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Physico-chemical properties and mouth-feel in New Zealand Pinot Noir wines

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Master of Horticultural Science

at

Lincoln University

by

Chao Dang

Lincoln University

2013

Declaration Page

Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Horticultural Science

Physico-chemical properties and mouth-feel in New Zealand Pinot Noir wines

By

Chao Dang

A number of chemical and physical parameters that were suggested to be involved in the astringency and body, aspects of mouth-feel, including alcohol, organic acids, pH, titratable acidity, reducing sugar, tannins, phenolics, polysaccharides, proteins, glycerol, viscosity, specific gravity, extract, and the reactivity of tannins with protein, were determined for 18 New Zealand Pinot Noir wines of six producers from the 2010 and 2011 vintages. The wines were previously tasted by a professional palate, M. Cooper MW, for astringency and body evaluation. A sensory analysis of the wines was also conducted at Lincoln University, New Zealand, using the Shapley ranking method to evaluate the intensity of the wines when tasted with and without visual and olfactory influence. Statistical analysis using principal component analysis, correlation analysis and canonical variate analysis showed the following results: (a) viscosity, alcohol and glycerol were the major contributors to wine body, although the differences of wine body between the samples were not substantial; (b) producer was the most influential factor in grouping the wines when considering the variance of mouth-feel related parameters between samples.

Key words: astringency, mouth-feel, Pinot Noir, Shapley ranking, wine body.

Acknowledgements

I would like to thank Dr. Roland Harrison and Dr. Sue Mason, as supervisors for this project, for their wisdom, patience and guidance throughout the entire process of producing this thesis at Lincoln University.

I am very grateful to Dr. Ali Reza Nazmi at Canterbury University for his kindness, and for providing access to the ITC equipment, his help and opinions for parts of this study.

I would like to express my gratitude to the wine producers that were involved in this study, Escarpment, Mount Difficulty, Neudorf, Pegasus Bay, Saint Clair, and Villa Maria, for their generosity in providing the wines for analyses.

I would like to thank Dr. Simon Hodge for his support in the statistical analysis.

I would also like to thank Michael Cooper MW for providing his precious tasting notes and ranking of the sample wines.

Finally, I want to thank my parents in China, who supported me spiritually and economically throughout the whole time while I was studying in New Zealand.

These acknowledgements also extend to all my friends who around the world who have been my constant support.

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Chapter 1 Introduction

New Zealand has been known for its unique and cheerful Sauvignon Blanc for more than 30 years. However, the industry has been looking for another successful variety. Currently wines from Pinot Noir, a red variety, have become some of the most in-demand wines from New Zealand. From a cultivar that thrives in a cool climate, good Pinot Noir wines has a soft, silky-smooth mouth-feel with fruity characters reminiscent of strawberries, raspberries and cherries (Cooper 2011). Few places outside Burgundy are known to have the ideal climate condition for producing high quality Pinot Noir grapes and varietal wines. Despite the significant percentage of Pinot Noir grapes used for sparkling wines, New Zealand has produced many high quality Pinot Noir varietals. In general, New Zealand Pinot Noir wines are fruit driven, forward, and early maturing during bottle aging (Saker 2011). These characters are represented by wines from different regions throughout the north-south aligned country.

During wine tasting, mouth-feel is without doubt one of the most crucial sensory aspects used to judge the quality of wine. Many studies have been carried out to investigate the mechanism, influencing factors and manipulation of this complex red wine mouth-feel perception; however, much of it remains unclear (Gawel 1998). Taking two of the most important mouth-feel aspects, astringency and body, as research objectives, the present study had the following hypotheses:

1. Chemical and physical parameters of red wines contribute to astringency and body mouth-feel and their differences contribute to the perceived mouth-feel difference of the wines.
2. There are differences in mouth-feel of New Zealand Pinot Noir wines from different producers, price point and producer-defined quality. These factors can be used as factors to predict the mouth-feel quality and related parameters of the wines.

The objectives of this study were to explore the relationship between measured parameters and mouth-feel of New Zealand Pinot Noir wines, to use statistical methods to examine the variance of the samples according to producers, price point and producer-defined quality, and to correlate the chemical and physical measurements with sensory analysis.

Chapter 2 Literature review

Previous research on red wine mouth-feel, especially astringency and body, have shown differences in scientific focus on these different mouth-feel aspects. Red wine body has been less well studied than red wine astringency. This may be due to the dominant influence of astringency on overall red wine quality during tasting, which also contributes to the main separation between red wines and white wines. Astringency descriptors, such as firm, soft, harsh and silky, are constantly used during red wine tasting. Despite the lesser focus on red wine body, full, medium and light body descriptions also appear frequently during tasting. Recently, red wine body has begun to receive the attention of researchers because it has been suggested that some potential red wine body components, including phenolics, have the potential to interact with the astringent aspect of red wine mouth-feel and influence overall wine quality (Smith et al. 1996; Gawel 1998; Yanniotis et al. 2007; Jackson 2009).

2.1 Red wine mouth-feel

Jackson (2009) referred to wine mouth-feel as sapid sensations with the involvement of several types of stimulations of trigeminal nerve endings. These nerves are distributed on the tongue and the mouth interior (Gawel 1998). The mouth-feel sensations include body, astringency, temperature, prickling, and burning (Gawel et al. 2000). Two major aspects among these mouth-feel sensations are body and astringency. Wine body is particularly important for white wine perception and quality, and astringency is the predominant mouth-feel that contributes to red wine quality (Jackson 2009). For this reason, studies on wine body were mostly done with white wines (Runnebaum et al. 2011), whereas the nature of the body of red wine remains mostly unknown (Jackson 2009). In terms of red wine astringency, McRae and Kennedy (2011) and Gawel (1998) reviewed the large number of studies that

were related to this aspect of red wine mouth-feel.

2.2 Wine body

2.2.1 Wine body as a mouth-feel character

Wine body, or fullness, was reported to be an important mouth-feel aspect of white wine quality (Gawel et al. 2000). This sensation is often linked with the sensations of density, viscosity, roundness, thickness and slipperiness (Jackson 2009; Runnebaum et al. 2011). Langstaff and Lewis (1993) categorized density and viscosity as sub-qualities of the fullness of beers. Jackson (2009) suggested the synonym of wine body to be weight, and that it may have a sense of viscosity. According to Gawel (1997), experienced tasters showed good consensus when judging the body of wines. Often, a fuller body is considered to be a positive character contributing to the quality of the wine, whereas the descriptors, “watery” or “thin”, indicate that the lesser body of the wine has negatively impacted the wine’s mouth-feel. The ambivalent role of wine body, or fullness, has seldomly been investigated by researchers despite its frequent usage during red wine tasting and its importance to white wine. Most of the existing findings center around white wines (Nurgel and Pickering, 2005; Runnebaum et al., 2011; Szczesniak, 2002; Gawel et al., 2000).

2.2.2 Physical and chemical contributors of wine body

A few studies have investigated components that contribute to wine body. Most have used white wines as samples (Noble & Bursick 1984; Nurgel & Pickering 2005; Gawel et al. 2007; Yanniotis et al. 2007; Runnebaum et al. 2011) with very few studies of red wine body (Yanniotis et al. 2007). However, many of the body-related constituents that have been studied in white wine are also present in red wine. Therefore, some of these results may cast some light on the investigation of red wine

body. In some earlier studies, viscosity, which appears to have a narrower significance, was investigated in the place of body and the influence of a few wine chemical components on the perception of viscosity was examined (Soesanto & Williams 1981; Noble & Bursick 1984; Burns & Noble 1985). Sugar, alcohol and glycerol have been studied as the main contributors to wine body because of their viscous solutions (Noble & Bursick 1984; Pickering et al. 1998; Nurgel & Pickering 2005). Understanding the relationship between other chemical and physical properties and the perception of wine body is still limited. Skogerson et al. (2009) and Runnebaum et al. (2011) attempted to establish correlations between perceived wine body and physical or physico-chemical measurements, such as gas-chromatography mass spectrometry (GC-MS) metabolite profiling, nuclear magnetic resonance spectroscopy (NMR) profiling, and viscosity. These two studies reported correlations between wine body and viscosity, total extract, NMR and GC-MS models, as well as chemical constituents, such as lactic acid. Jackson (2009) summarized that sugar is generally the most important contributor of the body of sweet wines, whereas alcohol and glycerol could be associated with the body of dry wines. As for red wine, tannins, the major component responsible for astringency, could be involved in the perception of wine body through physical characteristics of their macro-molecular structure and chemical interactions with potential wine body compounds, such as proteins, pigments, and polysaccharides (Baxter et al. 1997).

2.2.2.1 Wine viscosity

Wine viscosity is considered to be an important physical parameter that correlates well with the perception of wine body (Runnebaum et al. 2011). The difference between low viscosity and high viscosity beverages lies in the spreading speed and the distance covered in the mouth (Szczesniak 2002). Beverages with higher viscosity tend to be more resilient to spreading and dispersion due to the higher shear stress of the liquid (Shama & Sherman 1973). The importance of viscosity is not limited to

the sought-after sensations of roundness and smoothness, but also influences other tastes (Smith et al. 1996; Hollowood et al. 2002). Viscosity can be measured by instrumental analysis and well-designed sensory analysis. Several types of viscometer were used to measure the viscosity of wine, including the capillary viscometer, the falling ball viscometer, and the rotary viscometer (Table 1). Since viscosity is a function of temperature, with higher temperature resulting in lower viscosity, the wine viscosity measured varied in different studies depending on the temperature regime. In the study by Yanniotis et al. (2007), the viscosities of two dessert (sweet) wines were 3.04 ± 0.1 and 3.16 ± 0.1 mPa s at 16 °C while the measurements of two dry red and two dry white wines gave a range between 1.71 ± 0.02 and 1.92 ± 0.07 mPa s. Runnebaum et al. (2011) tested the viscosity of 17 white wine samples at 30 °C, and the viscosity ranged from 1.232 mPa s to 1.313 mPa s. Košmerl et al. (2000) measured the viscosity of 28 white wines and 12 red wines, at temperatures ranging from 20 to 50 °C and showed increasing viscosity with decreasing temperature. As for sensory analysis of wine viscosity, the study by Nurgel and Pickering (2005) showed quite good linearity between measured viscosity and perceived viscosity, and suggested that sensory perception could be predicted by physical measurements. The threshold, or the minimum value, of viscosity difference detectable by more than 75% of subjects in a sensory analysis was reported to be 0.141 mPa s by an early study (Noble & Bursick 1984). Later studies by Košmerl et al. (2000) and Runnebaum et al. (2011) showed similar results. Concluding from these results, the difference of viscosity in most dry wines does not meet the perceptible threshold.

Table 2.1 Viscometers used by previous studies of wine.

Reference	**Type of Viscometer**	**Type of wine or solution**
Nurgel and Pickering (2005)	Capillary viscometer.	Model wine solution with 150 – 300 g/L sugar or 8% – 10% ethanol.
Smith et al. (1996)	Capillary viscometer.	Extracted seed tannin solution with sweetener or thickener.
Runnebaum et al. (2011)	Capillary viscometer.	Dry white wine.
Košmerl et al. (2000)	Capillary viscometer.	Red wine and white wine from dry to sweet.
Yanniotis et al. (2007)	Falling ball viscometer.	Red wine and white wine from dry to sweet.
Peleg and Noble (1999)	Rotary viscometer (disk)	Cranberry juice with 12° Brix.

2.2.2.2 Reducing sugar

Reducing sugars in wine are mainly glucose and fructose from the grape juice that were not fermented in the winemaking process (Fernández-Novales et al. 2009). In wines that are not dry, the concentration of glucose and fructose can be higher than 50 g/L, whereas in dry table wines, this concentration is generally under 2 g/L, (Peynaud et al. 1996). One of the major contributions of reducing sugars is producing perceptible sweetness in non-dry wines. On the other hand, dry wines primarily contain sugars in pentose form, such as rhamnose and arabinose that are not reducing sugars (Dubois et al. 1956; Jackson 2008).

Another important contribution of the sugar content in wine is to the effect on viscosity, or body, particularly in sweet wines (Cliff et al. 2002; Nurgel et al. 2004). Chirife and Buera (1997) reported an increase in the viscosity of aqueous solutions containing higher sugar concentration, and the difference in the ability of different sugar forms to affect viscosity. It is clear that higher reducing sugar content can result in elevation of sweetness and body, as observed in ice wine and port wine (Lopes et al. 1995; Nurgel et al. 2004). Several studies investigated the separate effect of sweetness and viscosity on other sensations during wine tasting. Smith et al. (1996) and Smith and Noble (1998), by using carboxymethyl cellulose as a thickener and aspartame as a sweetener, reported the suppressing effect of sweetness on the intensity of bitterness, and the suppressing effect of viscosity on bitterness (not significant), astringency and sourness. Burns and Noble (1985) reported the sweetening and thickening effect of a non-reducing sugar, sucrose, and its suppressing effect on bitterness. These findings reveal the effect of reducing sugars, which can contribute to sweetness and the two sub-qualities of body, viscosity and density, (physical and perceived) of model wine solution, shown by Nurgel and Pickering (2005). In that study, the perceived viscosity and perceived density of model wine solution increased significantly in response to increasing sugar

concentration up to 80 g/L. At sugar concentrations above 80 g/L, the increase of perceived viscosity and density was not as significant as with the concentration below 80 g/L. In another study, which involved the comparison between the viscosity of dessert wines and dry wines, the viscosity of dessert wines was significantly higher than that of dry wines (Košmerl et al. 2000). All of these findings contribute to the fact that most dessert or sweet red wines are perceived as full bodied. For dry or off-dry red wines, which often have sugar levels below 15 g/L, it is reasonable to assume that residual sugars contribute to the perceived body, but the extent of this contribution is not clear.

2.2.2.3 Ethanol

Ethanol is the most important alcohol in wine, mainly produced from fermentable sugars during primary fermentation by the yeast. Generally, the ethanol concentration in table wines can reach up to 15% v/v. Higher concentrations of ethanol could result from fortification or addition of sugar during fermentation (Lea et al. 2003). The ethanol content is crucial to wine due to its function in the dissolution of hydrophobic compounds, which include a number of important flavor and mouth-feel compounds (Jackson 2008).

In the early wine sensory book, “Wines: their sensory evaluation”, by Amerine and Roessler (1976), it was mentioned that increasing concentration of ethanol can enhance the perception of wine body. This idea was later proven to have merit by Nurgel and Pickering (2005), who used model wine solutions as medium, and found moderate contributions of ethanol to perceived viscosity and density at 3, 7, and 15% v/v ethanol concentration. Gawel et al. (2007) found increasing body and perceived viscosity resulted from increasing alcohol concentration in the normal wine alcohol concentration range, but the effect was not statistically significant and it was influenced by the concentration of glycerol. On the other hand, an earlier study

conducted by Pickering et al. (1998) showed no statistical significance of the changes of perceived viscosity and density of white wine due to increasing ethanol concentration between 7% and 14%. Pickering et al. (1998) raised doubts about the strong anecdotal role of ethanol in the body of white wines. Further study, which also investigated the effect of ethanol on the viscosity of white table wines (Runnebaum et al. 2011), showed an increase in physical measurements of model wine solution but no or small contribution of ethanol to perceived wine body within normal concentrations in wine. For red wines, no study, so far, has attempted to find the role of ethanol content in red wine body.

2.2.2.4 Glycerol

Glycerol is the most abundant polyol in wine, and sometimes the third most abundant compound (by weight) in dry wines after water and ethanol (Ough et al. 1972). Glycerol has been found to be formed in winemaking grapes (Calull et al. 1992; López & Gómez 1996) and by yeasts during the fermentation process (Remize et al. 1999). Grape variety, berry development and disease, as well as yeast strain and stress during fermentation can influence the final concentration of glycerol in wine (Ough et al. 1972; López & Gómez 1996; Beleniuc et al. 2002). Red wines generally contain more glycerol than white wines, with around 10 g/L in red wines, as opposed to about 7 g/L in white wines (Jackson 2008).

The most notable characteristics of glycerol are the slight sweetness and high viscosity. For this reason, it has long been speculated as an important contributor to wine body. Noble and Bursick (1984) reported that the addition of at least 25.8 g/L of glycerol into table wine can give an observable increase of perceived white wine viscosity, suggesting that at normal table wine glycerol concentration, which is 1.0 to 10.6 g/L, glycerol cannot add to perceived viscosity. Nurgel and Pickering (2005) reported a similar 25 g/L concentration of glycerol to be the minimum requirement

in model wine solution to give a significant viscosity increase. The recent study by Runnebaum et al. (2011) reported no notable relationship between glycerol content and wine viscosity in dry white table wine. On the other hand, Gawel et al. (2007) reported statistical correlation between glycerol content within normal wine concentration range and perceived viscosity. So far, there is still no solid conclusion about the contribution of glycerol in wine body. Some sweet, botrytised wines, or ice wines may contain glycerol at concentrations above 25 g/L, which suggests that the glycerol content in these wines may contribute to the perceived wine body to a more significant degree (Nurgel et al. 2004). There has no literature about the role of glycerol in the perception of the body of dry red wine, but it is thought that the contribution of glycerol to red wine body is limited.

2.2.2.5 Other wine constituents

Apart from sugar, ethanol and glycerol, several other wine constituents also possess the potential to contribute to the perception of wine body. These compounds include polysaccharides (Vidal et al. 2004b; Carvalho et al. 2006), proteins (Ricardo da Silva et al. 1991; Maury et al. 2001; Runnebaum et al. 2011), lactic acid (Liu 2002; Skogerson et al. 2009; Runnebaum et al. 2011), anthocyanins and tannins (Vidal et al. 2003b; Vidal et al. 2004a; Vidal et al. 2004b).

Two recent studies (Skogerson et al. 2009; Runnebaum et al. 2011) have shown a significantly positive relationship between the lactic acid content and the body of white wine. Lactic acid exists in relatively low concentration in wine when it is mainly produced by yeast metabolism during primary fermentation, whereas it can become the most abundant organic acid in wine if malo-lactic fermentation, a practice used in making some white wines and the majority of red wines, is applied (Liu 2002). This process is primarily carried out by lactic acid bacteria, converting the dicarboxylic and ‘harsh’ malic acid to the monocarboxylic and ‘soft’ lactic acid, with L-lactic acid being

predominant (Davis et al. 1985; Kunkee 1991). The relationship found between lactic acid and white wine body by Skogerson et al. (2009) and Runnebaum et al. (2011) indicates that the body of red wines, which generally contain high levels of lactic acid as a result of malo-lactic fermentation, can be potentially modified through this practice. The “milky” sensation from malo-lactic fermentation, mentioned in the review by Davis et al. (1985), may be related to the effect of lactic acid and malo-lactic fermentation as part of red wine making.

Polyphenols, polysaccharides and proteins account for the majority of the macromolecule fraction in the wine matrix. These compounds have been studied and it has been suggested that they may have a role in the perception of wine body (Vidal et al. 2004b). As the most important mouth-feel contributors in red wine, phenolics could contribute to red wine body, but most of the studies on red wine tannins are focused on the mouth-feel of astringency with very few publications concerning the relationship of phenolics to wine body. Jackson (2009), in his book “Wine tasting: a professional book”, suggested that tannins may be involved in the perception of body, but no evidence was provided. Anthocyanins, another fraction of red wine phenolics, were pointed out by Brossaud et al. (2001) to have a moderate effect on the general wine mouth-feel, which may involve a contribution to the body. Further study by Vidal et al. (2004a) suggested that the influence of anthocyanins on red wine mouth-feel appears to result from polymerization between anthocyanins and tannins, which reduces perceived astringency of tannins, whereas the free anthocyanins do not contribute to the taste or mouth-feel of red wine (Vidal et al. 2004a). Some research on white wines found that extract, a general parameter representing the complete soluble solids in wine, could be correlated with wine body (Zoecklein et al. 1995; Runnebaum et al. 2011). Zoecklein et al. (1995) mentioned a 20 g/L threshold of dry extract for white wines, below which the wine is generally perceived as thin. Considering the total extract of red wine is much more influenced by phenolics, further studies can be carried out in this direction to understand red wine body.

The protein content in wine differs in red wine and white wine due to different winemaking directions. For whites, the indigenous pathogenesis - related proteins from the winemaking grapes are often removed from the wine due to their ability to form undesirable haze in the bottle (Ferreira et al. 2001; Waters et al. 2005; Pocock & Waters 2006). On the other hand, exogenous proteins, such as egg white and gelatin, are added to red wine as a fining method to precipitate excessive tannins and to reduce the level of astringency (Ricardo da Silva et al. 1991; Maury et al. 2003). In general, proteins are rare in red wines, with low concentrations of soluble proteins co-existing with tannins due to the formation of soluble complex that avoids tannin – protein precipitation (Vincenzi et al. 2005; Smith et al. 2011). There have been many reports about the astringency reduction effect of proteins deliberately added to red wine as a fining practice (Singleton & Esau 1969; Ricardo da Silva et al. 1991; Sarni-Manchado et al. 1999; Maury et al. 2001, 2003), whereas there is no literature so far for the contribution of protein to wine body. Runnebaum et al. (2011) suggested that proteins could be an aspect of future research into wine body.

The concentration of polysaccharides in red wine is generally higher than proteins, but lower than the polyphenols (Jackson 2008; Guadalupe et al. 2012). This fraction of macro-molecular compounds in wine has drawn some attention of researchers recently, in terms of their influence on the sensory properties of wine. Polysaccharides in wine started to be characterized in the 1990s with colorimetric analysis and then HPLC analysis methods (Pellerin et al. 1995; Segarra et al. 1995; Pellerin et al. 1996; Vidal et al. 2003a; Aguirre et al. 2009). Based on origin, wine polysaccharides can be categorized into grape polysaccharides, primarily arabinogalactan-proteins and rhamnogalacturonans, and yeast polysaccharides, mainly mannoproteins (Vidal et al. 2003a; Vidal et al. 2004b). The arabinogalactan-proteins and mannoproteins portions are neutral, whereas rhamnogalacturonans are acidic polysaccharides (Aguirre et al. 2009). Sensory properties of these polysaccharides and their influence on wine perception were not considered by researchers, until Vidal et al. (2004b) reported evidence that both

acidic and neutral portions of polysaccharides in wine can reduce astringency and affect the fullness of wine, with the influence of acidic polysaccharides being more significant. Further studies examined the influence of polysaccharides on the tannin-protein interactions that cause astringency and suggested models with complexes between polysaccharides and tannins that affect the overall mouth-feel of red wine (Carvalho et al. 2006; Poncet-Legrand et al. 2007a; Mercurio & Smith 2008; Aguirre et al. 2009), but there insights of the contribution of polysaccharides to wine body are still lacking.

2.3 Red wine astringency

2.3.1 Astringent as a tactile sensation

Astringency is considered to be a very important mouth-feel attribute associated with red wines, which generally involves oral sensations, such as dryness, puckering and rough feelings (Gawel 1998). According to the "red wine mouth-feel wheel" developed by Gawel et al. (2000), astringency of different intensities and characters can relate to different sensory descriptors and influence the judgment of overall wine quality by the taster. For example, excessive astringency can be described as aggressive or harsh, and too week astringency can result in wines being described as flat or insipid. It is clear that balanced and ripe astringency sensations have been constantly sought after in red wine tastings (Jackson 2009).

2.3.2 The tactile sensation and taste sensation debate

Astringency is predominantly viewed as a tactile sensation of the interior surface of mouth, generated by stimulations of the trigeminal nerve (Breslin et al. 1993; Green 1993; Gawel 1998). Nevertheless, an alternate hypothesis advocating that astringency is a taste sensation has also been proposed by some earlier

neurophysiological studies (Kawamura et al. 1969; Schiffman et al. 1992; Critchtey & Rolls 1996). In this argument, Breslin et al. (1993) provided evidence to support the tactile sensation theory that astringency could be lowered by the reduction of friction with the application of lubricating rinses of water, saliva, oil, sugar solution and other viscous liquids. However, their study could not rule out the involvement of astringency in taste sensations, because some other researchers showed that increasing lubricity and viscosity can also delay tastes, such as sweetness, sourness and bitterness, potentially indicating that they share the same sensational mechanism with astringency (Burns & Noble 1985; Ishikawa & Noble 1995; Smith et al. 1996; Smith & Noble 1998; Lesschaeve & Noble 2005). From another point of view, neurophysiological responses, which were found to link with tastes, were also found to be related to astringency, but this could not be viewed as solid evidence that astringency is a taste. This is because clear scientific results showed that some astringent compounds, such as polyphenols, can also elicit bitterness taste (Brossaud et al. 2001; Vidal et al. 2003b; Lesschaeve & Noble 2005; Landon et al. 2008). The most important difference found between astringency and taste, which refutes the classification of astringency as a taste, is that astringency tends to increase with multiple exposures of astringent agents, whereas all known tastes loose intensity upon repeated exposures (Guinard et al. 1986). In more recent literature, the idea of astringency being a tactile sensation seemed to be more supported, and polyphenol-salivary protein association and precipitation were observed to be the cause of astringency sensation (Charlton et al. 1996; Baxter et al. 1997; Lu & Bennick 1998; Sarni-Manchado et al. 1998; Prinz & Lucas 2000; Kallithraka et al. 2001; Bennick 2002; Charlton et al. 2002).

2.3.3 Mechanism

There can be several mechanisms involved in the perception of astringency. Although the major mechanism is widely acknowledged as the loss of oral lubrication, caused

by interactions between wine polyphenols and salivary proteins (Gawel 1998; Sarni-Manchado et al. 1998; Charlton et al. 2002), Santos-Buelga and de Freitas (2009) indicated that the study of polyphenol and salivary interactions needs to be refined to include influencing factors, such as supplement of viscosity the mouth by saliva flow (de Wijk & Prinz 2005; Lesschaeve & Noble 2005), interactions from constituents of the wine matrix (Kallithraka et al. 1997; Fontoin et al. 2008; Rinaldi et al. 2012a; Scollary et al. 2012), and interactions between polyphenols and non-salivary mouth proteins or taste receptors (Critchtey & Rolls 1996; Brossaud et al. 2001; Vidal et al. 2004a). Another important factor that needs to be considered in the study of astringency is the psychological and physiological variance between human individuals. This can involve their neural sensitivity (Dinnella et al. 2009b; Dinnella et al. 2011), saliva flow rate (Lyman & Green 1990; Smith et al. 1996), and psychological status influenced by personal preference and other perceptual conditions (Delwiche 2004). Higher saliva flow rate was found to reduce perceived astringency in the panel evaluation conducted by Condelli et al. (2006). Factors that influence the perception of polyphenols that cause astringency were reviewed by Lesschaeve and Noble (2005), including polymer size, the extent of galloylation, formation of derivatives of polyphenols, pH, ethanol, sweetness, viscosity and individual variance. Most of these factors will be discussed in detail in the later section of the present review.

2.3.4 Wine constituents contributing to astringency

According to Gawel (1998), the term “tannin”, which originates from a practice in the leather industry, was first used in 1796 to refer to the phenolic compounds in oak galls. In red wine, condensed tannins, also referred to proanthocyanidins, are the most important astringent agents, consisting of a group of polymerized phenolic compounds extracted from the grapes during the winemaking process (Singleton & Trousdale 1992). Other phenolic compounds, such as hydrolysable tannins from oak

cooperage, anthocyanins, monomeric and oligomeric flavan-3-ols also exist in red wine, and they may contribute to astringency, but their contribution to wine astringency is less significant than that of tannins (Pocock et al. 1994; McRae & Kennedy 2011).

Tannins are formed in grape berries through the polymerization of flavan-3-ol subunits, which mainly include (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate and (-)-epigallocatechin (Waterhouse 2002; Bogs et al. 2005). The polymerized flavan-3-ols undergo a series changes in concentration, size and conformation in the winemaking process after the grapes are harvested (Pérez-Magariño & González-San José 2004; Jaffré et al. 2009). The flavan-3-ols are analogous to flavonoids of the phenolic family and share a C15 triple ring structure whereby the C ring of the skeleton is saturated, as opposed to the other flavonoids, such as anthocyanins and flavonols that contain unsaturated C ring (Jackson 2008). Several studies have investigated the mechanisms and pathways of the formation of condensed tannins in grape berries, and reported that the polymerization is mainly facilitated by enzyme catalyzed formation of covalent linkages between the C4 carbon of the pyran ring and the C8 carbon of A ring, with branches being able to formed on the C6 carbon of A ring (Figure 1) (Downey et al. 2003; Xie et al. 2003; Bogs et al. 2005; Dixon et al. 2005; Bogs et al. 2007). This type are referred to as B type procyanidins, as opposed to the less common A type procyanidins, which contain the C4-C8 bond as well as C2-C7 bonding (Cheynier 2005; Cheynier et al. 2006).

In grape berries, tannins are formed primarily in skins and seeds, with trace amounts found in the flesh (Downey et al. 2003; Herderich & Smith 2005). A general sense among winemakers is that skin tannins are more desired than seed tannins, because skin tannins can provide riper astringency (Kennedy 2008). This idea has been controversial and even confusing, and seemingly contradictory research results have been presented, in terms of the relative contribution of skin and seed tannins to wine quality (Harbertson et al. 2002; Chira et al. 2008; del Llaudy et al. 2008;

Kennedy 2008). From a structural investigation angle, several studies have revealed some critical differences between skin tannins and seed tannins that may lead to perceptional differences: (1) skin tannins have higher degree of polymerization, or longer molecular chains, than seed tannins, with the mDP (mean degree of polymerization) having been reported to range from 3 to 83 for skin tannins (Brossaud et al. 2001; Kennedy et al. 2001; Vidal et al. 2004a; Cheynier et al. 2006) and 2 to 17 for seed tannins (Brossaud et al. 2001; Lee et al. 2008); (2) grape seed tannins do not contain epigallocatechin, a tri-hydroxylated flavan-3-ol subunit (Prieur et al. 1994; Souquet et al. 1996); (3) seed tannins have a higher galloylation level than skin tannins (Riou et al. 2002; Cheynier et al. 2006).

During the winemaking process, tannins are gradually extracted from the grapes, but the proportion of skin versus seed tannins may not necessarily be the same as that of the berries (Cortell & Kennedy 2006). Sacchi et al. (2005) reviewed the techniques and factors related to the extraction of skin and seed tannins during grape processing and fermentation. From this stage on, up until the wine is consumed, the extracted tannins further polymerize and change in structure as a result of a series of reactions, such as acetaldehyde induced polymerization and incorporation with anthocyanins forming polymerized pigments (Singleton & Trousdale 1992; Waterhouse 2002; Toit et al. 2006; Ortega-Heras et al. 2007). These reactions potentially influence the reactivity of tannins with proteins, which have further impact on the astringency.

2.3.5 Salivary proteins

Saliva is the gland-excreted aqueous fluid in the mouth, whose functions were summarized by Mandel (1987), including coating and lubricating the interior surface of mouth. Human saliva is mainly composed of proteins, mucin-glycoproteins, α-amylase, glycolipids, carbohydrates, serum transudates, and inorganic ions (Wu & Lavelle 1994; Gawel 1998; Monteleone et al. 2004; Condelli et al. 2006). The protein

fraction has been reported to be the most important participant of the perception of astringency in the saliva profile (Dinnella et al. 2009a). Proline-rich proteins and histidine-rich proteins, accounting for 70% and 2.6% of the total salivary protein composition respectively, are the major proteins that are responsible for the interactions with astringent agents in wine (Kauffman & Keller 1979; Lamkin & Oppenheim 1993; de Freitas & Mateus 2001; Charlton et al. 2002).

Figure 2.1 Flavonoid skeleton, unbranched A type procyanidin, and branched A type procyanidin (from left to right), from Jackson (2008). **Removed to comply with copyright.**

Proline-rich proteins (PRP) are characterized by relatively low complexity and a highly repetitive structure, comprising of a number of repeating units of proline, glycine, glutamic acid and glutamine, with proline contributing to around half of the total amino acids (Hay et al. 1988; Lamkin & Oppenheim 1993; Charlton et al. 1996; Croft & Foley 2008). This extended chain structure allow PRPs to have a number of binding sites available to interact with polyphenols with strong affinity (Hagerman & Butler 1981; Dangles & Dufour 2008). Three sub-categories of PRPs are basic, acidic and glycosylated PRPs (Kauffman & Keller 1979; Bennick 2002). Although acidic PRPs make up the largest portion (30%) of the total PRPs, basic PRPs, which amount to 23% of the total, were reported to be the major PRP involved in the interaction with astringent agents and the perception of astringency (Lu & Bennick 1998). Bacon and Rhodes (2000) and Pascal et al. (2008) reported bindings of acidic and glycosylated PRPs with hydrolysable tannins and condensed tannins. Sarni-Manchado et al. (2008) suggested that products of the interactions between glycosylated PRPs and tannins may remain soluble, while the binding between non-glycosylated PRPs and tannins will more likely result in precipitation.

Histidine-rich proteins (HRP), with histidine making up to around 25% of the total amino acids, are a group of salivary proteins that have smaller molecular sizes and lower molecular weights, comparing with PRPs (Oppenheim et al. 1988; Lamkin & Oppenheim 1993). The HRPs are even more capable of binding tannins, but they only account for 2.6% of the total protein fraction in saliva (Yan & Bennick 1995). Twelve HRPs have been identified and numbered, with histadin 1, 3 and 5 having been studied particularly due to their high ability to have hydrophobic interactions with tannins (Naurato et al. 1999)

2.3.6 Character of tannin – protein interactions

The knowledge that astringency mainly results from the loss of mucosal lubrication in

the mouth due to the interaction between astringent agents, mainly condensed tannins, and salivary proteins, has now been well established (Gawel 1998; Prinz & Lucas 2000). Studies have shown that hydrophobic interactions and hydrogen bonding are the major mechanisms involved in the interactions between tannins and salivary proteins (Luck et al. 1994; Baxter et al. 1997; Gawel 1998). Hydrophobic interactions refer to the coalescence of molecules in aqueous solution due to the shift of surrounding water molecules from a partially ordered state to a more ordered state (Luck et al. 1994). Therefore, hydrophobic interactions are entropy driven, with the appearance of Van der Waals force, or π bonds, between the nucleophilic B ring of the flavan-3-ol triple ring structure and the heterocyclic amide bonds of proline (Artz et al. 1987; Baxter et al. 1997; Bennick 2002; Simon et al. 2003; McRae et al. 2010). On the other hand, an enthalpy driven interaction, hydrogen bonding, was also observed to take part in the tannin and protein interaction and aggregation (Luck et al. 1994). Hydrogen bonding was believed to be more important than other mechanisms in the reaction between procyanidin and proteins, in an early study by Artz et al. (1987). Hydrogen bonding is facilitated by the electrophilicity and nucleophilicity of different hydroxyl groups of the tannins, carbonyl function of PRP, and the imidazole rings of HRP (Haslam 1974; Naurato et al. 1999; Simon et al. 2003; Jöbstl et al. 2004). It is worth mentioning here, that due to the complexity of the macromolecules, inner-molecular conformation changes can also take place along with the inter-molecular interactions. Jöbstl et al. (2004) reported the spherical forms of tannin/protein complex with inner-molecular congregation, resulting from interactions of the multiple binding spots on tannins. This may be applied to explaining the mouth-feel evolution during red wine aging. Red wines tend to soften, rather than become more astringent, during the aging process that results in more polymerized tannins, whereas higher mDP of flavan-3-ols has been reported to correlate with higher astringency (Vidal et al. 2004a). The finding by (McRae et al. 2010) seems to support the conformational change theory, as they reported a reduction in the reactivity of tannins extracted from aged wines in a tannin – protein reaction thermodynamics study.

Modeling simulations of the interactions between tannins and proteins suggested that there are three major stages in the tannins and proteins interactions (Dangles et al. 2006). According to Baxter et al. (1997) and (McRae et al. 2010), the three stages include an initial phase that forms the tannin – protein complex through hydrophobic interactions and hydrogen bonding, a second phase during which the complex further aggregates, and a third phase that leads to precipitation. Hydrophobic interactions and hydrogen bonding are involved in each stage, but to different extents, based on specific molecular and environmental condition (Artz et al. 1987; Luck et al. 1994; Gawel 1998; McRae et al. 2010). As both the entropy driven hydrophobic interactions and enthalpy driven hydrogen bonding cause changes in the thermodynamics of the whole system, observations of thermodynanics may provide insight of this astringency initiation reaction. Isothermal titration calorimetry (ITC) is a technique that has been used to study the thermodynamics of tannin – protein interactions. Using ITC, (McRae et al. 2010), (Poncet-Legrand et al. 2007b) and Frazier et al. (2003) reported influences of molecular ratio between tannins and proteins, molecular size (related to age) and galloylation of tannins, and hydrolysable tannin concentrations on the thermodynamics of the reactions that cause astringency. The merit of the ITC method is its ability to provide a model of astringency from the thermodynamic changes of the tannin – protein interactions without undertaking the complicated sensory analysis that is prone to a number of inevitable defects (Lesschaeve & Noble 2005). Another astringency modeling method, saliva precipitation index (SPI) with electrophoresis, different from the thermodynamic approach of ITC, has also been used recently (Gambuti et al. 2011; Rinaldi et al. 2012a, b). Comparing these two techniques, Rinaldi et al. (2012b) showed significant correlation between the SPI result and sensory evaluation of wine astringency, but to date there has been no information on the correlation between the ITC results and sensory properties.

2.3.7 Astringency and the wine matrix

Many factors in the wine matrix have influence on the perception of astringency. Generally, they can be categorized into two groups: 1) influences on the concentration and conformation of tannins; and 2) physical and chemical influences on the interaction and perception of astringency, which include physical exacerbation or compensation of the loss of lubrication and chemical impacts on tannin-protein binding and precipitation.

2.3.7.1 Oxygen and anthocyanins

The incorporation of moderate amounts of oxygen during the fermentation and post-fermentation phases has been reported to contribute to a more stable color and softened mouth-feel, and spawned a technique in the winemaking, micro-oxygenation (del Carmen Llaudy et al. 2006; Toit et al. 2006). del Carmen Llaudy et al. (2006) suggested that micro-oxygenation does not change the concentration of proanthocyanidins, but produces a slightly higher degree of polymerization and a drastically lower level of astringency. One possible explanation of this softening effect brought by oxygen treatment, may be a greater hydrophobicity and a higher level of intra-molecular bonding that reduce the ability of tannins to react with proteins (Zanchi et al. 2007). The ethyl linkage between polyphenol molecules can potentially provide better chances for tannins to precipitate from the wine, and thus reduce the perceived astringency (Vidal & Aagaard 2008; McRae et al. 2010).

It is known that purified anthocyanins do not contribute to the astringency of red wines (Kantz & Singleton 1990; Gawel 1998; Vidal et al. 2004a; Vidal et al. 2004b), whereas polymeric pigments are part of the wine components that produce astringency sensation (Gawel 1998). Despite the role in producing astringency, a

lowering effect of astringency may be implied by the formation of polymerized pigments due to the terminating effect of anthocyanins (Vidal et al. 2004a).

2.3.7.2 Wine body-related constituents

Some of the factors that influence red wine astringency, such as sugar, ethanol, glycerol, polysaccharides, and viscosity, could also be found in studies related to white wine body (Vidal et al. 2004b; Nurgel & Pickering 2005; Yanniotis et al. 2007). This may reveal some synergistic effect between red wine astringency and red wine body in the sensory perception of general mouth-feel.

Several studies reported the influence of viscosity and wine components contributing to viscosity on astringency, such as ethanol, polysaccharides and sugar. Smith et al. (1996), by using carboxymethyl cellulose as a thickener, found that the intensity and duration of astringency were reduced by viscosity. This result was supported by later studies in the astringency of different beverages (Smith & Noble 1998; Peleg & Noble 1999). It also explained the suppressing effect of sucrose on astringency reported in a previous study, which brought suspicion of the lowered astringency due to sweetness (Ishikawa & Noble 1995).

Ethanol was found to lower wine astringency with increasing concentration in several studies (Serafini et al. 1997; Demiglio & Pickering 2008; Fontoin et al. 2008; Rinaldi et al. 2012a), except for one study, which found ethanol to have to an enhancing effect on astringency with higher concentration (Obreque-Slíer et al. 2010). The mechanism of this suppressing effect of ethanol on astringency is still unclear, but it was suggested that it could be a two way effect; conformational changes of tannins due to a change in hydrophobicity with the presence of ethanol and the contribution to viscosity (McRae et al. 2010).

Polysaccharides, due to their presence in red wines and suspected influence on red wine astringency, were investigated by several studies (Pellerin et al. 1995; Escot et al. 2001; Vidal et al. 2003a; Vidal et al. 2004b; Carvalho et al. 2006; Aguirre et al. 2009). The characterization of polysaccharides in wine has been reviewed above. In terms of the role that polysaccharides play in wine body, Vidal et al. (2004b) provided evidence that polysaccharides could increase the fullness of model wine solution, as stated earlier. They also reported the significant suppressing effect of acidic polysaccharides on the perception of astringency, whereas neutral polysacchairdes were reported to have insignificant lowering ability. A later study by Carvalho et al. (2006) showed the same results about the suppression of astringency by different fractions of polysaccharides. Possible models describing the mechanisms of this inhibitory influence were suggested by Mateus et al. (2004) (Figure 2).

Summarizing from previous research on red wine astringency, its influencing factors, and wine body, it is reasonable to presume that some of the compounds involved in the perception of white wine body are contributors to red wine body, and that there is a synergistic effect between the two mouth-feel aspects of red wine, with the key role played by phenolic compounds.

Figure 2.2 Models of possible mechanisms of the inhibitory effect of carbohydrates on tannin– protein aggregation, from Mateus et al. (2004).
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Chapter 3 Materials and Methods

3.1 Wines

Wine samples were 18 New Zealand Pinot Noir wines from six producers, with three wines from each producer (Table 3.1). Wines of each producer represent three market ranges defined by the producer, according to their market price.

Before analysis, wines were divided into smaller portions by decanting the contents of a standard wine bottle (750 mL) in glass bottles (250 mL, 100 mL, 50 mL) and plastic tubes (15 mL) with closures. Inert gas (N_2) was sparged into each bottle/tube to fill the headspace before the bottle/tube was carefully sealed. All samples were labeled with the producer name and the producer defined quality grade: L (low), M (medium), and H (high). These were then stored at 4°C until required.

3.2 Reagents

Copper sulfate (pentahydrate), Folin-Ciocalteau reagent, sodium hydroxide, sodium potassium tartrate and sulfuric acid were obtained from Ajax Finechem Pty. Ltd. (Auckland, NZ). Acetic acid, p-hydroxydiphenyl, potassium iodide, sodium tetraborate and trichloroacitic acid were obtained from BDH Chemicals Ltd. (Poole, UK). Coomassie Brilliant Blue dye reagent was obtained from Bio-Rad Laboratories (New Zealand) Pty. Ltd. (Auckland, NZ). Acetone was obtained from LabServ, Biolab (Australia) Ltd. (Victoria, Australia). L-malic acid was obtained from Merck Millipore (Auckland, NZ). Ammonium acetate, ammonium sulfate, charcoal (Norit), citric acid, ethanol, gallic acid, galactose, galacturonic acid, glucose, methyl cellulose, potassium phosphate, poly-L-proline, sodium carbonate, sodium thiosulfate (pentahydrate), starch (soluble), succinic acid and L-tartaric acid were obtained from Sigma-Aldrich

Ltd. (Auckland, NZ). Ethyl acetate, hydrochloric acid, DL-lactic acid, methanol, phenol and phosphoric acid were obtained from Thermo Fisher Scientific (New Zealand) Ltd. (Auckland, NZ). All reagents were analytical grade.

Table 3.1 Pinot Noir wine samples.

Producer	Wine	Vintage	Recommended Retail Price	Quality grade
Escarpment	The Edge	2011	$24	L
	Escarpment	2010	$50	M
	Kupe	2010	$85	H
Mt Difficulty	Roaring Meg	2010	$28	L
	Bannockburn	2010	$45	M
	Long Gully	2010	$90	H
Neudorf	Tom’s Block	2010	$30	L
	Moutere	2010	$50	M
	Moutere Home Vineyard	2010	$80	H
Pegasus Bay	Main Divide	2010	$25	L
	Pegasus Bay	2010	$47	M
	Prima Donna	2010	$84	H
Saint Clair	Vicar’s Choice	2010	$22	L
	Marlborough Premium	2010	$27	M
	Pioneer Block 14 Doctor’s Creek	2010	$34	H
Villa Maria	Private Bin Central Otago	2010	$27	L
	Cellar Selection Marlborough	2010	$33	M
	Reserve Marlborough	2010	$51	H

3.3 pH and titratable acidity

A Suntex SP-701 pH meter (Suntex, Taiwan) was used for wine pH measurement. Sample pH was measured in duplicate at room temperature (20°C). Titratable acidity was determined on 10 mL aliquots (degassed immediately prior to use by boiling) with 0.1 M NaOH (standardized against 0.100 M HCl) to an end-point of pH 8.2. Determinations were carried out in duplicate and results expressed as g/L tartaric acid equivalents.

3.4 Organic acids

Organic acids were determined by an HPLC system (Shimadzu Corporation, Kyoto. Japan) consisting of system controller (CMM-20A), pump (LC-20 AD), degasser (DGU-20A5), auto-sampler (SIL-10AF), sample cooler, UV detector (SPD-20A), column oven (CTO-10 ASvp) and LC solution data processing software, using the method modified from Shi et al. (2011). Standard stock solution was prepared by dissolving L-tartaric acid, L-malic acid, DL-lactic acid, acetic acid, citric acid and succinic acid in 2.67% v/v ethanol at the concentration of 2 g/L. The standard stock solution was kept at 4°C prior to the analysis. Working standard solutions were made by diluting the stock solution with 2.67% v/v aqueous ethanol solution to 0, 1, 2, 5, 10, 20, 50, 100, 200, 500, and 800 mg/L. The HPLC column used for separation and analysing was a Prevail ™ organic acid column (250 x 4.6 mm, 5 µm particle size) with guard column (7.5 x 4.6mm, 5 µm particle size) (Grace Darison Discovery Sciences, Victoria, Australia). The mobile phase was 25 mM potassium phosphate (pH 2.5 adjusted with phosphoric acid) which was filtered through a 0.45 µm membrane (Merck Millipore, MA, USA). The flow rate was 0.6 mL/min and the column temperature was 50°C. The detection wavelength was 210 nm. Wines samples were diluted 5 times with pure water and filtered through a 0.2 µm Nylon membrane (Merck Millipore, MA, USA). Diluted samples were stored at 4°C before analysis. Sample injection volume was 20 µL. Identification of organic acids was done by comparing the retention time to the standards. Sample quantification was determined using the peak height of chromatogram with external calibration standard

curves. All data were processed using LC solution software.

3.5 Total phenolics

Total phenolics of the wine samples were determined using a micro Folin-Ciocalteu assay according to Singleton et al. (1999). Gallic acid standards were prepared using 5 g/L stock solution in 10% v/v aqueous ethanol solution, previously refrigerated at 4°C for less than one week before the experiment was conducted. Working standard concentrations were 50, 100, 250 and 500 mg/L. Wine samples were diluted by 10 times prior to the analysis. In 2 mL transparent cuvettes, 20 µL sample, blank (deionized water) or gallic acid standards were added. Then, 1.58 mL deionized water and 100 µL Folin-Ciocalteau reagent were added in sequence. Mixing was achieved through pipetting. The mixture was left for 5 minutes after which 300 µL 20% w/v sodium carbonate solution was added. The final mixture was incubated at room temperature (20°C) for 2 h before the absorbance at 765 nm was measured with a spectrophotometer (Helios Alpha, Unicam, England).

3.6 Methyl cellulose precipitable tannins

Methyl cellulose precipitable (MCP) tannins were determined according to Mercurio and Smith (2006). A standard curve was constructed using absorbance at 280 nm of epicatechin standard solutions (10, 25, 50, 75 and 100 mg/L). In a 1.7 mL eppendorf tube with 25 µL sample wine, 300 µL polymer (0.04% methyl cellulose solution) was added for the treatment group, whereas for the control group, pure water of the same volume (300 µL) was added in the place of polymer. Mixing was achieved by pipetting, and the solution was allowed to sit for 3 minutes. Then, for each group, 300 µL saturated ammonium sulfate solution and 475 µL deionized water was added in sequence. After 10 minutes of incubation, the mixture was centrifuged at 9,000 g for 5 min. The supernatant were poured into UV transparent cuvettes and the absorbance at 280 nm was measured. Tannin concentration was calculated from the difference between control and treatment groups according to the standard curve, with

consideration of the dilution factor (40). The tannin concentrations were expressed in mg/L epicatechin equivalents.

3.7 Isothermal titration calorimetry (ITC)

Tannin extraction

Tannins were extracted from the six wines of two producers: Neudorf and Pegasus Bay. The two groups of wines were selected based on their wide price range and small difference in tannin concentration. Solid phase extraction of tannins was carried out with 0.36 g C_{18} SEP-PAK cartridges (WAT051910, Global Science, Auckland, New Zealand) according to Kemp (2010)**.** Based on previously determined tannin concentrations, the amount of wine to deliver 9.9 mg of tannins was calculated and accurately measured. The volume of the samples ranged from 8.001 mL to 9.171 mL. Alcohol was removed by concentrating the samples to a volume of less than 0.5 mL in a centrifuge benchtop vacuum concentrator (Centrivap, Labconco, MO, USA) at 40°C for 12 h. Then 9 mL of deionized water was added to the concentrated alcohol free samples. SEP-PAK cartridges were activated by running 5 mL methanol, 7.5 mL ethyl acetate and 7.5 mL deionized water in sequence through the cartridge. Each sample was split into three equal portions to apply to three cartridges in consideration of the capacity of the cartridge. After the sample, 7.5 mL deionized water was applied to the cartridges for rinsing. Each cartridge was then dried with nitrogen gas at a flow rate of 1 L min^{-1} for 60 min. Ethyl acetate (5 mL) was then added to each dried cartridge to ensure all monomeric material was completely removed. The tannins were eluted with 5 mL of methanol for each portion, and the three portions of each sample were combined. Then, tannins were dried by rotary evaporation at 30°C and then dissolved in 150 µL ethanol.

Tannin-protein affinity assay by ITC

To simulate the thermodynamics of tannin-salivary protein interactions, isothermal titration calorimetry (ITC) was performed using a MicroCal VP-ITC (GE Healthcare Ltd, Auckland, New

Zealand) modified from the method described by (McRae et al. 2010). In that study, micro-titrations comprising 2 µL injections were used whereas in the present study, due to the larger capacity of the machine, 10 µL injections were used. The tannin–protein ratio was calculated according to McRae et al. (2010). In their study, a 2 mM tannin-buffer solution was used as titrant, with the molar concentration of extracted wine tannins calculated based on the molecular weight data from gel permeation chromatography (GPC) measurements. The average molar weight was 3325 ± 438 g for young Australian Shiraz wines from the 2007 vintage and 4304 ± 863 g for aged wines from the 2000 vintage. In the present study, molar weights of the extracted tannins from the samples were not determined. It was assumed that because the sample wines were of the same age (the 2010 vintage) it was acceptable to compare them at the same tannin mass concentration. A value of 3,300 g was assumed as the molar mass of the extracted tannins in the present study. This assumption was the basis for the quantification of 9.9 mg of previously extracted tannins. Poly-L-proline (PLP) was used to represent salivary proteins for the measurement (Poncet-Legrand et al. 2007b; McRae et al. 2010).

The extracted tannin in 150 µL ethanol were diluted to 1.5 mL with 11.1 mM aqueous ammonium acetate buffer (previously adjusted to pH 4.0 with 2 M HCl), creating a tannin solution with 10 mM ammonium acetate buffer and 10% v/v ethanol. The pH was then re-adjusted to 4.0 with 2 M HCL solution. A blank was prepared in the same way but without tannin. PLP (100 µM) buffered solution was made with 10 mM ammonium acetate pH 4.0 buffer and stored at 4°C overnight before use.

The buffered tannin solution or blank solution was used as titrant, titrating into the buffered PLP solution in the 1.448 mL sample cell. All solutions were degassed for ten minutes using a vacuum degasser immediately prior to the titration. The titration comprised of one 2 µL injection followed by seventeen 10 µL injections at 25°C, with each injection taking 20 seconds and each interval being 5 minutes. Titrations were carried out in duplicate.

The raw data (instant signal of system thermodynamics) and integrated ΔH (enthalpy change

of the reaction) by Microcal Origin (Microcal Software, Northhampton, MA) of each titration were extracted from the VP-ITC system.

3.8 Alcohol

The alcohol content (v/v) of the samples was determined by an ebulliometer (Laboratories Dujardin-Salleron, Noizay, France) based on the measurement of sample boiling temperature. A dial card was used to convert the boiling point to alcohol percentage using the reference boiling point of pure (reverse osmosis) water at room temperature (20°C). On the dial card, a 0.1°C difference in boiling temperature accounts for 0.3% v/v difference in alcohol (Figure 3.1), and the minimum scale of the thermometer is 0.1°C. Therefore, an estimation of half of the 0.1°C division was made during the reading. Although the lab temperature was controlled at 20°C throughout the whole experiment, the boiling point of pure water was measured at the beginning and middle of each set of tests. All samples were measured in duplicate.

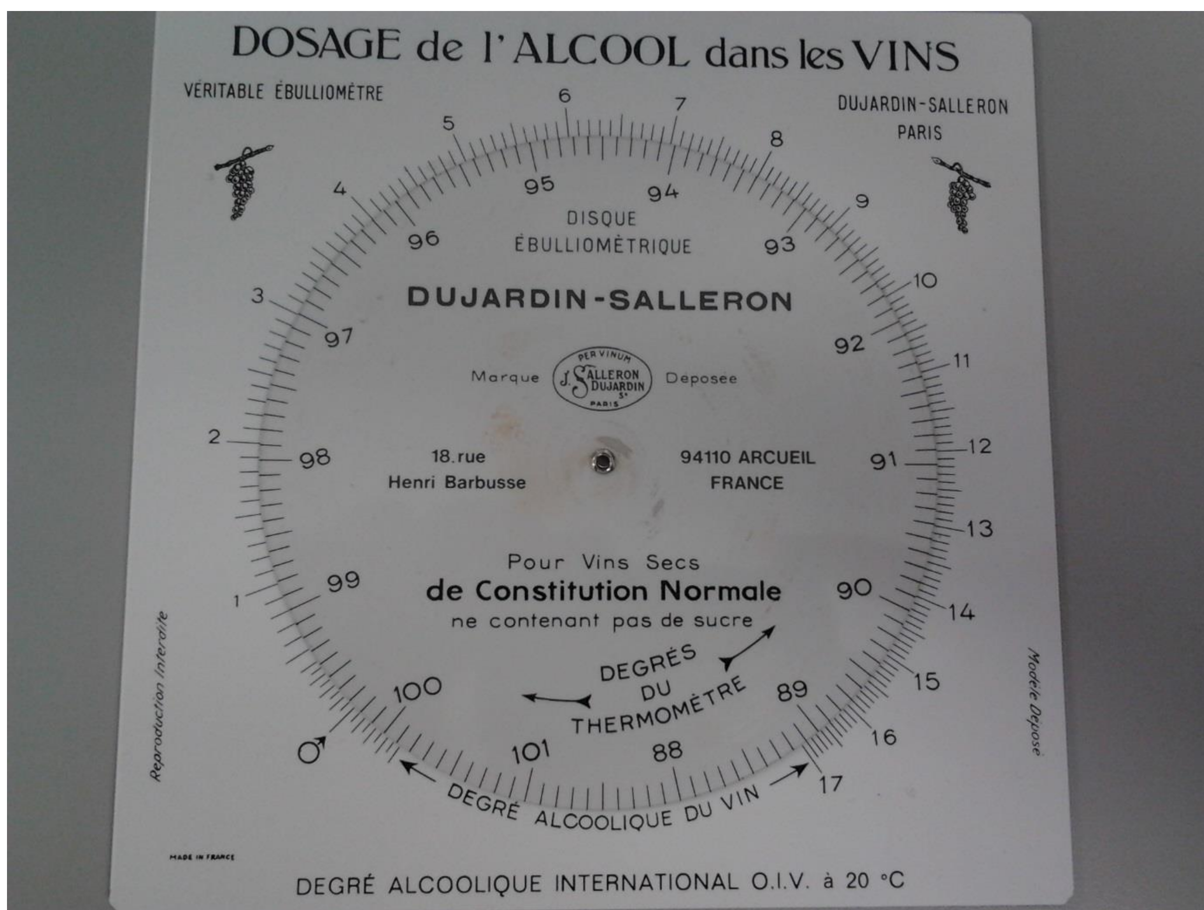


Figure 3.1 Dial card of ebulliometer to convert boiling temperature into alcohol content.

3.9 Glycerol

Wine samples were decolorized using “Norit” decolorizing charcoal. An amount of “Norit”, determined by trial and error to produced acceptable decolorized samples, was added to 10 mL of wine and the mixture was shaken thoroughly before it was filtered through 0.45 µm PES syringe filter (Merck Millipore, MA, USA). The same amount of “Norit” was used for each wine sample. The glycerol content of the decolorized samples was analyzed using the Randox glycerol colorimetric analysis kit (GY-105) with RX-Daytona benchtop clinical chemistry analyzer (Randox, Ireland), as described by Fossati and Prencipe (1982).

3.10 Reducing sugar

The reducing sugar content of wine samples was determined using the Rebelein method as

described by Iland (2004). The method is based on determination of excess Cu^{++} with iodide and starch after reacting with reducing sugars in wine.

Six reagents (Z1-Z6) were prepared with 1 L of each. Acidified copper solution (Z1) was prepared with 41.92 g of copper sulfate pentahydrate dissolved in reverse osmosis water with 1 mL concentrated sulfuric acid. Alkaline potassium tartrate solution (Z2) was made with 250 g sodium potassium tartrate and 80 g sodium hydroxide. Alkaline potassium iodide solution (Z3) was made with 100 mL 1 M sodium hydroxide and 300 g potassium iodide. Sulfuric acid solution (Z4) was carefully prepared with 175 mL of concentrated sulfuric acid added to cold reverse osmosis water. Potassium iodide/starch solution (Z5) was prepared using method 2, according to Iland (2004), with 20 g of potassium iodide and 10 g of soluble starch. A 0.055 M sodium thiosulphate solution (Z6) was prepared using 13.78 g sodium thiosulfate pentahydrate and 50 mL 1 M NaOH. A 10 g/L glucose solution was used as a spike to determine recovery for the assay.

The wines were decolorized using the same method as glycerol analysis with "Norit" decolorizing charcoal and SPE syringe filter (section 3.8). A mixture containing 10 mL Z1, 5 mL Z2, and 2 mL deionized water (blank), glucose standard or sample, was heated to boiling in a conical flask. The mixture was cooled and 10 mL of Z3, Z4 and Z5 were added in sequence. The titration was carried out with Z6 being the titrant. The end point was recorded when the solution turned to a milky white color. The sugar content was calculated using the difference between the blank titre volume (mL) and sample titre volume (mL), according to Iland (2004). A recovery assay was carried out with 10 g/L glucose solution with acceptable recovery being between 95% and 105%.

3.11 Polysaccharides

Total polysaccharides in wine were determined using methods based on Segarra et al. (1995).

The method combines determination of experimental total polysaccharides using the phenol-sulfuric acid method (Dubois et al. 1956), acidic polysaccharides using the hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen 1973). Calculated total polysaccharide is based on these two measured parameters.

Polysaccharides extraction

Wine samples (1 mL) were centrifuged at 2,500 g for 15 minutes. The supernatants were mixed with 5 mL ethanol and 50 µL 1 M HCl in 15 mL centrifuge tubes. The tubes were sealed and incubated in a water bath at 22°C for 18 hours before they were centrifuged at 1,800 g for 20 minutes. The supernatant was carefully discarded and the precipitates were washed 3 times with ethanol. Then, the precipitates were dried with nitrogen gas and re-solubilized in 5 mL deionized water.

Determination of experimental total polysaccharides

For experimental total polysaccharides, galactose solutions were used as standards at concentrations of 20, 40, 80, 120, 160 and 200 mg/L. Phenol reagent (50 µL 5% w/v freshly prepared) was added to 2 mL of extracted sample polysaccharides solution or galactose standard solution in a glass test tube. Concentrated sulfuric acid (5 mL) was directly pipetted to the surface of the solution to allow good mixing. Heat was generated in the process, and extreme care was required due to the possibility of splash. The test tubes were incubated for 10 minutes at room temperature and carefully shaken to mix and then incubated in a water bath at 25°C for 15 minutes. Absorbance was measured at 490 nm with the spectrophotometer (Helios Alpha, Unicam, England).

Determination of acidic polysaccharides

For acidic polysaccharides, the standards used were galacturonic acid solutions at concentrations of 10, 20, 40, 60, 80 and 100 mg/L. Sodium tetraborate (borax) was dissolved in 100 mL concentrated sulfuric acid to a concentration of 0.0125 M. The prepared sodium tetraborate/sulfuric acid solution (1.2 mL) was added to 0.2 mL galacturonic acid standards or extracted polysaccharides solution in 1.7 mL eppendorf tubes which were placed in ice.

Then the tubes were vortex mixed and incubated at 100°C for 5 minutes. The tubes were cooled in crushed ice. Instead of *o*-hydroxydiphenyl (Segarra et al. 1995), *p*-hydroxydiphenyl was used in the present study. The sensitivity of *p*-hydroxydiphenyl was recorded to be lower than *o*-hydroxydiphenyl and *m*-hydroxydiphenyl by Blumenkrantz and Asboe-Hansen (1973). *p*-Hydroxydiphenyl (0.15 g) and sodium hydroxide (0.05 g) were dissolved in reverse osmosis water, to give a 30% w/v solution with 10% w/v NaOH. The solution was diluted 20 times to give 1.5% *p*-hydroxydiphenyl in 0.5% NaOH, and further diluted with 0.5% w/v NaOH solution by 10 times. The final solution was 0.15% *p*-hydroxydiphenyl in 0.5% NaOH. A blank solution was produced without the addition of *p*-hydroxydiphenyl to account for the formation of coloured species by carbohydrates and the sodium tetraborate/sulfuric acid mixture at 100 °C. In the cooled eppendorf tubes, 20 µL of the indicator or blank was added. The tubes were shaken and the absorbance at 520 nm was measured within 5 minutes. Readings of the blank were subtracted in the final results.

Correction for total polysaccharides

According to Segarra et al. (1995), the response of neutral polysaccharides to the phenol method is 2.5 times higher than that of acidic polysaccharides. Therefore, the experimental total polysaccharides determined with galactose standard curve need to be corrected. The formula provided by Segarra et al. (1995) was used for the correction of total polysaccharides in the present study:

TPS (total polysaccharides) = experimental TPS + 0.6 APS (acidic polysaccharides).

3.12 Protein

Protein content was analyzed according to Smith et al. (2011) who tested different precipitation and quantification methods for protein in Pinot Noir wines. They recommended TCA/acetone precipitation and Bradford quantification with yeast invertase as protein standard. The Bradford assay in the present study was carried out as described by

Hung et al. (2013).

TCA/acetone precipitation

The TCA/acetone mixture was prepared by dissolving 10% w/v trichloroacetic acid in -20°C acetone 1 hour prior to the experiment, and was stored at -20°C before the analysis. To a 1.7 mL eppendorf tube containing 0.5 mL filtered sample (0.45 µm PES syringe filter, Merck Millipore, USA), 1 mL of -20 °C TCA/acetone was added. A blank, comprising reverse osmosis water rather than the sample, was included. The mixture was incubated at -20°C for 45 minutes and then centrifuged at 22,000g at 4°C for 15 minutes (5810R refrigerated centrifuge, Eppendorf, USA). The supernatant was carefully discarded, and the pellet was washed once with -20°C acetone. The pellet was dried with nitrogen gas and re-solubilized in 0.5 mL deionized water.

Bradford assay

Yeast invertase solutions were prepared as the standards at concentrations of 50, 100, 150, 200, 250 and 300 mg/L. To 200 µL re-dissolved sample protein and yeast invertase standards in eppendorf tubes, 100 µL 1 M NaOH was added. The mixture was incubated for 5 minutes and centrifuged at 10,000 g for 5 minutes. The alkalized sample protein (60 µL) and 1200 µL five-time diluted Coomassie Brilliant Blue dye reagent were pipetted into 1.5 mL transparent cuvettes. Mixing of the solutions was achieved by pipetting. After 10 min incubation at room temperature, the absorbance at 595 nm was measured against the blank with a spectrophotometer (Helios Alpha, Unicam, England). Determinations were carried out in duplicate.

3.13 Viscosity

The viscosity of sample wines was measured using a rotary viscometer, following the method described by Lopez et al. (1989). The instrument was a DV-*II*+ Brookfield rotary viscometer (Brookfield Engineering Laboratories Inc., MA, USA) with an adapter for low

viscosity measurements. Measurements were carried out in a water bath (TW8, Julabo, Seelbach, Germany) set at 20°C in a thermostatically controlled room at 16°C. The machine was zeroed, but not calibrated due to the lack of standard viscosity solution. Reverse mosis water was used as a reference for the measurement. The spinning speed was programmed to be from 40 to 200 rpm. Different torque values were generated at different spinning speeds, and the viscosity were calculated by the machine automatically based on the torque value. Only measurements within the range from 10% to 100% torque were recognized as valid values.

3.14 Specific gravity

Specific gravity of the samples was measured with 10 mL specific gravity bottles (H. J. Elliott Ltd., UK) according to Pomeranz and Meloan (2000). The specific gravity bottles were dried and weighed carefully at room temperature (20°C). The bottles were then filled with reverse osmosis water and incubated at 20°C for 15 minutes. The weight of each bottle with pure water was weighed after ensuring that no surplus water was left on the surface. The bottles were dried, and used for measuring the weight with wine samples following the same steps. The specific gravity was calculated using the equation:

Specific gravity = $m_{wine+bottle} – m_{bottle} / m_{water+bottle} – m_{bottle}$

3.15 Extract

Extract was calculated according to the method described by Amerine and Ough (1988). The first step was to find the density of residue of the sample using the following equation:

$$d_r = d_w - d_a + 1.0000.$$

where, d_r is the density of residue, d_w is the specific gravity of wine and d_a is the specific gravity of aqueous ethanol solution containing the same ethanol concentration as the wine (Table 3.4). The extract value (g/100 mL) was then determined using Table 3.5 provided by Amerine and Ough (1988).

Table 3.2 Specific gravity of aqueous alcohol at 20°C, from Amerine and Ough (1988), Table 8.

Alcohol (vol %)	Specific gravity
8.00	0.98894
8.50	0.98832
9.00	0.98771
9.50	0.98711
10.00	0.98650
10.50	0.98590
11.00	0.98530
11.50	0.98471
12.00	0.98412
12.50	0.98354
13.00	0.98297
13.50	0.98239
14.00	0.98182
14.50	0.98127
15.00	0.98071
15.50	0.98015
16.00	0.97960
16.50	0.97904
17.00	0.97850

Table 3.3 Specific gravity and extract at 20°C, from Amerine and Ough (1988), Table 9.

Specific gravity	Extract (g/100mL)
1.000	0.00
1.001	0.26
1.002	0.51
1.003	0.77
1.004	1.03
1.005	1.29
1.006	1.54
1.007	1.80
1.008	2.06
1.009	2.32
1.010	2.58
1.011	2.84
1.012	3.10
1.013	3.36
1.014	3.62
1.015	3.88
1.016	4.13
1.017	4.39
1.018	4.65
1.019	4.91
1.020	5.17

3.16 Sensory analysis

3.16.1 Shapley ranking

Thirteen panelists, including seven male and six female of age between 20 and 32, from Lincoln University, New Zealand, were selected based on their wine related experience and interests. All the panelists had adequate previous red wine tasting experience. Most of the panelists were final year students of the Viticulture and Oenology program with academic knowledge in related fields. The sensory analysis was carried out in a temperature controlled (20°C) purpose-built sensory room (RFH Building, Lincoln University). Data was captured using the Lincoln University online Qualtrics Survey System. Nine wines from three producers (Escarpment, Neudorf and Pegasus Bay) were chosen for the analysis.

Shapley ranking of the wines was conducted as described by Ginsburgh and Zang (2012). The panelists were split into two groups, with one group conducting the analysis at 9:30 in the morning and the other at 3:30 in the afternoon. Two sets of tastings were performed by each panelist. In order to separate the effects of mouth-feel from those of other sensations, in one tasting the panelists performed the evaluation whilst wearing a nose clip and under red light, thus depriving them of color and smell sensations. Half the panelists (randomly chosen) performed the evaluation under normal conditions in the first tasting, whilst the other half performed it with nose clips and under red light. This was reversed for the second tasting. The wines were presented in random order to the panelists in conventional XL5 standard wine tasting glasses with each sample designated by a three digit code. Panelists were given specific instructions on using water to rinse their mouths before tasting each sample and to leave a 30 s break between each sample. The panelists were instructed to separate the wines into two groups: one of wines with greater intensity of sensation in the mouth and the other of wines with lesser intensity of sensation in the mouth, with no limit on the number of wines in each group. For each panelist, the greater intensity group was allotted one point, which was equally distributed to every wine in that group. For example, if a panelist selected one wine for the greater intensity group, that wine gets one point, and if a panelist selected three wines for this group, each of the selected three gets 1/3 of a point. Finally, a third evaluation was carried out in which 8 panelists (randomly selected) were asked to score all of the 9 wines from 1 to 10 in terms of their intensity of sensation.

3.16.2 Sensory evaluation by Michael Cooper MW

Sixteen of the 18 sample wines had previously been tasted and evaluated by Mr. Michael Cooper MW. Descriptions of these wines are available in his book “Buyers Guide to New Zealand Wines”. Of relevance to the present study particularly, he ranked the 16 wines according to their astringency and wine body qualities. A three level grading system was used for both astringency and body, with A being soft and silky astringency or light body, B

being moderate astringency or medium body, and C being firm astringency or full body. Tasting notes from his book “Buyer’s Guide of New Zealand Wines” were also used as a reference for the present study.

3.17 Statistical analysis

Correlation analysis was used to analyze the linear correlation between variables. Principal component analysis (PCA) was conducted to explore the linear combinations of the data that could better explain the variance between sample wines. The value of PC1 and PC2 of each sample was calculated and the sample scatterplot of PC1 versus PC2 was used to compare the distance between wines from the same producer or similar price ranges. The variable loading plot of PCA was also produced to analyze the distance and clustering between variables. Canonical variate analysis (CVA) was also carried out to find the best combinations of data to better differentiate the wines according to certain factors, such as producer, price range ($0-30, $30-60, $60+)or producer defined quality grade. For data from each analysis, general linear model (GLM) was used with producer being as the model and market price being as a covariate to analyze the significance level of difference between data of different samples.

Chapter 4 Results

4.1 pH and titratable acidity

Wine pH ranged from 3.43 to 3.87 across the 18 samples. Significant differences in pH were found between producers ($P < 0.05$), whereas there was no significant difference between wines according to price range (Table 4.1). Similar patterns were observed with titratable acidity (TA). Escarpment and Saint Clair had the highest and lowest mean pH, respectively. Villa Maria and Neudorf wines, on the other hand, had the highest and lowest TA, respectively.

4.2 Organic acids

Lactic acid was the most abundant acid in all wines, followed by tartaric acid (Table 4.2). Higher concentrations of lactic acid in red wines, likely the result of malo-lactic fermentation during winemaking, was also observed in several other studies (Pérez-Ruiz et al. 2004; Valentão et al. 2007; Pereira et al. 2010). The practice of malo-lactic fermentation, converting diprotic malic acid into monoprotic lactic acid, was also reflected in the significant inverse correlation found between lactic acid and malic acid ($P < 0.01$). Tartaric acid was significantly different ($P < 0.001$) between producers (Table 4.2) with Villa Maria and Saint Clair wines having higher tartaric acid concentrations than the others. As for the other organic acids, citric acid was observed to be significantly different by price (Table 4.2).

Table 4.1 Results of analyses of pH and titratable acidity for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	pH	Titratable acidity (g/L)
Escarpment	L	$24	3.58	4.92
	M	$50	3.80	5.77
	H	$85	3.87	5.31
Mt Difficulty	L	$28	3.66	5.02
	M	$45	3.64	5.45
	H	$90	3.62	5.56
Neudorf	L	$30	3.74	5.20
	M	$50	3.68	5.31
	H	$80	3.75	5.13
Pegasus Bay	L	$25	3.53	5.34
	M	$47	3.45	5.81
	H	$84	3.43	5.91
Saint Clair	L	$22	3.49	6.02
	M	$27	3.55	5.70
	H	$34	3.57	5.81
Villa Maria	L	$27	3.71	5.45
	M	$33	3.63	5.74
	H	$51	3.53	6.23
Significance	Producer		*	*
	Price		ns	ns (P=0.059)

Significance[1]: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

Table 4.2 Results of analyses of individual organic acids for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Tartaric acid (g/L)	Malic acid (mg/L)	Citric acid (mg/L)	Lactic acid (g/L)	Acetic acid (mg/L)	Succinic acid (mg/L)
Escarpment	L	$24	1.94	305	572	2.22	403	813
	M	$50	1.94	177	630	3.15	503	672
	H	$85	1.64	75.0	848	3.68	642	472
Mt Difficulty	L	$28	1.72	134	644	2.73	559	674
	M	$45	1.69	166	825	3.29	653	844
	H	$90	1.76	170	804	3.17	646	721
Neudorf	L	$30	1.57	135	711	3.06	563	821
	M	$50	1.56	146	787	2.84	540	900
	H	$80	1.34	134	857	2.90	611	887
Pegasus Bay	L	$25	1.20	140	825	3.09	572	704
	M	$47	1.47	162	886	2.78	616	842
	H	$84	1.51	164	831	2.36	524	807
Saint Clair	L	$22	2.39	108	641	3.03	436	635
	M	$27	2.67	121	561	2.75	414	604
	H	$34	2.62	119	785	2.98	459	656
Villa Maria	L	$27	2.04	202	591	2.41	531	1003
	M	$33	1.77	179	691	2.89	602	643
	H	$51	2.21	165	661	2.63	522	399
Significance	Producer		***	ns	ns	ns	ns	ns
	Price		ns	ns	*	ns	ns	ns

Significance[1]: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

4.3 Total phenolics, methyl cellulose precipitable tannins and isothermal titration calorimetry (ITC)

4.3.1 Total phenolics and methyl cellulose precipitable tannins

Results for total phenolics and methyl cellulose precipitable (MCP) tannins were highly correlated ($r = 0.96$; $P < 0.001$). Total phenolics in the samples ranged between 1.62 and 3.52 g/L gallic acid equivalents (Table 4.3) similar to the results of previous total phenolics quantification in Pinot Noir wines (Landrault et al. 2001). MCP tannins were between 0.82 and 1.74 g/L epicatechin equivalents (Table 4.3), which is consistent to the findings in previous research in Pinot Noir wines (Cortell et al. 2005). Both phenolic and tannin concentrations were significantly different by producer. Mt Difficulty had the highest mean concentration of total phenolics and tannins, and Saint Clair had the lowest. On the other hand, neither total phenolics nor MCP tannins showed any significant difference by price.

4.3.2 Isothermal titration calorimetry (ITC)

The tannin – protein affinity assay was carried out on six wines from two producers, Neudorf and Pegasus Bay. These wines were selected due to their similar tannin concentrations and wide market price range (Table 4.3). Raw data for ITC titrations for sample and blank are illustrated in Figure 4.1. Negative values in the graph indicate exothermic signals, and positive values indicate endothermic signals. Each peak of the curve conveys the instant thermodynamic information of the titration system at a certain time point. Figure 4.2 shows the integrated enthalpy (ΔH) of each titration, obtained from the Microcal Origin software package (Microcal Software, Northampton, MA, ver. 7). Due to the complexity of the interaction between larger molecules, such as tannins and proteins, the interpretation of the ITC data is somewhat generalized in many cases (Poncet-Legrand et al. 2007a; McRae et al. 2010).

Table 4.3 Results of analyses of total phenolics, MCP tannins and ITC (Isothermal titration calorimetry) for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Total phenolics (g/L)	MCP tannins (g/L)	ITC enthalpy (mcal)
Escarpment	L	$24	2.48	1.14	
	M	$50	2.80	1.17	
	H	$85	2.61	1.23	
Mt Difficulty	L	$28	3.22	1.44	
	M	$45	3.41	1.74	
	H	$90	3.52	1.52	
Neudorf	L	$30	2.85	1.15	-2.63
	M	$50	2.88	1.09	-2.71
	H	$80	2.75	1.08	-2.46
Pegasus Bay	L	$25	2.69	1.23	-2.68
	M	$47	2.92	1.21	-2.42
	H	$84	2.77	1.24	-2.36
Saint Clair	L	$22	1.62	0.41	
	M	$27	1.81	0.53	
	H	$34	1.84	0.50	
Villa Maria	L	$27	3.24	1.64	
	M	$33	2.27	0.83	
	H	$51	2.21	0.82	
Significance[1]	Producer		***	**	ns
	Price		ns	ns	ns

[1]Significance: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

For all six samples, the exothermic signal increased in the first 4 to 8 injections, then decreased. The final injections were endothermic with relatively constant values. This pattern is similar to previously reported results (Poncet-Legrand et al. 2007b). The initial exothermic response has been ascribed to co-operative binding of tannins to PLP. Subsequently, the exothermic signal is reduced as binding sites for tannins with PLP are reduced. The endothermic component has been ascribed to the dilution of tannins once all the PLP binding sites are saturated.

The results of the titrations were similar except for the Pegasus Bay H wine. This was clearly different from the others with the exothermic signal decreasing more rapidly and with a greater endothermic plateau at the end of the titration (Figure 4.2). As indicated above, this is considered to result from dilution of the tannin titrant. Conventionally, a titration into buffer solution without PLP is carried out to support this theory; however, in the present study, due to a shortage of the titrant material, this blank was not possible. To compare integrated ΔH values for the interaction of different samples with PLP, a horizontal line was drawn at the point where the integrated curve started to plateau, and the ΔH value of that point was considered zero. The points before that were regarded as components of the tannin-protein interaction, and their ΔH were summed (Table 4.3). Neudorf M and Pegasus Bay H had the highest and the lowest ΔH (absolute value), which were -2707 µcal (0.113 J per 0.1448 mol PLP) and -2357 µcal (0.099 J per 0.1448 mol PLP), respectively.

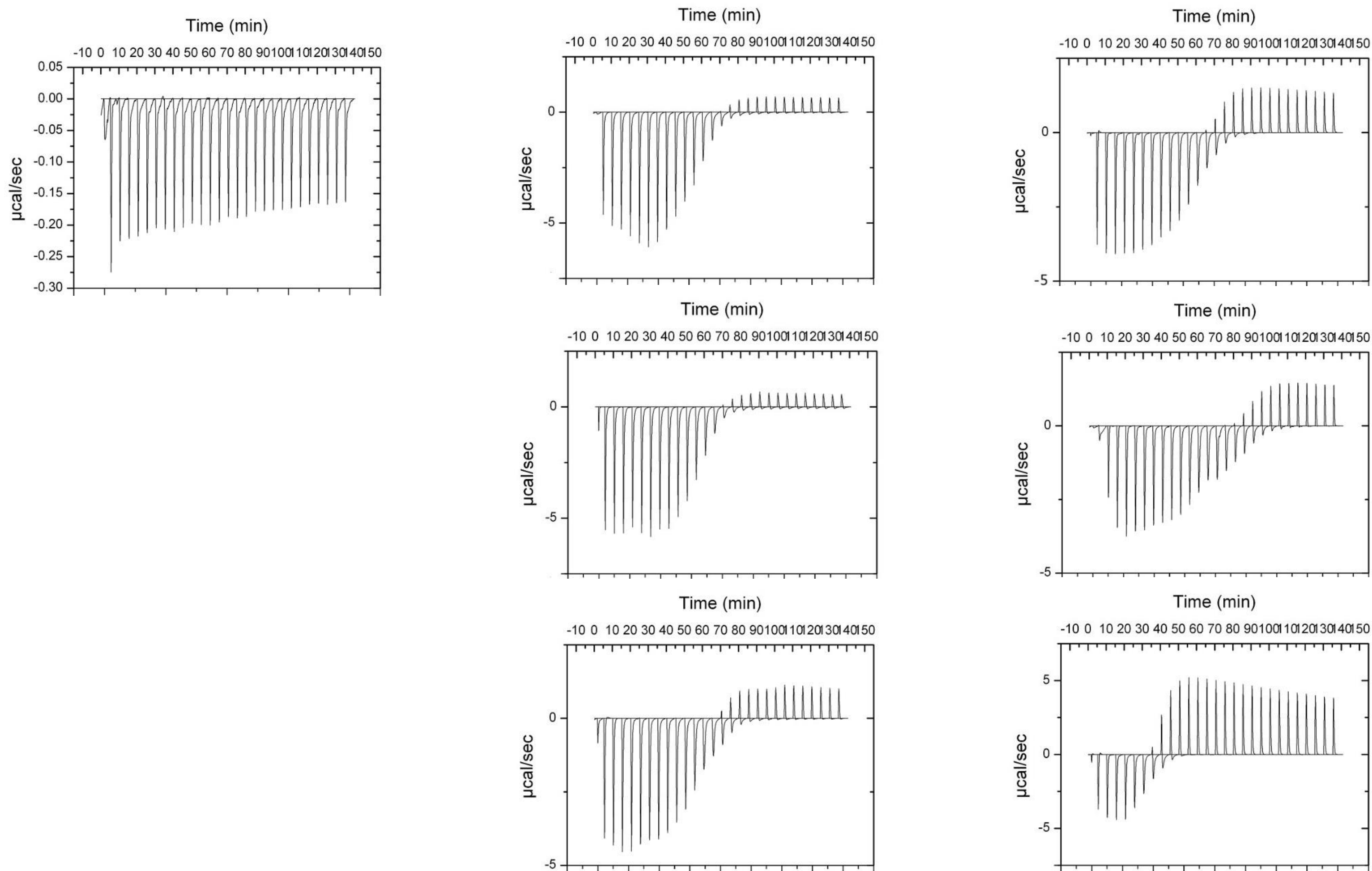


Figure 4.1 Raw data of tannin – protein affinity assay using ITC. Results are: blank titration (left), Neudorf wines (middle) and Pegasus Bay wine (right), and for L, M and H (top to bottom) quality grades. Note the change in scale between blank and sample titrations.

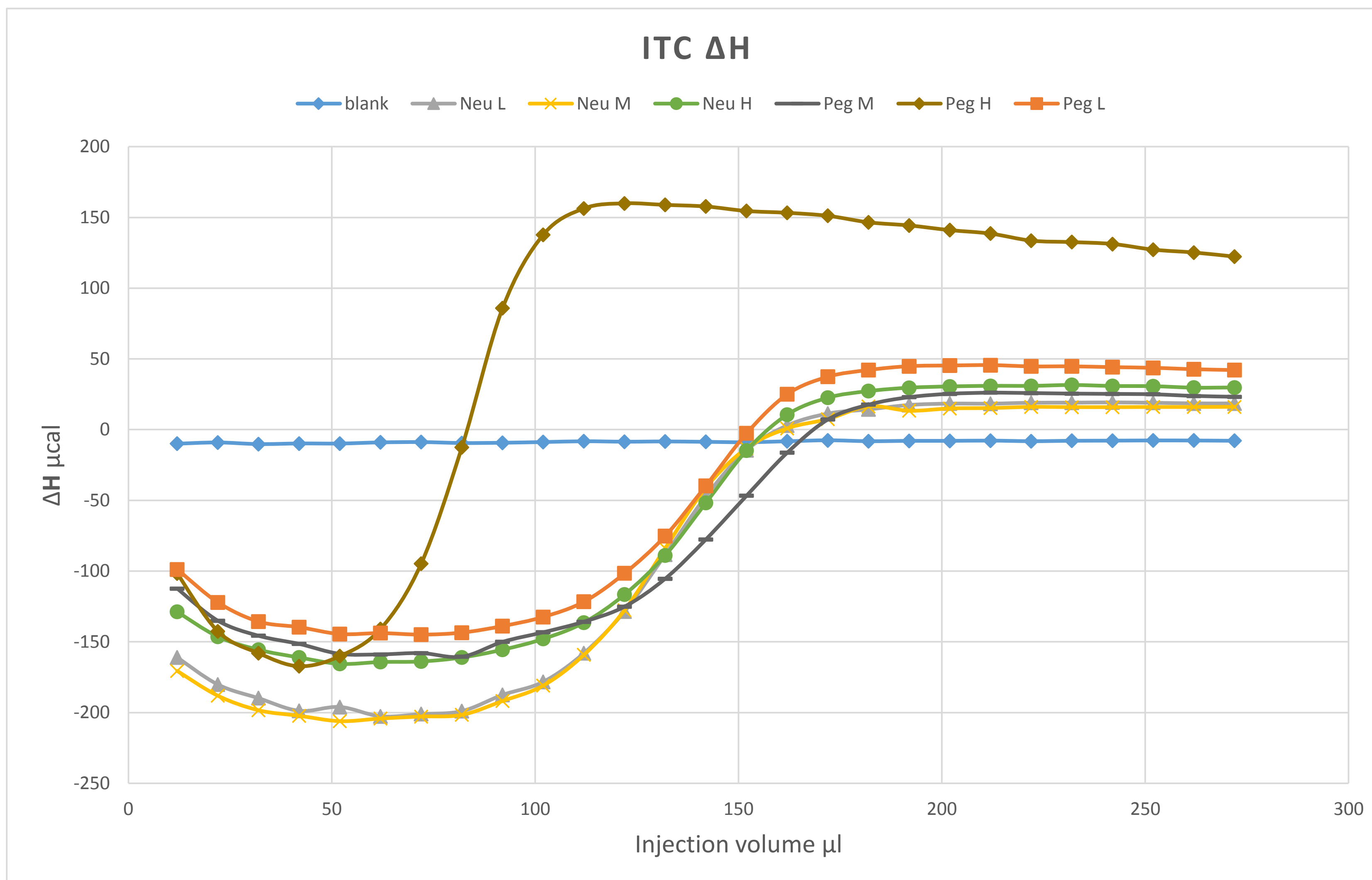


Figure 4.2 Integrated ΔH from ITC (Blank (no tannin), Neu L, M, H: Neudorf L, M, H; Peg L, M, H: Pegasus Bay L, M, H).

4.4 Alcohol and glycerol

The alcohol content of the Pinot Noir wines in the present study ranged from 13.0% to 15.0%, and was significantly different by producer (Table 4.4). Pegasus Bay was the producer that had the highest average alcohol content (14.6%), and Saint Clair was the one with the lowest (13.2%).

Red wine glycerol concentration was reported to be around 10 g/L in previous studies (Ough et al. 1972; Nurgel & Pickering 2005; Gawel et al. 2007). The glycerol content measured in the present study was between 7.59 and 10.88 g/L (Table 4.4), falling below the 25 g/L suggested concentration requirement for a significant increase in wine viscosity (Nurgel & Pickering 2005).

4.5 Reducing sugar, polysaccharides and protein

The Pinot Noir wine samples in the present study were dry wines, with reducing sugar content (mainly glucose and fructose) up to 3.20 g/L (Table 4.5). Reducing sugars at this concentration in red wine give hardly noticeable sweetness under the influence of tastes of other wine constituents, such as acids and phenolics (Jackson 2009). Polysaccharides were also quantified. Generally, there are two major groups of total polysaccharides in wine comprising of neutral and acidic fractions (Vidal et al. 2003a). Grape-derived type II arabinogalactan proteins and yeast derived mannoproteins contain the major neutral polysaccharides in wine. Rhamnogalacturonans are the main component of the acidic fraction (Vidal et al. 2004b). In the present study, total polysaccharides in Pinot Noir wines were between 0.94 and 1.53 g/L (Table 4.5). This was consistent with previous results presented by Guadalupe et al. (2012) using gas-chromatography mass spectrometry and size exclusion chromatography. The acidic polysaccharide fraction which was used to correct the result for total polysaccharides, was between 71.6 and 138 mg/L (Table 4.5). Acidic polysaccharides and total polysaccharides were significantly correlated ($r = 0.69$, $P < 0.01$). A

significant difference was found in reducing sugar content by producer, whereas there was no significant difference for polysaccharide fractions.

The protein concentrations in the wines were found to vary significantly, ranging from 20.2 to 122.0 mg/L. The average concentration of protein in all samples was 46.5 mg/L, which is lower than the approximate 80 mg/L average concentration in Pinot Noir wines reported by Smith et al. (2011). The concentration of proteins was relatively small when compared with other macro-molecular fractions of red wine, such as polyphenols and polysaccharides. Protein content was significantly different for producers ($P < 0.05$), and not significantly different by price ($P = 0.051$) (Table 4.5).

4.6 Viscosity, specific gravity and extract

Viscosity of Pinot Noir wines, measured by a rotary viscometer in the present study, ranged from 1.79 to 1.95 mPa s at 20°C for 18 samples (Table 4.6), a range slightly higher than the average 1.55 mPa s recorded by Košmerl et al. (2000) for Slovenian red wine at the same temperature. Generally, it is hard to compare the results for Pinot noir wines from the present study with other published data because different temperatures were used (Nurgel & Pickering 2005; Yanniotis et al. 2007). Despite this, it was considered acceptable to compare the viscosity between samples within this study. The three least viscous wines, Escarpment L, Saint Clair L and Saint Clair H, and the three most viscous wines, Pegasus Bay L, M and H, have a viscosity difference just at the level of the threshold of perceptional difference of viscosity. Statistically, there was a significant difference in the viscosity of wines from different producers ($P < 0.01$).

Specific gravity was similar for all the wines, ranging from 0.99193 to 0.99378. The specific gravity did not show significant differences for producer or price. Combined with the alcohol results, specific gravity was used to calculate the value of extract of each wine. The extract concentration of the Pinot Noir wines in this study was calculated to be between 23.2 and

31.0 g/L. This result is similar to the red wine extract value provided by Mattivi (1993). Significant differences were found in the extract value of sample wines according to producer and price ($P < 0.05$).

Table 4.4 Results of analyses of alcohol and glycerol for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Alcohol (% v/v)	Glycerol (g/L)
Escarpment	L	$24	13.1	7.59
	M	$50	14.0	9.16
	H	$85	13.6	8.69
Mt Difficulty	L	$28	13.8	9.27
	M	$45	13.5	8.28
	H	$90	13.6	8.36
Neudorf	L	$30	13.9	8.83
	M	$50	13.9	9.13
	H	$80	14.1	9.42
Pegasus Bay	L	$25	14.5	10.75
	M	$47	14.5	10.40
	H	$84	14.8	10.88
Saint Clair	L	$22	13.2	8.00
	M	$27	13.1	7.99
	H	$34	13.2	7.87
Villa Maria	L	$27	13.4	8.63
	M	$33	13.7	9.27
	H	$51	13.7	9.02
Significance	Producer		***	***
	Price		ns	ns

Significance[1]: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

Table 4.5 Results of analyses of reducing sugar, polysaccharides (total polysaccharides and acidic polysaccharides) and protein for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Reducing sugar (g/L)	Total polysaccharides (g/L)	Acidic polysaccharides (mg/L)	Protein (mg/L)
Escarpment	L	$24	1.85	0.94	81.6	33.5
	M	$50	2.40	1.21	95.9	76.0
	H	$85	1.90	1.31	114	122
Mt Difficulty	L	$28	2.20	1.11	116	68.5
	M	$45	2.15	1.12	105	51.8
	H	$90	2.20	1.40	103	108
Neudorf	L	$30	2.30	1.14	103	34.3
	M	$50	2.70	0.99	94.4	23.5
	H	$80	2.75	1.10	97.3	41.8
Pegasus Bay	L	$25	3.20	1.53	138	41.0
	M	$47	2.95	1.17	99.4	29.3
	H	$84	3.05	1.19	111	21.0
Saint Clair	L	$22	2.25	1.17	71.6	29.3
	M	$27	3.20	1.15	83.7	20.2
	H	$34	2.30	1.18	93.7	24.3
Villa Maria	L	$27	1.80	1.35	112	49.3
	M	$33	2.55	1.35	107	29.3
	H	$51	2.25	1.46	125	31.8
Significance[1]	Producer		*	ns	ns	*
	Price		ns	ns	ns	ns(P=0.051)

[1]: Significance * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

Table 4.6 Results of analyses of viscosity, specific gravity and extract for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Viscosity (mPa s)	Specific gravity	Extract (g/L)
Escarpment	L	$24	1.79	0.99193	23.2
	M	$50	1.87	0.99302	28.4
	H	$85	1.86	0.99409	31.0
Mt Difficulty	L	$28	1.90	0.99298	28.4
	M	$45	1.86	0.99334	28.4
	H	$90	1.90	0.99346	28.4
Neudorf	L	$30	1.87	0.99224	25.8
	M	$50	1.87	0.99228	25.8
	H	$80	1.89	0.99259	28.4
Pegasus Bay	L	$25	1.93	0.99322	31.0
	M	$47	1.93	0.99316	31.0
	H	$84	1.95	0.99275	31.0
Saint Clair	L	$22	1.81	0.99243	23.2
	M	$27	1.82	0.99340	25.8
	H	$34	1.81	0.99300	25.8
Villa Maria	L	$27	1.83	0.99349	28.4
	M	$33	1.88	0.99261	28.4
	H	$51	1.88	0.99378	31.0
Significance	Producer		**	ns	*
	Price		ns(P=0.057)	ns	*

Significance[1]: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

4.7 Sensory evaluation

4.7.1 Astringency and body grading by M. Cooper MW

Mr. M. Cooper MW, as a highly trained professional palate, tasted most of the sample wines in the process of writing his book “Buyer’s Guide to New Zealand wines”. Detailed comments and descriptions of the wines are available in the book: 16 out of 18 sample wines, Saint Clair L and Villa Maria L were not included, were ranked by Cooper using a three level grading system to characterize the astringency and body mouth-feel (Table 4.7). For astringency, he ranked Mount Difficulty M and H as grade A (soft and silky tannins), Mount Difficulty L, Escarpment L, M, H, Neudorf H, Saint Clair M, H, Villa Maria H and Pegasus Bay L as grade B (moderate tannins), and Neudorf L, M, Villa Maria M, Pegasus Bay M and H as grade C (firm tannins). As for body, most of the wines fall into grade A (light body), with two wines, Mount Difficulty L and Saint Clair M, being exceptional and categorized as grade B (medium body). There was no clear relationship found between these rankings and the chemical analyses.

4.7.2 Shapley ranking by Lincoln University students

The Shapley ranking values are given in Table 4.7. The values for the normal tasting ranged between Escarpment L being the lowest (0.458) and Pegasus Bay M being the highest (2.358). The Shapely values for the tasting under red light and with nose clips showed different extremes with Neudorf L receiving the lowest value (0.367), and Pegasus Bay H receiving the highest (1.983). A statistically significance difference was found in the normal tasting Shapley ranking values according to producer (P=0.004). The Shapley ranking values where the wines were tasted under red light with nose clips were not significantly different according to producer or price (P=0.052 for price).

Table 4.7 Results of sensory analyses for the 18 study wines.

Producer	Quality grade	Recommended Retail Price	Astringency grading[1] (M. Cooper)	Body grading[2] (M. Cooper)	Shapely ranking values (Normal tasting)	Shapely ranking values (Sensation partially deprived tasting)
Escarpment	L	$24	B	A	0.458	1.717
	M	$50	B	A	1.025	1.517
	H	$85	B	A	0.992	1.933
Mt Difficulty	L	$28	B	B		
	M	$45	A	A		
	H	$90	A	A		
Neudorf	L	$30	C	A	1.392	0.367
	M	$50	C	A	1.408	1.450
	H	$80	B	A	1.317	1.850
Pegasus Bay	L	$25	B	A	1.817	1.150
	M	$47	C	A	2.358	1.033
	H	$84	C	A	2.108	1.983
Saint Clair	L	$22				
	M	$27	B	B		
	H	$34	B	A		
Villa Maria	L	$27				
	M	$33	C	A		
	H	$51	B	A		
Significance[3]	Producer				**	ns
	Price				ns	ns (P=0.052)

[1]Astringency grading (M. Cooper): A – soft, silky ; B – moderate; C – firm;

[2]Body grading (M. Cooper); A – light; B – medium; C – full.

[3]Significance: * (P<0.05); ** (P<0.01); *** (P<0.001); ns (non-significant, P>0.05).

4.8 Multivariate analyses

Several significant correlations were observed (Table 4.8). Total phenolics and MCP tannins ($P<0.001$), alcohol and glycerol ($P<0.001$), and titratable acidity and pH ($P<0.05$), as expected (Jackson 2008), were found to be significantly correlated with each other. Tartaric acid was found to be significantly inversely correlated to a number of variables, including pH ($P < 0.01$), alcohol ($P < 0.001$), total phenolics ($P < 0.01$), tannins ($P < 0.01$) and viscosity ($P < 0.001$). Viscosity was found to be mostly correlated with alcohol ($P<0.001$) and glycerol ($P<0.001$), as well as other parameters including tartaric acid (inversely, $P<0.01$), acetic acid ($P<0.01$), citric acid ($P<0.01$), reducing sugar ($P<0.05$) and acidic polysaccharides ($P<0.01$). Viscosity also showed significant correlation with extract ($P<0.001$), but not with specific gravity. The Shapley ranking values for the different trials showed different characteristics in the correlation analysis (Table 4.9). The Shapley ranking values for the normal tasting showed high correlations with alcohol ($P<0.001$), glycerol ($P<0.001$), reducing sugar ($P<0.001$) and viscosity ($P<0.001$), and significant correlations with total phenolics ($P<0.05$), citric acid ($P<0.05$), tartaric acid ($P<0.05$), TA ($P<0.05$) and extract ($P<0.05$).

Results from PCA found that the first six principal components, each consisting of a linear combination of the variables involved in the analysis, could explain 91.7% of the total variance in sample wines (Table 4.10). Figure 4.3 showed the scree plot of the principal components against the component number; the eigenvalue became small after principal component number 6. The first two principal components accounted for 57.2% of the total variance. The variance proportion of PC1 was 39.1% (Table 4.10), and it was correlated most strongly with alcohol ($P<0.05$), glycerol ($P<0.05$), viscosity ($P<0.05$) and acidic polysaccharides ($P<0.05$) (Table 4.8). The variance proportion of PC2 was 18.1% (Table 4.10), and it was most strongly correlated with tartaric acid ($P<0.05$) (Table 4.8). The variable loading plot of PC1 versus PC2 (Figure 4.4) showed that clusters of the variables could be found. On PC1 tartaric acid was well separated from most variables, and on PC2 sugar, titratable acidity (TA), glycerol, alcohol and viscosity were well separated from protein, lactic acid, pH, tannins and total phenolics. The sample scatterplots (Figure 4.5, 4.6 and 4.7) of PC1 versus PC2 with different legends showed the effect of producer, price range, and quality grade factors in separating the sample wines according to the first two principal

components. Producer appeared to be the best factor to differentiate the samples in PCA, whereas price point and quality grade factors separated the wines poorly.

Canonical variates obtained using producer, price range and quality grade as grouping factors differ. Table 4.11 showed the correlations between the variables and canonical variates for each grouping factor. Figure 4.8, 4.9 and 4.10 showed the sample scatterplot of CV1 versus CV2 by the three grouping factors. Linear combinations of variables separated the sample wines most clearly according to producer. Price range and quality grade, on the other hand, provided linear combinations of variables that could show clear separations between the samples according to these two factors. CV1 and CV2 for the producer factor showed highest correlations with alcohol (P<0.01), total phenolics (P<0.001), tannins (P<0.001), tartaric acid (P<0.001), acetic acid (P<0.01), citric acid (P<0.05), glycerol (P<0.01), pH (P<0.05), acidic polysaccharides (P<0.01), viscosity (P<0.01), extract (P<0.05), pH (P<0.05), sugar (P<0.05) and protein (P<0.01). CVA for price range showed canonical variates with higher correlations with acetic acid (P<0.05), citric acid (P<0.01), protein (P<0.05), extract (P<0.05). CVA for quality grade showed CV1 and CV2 with higher correlations with citric acid (P<0.05).

Table 4.8 Correlations (r value) between variables and principle components (PC1 and PC2).

	PC1[1]	PC2	PRI	ALC	TPH	TAN	TAR	MAL	LAC	ACE	CIT	SUC	GLY	pH	TA	SUG	TPA	APA	PRO	VIS	EXT	SPE
PC1	1.00																					
PC2	0.00	1.00																				
PRI	0.10	0.03	1.00																			
ALC	0.54	-0.01	0.37	1.00																		
TPH	0.02	-0.18	0.36	0.37	1.00																	
TAN	-0.08	-0.16	0.30	0.31	0.96	1.00																
TAR	-0.39	0.26	-0.39	-0.77	-0.65	-0.62	1.00															
MAL	-0.31	0.13	-0.21	-0.10	0.24	0.30	-0.05	1.00														
LAC	-0.06	-0.28	0.29	-0.04	0.07	0.04	-0.15	-0.66	1.00													
ACE	0.31	-0.30	0.55	0.45	0.68	0.63	-0.70	-0.25	0.53	1.00												
CIT	0.36	-0.26	0.62	0.61	0.33	0.27	-0.63	-0.38	0.45	0.71	1.00											
SUC	0.00	0.15	-0.06	0.23	0.53	0.50	-0.40	0.39	-0.35	0.12	0.15	1.00										
GLY	0.57	-0.02	0.27	0.97	0.29	0.26	-0.71	-0.13	-0.09	0.41	0.52	0.15	1.00									
pH	0.02	-0.29	0.40	0.38	0.43	0.40	-0.67	-0.23	0.60	0.56	0.39	-0.05	0.28	1.00								
TA	0.12	0.49	0.14	0.20	-0.13	-0.11	0.31	-0.06	-0.20	-0.09	0.03	-0.06	0.27	-0.51	1.00							
SUG	0.28	0.00	0.05	0.60	-0.20	-0.28	-0.23	-0.27	-0.09	-0.02	0.32	0.04	0.63	-0.09	0.29	1.00						
TPA	0.33	-0.19	0.16	0.19	0.07	0.12	-0.07	-0.20	0.28	0.38	0.15	-0.41	0.29	0.01	0.43	0.05	1.00					
APA	0.45	-0.33	0.18	0.53	0.41	0.46	-0.50	-0.12	0.12	0.54	0.33	-0.17	0.61	0.29	0.17	0.10	0.69	1.00				
PRO	-0.21	-0.17	0.51	-0.08	0.48	0.49	-0.21	-0.19	0.61	0.52	0.18	-0.25	-0.12	0.60	-0.22	-0.47	0.33	0.25	1.00			
VIS	0.58	-0.10	0.47	0.92	0.48	0.40	-0.73	-0.18	0.04	0.61	0.60	0.09	0.91	0.34	0.20	0.54	0.35	0.63	0.09	1.00		
EXT	0.34	-0.14	0.51	0.68	0.40	0.43	-0.51	-0.22	0.18	0.65	0.54	-0.18	0.73	0.33	0.40	0.27	0.62	0.81	0.32	0.77	1.00	
SPE[2]	N/A	N/A	0.32	-0.02	0.15	0.22	0.13	-0.38	0.39	0.38	0.17	-0.46	0.06	0.08	0.41	-0.08	0.67	0.52	0.53	0.16	0.66	1.00

Abbreviations[1]: PC – principal component; PRI – price; ALC – alcohol; TPH – total phenolics; TAN – MCP tannins; TAR – tartaric acid; MAL – malic acid; LAC – lactic acid; ACE – acetic acid; CIT – citric acid; SUC – succinic acid; GLY – glycerol; TA – titratable acidity; SUG – reducing sugar; TPA – total polysaccharides; APA – acidic polysaccharides; PRO – protein; VIS – viscosity; EXT – extract; SPE – specific gravity.

SPE[2]: Not applied. Specific gravity was not applied to principal component analysis (PCA), for “extract” was calculated using specific gravity and alcohol value.

Table 4.9 Variables with significant ($P < 0.05$) correlations (r value) with Shapley ranking values from tasting (normal light, without nose-clip).

	r value
Alcohol	0.939
Total Phenolics	0.681
Citric acid	0.723
Tartaric	-0.676
Glycerol	0.918
TA	0.691
Reducing sugar	0.860
Viscosity	0.936
Extract	0.677

Table 4.10 Eigenvalues of principal components (PC1 – PC6) (above) and coefficients of variables (below) from PCA.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	7.04	3.25	2.86	1.91	0.97	0.472
Proportion	0.391	0.181	0.159	0.106	0.054	0.026
Cumulative	0.391	0.572	0.731	0.837	0.890	0.917

Variable	Variable Coefficient					
	PC1	PC2	PC3	PC4	PC5	PC6
Alcohol	0.309	0.250	-0.107	0.136	0.115	-0.180
Total Phenolics	0.250	-0.242	-0.281	-0.167	-0.170	-0.154
MCP Tannins	0.238	-0.253	-0.276	-0.244	-0.114	-0.105
L-Tartaric acid	-0.321	0.061	0.205	-0.189	-0.174	-0.190
L-Malic acid	-0.077	-0.027	-0.430	-0.299	0.260	0.083
Lactic acid	0.118	-0.275	0.389	0.229	-0.215	0.002
Acetic acid	0.315	-0.188	0.056	0.002	-0.250	0.290
Citric acid	0.271	0.017	0.067	0.269	-0.384	0.311
Succinic acid	0.053	-0.018	-0.499	0.088	-0.403	0.070
Glycerol	0.299	0.298	-0.064	0.066	0.170	-0.116
pH	0.223	-0.293	0.062	0.273	0.336	-0.280
TA	0.022	0.345	0.112	-0.362	-0.456	-0.387
Sugar	0.107	0.422	0.024	0.287	-0.026	-0.122
Total Polysaccharides	0.161	0.073	0.321	-0.420	0.025	0.293
Acidic Polysaccharides	0.280	0.056	0.112	-0.306	0.265	0.362
Protein	0.142	-0.409	0.197	-0.146	0.016	-0.446
Viscosity	0.334	0.196	-0.032	0.034	0.070	-0.144
Extract	0.317	0.119	0.148	-0.227	0.034	-0.105

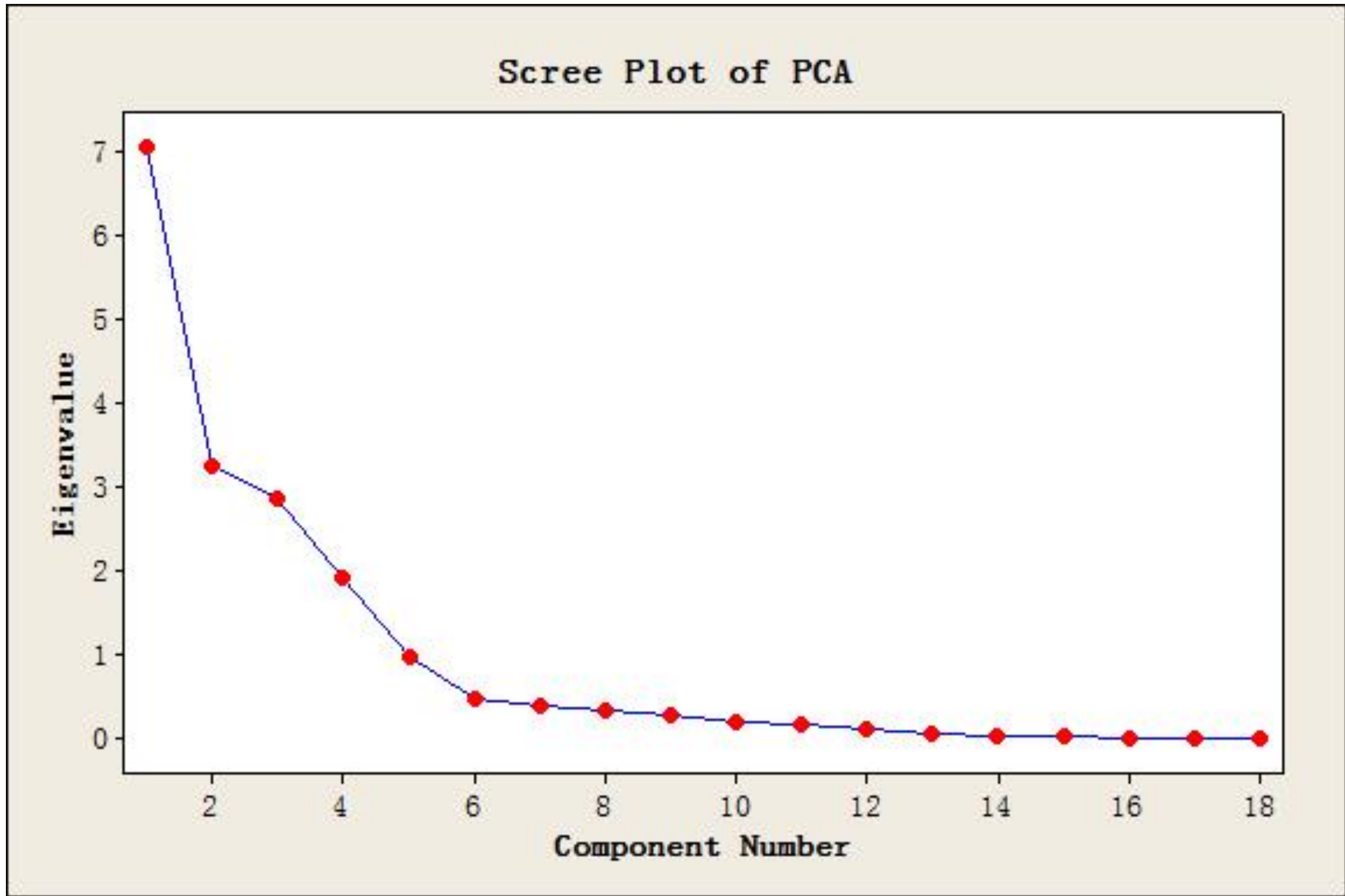


Figure 4.3 PCA Eigenvalue versus Component number plot.

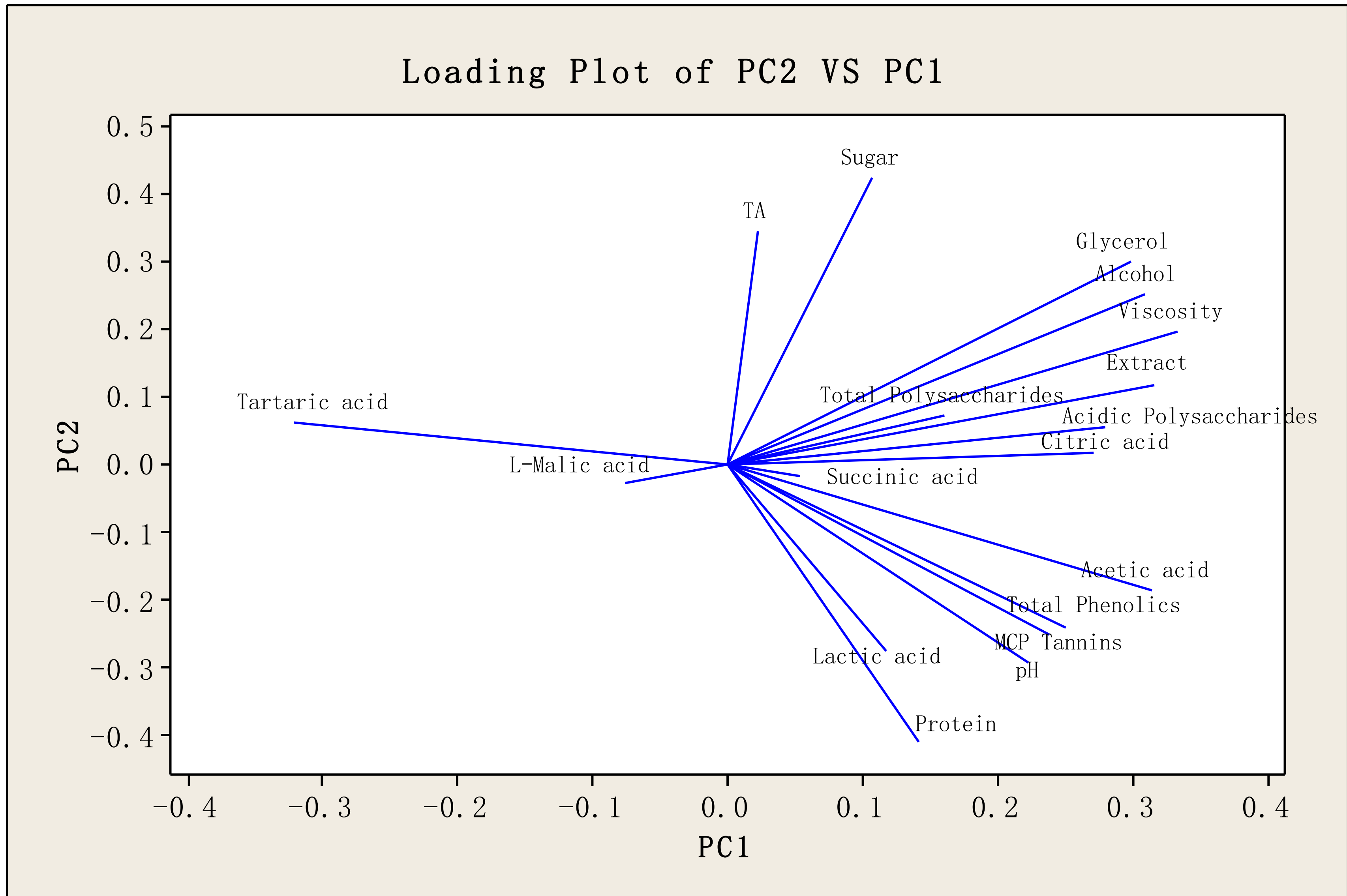


Figure 4.4 PC1 versus PC2 variable loading plot.

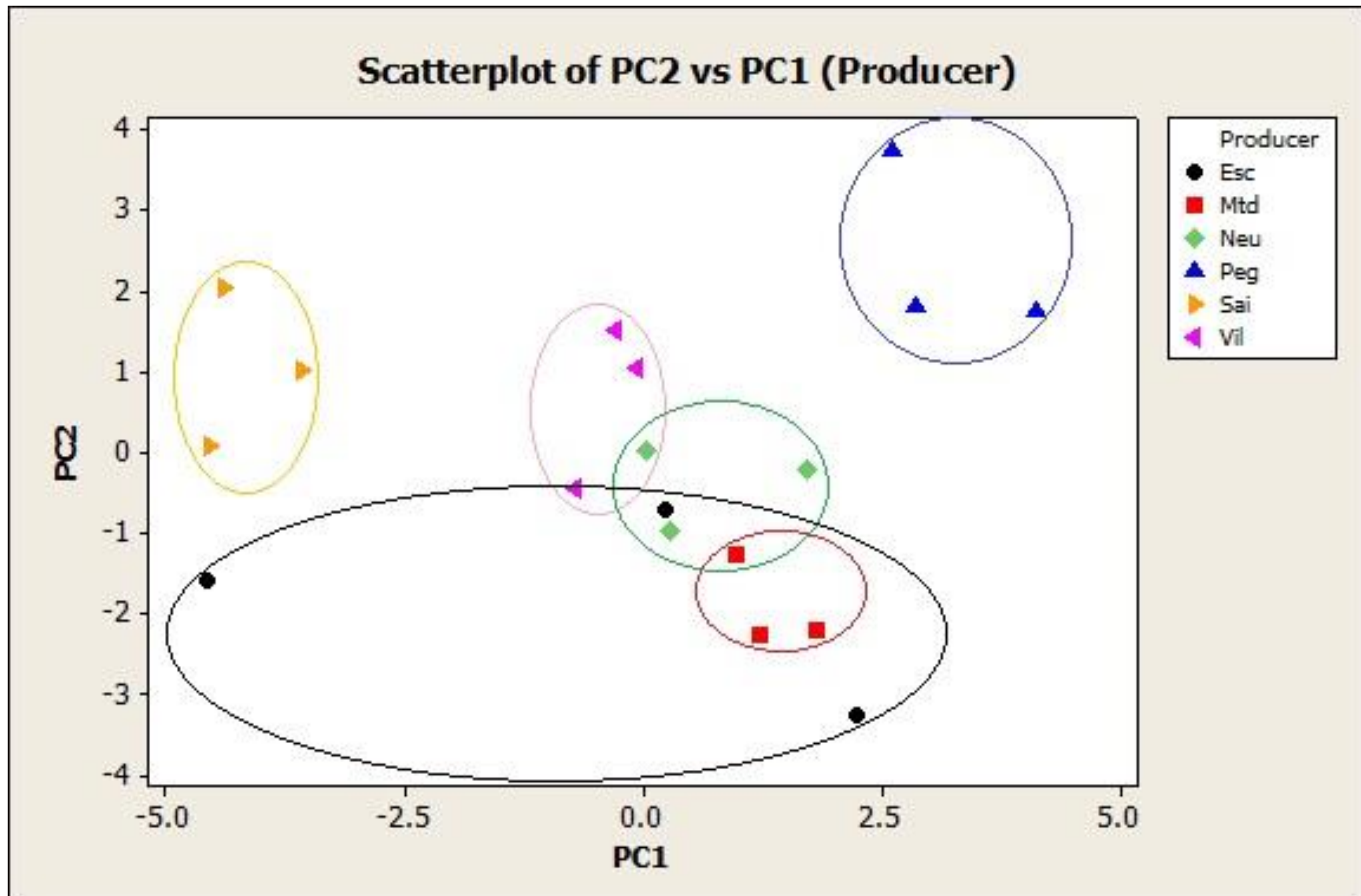


Figure 4.5 Sample scatterplot of PC1 versus PC2 with Producer legends (Esc: Escarpment, Mtd: Mt Difficulty, Neu: Neudorf, Peg: Pegasus Bay, Sai: Saint Clair, Vil: Villa Maria).

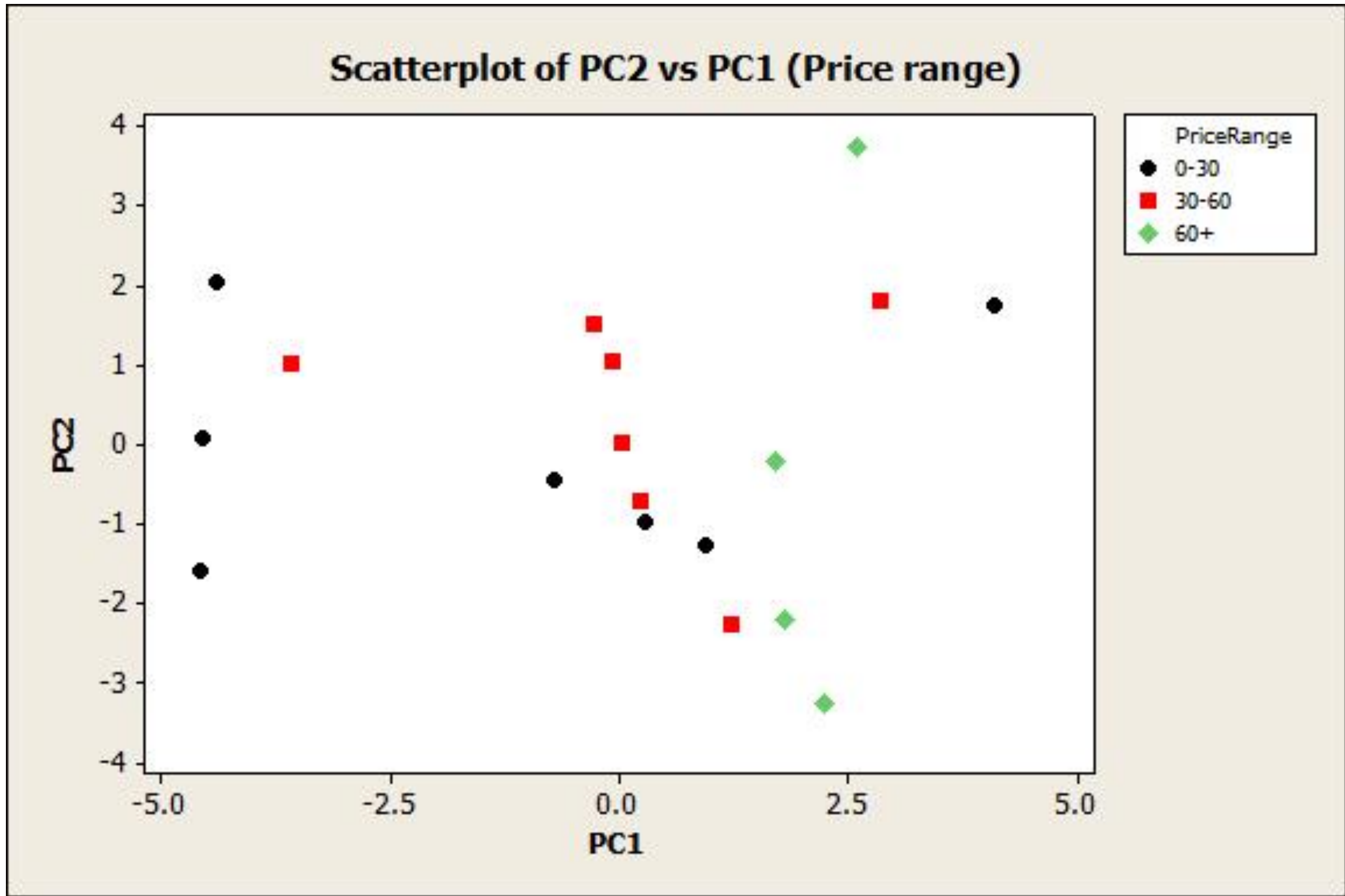


Figure 4.6 Sample scatterplot of PC1 versus PC2 with Price Range legends.

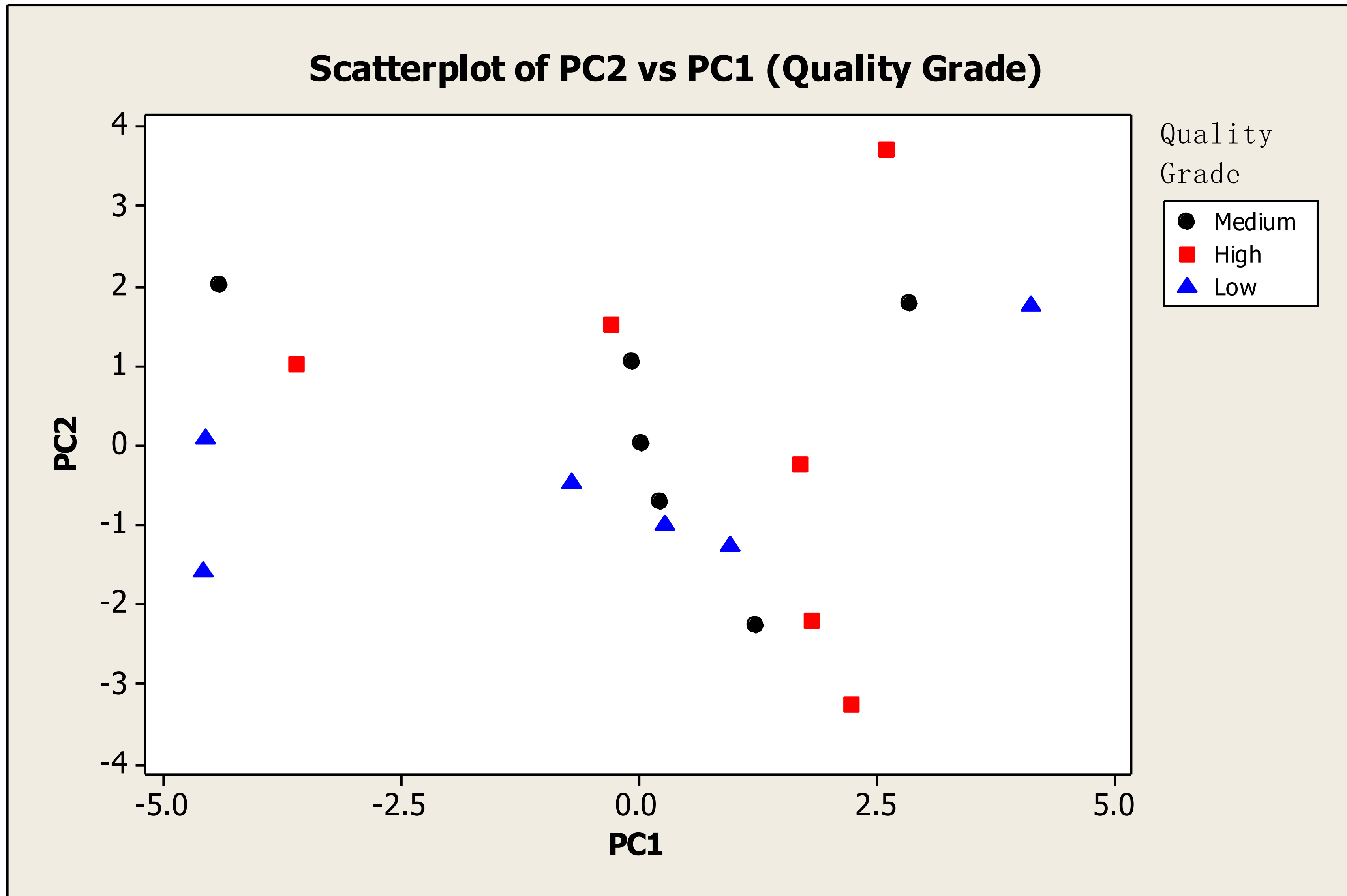


Figure 4.7 Sample scatterplot of PC1 versus PC2 with Quality Grade legends.

Table 4.11 Correlation (r value) between variables and canonical variates for producer, price range and grade.

CVA for producer	ALC[1]	TPH	TAN	TAR	MAL	LAC	ACE	CIT	SUC	GLY	pH	TA	SUG	TPA	APA	PRO	VIS	EXT
CV1	0.69	0.85	0.81	-0.89	0.23	-0.01	0.72	0.50	0.39	0.63	0.50	-0.18	0.10	0.13	0.59	0.26	0.76	0.58
CV2	0.29	-0.16	-0.24	0.02	-0.23	-0.30	0.04	0.15	0.19	0.37	-0.53	0.45	0.51	0.27	0.20	-0.64	0.33	0.15

CVA for price range	ALC	TPH	TAN	TAR	MAL	LAC	ACE	CIT	SUC	GLY	pH	TA	SUG	TPA	APA	PRO	VIS	EXT
CV1	-0.34	-0.27	-0.22	-0.37	0.24	-0.30	-0.55	-0.68	0.07	-0.26	-0.33	-0.16	-0.06	-0.16	-0.14	-0.48	-0.42	-0.49
CV2	0.11	-0.05	-0.12	0.09	0.04	0.15	0.18	0.23	-0.12	0.06	-0.03	0.33	0.07	0.01	0.02	-0.21	0.09	0.19

CVA for grade	ALC	TPH	TAN	TAR	MAL	LAC	ACE	CIT	SUC	GLY	pH	TA	SUG	TPA	APA	PRO	VIS	EXT
CV1	0.17	-0.04	-0.12	0.04	-0.28	0.27	0.33	0.52	-0.30	0.10	0.04	0.43	0.23	0.15	0.04	0.17	0.24	0.43
CV2	0.03	0.04	0.00	0.02	0.12	0.07	0.02	-0.15	0.21	0.02	0.01	0.07	0.33	-0.28	-0.28	-0.27	-0.01	-0.12

Abbreviation[1]: PC – principal component; PRI – price; ALC – alcohol; TPH – total phenolics; TAN – MCP tannins; TAR – tartaric acid; MAL – malic acid; LAC – lactic acid; ACE – acetic acid; CIT – citric acid; SUC – succinic acid; GLY – glycerol; TA – titratable acidity; SUG – reducing sugar; TPA – total polysaccharides; APA – acidic polysaccharides; PRO – protein; VIS – viscosity; EXT – extract; SPE – specific gravity.

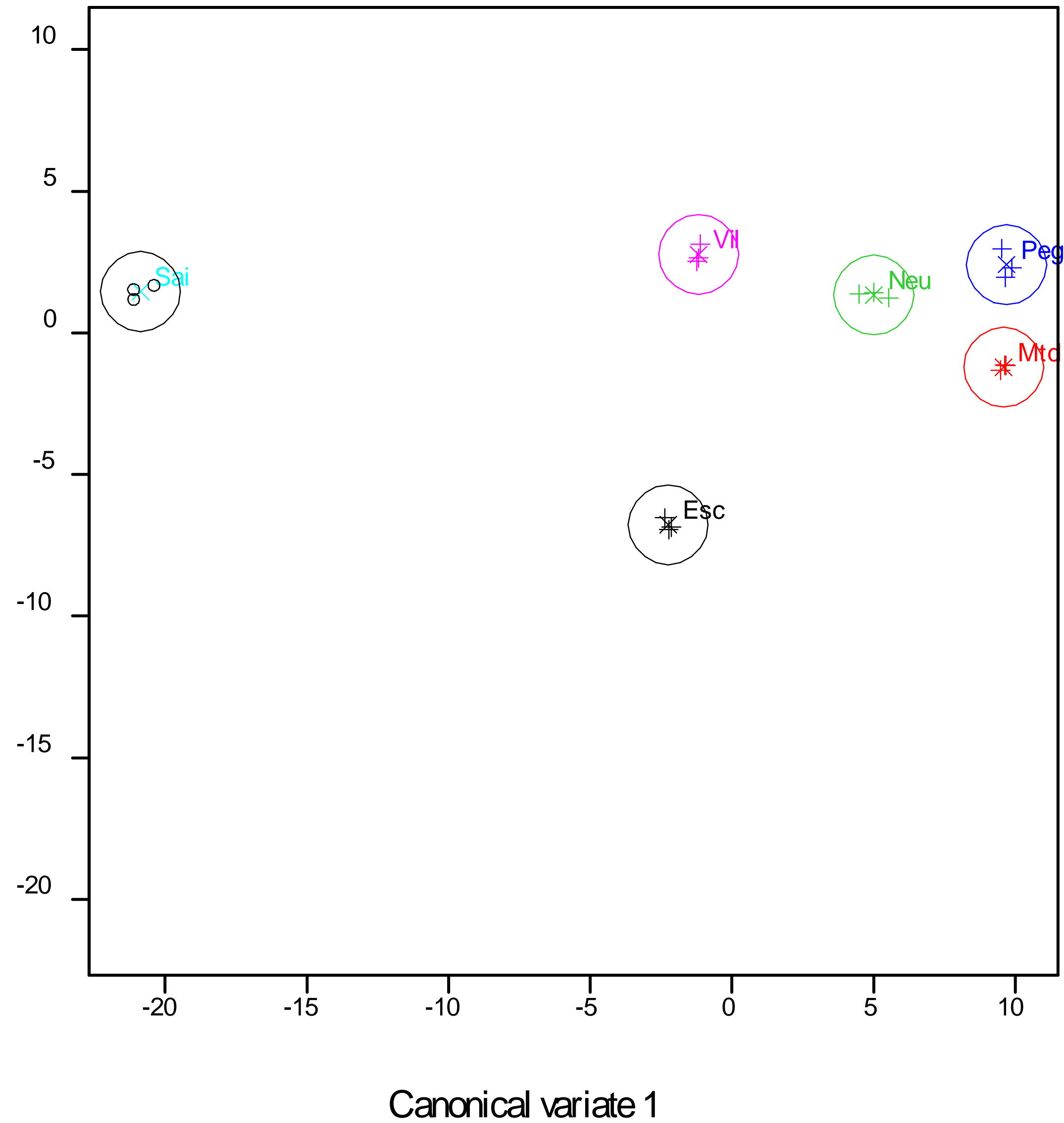


Figure 4.8 Sample scatterplot of CV1 versus CV2 for producer (Esc: Escarpment, Mtd: Mt Difficulty, Neu: Neudorf, Peg: Pegasus Bay, Sai: Saint Clair, Vil: Villa Maria).

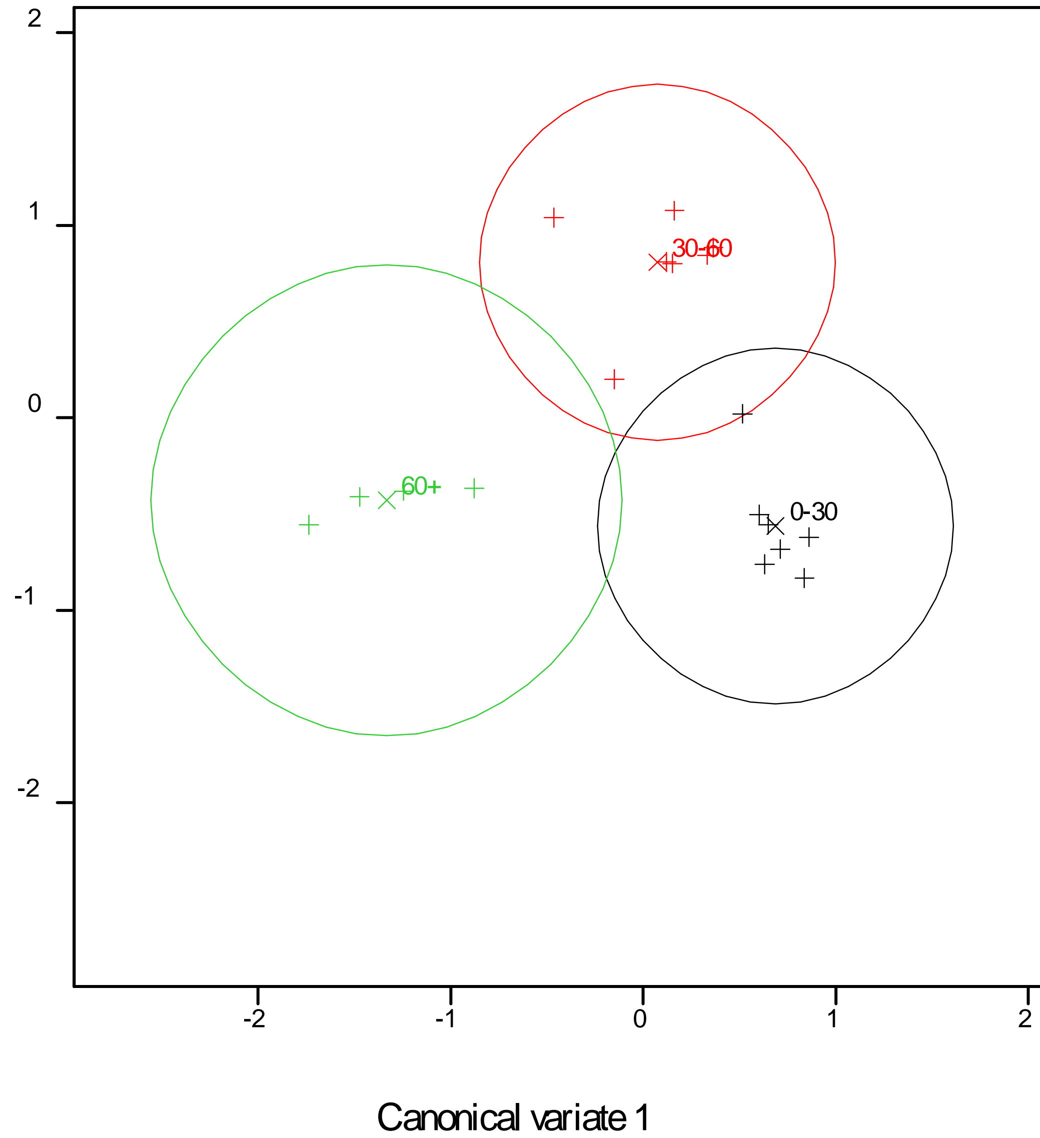


Figure 4.9 Sample scatterplot of CV1 versus CV2 for Price Range.

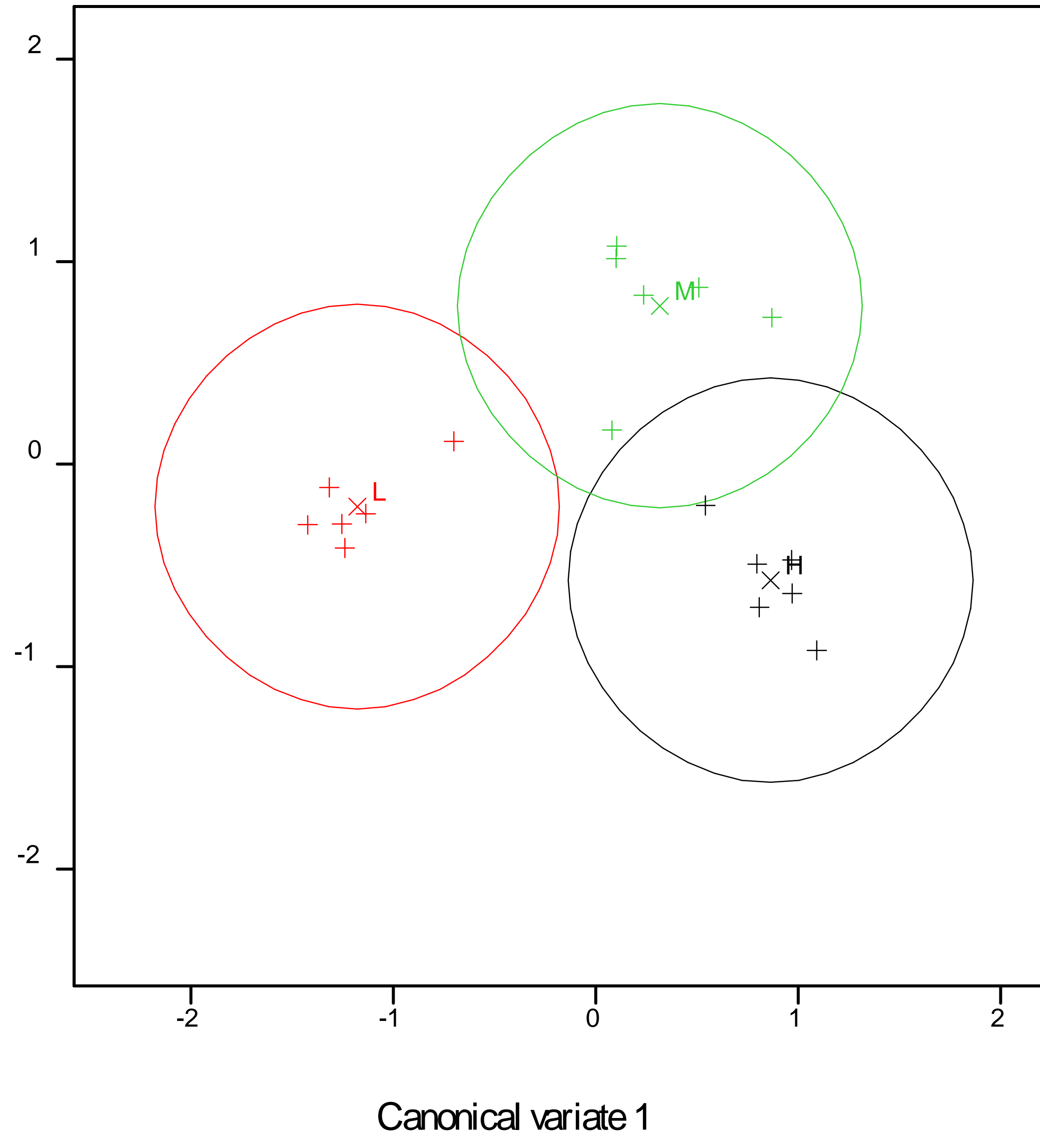


Figure 4.10 Sample scatterplot of CV1 versus CV2 for Quality Grade.

Chapter 5 Discussion

The dry, puckering mouth-feel of astringency was hypothesized to be correlated with the characters of the tannin profile and the reactivity of tannins with salivary proteins. It could also potentially be influenced by the wine body dimension of mouth-feel, which may involve components suggested by white wine studies. The difference in sensory evaluations and measured chemical and physical parameters of the Pinot Noir wines may be influenced by the producer, the price and producer defined market quality.

5.1 Astringency

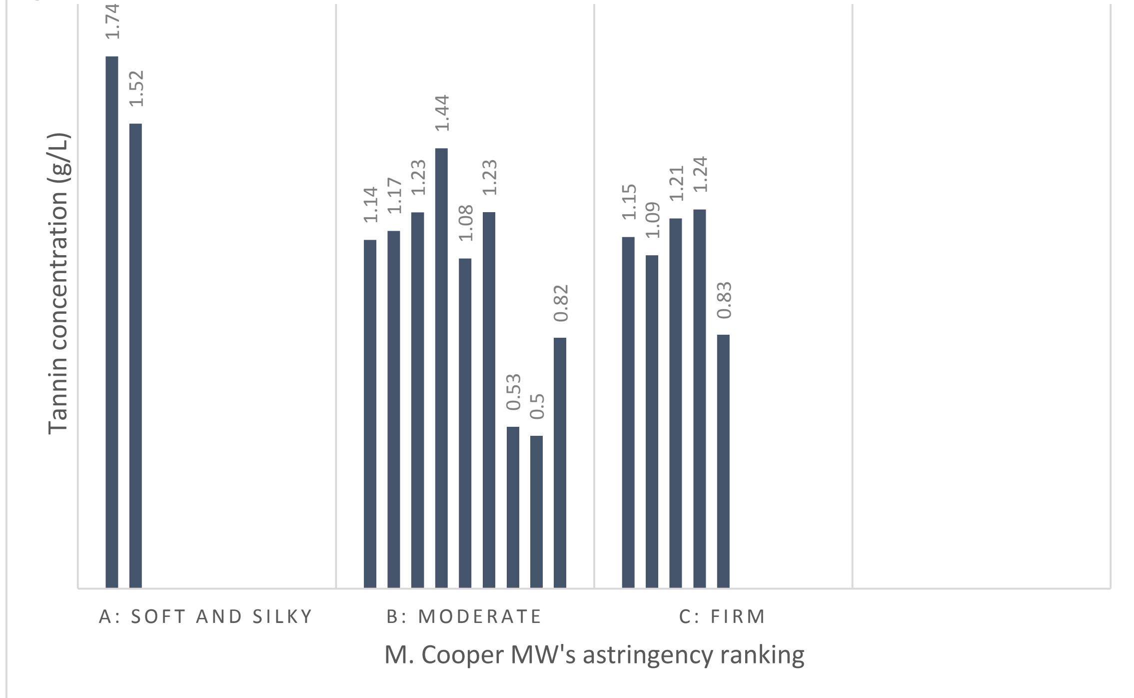
Astringency mainly results from the phenolics content in the wine, especially condensed tannins. It is clear that not only tannin concentration (Gawel 1998), but also tannin structure (Vidal et al. 2004a; Poncet-Legrand et al. 2007b; McRae et al. 2010) have influence on astringency. McRae et al. (2010) found that the tannin age of Shiraz wines, which could lead to conformational curling of tannin molecules, had an lowering effect on the reactivity of the tannin – protein interaction through the aging process. Poncet-Legrand et al. (2007b) and Vidal et al. (2004a) reported that higher tannin polymerization level is correlated with higher astringency. Poncet-Legrand et al. (2007b) also reported the effect of galloylation level in the tannin structure on astringency. In the present study, the total concentrations of phenolics and tannins were analyzed, but the structural characteristics of the tannins were not tested. In terms of astringency, the difference between the samples could result from the difference in tannin concentration or the difference in tannin structure. For this reason, ITC analysis, which was used by previous studies to evaluate the tannin – protein association (Frazier et al. 2003; Pascal et al. 2007; McRae et al. 2010), was adopted in the present study to evaluate astringency.

The ITC experiment for six selected samples (Neudorf L, M and H, Pegasus Bay L, M and H), using buffered tannin solution to titrate buffered PLP solution, was carried out using the same concentration of tannins in all samples, so the difference in thermodynamics of the reaction could only be the result of conformational differences in the extracted tannins of the wines. The ITC raw data and integrated data curves in Figure 4.1 and 4.2 showed the instantaneous thermodynamics of the reaction throughout the titration and the calculated enthalpy. For all the raw data curves in Figure 4.2, exothermic signals increased in the first several injections and peaked at about the 4th to 8th titration. Then the exothermic signals reduced, and the curves went on the endothermic direction until going to another peak and a plateau following the peak. The exothermic signals were regarded as the tannin – protein reaction signals resulting from hydrophobic interactions and hydrogen boding involved in the interaction (Bennick 2002), and the endothermic plateau was regarded as the dilution effect of tannin solution titrating into buffer solution without available PLP. The point when the curve reached the plateau indicated the amount of tannins used to fully titrate the PLP in the sample cell. It could be observed, from Figure 4.1 and 4.2, that the curves share some similarities in shape and value, except for Pegasus Bay H, which stood out particularly from others and showed a lesser amount of tannin needed to titrate the PLP with a stronger endothermic signal at the final plateau. The uniqueness of Pegasus Bay H cannot not be explained until further analysis in the sample tannin structure is conducted, whereas the similarities between curves of the other samples indicated that the difference of astringency between these wines, which are all from the 2010 vintage and from New Zealand producers, would more likely be resulting from the tannin concentration. The total phenolics and total tannins analysis on the 6 selected wines showed a range of 2.69 to 2.92 g/L for total phenolics and a range of 1.08 to 1.24 g/L for total tannins. As for the other samples, the total phenolics and total tannins (Table 4.3) showed that Saint Clair L, M and H had significantly lower phenolic and tannin contents comparing with the other samples. This indicated that it was likely that the three wines from Saint

Clair would give less astringency during tasting.

In the sensory analysis, M. Cooper MW tasted 16 out of the18 sample Pinot Noir wines and ranked astringency using a three level categorization. However, the ranking did not significantly correlate with the tannin concentration of the samples (Figure 5.1). The Shapley ranking analysis conducted by the present study at Lincoln University, New Zealand showed that the nine selected wines received different points according to their "intensity of sensation in the mouth" (Table 4.7), but did not show a significant correlation between the scores of of the two tasting sets (normal and sensory deprived). Although a significant correlation between the scores of the normal tasting group and the total phenolic content in the wines was shown in the data analysis, it was hard to correlate the scores with the astringency character of the wines. This may be due to complex interactions between tannins and other components in the wine matrix. The alcohol content, which ranged from 13.1% to 14.8% in the samples of this study (Table 4.4), has been found to have a lowering effect on astringency (Serafini et al. 1997; Lesschaeve & Noble 2005; Demiglio & Pickering 2008; Fontoin et al. 2008; Rinaldi et al. 2012a). The wine pH, ranging from 3.43 to 3.87 in the present study, was also studied in several research laboratories and showed that lowering pH can result in higher astringency and greater tannin-protein astringency (Peleg & Noble 1999; Demiglio & Pickering 2008; Fontoin et al. 2008; Rinaldi et al. 2012a). It was also found that acidic polysaccharides have a significant suppressing effect on the perception of astringency, whereas neutral polysacchairdes have insignificant effects (Vidal et al. 2004b).

Figure 5.1 Tannin concentration for 16 wines with M. Cooper MW's astringency ranking.



Combining the results of the ITC and sensory analyses, it appeared that the sample wines differed from each other in the astringency mouth-feel aspect, and it could be deducted that the difference in astringency was not likely due to the difference in tannin structure. However, there was not a linear correlation with tannin concentration either. The not-fully understood interactions in the complex process of perceiving astringency could have contributed to this matter.

5.2 Body

Red wine body is a less studied wine character than astringency. Previous research suggested (Jackson 2009; Runnebaum et al. 2011) that viscosity can be regarded as a major representative parameter of wine body, and specific gravity could be a sub-quality of wine body. Chemical constituents that contribute to red wine body are not fully known. Some physical and chemical parameters, such as extract, specific gravity, viscosity, alcohol, glycerol, lactic acid, phenolics, polysaccharides, protein and reducing sugars have been suggested to be involved in wine body (Noble & Bursick

1984; Vidal et al. 2004a; Vidal et al. 2004b; Nurgel & Pickering 2005; Gawel et al. 2007; Yanniotis et al. 2007; Jackson 2009; Runnebaum et al. 2011). Specifically, parameters that have significant correlations with or contributions to viscosity can be expected to be involved in wine body. In this study, alcohol and glycerol showed the highest correlation with viscosity (Table 4.8). Some earlier studies that investigated the role of alcohol in wine body showed statistically significant increase in instrumentally measured viscosity with increasing level of alcohol content in wine or model solution, but no significant increase in perceived viscosity (Runnebaum et al. 2011), whereas other studies reported moderate increase in perceived body or viscosity with increasing level of alcohol (Nurgel & Pickering 2005; Gawel et al. 2007; Yanniotis et al. 2007). As for glycerol, its concentration in wine was reported to be too low to significantly contribute to the perceived viscosity in wine (Noble & Bursick 1984; Nurgel & Pickering 2005; Runnebaum et al. 2011). In the present study, the significant correlation found between alcohol, glycerol and viscosity confirmed alcohol and glycerol content to increase the physical viscosity in commercial wines within normal ranges.

Other variables were found to be significantly correlated with viscosity to a lesser degree, included total phenolics, tartaric acid (inversely), acetic acid, citric acid, reducing sugar, acidic polysaccharides and extract (Table 4.8). Some of these parameters may correlate without making any actual contribution to viscosity, such as the minor organic acids (acetic acid and citric acid) and reducing sugar in dry red wine samples in the present study. The role of phenolics in red wine body is still not certain due to a lack of focus of research. Yanniotis et al. (2007) mentioned that the physical viscosity of dry red wines was higher than that of dry white wines. Considering the correlation identified in this study and the major contribution of phenolics to the differences between red wines and white wines, it is possible that the interactions between red wine phenolics and other macro-molecular constituents in the wine matrix (Chapter 2) could subtly influence wine body. Further in depth research is required to investigate any relationship between red wine

phenolics and red wine body. The strong inverse correlation between tartaric acid concentration and several other variables indicative of grape ripeness suggested that the gradual decrease in tartaric acid concentration during ripening (Lamikanra et al. 1995; Jackson 2008) had a counteracting effect on wine body. The correlations between viscosity and the other two parameters, acidic polysaccharides and extract, which were mentioned in previous research (Vidal et al. 2004b; Yanniotis et al. 2007), were further confirmed by the correlation analysis in the present study.

PCA provided another angle to analyze the composition of Pinot Noir wine body. Although interpreting the principal components is somewhat subjective, the first principal component (PC1), which explained 39.1% of the total variance, could be interpreted as representing “wine body”. Thus, PC1 was most strongly correlated with viscosity, glycerol, alcohol and acidic polysaccharides (Table 4.10). The PCA variable loading plot of PC1 versus PC2 (Figure 4.4) showed clear clustering of glycerol, alcohol, viscosity, extract, and acidic polysaccharides with tartaric acid standing out at the opposite direction of PC1.

Results of the present study did not show significant difference in wine viscosity across 18 New Zealand Pinot Noir wines from similar vintages. However, the difference between the highest and the lowest viscosity observed in sample wines was very small (Table 4.6), and there were only three wines (Peg L, M and H) that had viscosity value higher than that of the least viscous wine (Esc L) with a difference big enough to exceed the sensorial threshold (0.141 mPa s), reported by Noble and Bursick (1984). Specific gravity was also very similar (Table 4.6) in all samples.

In terms of sensory analysis, M. Cooper MW’s wine body rating showed similarity of body of sample wines’ comparing with the physical analysis. In his tasting experience, most of the sample wines had a light body (rating A) and two of them had medium (rating B) body.

As for the Shapley ranking analysis of nine sample wines carried out at Lincoln University, it was not possible to straightforwardly extract the role of wine body because the criteria used by the panelists to separate the wines was "the intensity of sensation in the mouth". The values from the tasting which excluded visual and olfactory effects in order to focus on taste and mouth-feel sensations did not show any significant correlation with other variables. This was consistent with the idea that there is little difference in body between the wines. On the other hand, the scores from the tasting which included visual and olfactory cues showed significant correlations with a number of variables (Table 4.8.2) including viscosity. Combined with the results from M. Cooper MW, it is hard to draw the conclusion that panelists could sense the difference of wine body of the samples with the help of visual and olfactory sensations, but it was clear that these sensations influenced their judgment significantly.

5.3 Producer, producer-defined quality and price

The 18 New Zealand Pinot Noir wine samples in the present study had the following pre-set attributes: six different producers, different recommended market price ranging from \$20 to \$90, and three quality grades within each producer (Low, Medium and High) (Table 3.1). ANOVA, PCA and CVA were used to explore the relationship between these attributes and the chemical and physical measurements.

The results presented in Chapter 4 (Tables 4.1 – 4.7) show the significance level for each variate with producer as a factor and price as a covariate. Titratable acidity, pH, total phenolics, tannins, alcohol, glycerol, reducing sugar, protein, viscosity, and extract were found to be significantly different among the samples according producer, whereas citric acid and extract were the only two parameters that were found to be significantly different according to price. These results indicated the importance of producer vis-à-vis price in relation to differences in chemical

composition and physical parameters.

In the statistical analysis, PCA and CVA were used to investigate producer, price range and quality grade factors of the sample wines. The difference between these techniques is that PCA uses standardized data to find the best linear combinations of the measurements that can explain the most variance, whereas CVA tries to find the combinations that have highest correlation with certain factors (Brand 2013). The sample scatterplots of PCA and CVA (Figures 4.5 – 4.10) showed that producer was the best factor to differentiate the wines with the knowledge of their chemical parameters used in this study. The sample scatterplot of CVA also well separated the samples according to price range and quality grade.

The grouping of sample wines according to producer in PCA (Figure 4.5) indicated that the body factor (PC1) and the tartaric acid factor (PC2) tend to be similar among wines from the same producer, rather than the same price range or quality grade. It then can be assumed that if a wine consumer wants to purchase a wine with similar body mouth-feel as the wine they like, they should purchase another wine from the same producer, rather than a wine from a different producer with similar price point.

CVA according to producer, price range and quality grade showed different levels of separation of the samples. Comparing the CV1 and CV2 in these analyses, CVA for producer was clearly the most successful separation with a number of parameters being significantly correlated with CV1 and CV2, whereas the CVA for the other two factors had fewer parameters that were significantly correlated with the variates. An interesting observation is that citric acid was the only significantly correlated parameter that appeared in all three analyses. This indicated that citric acid contributed significantly in differentiating the wines according to producer, price point and quality grade.

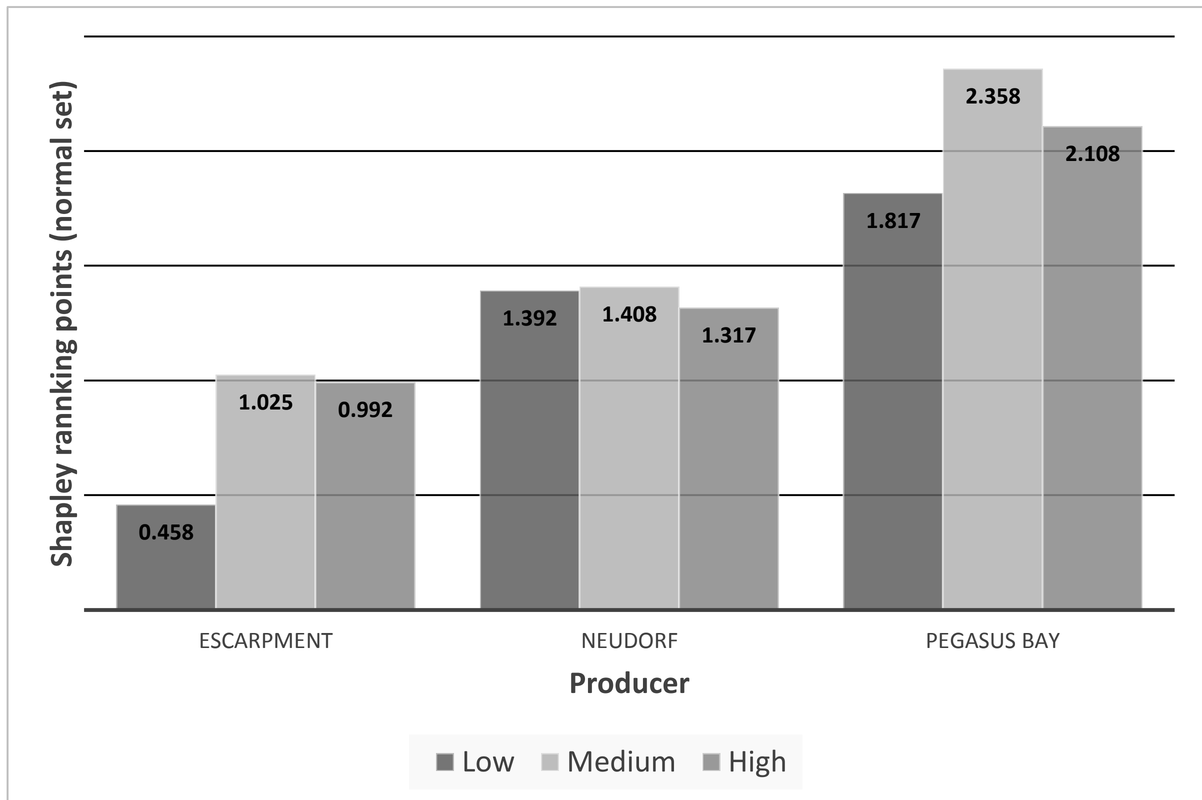


Figure 5.2 Shapley ranking points of normal tasting set for 9 sample wines.

5.4 Conclusion

Results of the present study suggested that young (1-2 year old) New Zealand Pinot Noir wines differ statistically in the chemical and physical parameters involved in red wine body, whereas the difference of wine body between the wines may not be obvious in the perception of the wine. Viscosity, alcohol and glycerol were particularly significant contributing to this statistical difference. The Pinot Noir wines from similar vintages tend to have similar tannin structures, indicating the difference in perceived astringency is more likely the result from difference in concentration and interactions with the wine matrix. On the overall mouth-feel quality level, the Pinot Noir wines differentiate the most according to producer, rather than price and quality grade This may be attributed to the common practice of selecting of the best vineyard or block to make the higher end wines, leaving the rest of the grapes carrying similar characteristics, but lower quality, to make wines at lower ranges.

5.5 Recommendations for future study

In this study, mouth-feel related chemical and physical parameters were used to explore the group the wines according to different factors and to correlate to the sensory characteristics of the wine. Several aspects of this study can be expanded and further investigated.

1. To explore the role of phenolics, especially tannins and anthocyanins, in the perception of red wine body or fullness.

2. To explore the difference of red wine body in different varieties and the difference in related chemical and physical parameters.

3. To characterize the difference of related parameters in red wine according to a more objective quality specification, instead of producer, price or producer defined market quality grade.

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