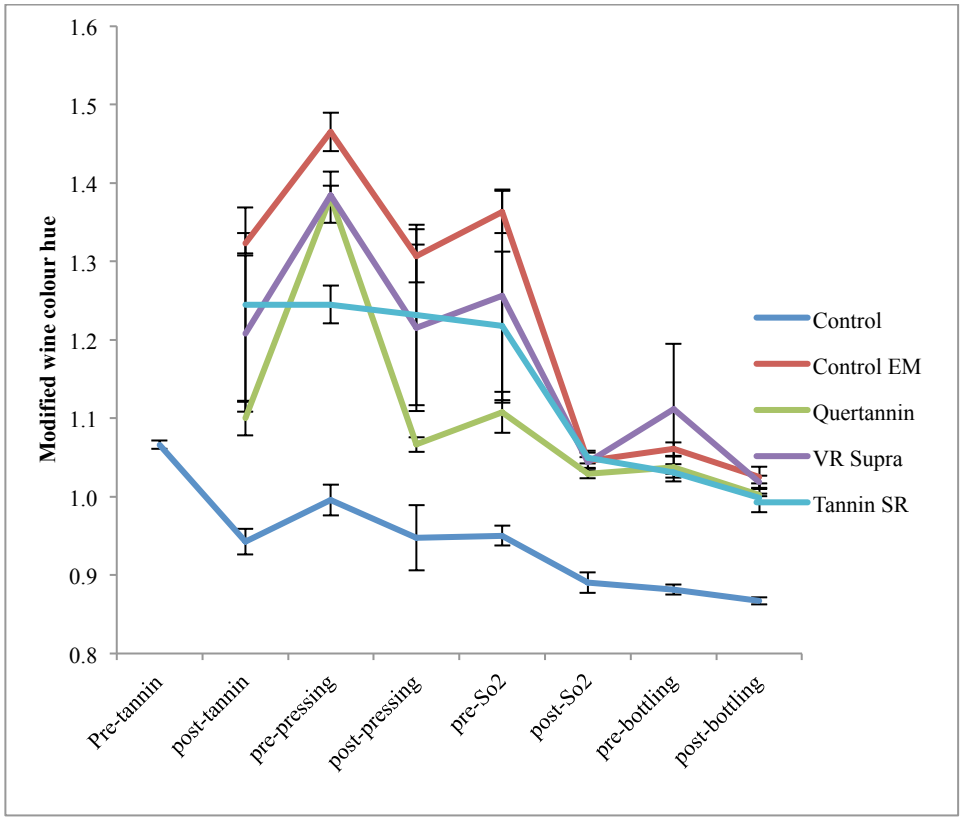


Figure 3.11: Spectrophotometric analysis of modified wine colour hue in fermentation and wine samples. Modified wine colour hue was determined as $A_{520acetal}$ / $A_{420acetal}$. Error bars represent ± standard error (n=3) divided by the square root of n.

On observing the modified wine colour hue as illustrated in Figure 3.11, the treatments varied considerably to the control and also that the treatments behaved similarly through the main winemaking stages.

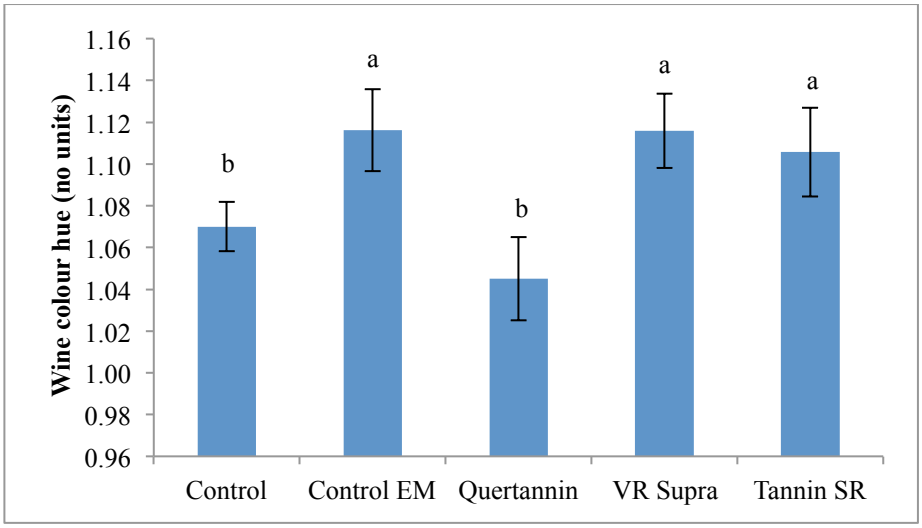


Figure 3.12: Spectrophotometric analysis of wine colour hue post-bottling. Wine colour hue was determined as $A_{420buffer1}$ / $A_{520buffer1}$. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

Figure 3.12 shows that the Quertannin treatment had the lowest value (1.04) for wine colour hue post-bottling. The Control EM had similar values to VR Supra and Tannins SR for wine colour hue.

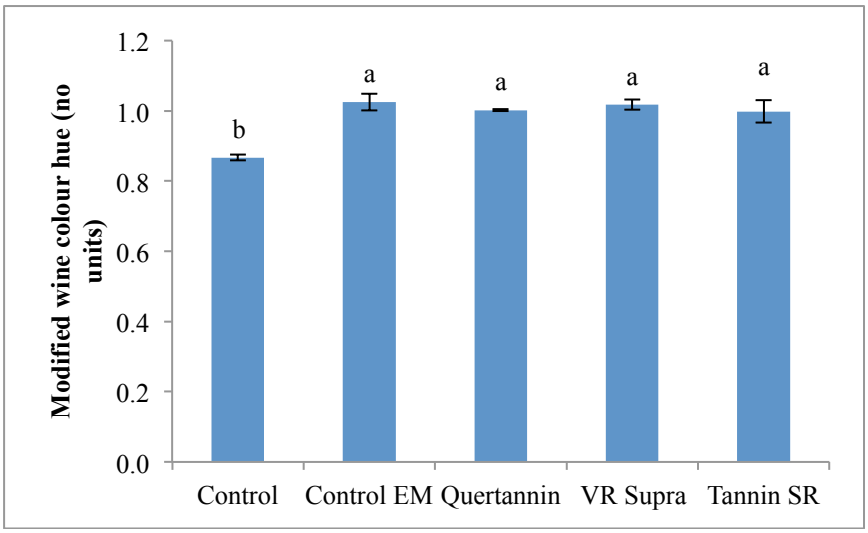


Figure 3.13: Spectrophotometric analysis of modified wine colour hue post-bottling. Modified wine colour hue was determined as $A_{520acetal}$ / $A_{420acetal}$. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

The modified wine colour hue post bottling, as seen in Figure 3.13 was similar between treatments and also between the treatments and the Control EM wines. There

was however a statistically significant difference between the Control wine and the Control EM wines and treatment wines.

3.4.5 Degree of red pigment colouration

At wine pH and sulfur dioxide levels, the degree of red pigment colouration gives the percentage of the total wine pigments that are coloured (Iland et al., 2000).

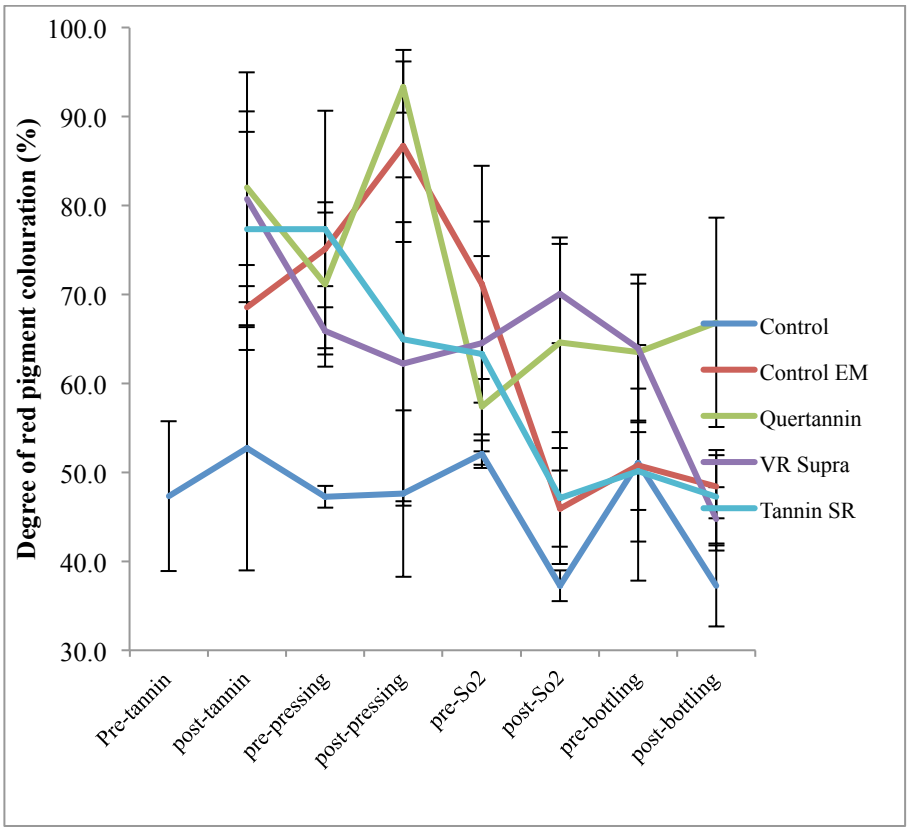


Figure 3.14: Spectrophotometric analysis of degree of red pigment colouration in fermentation and wine samples expressed as percentage (%). Degree of red pigment colouration was determined as $\{[(A_{520buffer1} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$. Error bars represent ± standard error (n=3) divided by the square root of n.

Figure 3.14 shows that there was an increase in the degree of red pigment colouration after the addition of tannin, with the highest increase exhibited for Quertannin (82.0%). There was an increase in the degree of red pigment colouration during pressing for the Control EM and the Quertannin treatments, which was lost upon the

addition of sulfur dioxide as the value for Quertannin treatments dropped from 93.3% to 57.4% (Figure 3.14). Also, the bottling process decreased the degree of red pigment colouration for the VR Supra treatment quite considerably as it was at 63.9% before bottling which was a similar to Quertannin (66.8%), which decreased to 44.7% post-bottling (Figure 3.14). Figure 3.15 shows that most of the treatments display patterns similar to the degree of red pigment colouration when compared with the modified degree of red pigment colouration. Tannin SR shows a linear trend from the pressing stage through to the bottling stage.

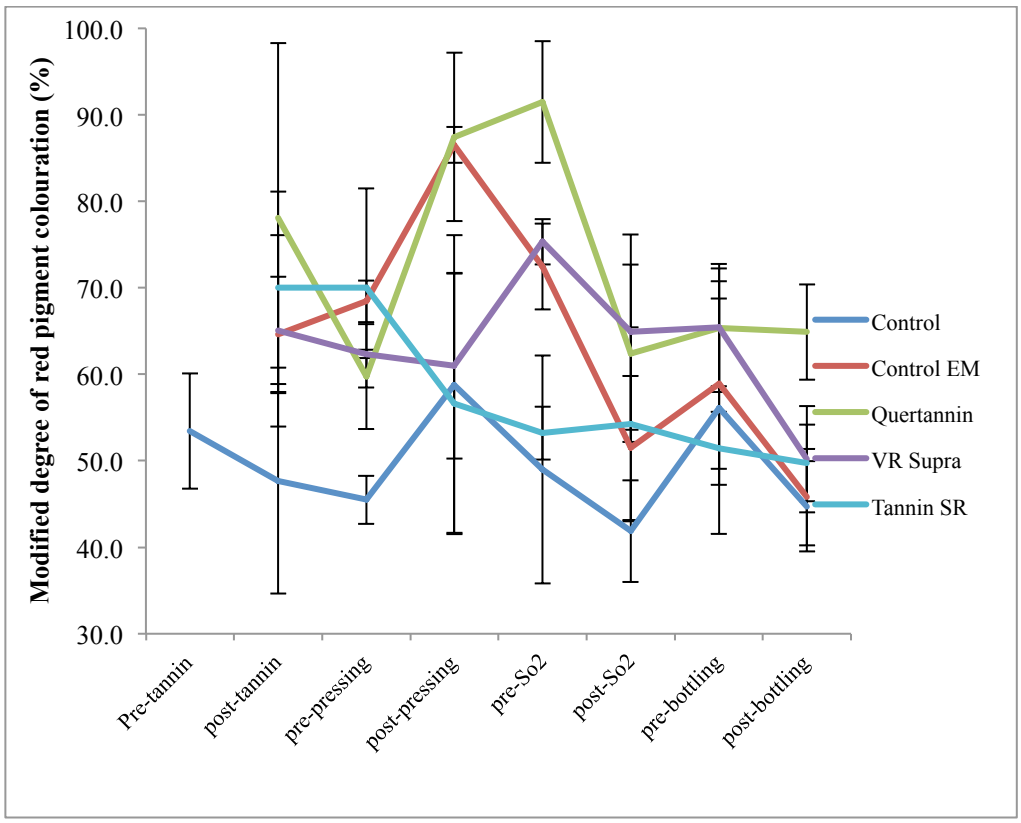


Figure 3.15: Spectrophotometric analysis of modified degree of red pigment colouration in fermentation and wine samples expressed as percentage (%). Modified degree of red pigment colouration was determined as $\{[(A_{520acetal} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$. Error bars represent ± standard error (n=3) divided by the square root of n.

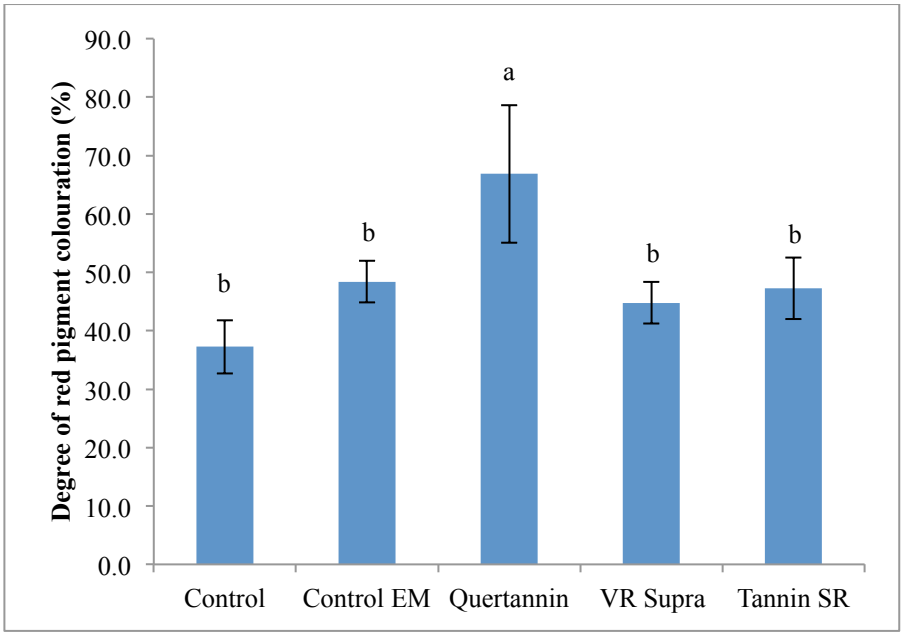


Figure 3.16: Spectrophotometric analysis of degree of red pigment colouration post bottling expressed as percentage (%). Degree of red pigment colouration was determined as $\{[(A_{520buffer1} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

Figure 3.16 illustrates the degree of red pigment colouration for all the wines post bottling. Quertannin exhibits the highest degree of degree of red pigment colouration (66.8%). All the other treatments, including the control, have values below 50%, the lowest being the control (37.3%) and the Control EM (48.4%) being the highest. The modified degree of red pigment colouration (Figure 3.17) also shows similar trends, when compared to the degree of red pigment colouration (Figure 3.16), with Quertannin showing the highest value (64.9%) and the other treatments along with the Control being in the range of 44.7% (Control) to 50.1% (VR Supra).

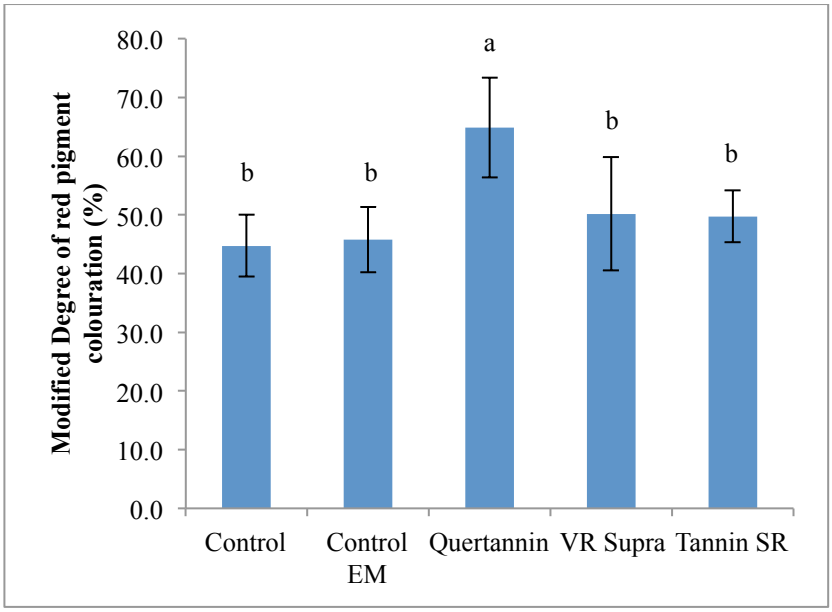


Figure 3.17: Spectrophotometric analysis of modified degree of red pigment colouration post bottling expressed as percentage (%). Modified degree of red pigment colouration was determined as $\{[(A_{520acetal} \times 10) - (A_{520sulfite} \times 10)] / [(A_{520HCl} \times 50) - (1.6667 \times (A_{520sulfite} \times 10))]\} \times 100$. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

3.5 Analysis for the presence of Laccase

As illustrated in Figure 3.18, both the control and the Quertannin treatment do not show a significant change in wine colour density after being left on the bench overnight. The wine colour density for the Control wines was 0.8 a.u. on the first day and 0.8 a.u. on the second day. Quercetin treatment wines were 1.9 a.u. on the first day and 1.9 a.u. on the second day.

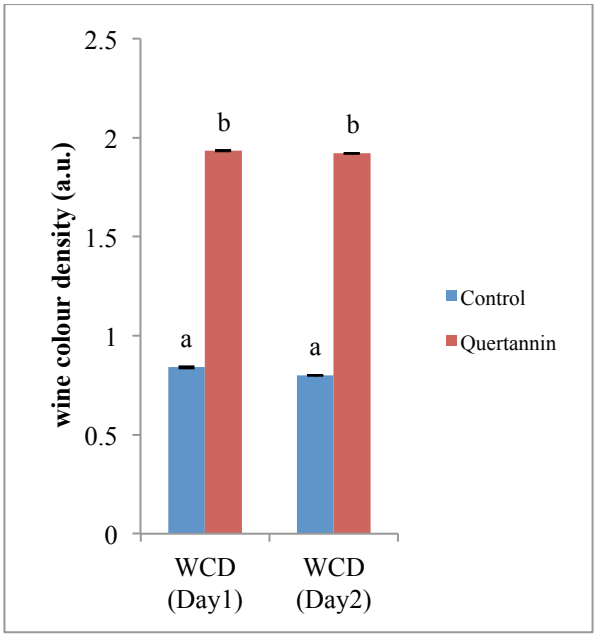


Figure 3.18: Spectrophotometric analysis of wine colour density for bottled wines of Control and Quertannin treatments for the presence of laccase expressed in a.u. Wine colour density was determined as ($A_{420buffer1}$ + $A_{520buffer1}$) × 10. Error bars represent ± standard error (n=3). Significance of differences between treatments is indicated by different letters.

4. Discussion

4.1 Basic wine parameters

The initial must analysis exhibited low TSS and the condition of the fruit was fairly diseased as reported in South Australia for vintage 2011 (WFA Vintage report, 2011). The low TSS was exhibited as the grapes had to be picked before they reached traditional harvest TSS range to prevent further damage from disease and rain. High rainfall also has shown to decrease the phenolic concentration of grapes (Piretti et al., 1980). Since there was no free sulfur dioxide in the grapes on receival, they had been exposed to a certain degree of oxidation, which resulted in browning of the juice as sulfur dioxide acts as an antioxidant in grape juice (Boulton, Singleton et al., 1996). Also, the presence of *Botrytis cinerea* in the grapes further made them sensitive to oxidation and bacterial contamination (Percival et al., 1994).

The high temperature of the fermentation (26 °C), as observed in Figure 3.1 resulted in a rapid fermentation and could have also decreased monomeric anthocyanins (Mazza et al., 1990) and thus resulted in lesser expression of colour.

4.2 Spectral measurements

4.2.1 Total phenolics

Figure 3.2 and Figure 3.3 show that post-fermentation extended maceration increases phenolic concentration of the wine, which is consistent with the findings of Scudamore-Smith et al., (1990), Auw et al., (1996) and Zimman, Joslin et al., (2002). The addition of exogenous tannins also increased the total phenolic concentration of the wines (Figure 3.2), which is consistent with the observations of Cichova et al., (2008). Although, it can also be observed from Figure 3.2, that this effect decreased

towards the end of vinification. At the sulfur dioxide addition stage, the total phenolic concentration also decreased to almost the same value as at the end of pressing. Thus, although there was no significant loss when compared to the phenolic concentration prior to pressing, there was a large increase in phenolic concentration after the pressing stage, which should have been preserved, possibly by oenotannin addition. As VR Supra treatment showed the most significant increase in total phenolic concentration, it is evident that a blend of ellagic tannins and proanthocyanidic tannins are more suitable for increasing the total phenolic concentration.

4.2.2 Sulfur dioxide resistant pigments

Polymeric pigments which form due to oxidation, are less sensitive to the decolouration (bleaching) effect of sulfur dioxide than anthocyanins (Somers and Evans 1974). Therefore it can be interpreted that a wine with a high level of sulfur dioxide has more polymeric pigments and thus is exposed to a greater degree of oxidation. A high level of sulfur dioxide resistant pigments in a young wine indicates rapid ageing and therefore is not desirable for wine colour quality. The sulfur dioxide resistant pigments increased after pressing (Figure 3.4) and this can be attributed to the polymerisation of the anthocyanins, which were released into the wine, with the tannins and other compounds present in the wine (Adams et al., 2004; Lee et al, 2004; Saucier, Lopes et al., 2004; Somers and Evans, 1974). The sulfur dioxide resistant pigments also decreased steadily till the addition of sulfur dioxide and then stabilised (Figure 3.4). This could be attributed to the rapid degradation of ethyl-linked pigments post-pressing (Lee, Swinny et al., 2004) or the precipitation of the polymeric pigments formed during fermentation (Adams et al., 2004.). As observed in Figure 3.5, since the addition of tannins showed significant difference in the sulfur

dioxide resistant pigments, it can be interpreted that the wines are possibly too young to show significant differences in this parameter and the addition of exogenous tannins used in this study is not ideal to increase the amount of sulfur dioxide resistant pigments.

4.2.3 Wine colour density

The wine colour densities for all treatments decreased considerably after the pressing stages till the wines were bottled. This could possibly be due to the oxidation of the anthocyanins and polymerisation and co-pigmentation of the anthocyanins with tannins (Atanasova, Fulcrand et al., 2002; Gomez-Plaza et al., 2004; Somers, 1971; Somers and Evans, 1979).

The modified wine colour density decreased before sulfur dioxide addition, which was also the end of malolactic fermentation to almost the same value as at the beginning of fermentation, for the control wines (Figure 3.7). This is consistent with the findings of Gomez-Cordoves and Gonzalez SanJose, (1995), Mazza et al., (1999) and Sims and Bates, (1994). From Figure 3.7 and 3.8 we can conclude that Quertannin was the only exogenous tannin to increase wine colour density to a statistically significant value among the bottled wines while tannin SR did not show any effect towards increasing wine colour density as the values were statistically the same to the control wine.

4.2.4 Wine colour hue

Wine colour hue expresses the tint or shade of wine colour (Iland, Ewart et al., 2000). It is a value associated with ageing the wine and hence a high value of wine colour hue is not desirable in a young red wine as this indicates the formation of brown

pigments due to oxidation and polymerisation of colour pigments (Sartini, et al. 2007). Since the control treatment showed no significant increase in wine colour hue (Figure 3.9) or modified wine colour hue (Figure 3.10), we can interpret that the wines did not exhibit enzymatic browning. The decrease in wine colour hue over vinification for all the other treatments (Figure 3.9) was due to the increase in red coloured pigments (A_{520}) and decrease in yellow/brown pigments (A_{420}). It can also be concluded from Figure 3.10 and Figure 3.12 that since all the treatments had higher values for modified wine colour hue, which were almost the same, they had a higher yellow/brown colour pigment to red colour pigment ratio than the control. This increase in wine colour hue could mostly be due to increase in brown colour due to the oxidation of phenols (Castellari, Piermattei et al., 2001; Perez-Prieto et al., 2003) and the polymerisation of anthocyanins with tannins (Castillo-sanchez et al., 2008). These wines are possibly too young to show significant differences in this parameter and further trails should investigate their behavior preferably every 6 months.

4.2.5 Degree of red pigment colouration

Since the degree of red pigment colouration gives the percentage of total wine pigments that are coloured at the actual wine pH and sulfur dioxide level (Iland et al., 2000), this makes it the most important parameter to judge the effectiveness of the exogenous tannins which were used in this experiment. A higher degree of red pigment colouration in a wine increases the perceived intensity of the colour and thus it's market quality. It can be observed from Figure 3.13 and 3.14 that there was an increase in the degree of red pigment colouration after tannin addition and after pressing, this increase is probably because anthocyanins were still being extracted towards the end of maceration. It can be also be observed from Figure 3.13 and 3.14

that there was a loss of the degree of red pigment colouration before the addition of sulfur dioxide, which is also the end of malolactic fermentation and is especially evident with the Quertannin treatment. This loss could be attributed to the destructive effect of ethanol upon the coloured pigments extracted during pressing (Somers and Evans, 1979).

Another major step in the decrease of the degree of red pigment colouration as can be observed from Figure 3.13 and 3.14 is the addition of sulfur dioxide. This decolourisation of anthocyanins has been attributed to reaction of species such as HSO_3^- with the flavylium ion (Jurd, 1964; Timberlake and Birdle, 1967). Also, the decolourisation was less pronounced in the treatments of Quertannin and VR Supra, which is possibly due the higher concentrations of anthocyanins (Glories, 1984). This higher fraction of coloured anthocyanins can be due to their binding with tannin molecules with subsequent oxidation (Monagas et al., 2005). Also, it can be observed from Figures 3.15 and 3.16 that Quertannin was the only exogenous tannin to successfully maintain a higher degree of red pigment colouration (66.8%) and thus protect the colour of the wine. This is also consistent with the conclusions of Vivas and Glories, (1996) who found that ellagitannins slowed down the degradation processes of the colour pigments. Figure 3.15 and 3.16 also illustrate that post-fermentation extended maceration and the addition of VR Supra and Tannin SR had no contribution to increase the degree of red pigment colouration as their values, which were all below 50%, were statistically similar to the control wines (37.2%).

4.3 Presence of Laccase

Figure 3.17 showed that the decrease in the wine colour density on the second day of the Control wine and the Quercetin treatment wines was not significant enough to employ the syringaldazine method. If there had been laccase present in the wine, the wine colour density for the control wines would have decreased significantly overnight. Thus, it was concluded that the poor expression of colour and browning in the control wines was not due to the presence of laccase.

5. Conclusion

The key goal of this project was the identification of the effects of the addition of different types of exogenous tannins during post-fermentation extended maceration and to find out which exogenous tannin is most effective in contributing towards the colour stability of the wine. Previous study by Joscelyne, (2009) have shown that

post-fermentation extended maceration decreased red pigment colouration. The study by Joscelyne, (2009) further showed that post-fermentation extended maceration decreased wine colour density. While the results have contradicted this, they have also shown that post-fermentation extended maceration increased wine colour hue, which is not desirable in a young red wine. One more important factor is that, though there has been an increase in wine colour density and the degree of red pigment colouration, winemakers need to weigh these results and the benefits they contribute to the wine against the capital they would be spending on their wine for undergoing post-fermentation extended maceration. Finally, in a difficult vintage like the one in 2011, post-fermentation extended maceration is not advisable as the nature of the process exposes the wine to bacterial and fungal activity, which further decreases wine quality.

One of the most significant findings of this study was that Quertannin, which is composed of ellagic tannins derived from oak heartwood, was the most effective exogenous tannin among the three different types of tannins used. This outcome can be attributed to the higher capacity of ellagic tannins for oxygen consumption than condensed tannins (Du Toit, Marais et al. 2006). The greater consumption of ellagic tannins to consume oxygen is due to the presence of several hydroxy functions in *ortho* which induce an increased oxidizing power, which in turn causes a large quantity of acetaldehyde to be produced (Vivas and Glories 1996). The production of hydroperoxides makes this possible as these molecules have a highly oxidizing R-OOH function (Vivas and Glories 1996). The results obtained in this experiment are also consistent with the ones obtained by Vivas and Glories (1996) where the presence of ellagitannins enhanced the colour of the wine and at absorbance 420 nm,

which is associated with yellow/brown pigments and the control wine had a higher value, while the wine containing ellagitannins remained constant. Thus, the presence of ellagitannins made it possible to regulate oxidation mechanisms in the wine. Instead of undergoing oxidation reactions with oxygen directly, as a harsher and more destructive process, they produce a slowly consumed hydroperoxidizing pool (Vivas and Glories 1996). Ellagitannins are also found to produce larger amounts of acetaldehyde than condensed tannins, which lead to higher levels of polymerisation, resulting in the induction of tannin-anthocyanin polymerisation. (Du Toit, Marais et al. 2006).

Furthermore, ellagitannins have a greater capacity to consume oxygen than the other exogenous tannins used in this experiment, which composed of proanthocyanidic tannins and catechins and thus, this is why they were more effective in protecting the wine colour. From this study it has been established that ellagitannins are the most effective to improve colour for Pinot noir in a bad vintage but further research into its effects for improving colour in a good vintage and also a comparison of its effects on fruit from different regions would provide a better understanding of its effectiveness.

The results also illustrated that pressing, sulfur dioxide addition and bottling, are the major steps where colour pigments are lost. It is clear that further work is required to observe the effect of adding oenotannins, and particularly ellagitannins, during these stages. Distributing additions of oenotannins over each of these stages should also be investigated.

The long-term effects of the addition of exogenous tannins should also be investigated by analysing these wines 6 months post-bottling and thereafter during further ageing of the wine. Detailed analysis of the phenolic composition, particularly for anthocyanins, pigmented polymer and tannins would further provide better understanding of the chemical changes the wines underwent as a result of oenotannin addition. Furthermore, the effectiveness of ellagitannins for the retention of colour should also be confirmed by investigating its effects at different doses. A comparison of similarly formulated oenotannins from different manufacturers would also provide further insight into their effectiveness. Although an informal sensory analysis on the wine was conducted which demonstrated that the wine treated with Quertannin showed superior sensory qualities, a dedicated sensory analysis of the wines produced in this study would also provide a definitive understanding of the correlation between improving the colour of wine by addition of exogenous tannins during post-fermentation extended maceration and its effects on the sensory quality of the wine.

From this study, some outcomes of the addition of exogenous tannins during post-fermentation extended maceration and their effect on improving the colour of the wine have been identified that may be useful to winemakers. While it is evident that post-fermentation has its benefits and limitations for contributing towards colour stability, it can also be concluded that exogenous tannins, particularly ellagitannins, can be a possible aid to improve wine colour through more effective post-fermentation extended maceration.

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