The Australian Wine Research Institute

GWRDC GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

# Identification of the major drivers of 'phenolic' taste in white wines



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## i Abstract

The effects of the phenolic composition, alcohol and acidity levels of white wines on their mouth-feel and taste (astringency, bitterness, viscosity, oiliness, and hotness/pungency) were assessed. Phenolic composition impacts on these attributes, but the size of the effect depends on wine alcohol and pH. Conversely, some phenolics reduced the astringency and hotness of white wine that directly resulted from their low pH and high alcohol levels. Higher bitterness can be attributed to phenolics, but the specific types responsible are yet to be identified. Lastly phenolics were important in defining style and quality of white wines, but were perceived to be less important by consumers.

## ii Executive Summary

The term 'phenolic taste' is ill-defined, but is proposed to include the taste and textural attributes of astringency, bitterness, viscosity, oiliness, metallic and pungency/burning. The molecules responsible for causing 'phenolic taste' also remain unknown and, indeed, debate continues as to whether these molecules are even phenolic. While some tastes and textures in white wine such as 'hotness' can be confidently attributed to major features of the wine matrix such as alcohol (Gawel *et al.* 2008), others such as bitterness and astringency have been associated with the presence of phenolic compounds. The cause of other mouth-feel characters such as viscosity, oiliness, metallic or pungency/burning is unclear, but these attributes are often found in wines that were made using processes that encourage phenolic pick-up during winemaking.

This project investigates the role of phenolics on white wine character and style. Specifically its objectives were to identify the molecular basis of 'phenolic' taste by broadly considering all potential compounds, phenolic and non-phenolic, that may be involved in the different tastes and textures believed to be associated with phenolics in white wine, and use winemaking practice to create different phenolic styles. The role that pH and alcohol play on the perception of phenolic taste and texture was also explored. This work will allow winemakers to make better informed decisions about how to manage 'phenolic' taste in their white wines to achieve their desired wine style.

To achieve these objectives we made experimental wines which clearly exhibit a range of 'phenolic' character and used these wines to identify correlations with sensory ratings and to isolate phenolic compounds. We also used reconstitution or back addition studies of compounds suspected to be responsible from literature or identified in our ongoing work. The effects from matrix compounds such as residual sugar, ethanol, and acidity at realistic levels are included in our studies.

Before beginning detailed chemical analysis we investigated whether phenolic concentration did actually impact on the overall impression of what winemakers refer to as 'phenolic' taste**.** We established in an Australian scenario that overall 'phenolic' taste amongst Australian wines can be distinguished, at least by highly experienced wine assessors. Though it remained to be established whether, and in what ways, these different wine phenolic profiles might influence phenolic tastes. Using 'total phenolics' isolated from commercial wines we, for the first time, demonstrated that wines with different phenolic composition, when presented at wine-like concentrations, can display different textures when tasted in the same matrix (alcohol, pH, TA etc). This suggests that phenolic composition influences textural differences in white wines.

We also established that alcohol concentration positively enhanced four major taste/textural attributes (astringency, viscosity, bitterness and hotness) in white wine, and that phenolics and alcohol contributed in an additive way to these attributes. Interestingly, research into stylistically different whole wines demonstrated that the astringency of Pinot Gris/Grigio wines was mostly associated with low pH. In order to further explore the influence and interactions of phenolics with the key matrix elements of alcohol and pH on taste/textural attributes, we demonstrated that variation in white wine tastes and textures could be attributed to both phenolic composition and the interaction with the wine matrix. These were important steps towards understanding the molecular basis of textural perception

in white wine, as the identity of the phenolic molecules (or groups of molecules) that caused differences in tastes and textures remained unclear.

To tackle this, the two most dominant phenolic molecules in Australian white wines were isolated and then tasted. The first was caftaric acid. It is usually the most abundant hydroxycinnamate in both juice and wine. The second was 2-S-glutathionyl caftaric acid (better known as Grape Reaction Product (GRP). It is a derivative of caftaric acid and can be increased in white wine by using oxidative juice handling, but only at the expense of caftaric acid. Caftaric acid was shown to reduce the burning hotness from alcohol and GRP was shown to increase palate oiliness.

An advanced HPLC method has been developed and now allows separation of more than 80 identified (40 of which can currently be quantified) phenolics in white juices and wines. This is a notable advancement on the previous methods available, in particular because it requires minimal sample preparation. This was a challenging aspect to the project that was critical to analysing the winemaking experiments described next.

We produced a set of white wines that varied greatly in phenolic composition, so as to elicit measurable differences in both taste and texture. A mix of both conventional and less practiced white wine making techniques were used over three vintages and most winemaking treatments were repeated over two of the years (2010 and 2011). The winemaking experiments, together with the outcomes of the previous experiments in the project, allow insight into the molecular basis of 'phenolic' taste which is summarised below.

'**Astringency'** ratings in white wines were found to be strongly negatively correlated with pH (i.e. lower pH gives higher astringency). Aggregate measures of phenolics, various complex mixtures of phenolic compounds, and the two major individual phenolics (caftaric acid and GRP) were, in general, not particularly associated with astringency. A striking example of this is the minimal difference in astringency between low phenolic hyper-oxidised wines and the high phenolic macerated and skin contact wines made in 2011. These are among several findings of significance that contradict the widely held assumption that phenolics are the main cause of astringency in white wines.

'**Viscosity'** ratings in white wines were found to be strongly positively correlated with pH (i.e. lower pH gives lower viscosity), further emphasising the importance of this matrix effect on the perception of mouth-feel in white wines.

'**Hotness'** and '**burning after taste'** are most highly associated with alcohol concentration, not aggregated phenolic measurements or caftaric acid. Phenolics have anecdotally been implicated in these sensory characteristics, but mostly phenolics seem not to be positively related to these heat attributes, with the exception of some small effects from GRP and GRP-like compounds. In a further discovery, these measures of heat are indeed generally suppressed by the presence of phenolics, including caftaric acid. This shows that, in the absence of variations to matrix composition, phenolics may allow winemakers to 'dial down' white wine hotness in some circumstances.

**Bitterness** was, however, generally shown to be positively associated with phenolics. However, the two major phenolics in Australian white wines (GRP and caftaric acid) do not contribute to bitterness. This means some other phenolic or phenolic class in white wine does, but their identity remains

unknown. However, various correlative studies consistently implicated flavanol and glycosylated flavonols as candidates for these bitter tasting compounds.

Observations from winemaking treatments in 2010 show that, as anticipated, increased skin contact increases phenolics in the wines. However, somewhat unexpectedly astringency decreased and viscosity increased and this is mostly due to the pH increase (caused by potassium extraction from skins). Therefore, the desired phenolic outcome of any given winemaking treatment needs to be carefully considered from the perspective of the concomitant effect of pH on astringency and viscosity. Observations from winemaking treatments in 2011 (which were adjusted to similar pH’s) show that skin contact before and during fermentation did not generally differ in much phenolic taste compared to whole bunch pressed, free run or hyper-oxidised wines despite large differences in phenolics.

Further opportunities for research into the molecular basis for phenolic taste exist. Of particular relevance is bitterness, which was one of the few sensory attributes clearly attributable to phenolics but the molecular drivers remain unknown. Suppressive effects on alcohol hotness and increases in oiliness from GRP-like compounds may also present opportunities.

We gratefully acknowledge the financial contribution from Orlando Wines and their support in providing grapes and juice. In addition the feedback, discussions and guidance provided by the Industry Reference Group members from Orlando Wines, Constellation Wines, Treasury Wine Estates, Yalumba, Wirra Wirra and Peter Leske is gratefully appreciated.

## iii Background

The conventional New World approach to white wine-making typically involves using techniques and processes that minimise the concentration of phenolic compounds in the finished wine. The acceptance of grape processing and wine-making strategies that limit phenolic concentration probably arose from the notion that their presence is thought to detract from varietal character, accelerates oxidation, and can result in palate hardness (McLean 2005).

However, many Australian winemakers have recently shown a greater willingness to incorporate phenolics into their white wines, referring to what they perceive as improved palate texture that complements and encourages the responsible consumption of wine with food. Mark Lloyd, a long time exponent of Italian varieties in Australia recently discussed what he saw to be the positive aspects of the phenolic character that he typically sees in the Italian variety Fiano - "There is an inherent conflict between the phenolic or 'pithy' character of the wine which is, on the one hand, the basis of its individuality, and yet, can be seen as a fault in contemporary winemaking circles" (Lloyd, 2010, p70).

The acceptability or otherwise of phenolic tastes in white wines clearly depends on the wine style that the wine-maker intends to produce. Phenolic characters are generally considered to be undesirable elements in elegant lighter bodied styles such as Riesling, unwooded Chardonnay and most New World interpretations of Sauvignon Blanc. Their presence is generally thought to detract from the fresh fruit characters that are keenly sought by purchasers of these styles. In addition, the presence of deeper hues and enhanced astringent, oily and bitter taste characters that are frequently associated with higher phenolics are not well regarded in lighter bodied styles.

On the other hand, some varieties that can be vinified in a way to intentionally incorporate higher phenolic levels (presumably with the view to creating more textural wines), have recently experienced significant sales growth. Winemakers are also showing interest in some Italian, Spanish and Greek varieties as they have been reported to acquire adequate flavour at lower grape maturity than do traditional cultivars, and therefore seem better suited to the production of flavoursome, lower alcohol wines in warm to hot climates (Tassie *et al.* 2010).

However, even in fuller bodied styles, the boundary between what is perceived to be positive 'textural' and what constitutes undesirable 'coarseness' is ill-defined. A systematic investigation into the scientific basis of perceived variations in white wine texture has not yet been attempted. By applying rigorous sensory testing in conjunction with traditional and newly developed analytical techniques we investigate the role of phenolics on white wine character and style. Specifically our objective is to identify the phenolic compounds responsible for the different phenolic tastes and textures in white wine, and to determine the effect of winemaking practice on these. The role that pH and alcohol play on the perception of phenolic taste and texture is also explored. This work aims to allow winemakers to make better informed decisions as to how to manage phenolics in their white wines to achieve their desired wine style.

# 1 General Introduction

From the moment of grape harvesting, the juice that is expressed from the pulp of the white grape that ultimately makes up the majority of the final wine is subjected to processes that affect its final phenolic composition. Harvesting techniques, juicing methods, pre-ferment skin contact and the creation and use of press fractions influence both the total amount and type of phenolics that are found in the finished wine. The phenolic content can be further modified both quantitatively and qualitatively either by fining juice or wine with proteinaceous fining agents, subjecting the juice to oxygen, or less commonly by the addition of commercial preparations of phenolics.

The use of pre-fermentation skin maceration and the judicious use of press fractions have been traditionally used by winemakers to achieve good expression of varietal flavour. These strategies are based on the knowledge that many of the volatile compounds responsible for flavour in these cultivars are mostly found in the fleshy cells immediately below the skin. However, varietal expression (or typicity as it is called in a broader sense) involves more than just aroma and flavour characteristics. The typicity of most varieties also depends on in-mouth textural characteristics. The three varieties used in this study – Viognier, Riesling and Chardonnay provide good examples. High quality examples of Viognier are as much defined by their oily, rich in-mouth texture as by their distinctive peach and apricot like aromas and flavours. Conversely a typical high quality example of an Australian or Germanic dry Riesling wine is typified by a palate structure that is light bodied and generally 'lean' in character. Finally, winemakers worldwide invest in high cost methods in an attempt to create the creamy and viscous texture that defines great examples of fuller bodied oaked Chardonnay styles.

While some textures in white wine such as 'hotness' can be confidently attributed to major features of the wine matrix such as alcohol (Gawel *et al.* 2008), others such as bitterness and astringency have been associated with the presence of phenolics (Singleton *et al.* 1975). The cause of other mouth-feel characters such as viscosity, oiliness, metallic or pungency/burning are less clear, but are often found in wines that were made using processes that encouraged phenolic pick-up during winemaking. While understanding that the term 'phenolic taste' is ill-defined, we initially include the textural attributes of astringency, bitterness, viscosity, oiliness, metallic and pungency/burning as sub-attributes of 'phenolic taste' in white wine.

## 1.1 Classes of Phenolics in White Wine

White wine phenolics can be broadly categorized into the non-flavonoids and the flavonoids. Non-flavonoids include the hydroxybenzoic and hydroxycinnamic acids. The flavonoids include flavonols and flavanols.

### 1.1.1 Hydroxycinnamates

The major non-flavonoids in white wine are the hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acids (HCA's) and their derivatives, collectively referred to as hydroxycinnamates, are the most abundant phenolic compounds in white wines. The hydroxycinnamic acids present in wine are mainly derived from caffeic, *p*-coumaric, ferulic, and sinapic acids. While they can be present in both *cis*- and *trans* forms, the *trans* form is more prevalent. The concentrations of HCA in their free form are low in comparison to their esters of l-(+)-tartaric acid – the most important being the ester of caffeic acid known as caftaric acid. (Ong and Nagel 1978; Singleton *et al.* 1978; Somers *et al.* 1987). Caftaric acid is the most abundant hydroxycinnamate in both juice and wine, but the tartaric esters of *p*-coumaric acid and ferulic acid are also present (Somers and Ziemelis 1985; Vérette and Somers 1988; Crothers 2005).

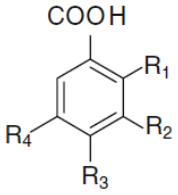
An important derivative of caftaric acid that has significant winemaking implications is 2-S-glutathionyl caftaric acid, better known as Grape Reaction Product (GRP). It forms when polyphenol oxidase (PPO) enzymes oxidise caftaric acid to the quinone which then chemically (i.e. non-enzymatically) react with the grape peptide glutathione. Similar GRP-like conjugates involving other hydroxycinnamic acids and peptides including cysteine and glutamine are also formed by the same mechanism. However, these are far less abundant than glutathionyl caftaric acid.

Numerous other derivatives of HCAs have been found in white wine. These are the ethyl esters of caffeic acid and coumaric acid, the ethyl esters and diethyl esters of caftaric acid and the glucosides of all the major free hydroxycinnamic acids (Baderschneider and Winterhalter 2001; Somers *et al.* 1987; Monagas *et al.* 2005).

The most prevalent benzoic acids in white wine are gallic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid and salicylic acid. Unlike the hydroxycinnnamic acids, they are usually found in their free form, and in comparatively low concentrations (Baderschneider and Winterhalter 2001). Of these gallic acid is the most abundant. While it is found in the grape itself, it also is released following the hydrolysis of the gallic acid esters of flavan-3-ols. Ethyl and methyl esters of vanillic and protocatechuic acids, and the glucose ester of vanillic acid have also been isolated from white wines.

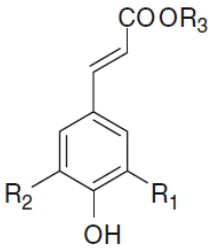
Hydroxycinnamic acids, benzoic acids and their derivatives are mostly found in the pulp of white grapes with up to 200 mg/L being reported in free run juice (Ong and Nagel 1978). White skins have also been reported to contain up to 45 mg/kg of hydroxycinnamates (Rodriguez Montealegre *et al.* 2006). Given that white wines are primarily made from the pulp of grapes, it is not surprising that the combined level of hydroxycinnamates can be substantial relative to other phenolic compounds found in white wine. As an example, a survey of 26 Greek white wines found an average hydroxycinnamate level six times higher than the combined concentration of the other phenolics present (Makris *et al.* 2003). Similarly, the combined concentration of caffeic and coumaric acids in dry Muscat wines was found by (Karagiannis *et al.* 2000) to be between 6 and 16 times higher than the combined concentrations of the important flavan-3-ol monomers, catechin and epicatechin.

**Table 1-1 : Structure of hydroxycinnamates found in white wine (from Rentzsch 2009).**




| Hydroxybenzoic acids | $R_1$ | $R_2$ | $R_3$ | $R_4$ | MW |
|---|---|---|---|---|---|
| Gallic acid | H | OH | OH | OH | 170 |
| Gentisic acid | OH | H | H | OH | 154 |
| p-Hydroxybenzoic acid | H | H | OH | H | 138 |
| Protocatechuic acid | H | OH | OH | H | 154 |
| Salicylic acid | OH | H | H | H | 138 |
| Syringic acid | H | $OCH_3$ | OH | $OCH_3$ | 198 |
| Vanillic acid | H | $OCH_3$ | OH | H | 168 |

**Fig. 9C.1** Structures of hydroxybenzoic acids in wine




| Hydroxycinnamic acids | $R_1$ | $R_2$ | $R_3$ | MW |
|---|---|---|---|---|
| Caffeic acid | OH | H | H | 180 |
| Caftaric acid | OH | H | Tartaric acid | 312 |
| p-Coumaric acid | H | H | H | 164 |
| p-Coutaric acid | H | H | Tartaric acid | 296 |
| Ferulic acid | $OCH_3$ | H | H | 194 |
| Fertaric acid | $OCH_3$ | H | Tartaric acid | 326 |
| Sinapic acid | $OCH_3$ | $OCH_3$ | H | 224 |

**Fig. 9C.2** Structures of hydroxycinnamic acids in wine

## 1.1.2 Flavonols

The first of the two major classes of flavonoid compounds are the flavonols. They are found both in grapes and wine mostly in a glycosylated form. Of these, quercetin glucoside and quercetin glucuronide are usually present in the highest concentrations, while myricetin, kaempferol and isorhamnetin glycosides are also frequently present (Vilanova *et al.* 2009). However as conventional white winemaking involves fermenting juice extracted from the grape pulp which is relatively devoid of flavonols, it is not surprising that their concentration in white wines is low. Makris *et al.* (2006) reports that 1-3 mg/L is typical in white wine, but up to 10 mg/L has been observed.

The flavonols in the skins of grapes increase with exposure to sunlight, which suggests that they play a role in protecting the grape from physiological damage resulting from sun-burn. Light exposure increased the flavonols in Australian Chardonnay wine by nearly 6 fold while only doubling the concentration of the major hydroxycinnamates (Crothers 2005). The limited information available to date indicates that flavonols are found in relatively high concentrations in Australian wines compared to wines from less sunny climates (Goldberg *et al.* 1998). The concentration of quercetin glucoside in grape skins can be high with up to 70 mg/L found in Viognier juice (Rodriguez Montealegre *et al.* 2006), and up to 28 mg/kg in juices from eight Spanish commercial cultivars (Vilanova *et al.* 2009).

In a recent survey of 22 white grape varieties, the total flavonol content varied between 8 mmol/kg to 160 mmol/kg. The quercetin derivatives comprised between 60 and 91% of the total, and Kaempferol derivatives between 9 and 37%. The glucosides and glucuronides were the dominant sugar moieties accounting for no less than 85% of the total flavonol content (Castillo-Munoz *et al.* 2010).

## 1.1.3 Flavanols

The second of the two major classes of flavonoid compounds in wine are the flavanols. The predominant monomeric flavanols in white wines: catechin; epicatechin; epigallocatechin; and the gallate ester epicatechin gallate derive mainly from the skins and seeds of grapes (Rodriguez Montealegre *et al.* 2006). In a survey of 57 French white wines, the average concentration of catechin and epicatechin was 10 and 5 mg/L respectively (Carando *et al.* 1999). Other researchers have reported a greater abundance of these two monomers in white wine. Catechin levels in 11 Greek white wines were found to range between 12 and 40 mg/L (Proestos *et al.* 2005), while the combined concentration of catechin and epicatechin in Portuguese white wine was found to range up to around 30 mg/L (De Limai *et al.* 2006). These low levels compared with the hydroxycinnamates most likely reflect the minimal contact the white must generally has with the flavanol rich skins and seeds during white winemaking.

## 1.1.4 Flavanonols (dihydroxyflavonols)

Glycosylated flavanonols are found in the skin and stems of grapes. Dihydroquercetin and dihydrokaempferol rhamnosides have been reported in stems and skins (Souquet *et al.* 2000; Singleton and Trousdale 1983), and the galactoside and glucoside of dihydroquercetin has been identified in skins (Masa *et al.* 2007). Grapes sampled from 8 different Spanish varieties over seven vintages showed that dihydroquercetin rhamnoside was the most abundant flavononol ranging from between 0.3 mg/L in Torrontes to 34 mg/L in Albarino. While most of the varieties contained non-detectable or

low concentrations of other members of the flavanonol class, Albarinho had around 8 times the dihydrokaempferol rhamnoside level as the average of the other 7 varieties. However like many other phenolics that are localised in the skin, their final concentration in wine is much lower than in the grape skin. Thirty commercial white wines from the south-west of France were sampled and gave an average level of dihydromyricetin rhamnoside of 3 mg/L (Vitrac *et al.* 2002). Neveu *et al* (2010) surveyed the literature and found that while the average concentration of dihydroquercetin rhamnoside in wine was also 3 mg/L, it ranged from 0.7 to 13 mg/L. The significant variation in flavanonol level between varieties warrants further investigation of this phenolic class.

### 1.1.5 Tyrosol

Tyrosol is thought to be formed from tyrosine by yeast during fermentation and as such its concentration depends on yeast strain and on the initial concentration of sugars and tyrosine in the must (Pena-Neira *et al.* 2000). Tyrosol has been estimated to comprise 10% of the total phenol content of white wine (Myers and Singleton 1979), and more recently it was found to dominate the phenolic profile of white wines from the Carinena region of Spain (Pena-Neira *et al.* 2000). Other winemaking practices such as oxidative must handling (Ritter *et al.* 1994) and juice solids (Konitz *et al.* 2003) also affect wine tyrosol concentration. The possible relationship between astringency perception and tyrosol concentration is worthy of further investigation given its reported prevalence in white wine and that its level might be influenced by the type of yeast strain chosen by the winemaker.

## 1.2 Sensory Impact

While the sensory properties of some of the phenolic compounds found in white juices and wines are known, the relative importance of individual compounds or groups of compounds has not been determined. It is not clear whether individual compounds, as opposed to classes of compounds, are drivers of 'phenolic taste' in white wines. The answer to the question of the relevance of individual phenolic compounds to the taste or mouth-feel of a white juice or wine is further complicated by the use of varying methodology in the different sensory studies that have been performed, making the results difficult to compare across studies.

### 1.2.1 Hydroxycinnamates

The hydroxycinnamates are the most abundant class of phenolics in white juices and wines, so they make an obvious target for analysis and sensory research. Most hydroxycinnamates are thought to have both astringent and bitter qualities (Singleton and Noble 1976; Makris *et al.* 2006b). Using qualitative flavour profiling, Dadic and Belleau (1973) noted that many common hydroxybenzoic and hydroxycinnamic acids present in beer were predominantly bitter in 5% aqueous ethanol when presented at threshold levels. Only protocatechuic acid and gentisic acid were described as being astringent. Whilst still predominantly bitter at suprathreshold concentration, many of these acids elicited minor levels of astringency together with sour tastes. However, as ethanol has bitter qualities itself (Scinska *et al.* 2000), a possible influence of the base solution used in this study on the perception of bitterness cannot be discounted. More recently, Boselli *et al.* (2006) found associations between the concentrations of caftaric acid with the astringency of Verdiccio and Passerina white

wines. They also found that the concentrations of gallic and caffeic acid were correlated with the perceived bitterness of these wines. However, such associations could be the result of co-correlation with other phenolic compounds.

While the threshold estimates of the hydroxycinnamic acids and their tartaric acid esters differ greatly between studies summarised in Gawel (1998), they are typically present in white wine at concentrations near or below their detection threshold in water. This would indicate that individually they have little or no sensory impact on wine. Using duo-trio difference testing Vérette *et al.* (1988) reported that caffeic, coumaric and caftaric acids were undetectable in white wine when present in the upper range of white wine concentrations. However, the effect of using a small number of tasters combined with applying a relatively insensitive test method suggests this study should be revisited. Furthermore, even if the concentrations of individual phenolic species were insufficient to induce an astringent or bitter sensation in white wine, they may do so in combination. The thresholds of mixtures of two or three hydroxycinnamic and hydroxybenzoic acids were in most cases found to be lower than the minimum threshold of each of the components (Maga and Lorenz 1973). This result is not surprising as the intensity of mixtures of similar tasting bitter substances is generally additive (Keast *et al.* 2003). Therefore, although it appears that single species of hydroxycinnamates and their derivatives are not bitter at the concentrations found in wine, the possibility that the combined impact of the entire hydroxycinnamate pool can elicit a perceptible bitterness in white wine cannot be ruled out. Nagel et al. (1997) have suggested that hydroxycinnamates might not be bitter, explaining that previous sensory assessors might have confused bitterness with the compounds' sourness. They noted that after masking the acidity of a hydroxycinnamate with potassium bitartrate, tasters were unable to discriminate bitterness in the sample. However, the possible masking role of the added salt on the bitterness of the test sample was not considered.

The oxidation product of caftaric acid, trans-2-S-glutathionyl caffeoyl tartaric acid (grape reaction product, or GRP) was also not perceptibly bitter when tasted at levels typically encountered in white wine (Vérette *et al.* 1988). A study of six wines produced from unoxidised juice and the corresponding oxidised juice gave inconclusive results regarding the role of GRP and bitterness (Nagel and Graber 1988).

### 1.2.2 Flavonols

Quercetin glycones are the main flavonols in white wine, although quantitative data in a representative range of Australian white wines is not available in the published literature (McDonald *et al.* 1998; Boselli *et al.* 2006). The thresholds of flavonol aglycones are around 10-20 mg/L, determined in 5% aqueous ethanol (Dadic and Belleau 1973), with descriptors such as 'bitter', 'harsh', and 'astringent'. Therefore, it might be that flavonols and their glucosides are important either individually or cumulatively, to the mouth-feel of white wine.

The impact of quercetin on white wine astringency is unclear. Dadic and Belleau (1973) reported that it elicits a bitter taste with only a weak astringency when presented in water. However, rutin which is one common quercetin glycoside has recently been reported to elicit an astringent like sensation even at extremely low concentrations when found in black tea (Scharbert *et al.* 2004). The reason for these conflicting results is unclear. It might have been due to the two studies using free and glycosidically bound quercetin, as the astringency of quercetin glycosides appears to depend on the structure of the

glycosidic moiety (Scharbert *et al.* 2004), or alternatively, the differences might have been due to the studies employing different media (i.e. water versus black tea) (Dadic and Belleau 1973). It is also worthwhile to note that the presence of rutin in grape skins of *Vitis vinifera* has been well documented; however, its existence in wine has been debated amid possible confusion with quercetin-3-glucuronide. In a recent survey of Australian wine, and contrary to literature reports of rutin in wine, rutin was not found in any of the wines analysed, and further experiments showed that rutin was rapidly degraded in wine to yield the aglycone quercetin (Jeffery *et al.* 2008).

No sensory data on the effect of glycosylated flavanonols have been reported.

### 1.2.3 Flavanols

While the concentration of flavanols in white wines is low, so too are their detection thresholds (summarised in Gawel 1998), suggesting that they might play a role in eliciting phenolic tastes. The major flavanol in wine, catechin has been described as being both astringent and bitter (Robichaud and Noble 1990). However, aqueous catechin solutions at (up to 200 mg/L) could not be distinguished from water when applied to the surface of the mouth while being restricted from contacting bitterness receptors in the tongue (Gawel, unpublished data). This result suggests that catechin does not elicit astringency. It is possible that catechin has been perceived as astringent in other studies when the compound came in contact with the tongue as well as the surface of the mouth due to a carryover effect from the perception of bitterness. Alternatively, the catechin used in some model studies might have been contaminated with tannins. If catechin is not astringent, then its reported detection thresholds are most likely to represent bitterness thresholds, rather than astringency. Furthermore, the bitterness of the other common monomeric flavan-3-ols such as epicatechin, epicatechin gallate, and epigallocatechin are likely to further contribute to the bitterness of white wines, particularly if they are made with intentional or unintentional skin and seed contact prior to fermentation.

### 1.2.4 Tyrosol

The 'tannin taste' of Riesling wines from the same juice with different levels of must solids correlated most strongly with their tyrosol concentration, while being poorly correlated with hydroxycinnamic acid and flavan-3-ol concentration (Konitz *et al.* 2003).

## 1.3 Matrix Effects

When considering the taste or mouth-feel of white juices or wines, we need to examine not only the phenolic compounds themselves, but also the matrix of the wine.

Alcohol is known to be bitter, hot and to a lesser extent viscous when at wine concentration (Scinska *et al.* 2000; Gawel *et al.* 2007). Higher alcohol also enhanced the perception of a metallic character (Jones *et al.* 2008), and reduced the astringency (Fontoin *et al.* 2008) of model wine. From these studies it appears that alcohol can directly affect wine textures, some of which are normally associated with the presence of phenolics.

Organic acids have been shown to be astringent in their own right (Rubico and McDaniel 1992, Hartwig and McDaniel 1995). The astringency of equi-normal binary mixtures of organic acids increased as pH decreased, but remained unchanged when the normality of equi-pH solutions was increased. This result suggests that pH rather than titratable acidity influences perceived astringency (Lawless *et al.* 1996). Furthermore, the astringency of organic acids has been shown to be independent of anion species again highlighting the influence of pH. The increased astringency of phenolics in lower pH wines may be due to direct changes in salivary rheology (Nordbo *et al.* 1984) or through enhancing interactions between lubricating salivary proteins and phenolics (Obreque-Slier *et al.* 2011).

Glycerol is an important compound in white wine, being the third most abundant after water and ethanol (Gawel *et al.* 2007). It is equi-sweet to glucose but despite its physical viscosity when in a pure form, it does not increase the perceptual viscosity of dry table wine (Noble and Bursick 1984; Gawel and Waters, 2008). However, due to their sweetness, both glycerol and residual sugar may play a role in moderating astringency (Lyman and Green 1990, Smith *et al.* 1996).

## 1.4 Winemaking

Prior to fermentation, juice for white wine production is typically expressed and separated from grapes by a sequence of unit processes including destemming, crushing, draining and pressing. The final phenolic composition of a wine depends not only on grape composition but also on the conditions that influenced their extraction into the must, and on subsequent fining, and pre and post bottle aging.

The compartmentalisation of particular phenolics in different parts of the grape bunch present winemakers with the ability to tailor the phenolic profile of their juices with the view to creating wines of a specific style. Free run juice is largely comprised of the hydroxycinnamates from the pulp, whereas pressings contain more flavonoids because of their greater abundance and consequent extraction from seeds and skins (Patel *et al.* 2010). They also showed that pressings contain less glutathione and caftaric acid, but more GRP than free run juice. This change in phenolic profile following pressing can be attributed to oxygen uptake during the press cycle.

Extraction of flavonoids is increased by longer skin contact times at higher temperatures and the length and pressure applied during the press cycle. The effect on individual phenolic compounds in white wines with different pre-fermentation maceration conditions, including contact time (2 to 24 hours) and temperature (5, 10 or 20ºC), has been studied both on both an experimental and industrial-scale (Gomez-Miguez *et al.* 2007; Hernanz *et al.* 2007). These studies showed that individual compounds and classes of phenolics vary widely in their extraction dynamics and response to both temperature and time.

Pressings also have higher pH values resulting from greater extraction of potassium, and slightly lower titratable acidities and slightly higher total soluble solids than do free-run juices (Singleton *et al.* 1975).

Once the grapes are pressed, there are many chemical reactions that can occur, with enzymatic and non-enzymatic oxidation resulting in major changes to phenolics. Hyperoxidation is a technique practiced on must prior to the start of fermentation. It involves saturating an unsulfited must with

oxygen with the aim of oxidising phenolic compounds that might later contribute to phenolic tastes and browning of the finished wine. While almost all phenolic compounds in the juice decrease with hyperoxidation leading to their polymerisation and sedimentation, the polyphenol oxidase induced enzymatic oxidation of hydroxycinnamic acids to form grape reaction products with glutathione and other peptides are the most significant (Cejudo-Bastante *et al.* 2011). GRP is colourless and relatively unreactive to oxygen. Therefore hyperoxidation generally produces lighter coloured wines that are more resistant to browning than those produced using conventional non-oxidative juice handling methods.

While fermentation on skins is rarely practiced in white winemaking on a large scale, some producers make small parcels of wine as blending components in this way. Increased phenolic extraction is achieved by re-incorporating a small proportion of the skins following draining back into the must before yeast inoculation. To date, only small scale laboratory ferments have been used to investigate the effect of skin fermentation on phenolic character. Fermenting in the presence of 100% skins and seeds increased the total phenolic concentration three fold in Ribolla Gialla and two fold in Malvasia wines as compared with a free run control (Bavcar *et al.* 2011). The catechin and epicatechin concentration of a Chardonnay wine fermented on 0.64 kg/L of skins and seeds was three times higher than in the free run control wine. The skin and seed fermented wines were significantly more astringent and slightly more viscous than the control wine (Oberholster *et al.* 2009).

Fining with proteinaceous agents can be performed on white wines or juices. All have the objective of reducing phenolic content in white wine. They can be chosen in order to remove undesirable coarseness, to protect against future browning, or to remove less soluble phenolics that may cause haziness. Different fining agents have different specificity towards phenolic compounds, which is not surprising given their various modes of chemical binding. As examples, PVPP has been shown to be more effective in removing quercetin compared to its glucoside (LaBorde *et al.* 2006), and gelatine based agents are more effective at removing non-flavonoids, and less effective in removing monomeric flavanols than casein based fining agents (Braga *et al.* 2007).

Little research has been conducted on the effect of fermenting in the presence of higher levels of suspended grape solids on phenolic content and taste. Singleton *et al.* (1975) showed that although wines made from juices with higher suspended grape solids were consistently rated as being more bitter and astringent than those made from clarified juice, their total phenolic content did not vary. This result suggests that either fermentation of juices high in solids produces non-phenolic compounds that elicit bitterness and astringency, or fermentation on solids favoured the production of phenolic compounds that better express these sensory characteristics. Alternatively, the extra yeast nutrients provided by the solids can enhance fermentation performance and result in lower residual sugars and higher alcohol content in the finished wine. Both these have the potential to affect taste perception.

## 1.5 Conclusion

White wine contains a diverse set of phenolic substances, all of which could conceivably contribute to ‘phenolic tastes’ in white wine. The possibility that other non-phenolic wine components either directly contribute to or accentuate ‘coarseness’, or accentuate it through wine matrix effects cannot be discounted and also requires further investigation. It also remains to be determined whether individual compounds, or broad classes of phenolic compounds are the key drivers of white wine taste. Without this essential information, it is difficult to define desirable phenolic profiles and to translate detailed phenolic composition into meaningful processing options for the winemaker. Relationships between chemical composition, sensory attributes, and consumer preference need further investigation. With this information, further research could be targeted to key positive or negative sensory characters, enabling the winemaker to better control style and understand consumer preference.

# 2 Does Phenolic Concentration Influence 'Phenolic Taste' in White Wine?

## 2.1 Introduction

One early study (Singleton *et al.* 1975) investigated the impact of phenolic concentration on the taste properties of white wines made using a range of solids and skin fermentations. Before doing further research we investigated whether phenolic concentration did impact on the overall impression of what winemakers refer to as 'phenolic taste'. Wines were made using currently accepted industry practices with different levels of pressings and fining treatments. Following this the phenolic content was measured and the wines tasted. The experiments were designed to elucidate to the extent of variability in sensory and compositional effects that could be expected when making white table wines using standard commercial practice. It was anticipated that these treatments would show the possible range of white table wines and give a logical basis for refinement of winemaking practices to highlight different taste profiles and styles of wine in future studies.

## 2.2 Methods

### 2.2.1 Grape Source, Juice Preparation and Fermentation

Riesling grapes were machine harvested from the Orlando Wyndham St Helga vineyard in Eden Valley, South Australia in 2009. The juice was processed at the Rowland Flat winery of Orlando Wyndham Ltd and fermented at the Hickinbotham Roseworthy Wine Science Laboratory (HRWSL). Three fractions of Riesling juice were made using standard commercial practice: free run (FR), light pressings (LP) and heavy pressings (HP). These were divided into triplicate 80 L stainless steel kegs, to which pectolytic enzymes were added (Ultrazyme CPL, 3 mL/hL). Three additional 80 L kegs of HP juice were gelatine fined (Liquifine) at both 200 ppm and 1,000 ppm. All juices were cold settled for three days before being racked into new 80 L kegs. After warming to 15°C, the kegs were inoculated with PDM yeast. At the conclusion of fermentation, the wine was heat and cold stabilised, before being filled into 750 mL antique green bottles and sealed with Saran-tin ROTE screwcaps.

In summary the treatments were:

- Free run (FR)
- Light pressings (LP)
- Heavy pressings (HP)
- Heavy pressings + 200 ppm gelatine fining (HP200G)
- Heavy pressings + 1000 ppm gelatine fining (HP1000G)

## 2.2.2 Treatments

Two tasting trials were conducted. The first compared 2009 Riesling wines that were made identically from free run juice and juice from light and heavy pressings. The wines were made as described in Section 2.2.1. The second tasting compared the heavy pressing wine with those made from the same heavy pressing juice that had been fined prior to fermentation with 0.2g/L and 1.0g/L of a gelatine-based fining agent (Liquifine). These levels were chosen to represent standard and very high fining rates in a commercial context. The wines tasted had basic analysis in the following ranges: pH (3.22-3.26), titratable acidity (6.3-6.5 g/L), alcohol (11.8-12.1% v/v) and residual sugar (0.4-0.9 g/L). This suggests that the wines were very similar in their basic structure.

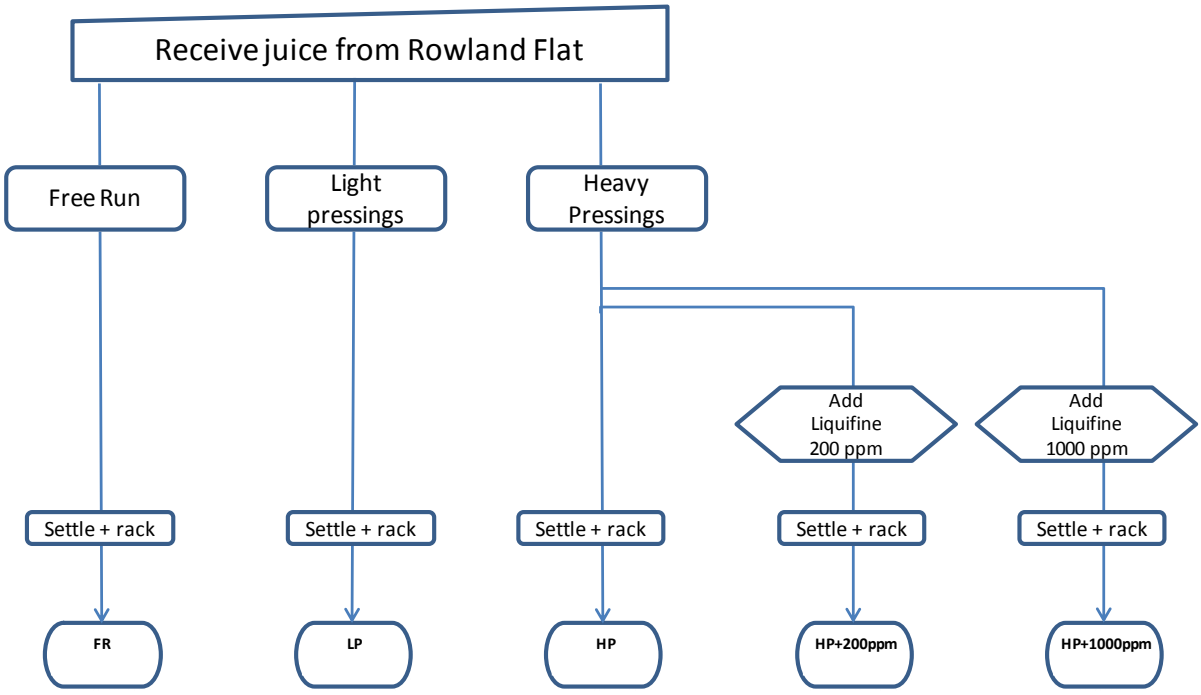



**Figure 2-1: Schematic of winemaking (2009)**

## 2.2.3 Tasting Conditions and Experimental Design

40 mL of wine were presented in clear ISO tasting glasses under natural light and at room temperature (23°C). While the assessment was not conducted in individual booths, the tasters did not communicate with each other during the duration of the tasting. The small number of tasting samples and tasters made it feasible to present the samples in an order which could control for possible presentation order effects resulting from bitterness carry-over between samples. A cross-over experimental design whereby each sample is tasted after each other sample the same number of times, and each sample appears in the same position the same number of times was used to this end.

### 2.2.4 Tasting Panel

Five of the six tasters were winemakers, each with over 25 years of winemaking experience. The sixth taster had undertaken vintage experience both in Australia and Europe and had over ten years of wine tasting experience as part of his profession.

### 2.2.5 Tasting Methodology

The assessors undertook a general discussion as to what they felt constituted 'phenolic taste' in white wine just prior to the tasting. The tasters were presented with duplicate sets of three wines in the orders dictated by the design. They were instructed to taste the three samples in each set in the order in which they were presented and rank them for overall 'phenolic taste' with a ranking of 1 indicating highest 'phenolic taste' and 3 lowest. While repeat tastings were not forbidden, the assessors were discouraged from doing so.

### 2.2.6 Statistical Analysis

Friedman's two way analysis of rank data was used to assess overall treatment effects. A rank separation method (Meilgaard *et al.* 1991) was used to compare treatment ranks.

## 2.3 Results and Discussion

The 2009 Riesling wines were analysed before a comprehensive HPLC analytical tool had been fully developed, so spectral UV analysis as described by Somers and Pocock (1991) were used to determine the approximate phenolic composition of the wines. Under this method, the 'total phenolics' values estimates all compounds containing phenol rings absorbing at 280 nm ($A_{280}$) corrected for the presence of peptides. 'Total hydroxycinnamic acids' (HCA) estimates $C_6$-$C_3$ compounds including GRP molecules absorbing at 320 nm ($A_{320}$), and the 'flavonoid extractibles' is an estimate of flavonol-like compounds represented by $A_{280}$ less the contribution from hydroxycinnamates.

As expected, the free run wine contained lower total phenolics, total HCA and flavonoid extractables than did either of the pressings wines (Table 2-1). Unexpectedly, the light and heavy pressings wines did not differ significantly in total phenolics. However the light pressings wines contained more total HCA and fewer flavonoid extractibles than did the heavy pressings wines.

Fining juices with a gelatine based fining agent at either level did not affect a practically significant change in any phenolic measure. Fining at a commercially realistic level of 200 ppm only reduced the total phenolic content by at best 5%, and heavy fining at 1000 ppm only resulted in a decrease in total phenolics in the order of 8%.

**Table 2-1: Somers' measurments for 2009 experimental wines (Mean of duplicate spectral measurements for each wine triplicate ferment, Standard errors in parentheses).**

| Press fraction/fining rate | Total Phenolics (au) | Total HCA (au) | Flavonoid Extract. |
|---|---|---|---|
| Free Run (FR) | 5.97 (0.086) | 6.35 (0.114) | 1.75 (0.016) |
| Light pressings (LP) | 10.19 (0.004) | 10.85 (0.004) | 2.97 (0.004) |
| Heavy pressings (HP) | 10.22 (0.029) | 8.39 (0.024) | 4.63 (0.012) |
| HP + 200 ppm Liquifine | 9.66 (0.008) | 8.04 (0.008) | 4.30 (0.004) |
| HP + 1000 ppm Liquifine | 9.34 (0.016) | 7.80 (0.016) | 4.14 (0.004) |

The consensus view amongst the experienced winemaker panel was that 'phenolic taste' was a multivariate sensory attribute that comprised aspects of bitterness, astringency (or 'grip'), and oiliness/viscosity. Some tasters also suggested that high phenolic wines displayed a specific flavour character but were unable to adequately describe it. Others felt that some phenolic wines also displayed hotness or burning sensation experienced at the back of the throat that detracted from overall wine quality.

**Table 2-2: Rank totals of perceived 'phenolic taste' (lower rank total implies higher intensity).**

| Treatment | Free Run | Light Pressings | Heavy Pressings | p | LSD |
|---|---|---|---|---|---|
| Rank totals | 32[a] | 22[b] | 18[b] | 0.013 | 9.6 |

| Treatment | HP | HP200G | HP1000G | p | LSD |
|---|---|---|---|---|---|
| Rank totals | 21[a] | 27[a] | 24[a] | 0.472 | n/a |

Singleton *et al.* (1975) fermented six different white grape varieties on total skins for one to five days before separating the skins from the fermenting must. The result of this highly unconventional white winemaking practice was to double total phenolics after five days of maceration. Making white wine as one would a red wine represents an extreme variant of traditional white winemaking with the potential to maximise total phenolic content. So although it was shown that both the astringency and bitterness of the wines were moderately correlated with total phenolic content by an expert tasting panel, the practical relevance of the results remain in question.

In this study, the wines being compared were a reasonable representation of commercial white winemaking practice. Despite being unable to fully agree as to the nature of 'phenolic taste' in white wine, the experienced winemakers were able as a group to differentiate the more phenolic wines made from light and heavy pressing juices from the free run wine (Table 2-2). They were unable to

distinguish the light from the heavy pressings wines, but this could be explained by them having similar amounts of total phenolics based on their $A_{280}$.

Juice fining using a gelatine fining agent at a medium and high rate did not affect the phenolic taste of the wines compared with a heavy pressings control. However, the fining agents as applied under the conditions of this study also did not affect either $A_{280}$ or $A_{320}$ suggesting that they were ineffective in fining total phenolics or total hydroxycinnamic acids from these wines. Others have also shown that gelatine fining of white juices is ineffective in reducing total phenolics in white wine. Adding 100 mg/L of gelatine to commercially produced Paradella juices did not influence the total phenolics of the finished wine (Puig-Deu *et al.* 1996), nor did adding 300 mg/L of gelatine to *V. rotundifolia* juices (Sims *et al.* 1995). Gelatine fining at a high rate of 500 mg/L did not reduced either total phenolics, non-flavonoids or flavonoids in various *V. vinifera* juices (Cosme *et al.* 2008). However, they showed that most small MW flavanols were reduced particularly when gelatine with a low molecular weight distribution was used.

In conclusion, the results of this trial indicate that differences in ‘phenolic taste’ amongst wines made using conventional white wine making practices can be distinguished, at least by highly experienced wine assessors.

# 3 Do Different Phenolic Profiles Affect the Taste Profiles of White Wines?

## 3.1 Introduction

It has been established in an Australian scenario that overall 'phenolic taste' amongst Australian wines can be distinguished, at least by highly experienced wine assessors (Chapter 2). However, the diversity of varieties and winemaking styles is known to result in wines with differing amounts and types of phenolics i.e. different 'phenolic profiles'. It remains to be established whether, and in what ways, these different wine phenolic profiles might influence phenolic tastes. In order to study this, whole phenolic fractions of differing phenolic composition were first isolated from commercial wine and characterised by HPLC. The whole phenolic fractions were then added to two commercial wines after which their phenolic taste profiles were quantified by a trained sensory panel. Wines created from a diversity of varieties and winemaking styles inherently have a range of different basic composition. Alcohol concentration can vary greatly and can be highly influential in the perception of phenolic tastes (see Chapter 5) and as such, its influence was also investigated as a variable with these whole phenolic fractions.

## 3.2 Methods

### 3.2.1 Sample Preparation

A current vintage commercial Riesling and unwooded Chardonnay were used as base wines. Whole phenolics were extracted from another three wines that were deemed by a panel of experienced tasters to exhibit phenolic tastes. They were a McLaren Vale Fiano, a Canberra District Viognier and an Alsatian Gewurztraminer. The whole phenolics from these wines were eluted off Amberlite FPX66 resin using 96% ethanol, the ethanol evaporated under vacuum and redissolved in water and freeze dried. All samples of whole phenolics were kept at -80°C before use.

Whole phenolics were extracted from a volume of wine as described above and then 50% of the whole phenolics that were collected were added back to the same volume of base wine just prior to tasting. An additional treatment factor of an additional 1% ethanol v/v (96%, Tarac Industries, SA) was included to assess the effect of alcohol concentration on the perception of phenolic tastes. The whole phenolic fractions were analysed using the HPLC method described in Section A.3.

### 3.2.2 Tasting Panel

The tasting panel comprised of four female and seven male employees of The Australian Wine Research Institute. All tasters had at least three years general wine tasting experience, and all but one had recent experience in rating the intensity of bitterness, astringency and hotness of white wines.

### 3.2.3 Tasting Conditions

30mL of sample was poured into ISO glasses and tasted at room temperature (22°C ± 1°C) under amber lighting. Three replicates were presented over three tasting sessions, with an entire set of treatments randomly presented to each taster during each session. Perceived astringency, bitterness, hotness and viscosity were rated on a 15 cm partially structured line scale with the word anchors "low" and "high" at 10% and 90% respectively. Assessors were asked to rinse their mouth with water and were required to wait 30 seconds before tasting the following sample. The ballots were served to tasters by the FIZZ v 2.46 sensory data acquisition software (Biosystèmes, Couternon).

### 3.2.4 Taster Training

Taster training comprised two, 45 minute sessions. In the first session, tasters were familiarised with the textural characters of astringency, bitterness, hotness and viscosity. This was achieved by tasting five white wines that had previously been deemed by an experienced wine tasting panel to vary substantially in palate texture. The training wines included the two base wines used in the study, an Australian Pinot Gris, and Italian Vermentino and Greco. The three commercial wines were selected as they were identified by an experienced wine tasting panel as displaying phenolic taste. The second training session involved assessing and discussing wines with added ethanol (+1% v/v), quinine sulfate (15 mg/L), grape seed tannin (200 mg/L) and carboxymethylcellulose (3 g/L). These were used as hotness, bitter, astringent and viscosity standards respectively.

### 3.2.5 Statistical Analysis

A three way ANOVA (Factors: base wine, phenolic source, alcohol) with assessors as a blocking variable was conducted using MINITAB v14. Means separation was determined with Fisher's Least Significant Difference.

## 3.3 Results

The effects on the taste and texture profiles of a Riesling and a Chardonnay wine at two alcohol levels following the addition of whole phenolics extracted from three commercial wines are shown in Figure

3-1. There were highly significant effects of adding the whole phenolic extracts on astringency (p=0.002), viscosity (p=0.004) and bitterness (p<0.001). Hotness was unaffected by phenolic addition (p=0.274).

Adding 1% v/v ethanol resulted in significant increases in all taste and textural characteristics including hotness (p<0.01). However it does appear that alcohol addition had less of an effect on viscosity than the other attributes (Figure 3-1). There were no significant interactions involving alcohol level and the source of the whole phenolic fraction indicating that the influence of alcohol on the taste and textural attributes was primarily additive. While not statistically significant, Figure 3-1 suggests that the differences in the effects of phenolic type became less pronounced at the higher alcohol level, particularly in the Chardonnay base wine.

The HPLC traces for the whole phenolics that were added are shown in Figure 3-2. The whole phenolic extract taken from the Viognier wine was high in GRP and had medium levels of caftaric acid and trans-coutaric acid as their primary phenolic species. The Fiano wine was high in caftaric and had comparatively lower levels of GRP and trans-coutaric acid. The Gewurztraminer whole phenolic extract did not contain significant quantities of either GRP or caftaric acid. Rather it contained a number of compounds in low concentrations that absorbed at 280 nm that were not in the other two whole phenolic extracts, particularly dihydroquercetin rhamnoside, as well as caffeic ethyl ester and some GRP-analogues. The addition of phenolics from the Alsatian Gewurtztraminer resulted in significantly higher bitterness, astringency and viscosity than the control wines (Figure 3-1). The addition of phenolics from the Australian Viognier only enhanced bitterness and astringency, while phenolics from the Australian Fiano contributed to the astringency and viscosity of the base wines but only if they contained low levels of alcohol. Therefore, the whole phenolic extracts from the three wines contributed differentially to the phenolic taste of the base wines. This suggests that phenolic composition is a determinant of textural differences in white wines.

Thus, for the first time it has been demonstrated that wines with the same matrix (alcohol, pH, TA etc) can display different textures resulting from different phenolic composition.

**Table 3-1 : Significance of ANOVA factors.**

| | Astringency | Viscosity | Bitterness | Hotness |
|---|---|---|---|---|
| Phenolics | **0.002** | **0.004** | **<0.001** | 0.274 |
| Alcohol | **0.001** | **0.010** | **<0.001** | **<0.001** |
| Base Wine | **0.067** | **<0.001** | 0.395 | **<0.001** |
| Phenolics x Alcohol | 0.511 | 0.570 | 0.944 | 0.833 |
| Base wine x Phenolics | 0.281 | 0.896 | 0.449 | 0.672 |
| Base wine x Alcohol | 0.770 | 0.701 | 0.738 | **0.087** |
| Base x Alcohol x Phenolics | 0.559 | 0.520 | 0.783 | 0.769 |

Bold highlight indicates statistical significance (p<0.10)

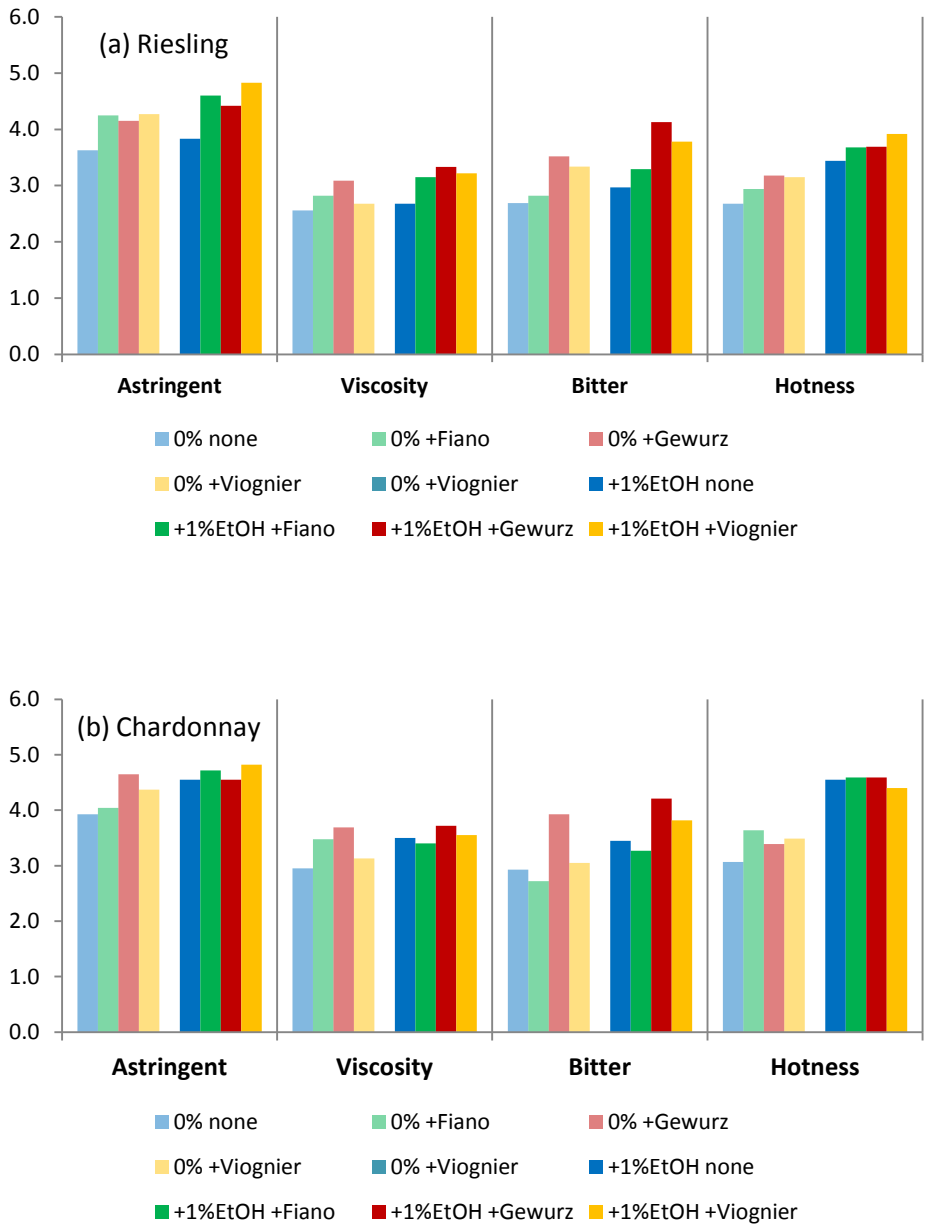



**Figure 3-1: Mean attribute intensities of phenolic fractions and alcohol added to (a) Riesling and (b) Chardonnay base wine.**

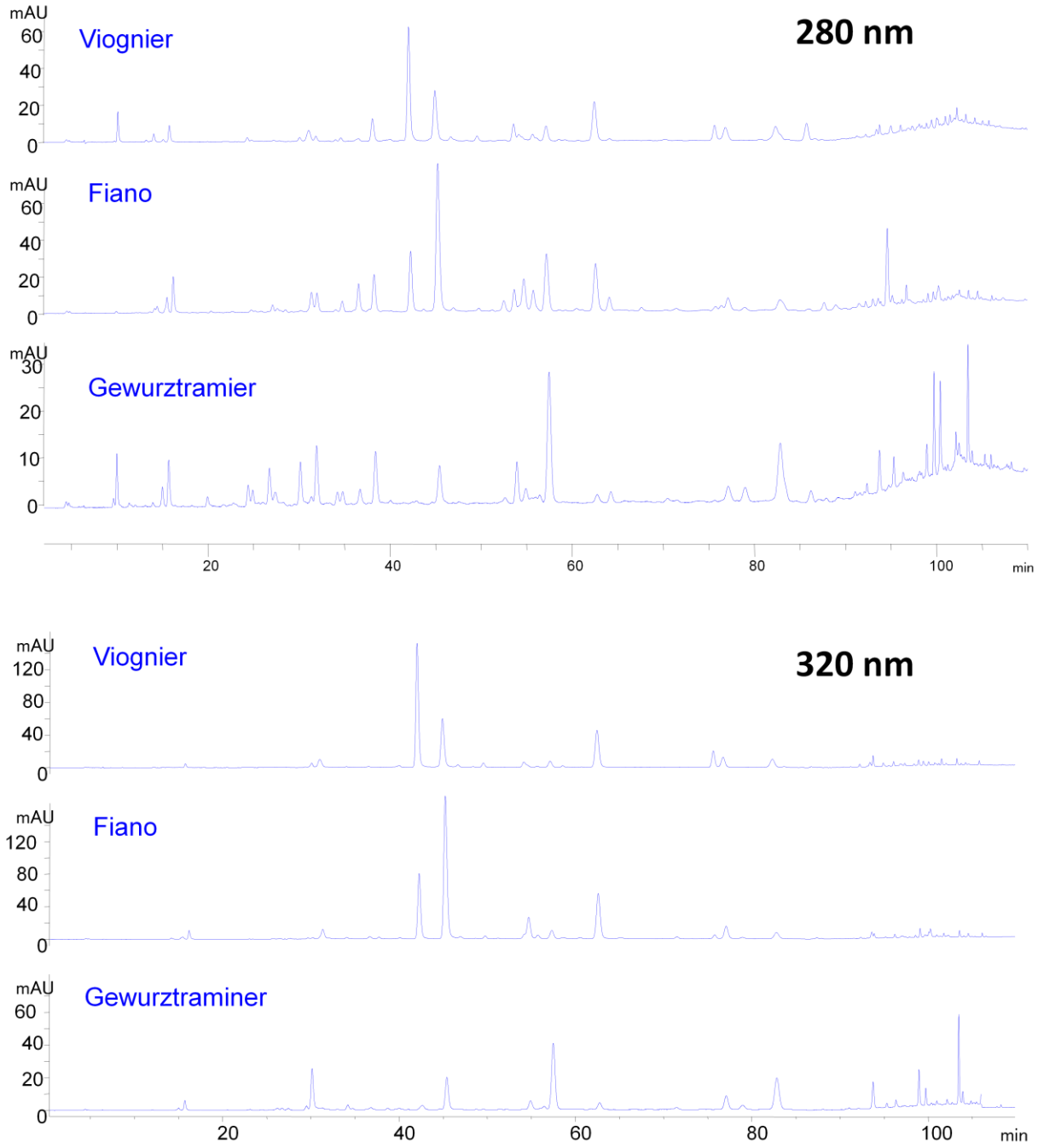



**Figure 3-2: HPLC traces of whole phenolic fractions from 3 wines recorded at 280 nm and 320 nm (in water).**

# 4 The Role of Phenolics in White Wine Style: Winemaker Perception of Quality, and Consumer Acceptance of Commercial White Wines

## 4.1 Introduction

As described in Chapter 3, the whole phenolics isolated from the three stylistically different wines (Fiano, Gewurtztraminer, Viognier) contributed to phenolic taste profiles when reconstituted in base wines. Thus, for the first time we demonstrated that different wines with different phenolic composition can display different textures when tasted in the same matrix (alcohol, pH, TA etc). This suggested that phenolic composition differences are a determinant of textural differences in white wines. However, of course, in ‘real’ wines these diverse phenolic profiles occur in the presence of a diverse range of matrices in terms of, for e.g., flavour, pH, acidity, alcohol, sugar etc. While it is widely accepted that specific combinations of flavour profiles, acidity and alcohol define specific styles, the role of phenolics is less well understood. So here we investigate the role of phenolics in contributing to various commercially acceptable styles produced from two important Australian white varieties. Furthermore, we explore how phenolics and other compositional factors influence winemaker perception of Riesling quality, and general Australian consumer acceptance of white wine.

## 4.2 Background

### 4.2.1 Study 1: Effect on ‘Pinot G’ Style

The variety known as Pinot Gris in Alsace, Pinot Grigio in North East Italy, Grauburgunder in the Baden region of Germany and increasingly in Australia as ‘Pinot G’ presents a useful case study for investigating the role of phenolics in white wine style. The Italian approach to the variety is to harvest it before it undergoes its characteristic loss of acidity at later ripening stages. This practice results in wines that are characterised by subtle varietal characters, light body and crisp acidity. The Alsatian and German approaches of using riper grapes, vinifying juices containing solids, often following pre-fermentation skin contact, results in more aromatic styles that are characterised by higher alcohol levels, richer flavours and fuller texture. While the higher alcohol style may be expected to be fuller when compared with their leaner Italian counterparts, it is unknown whether higher phenolics that might arise following later

harvesting and greater maceration are associated with any specific sensory attributes, or with general conformance to a particular style.

### 4.2.2 Study 2: Effect on Riesling Style and Perceived Quality

Riesling is the fourth most important Australian white grape variety in 2010 in terms of production (ABS Cat. 1329.0). Dry Riesling styles vary substantially - from the light bodied, low alcohol, high acid styles typical of the Ruhr/Rhine region of Germany through to fuller bodied, higher alcohol styles typical of the Alsatian region of France. Australian producers have generally produced leaner, aromatic wines with higher acidity in the Germanic style. In order to preserve the varietal character, Australian winemakers have generally avoided winemaking strategies that could potentially contribute to the phenolic content of the finished wine (McLean, 2005).

### 4.2.3 Study 3: Effect on Perceived Quality and Consumer Acceptance of Commercial Dry White Wines

The importance of phenolic content in dry white wines compared with other aspects of their composition such as alcohol, acidity and residual sugar is unknown. Here we investigate the role of phenolic content on winemaker perceived quality and consumer acceptability of commercial dry white wines.

## 4.3 Methods

### 4.3.1 Study 1: Effect on 'Pinot G' Style

#### Wine samples

22 commercial Pinot Gris/Pinot Grigio wines that made up the training set for the AWRI's Pinot Gris style project were used - 18 were Australian, three were from Alsace, France and one was from New Zealand. They formed a diverse set of styles representing that variety, ranging from a fuller bodied, higher alcohol style typically labeled in the marketplace as 'Gris', to the lighter bodied, lower alcohol, high acid style typically labeled as 'Grigio'. The 'Grigio' style that typifies Italian examples of the varietal, contrasts with the 'Gris' style that is typified by wines of Alsatian origin.

#### The tasters and sensory methods

The panel consisted of ten experienced tasters who were members of an AWRI tasting panel. They all had between 5 and 25 years continuous tasting experience as part of their profession and had undertaken the AWRI's Advanced Wine Assessment Course. Around half the tasters also had winemaking qualifications and/or had some practical winemaking experience.

After tasting and discussing the wines on two separate occasions, the panelists arrived at a set of descriptors that best differentiated the wines on the basis of 'Grigio' style as compared with the 'Gris' style. The wines were rated in duplicate on the intensity of the selected attributes using a 15 cm partially structured line scale with "low" and "high" word anchors at the 10% and 90% marks. Conformance to the 'Grigio' vs. 'Gris' style continuum was assessed using a 15 cm unstructured line scale with "Grigio like" and "Gris like" on the left and right extremes respectively. All wines were tasted in a random order at room temperature. Tastings were conducted in individual booths under amber lighting.

### Statistical Analysis

The conformance to Pinot Grigio vs. Pinot Gris style and the intensities of the individual taste and textural characters normally associated with phenolic character in white wine (astringency, oiliness, hotness, bitterness and viscosity) were modelled using Partial Least Squares (PLS) Regression analysis using ethanol concentration, pH, titratable acidity, residual sugar, and $A_{280}$ and $A_{320}$ as explanatory variables (the absorbances are proxies for total phenolic content, and total hydroxycinnamic acids respectively). To avoid over-fitting (i.e. including explanatory variables that do not contribute significantly to the explanatory power of the model), best subset regression was also used to model wine style on composition. Specifically, over-fitting was avoided by selecting models that were both closely fit (with a high adjusted $R^2$) and had a Mallows CP coefficient (Mallows, 1973) that was lower than the number of explanatory variables.

## 4.3.2 Study 2: Effect on Riesling Style and Perceived Quality

### Wine Samples

24 of the 100 best selling dry white wines in Australia in 2009 were selected by the AWRI's technical tasting panel on the basis of perceived variation in phenolic taste. Of these, the nine dry Rieslings were used in this study (details are given in Appendix B, Table B-2).

### The tasters and sensory methods

The selected Riesling wines were independently tasted blind in the same order by 20 practicing Australian white winemakers and two researchers employed by Australian wine companies. The tasters scored the wines for quality using the Australian 20 point system. In addition, the winemakers were asked to indicate if the wine displayed a phenolic taste which included bitterness, astringency, viscosity, oiliness, hardness, or an overall 'phenolic character'. Approximately 30 mL of each wine were tasted under natural light and at room temperature.

### Statistical Analysis

PLS regression was used to model winemaker quality scores of the Riesling wines on the same explanatory variables. Wine score was also correlated with taste attributes.

### 4.3.3 Study 3: Effect on Perceived Quality and Consumer Acceptance of Commercial Dry White Wines

#### Wine Samples

The 100 best selling dry white wines in Australia in 2009 were screened for phenolic character by the AWRI's technical tasting panel. Twenty four wines were selected to represent a range of 'phenolic character'. Seven were unoaked Chardonnay wines, five oaked Chardonnay, ten Rieslings, and two were Pinot Gris wines. Their analyses are given in Appendix B, Table B-2. A further subset of 14 of these wines was selected for a consumer acceptance study.

#### The tasters and sensory methods

The consumer panel comprised 203 Sydney consumers who drank white wine at least once per week, and who occasionally purchased $10-20 bottled white wine. Demographically, around half the tasters were aged over 40, half were female, around half were single, and over three quarters had undertaken some form of tertiary study. All preferred consuming white wines to sparkling, rose or red wines. The consumers were asked to taste the 14 wines and rate how much they liked them on a nine point Likert scale (Like extremely, very much, moderately and slightly – neither like nor dislike – dislike slightly, moderately, very much and extremely). 30 mL of the 14 wines were presented in a randomised order under natural lighting and at room temperature.

The winemaker panel and the tasting conditions used are the same as those used in Study 2.

#### Statistical Methods

Both consumer liking ratings and winemaker scores for all wines were correlated against alcohol concentration, pH, titratable acidity, residual sugar, $A_{280}$ and $A_{320.}$ Partial Least Squares (PLS) Regression was also used to relate these compositional variables to consumer liking and winemaker quality score. As it was assumed that winemakers applied style definitions when assigning quality scores, both winemaker quality score and liking for wines for a single recognised style (dry Riesling) were also regressed against composition.

The proportion of winemakers that indicated that the wine had a phenolic taste was correlated with alcohol, pH, titratable acidity, residual sugar, $A_{280}$, $A_{320}$ and average score. Binary logistic regression was applied to model the individual yes/no responses of winemakers to phenolic character with wine composition.

# 4.4 Results and Discussion

## 4.4.1 Study 1: Effect on 'Pinot G' Style

The PLS regression coefficients standardised for differences in scale are given in Figure 4-1. While viscosity and oiliness were mostly associated with residual sugar, oiliness was further associated with ethanol concentration. Palate hotness and bitterness were mostly determined by the level of ethanol. Consistent with these results, Gawel *et al*. (2007) found that higher alcohol concentration increased the viscosity of two of the three wines studied, and increased the perceived hotness of all three. The result that bitterness was associated with alcohol content contradicted the results of a model study using whole phenolic extracts from Riesling (Chapter 6), but was consistent with other model studies (Fontoin *et al.* 2008; Fischer and Noble 1994).

Wines that were lower in titratable acidity (TA) tended to be less astringent, viscous, oily, hot and bitter. Astringency in these wines was mostly associated with lower pH and also lower TA. pH and TA are usually moderately negatively correlated in grapes and wine. However under combination of low effects could be attributable to a situation where a wine went through either partial or no malolactic fermentation.

The PLS regression analysis also suggesed that wine phenolics (measured by $A_{280}$ and $A_{320}$) contributed much less to astringency, viscosity, oiliness, hotness and bitterness than the major compositional variables that reflect the wine matrix (pH, TA, residual sugar and alcohol). However, best subset regression suggested that the minimum number of analytical features required to best describe astringency and oiliness should also include $A_{280}$ and $A_{320}$ respectively (Table 4-1). The conclusions drawn from the two analyses are largely consistent as the PLS analysis revealed that absorbance at 280 and 320 nm were the most important phenolic indices in explaining astringency and oiliness respectively.

Conformance to a particular style implies that the wine has characteristics that, on the whole, can be recognised as being from a particular variety and have been made using specific wine-making processes (Cadot *et al.* 2010; Maitre *et al.* 2010). Conformance to defined styles has commercial importance as it provides consumers with the confidence to repeatedly purchase that wine, or seek other wines that are alike.

Both PLS and best subset regression suggested that Pinot Gris style could be differentiated from Pinot Grigio style on the basis of the combined effects of total phenolics ($A_{280}$), ethanol and residual sugar. Of note was the influence of total phenolics on style. While it was not a strong determinant of any particular sensory attribute, it was identified as being one of the most important determinants of the 'Gris' vs.'Grigio' style.

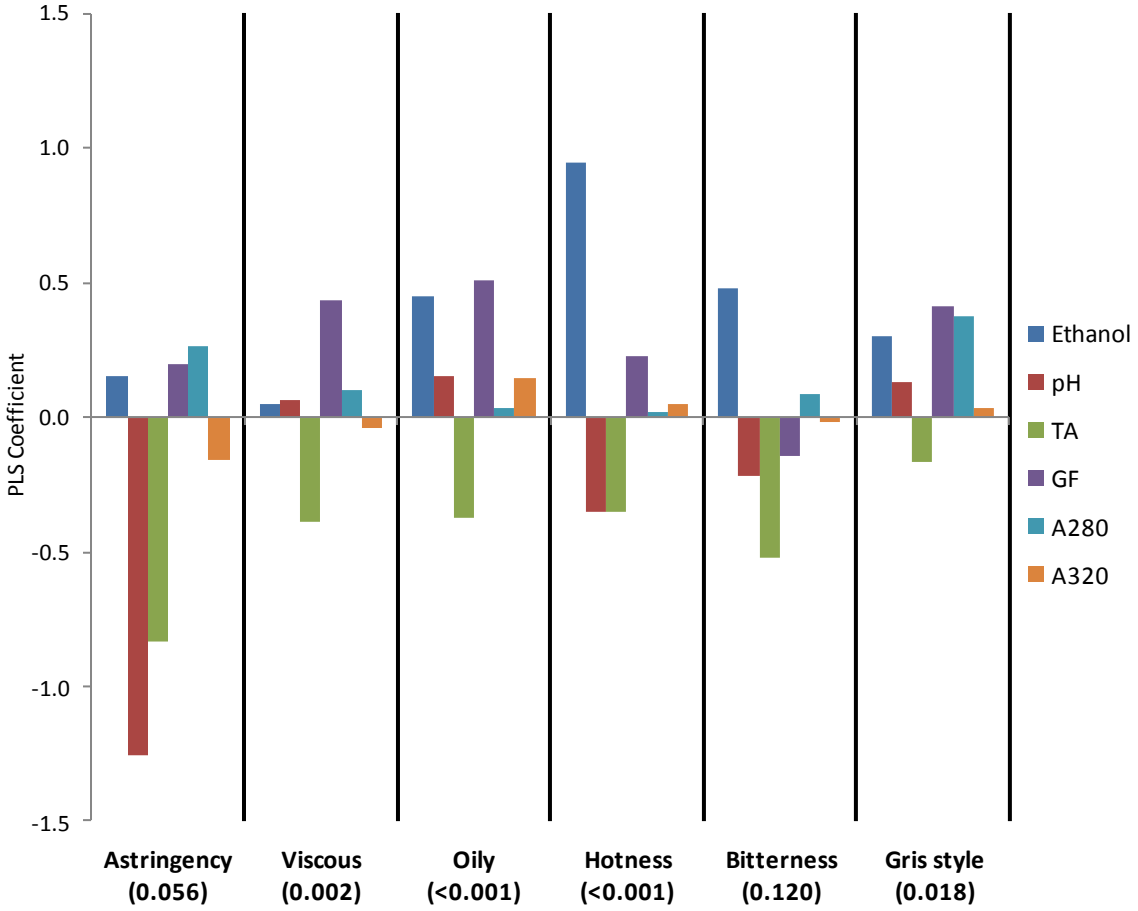



**Figure 4-1: Partial least squares regression coefficients modelling sensory attribute ratings on analytical parameters for 'Pinot G' wines. Significance of model fit is given in parenthesis.**

**Table 4-1 : Best subset regression for 'Pinot G' wines. Green fill indicates that the analytical parameter contributed significantly to the explanatory power of the model predicting the sensory attribute.**

| | Ethanol | pH | TA | Glu+Fru | $A_{280}$ | $A_{320}$ | Adjusted $R^2$ | Mallows CP |
|---|---|---|---|---|---|---|---|---|
| Astringency | | | | | | | 0.171 | 0.4 |
| Viscous | | | | | | | 0.580 | 2.4 |
| Oily | | | | | | | 0.660 | 4.1 |
| Hotness | | | | | | | 0.787 | 0.5 |
| Bitterness | | | | | | | 0.207 | 0.1 |
| 'Gris style' | | | | | | | 0.491 | 4.0 |

## 4.4.2 Study 2: Effect on Riesling Style and Perceived Quality

### Winemaker quality assessment

Simple correlation analysis (Table 4-2) showed that lower alcohol was very strongly associated with higher overall quality (r= -0.75). There was no significant association with pH, TA or residual sugar content. $A_{280}$ (r= -0.64) and $A_{320}$ (r= -0.58) were both strongly negatively correlated with perceived quality.

PLS regression showed that the perceived quality of these Riesling wines was more strongly associated with $A_{320}$ (positively) and $A_{280}$ (negatively) than with any aspect of the wine matrix (pH, TA and residual sugar) (Table 4-2). Australian winemakers have generally preferred making Riesling wines in the Germanic rather than the Alsatian tradition. In order to make these lighter bodied, higher acid styles, viticultural and winemaking strategies that control phenolic uptake and excessive fruit maturity are applied. The results of both correlation analysis and PLS regression analysis confirm that for this group of winemakers, that overall quality of Riesling wine depends on the wine having a overall low level of phenolics (as measured by $A_{280}$). The association of perceived quality with $A_{320}$ is intriguing. Australian Rieslings tend to have high hydroxycinnamic acid levels which manifest as high $A_{320}$. Either the compounds absorbing at 320 nm have a particular taste characteristic that are associated in the minds of winemakers with high quality Riesling, or other quality drivers are co-correlated with the compounds that absorb at 320 nm. The scope of data collected in this study does not allow either option to be explored.

**Table 4-2: Relationship between winemaker quality score for Riesling and analytical parameters.**

| | Ethanol | pH | TA | RS | $A_{280}$ | $A_{320}$ |
|---|---|---|---|---|---|---|
| Correlation | -0.75 | -0.13 | -0.12 | -0.03 | -0.64 | -0.58 |
| PLS Coefficients | -0.36 | +0.67 | +0.9 | +0.85 | -5.00 | +4.26 |

## 4.4.3 Study 3: Effect of Phenolics on Perceived Quality by Winemakers and Consumer Acceptance of Commercial Dry White Wines

### Relating Consumer Liking to composition

The consumers rated the 14 wines in a narrow range from 5.9 to 6.5 which may have contributed to the inability of the PLS regression model to adequately predict consumer liking from the compositional variables (p=0.794). However on a qualitative level, higher $A_{280}$ and lower $A_{280}$ and $A_{320}$ were positive predictors of higher consumer acceptance.

The correlations between wine composition and winemaker quality score differed from that of consumer liking (Figure 4-2: Correlations between consumer liking, winemaker quality score and basic wine composition of 24 commercial white wines.. Notably, consumer liking was positively correlated with

both alcohol (r= 0.37) and residual sugar (RS) content (r= 0.56), while the winemakers' perception of quality was negatively correlated with alcohol (r= -0.46), and was unrelated to residual sugar content (r= 0.03). Therefore it appears that consumers considered the basic wine matrix components were important in determining their liking of the wine, while winemakers saw alcohol level and phenolic levels as being important in their quality assessments of white wines.

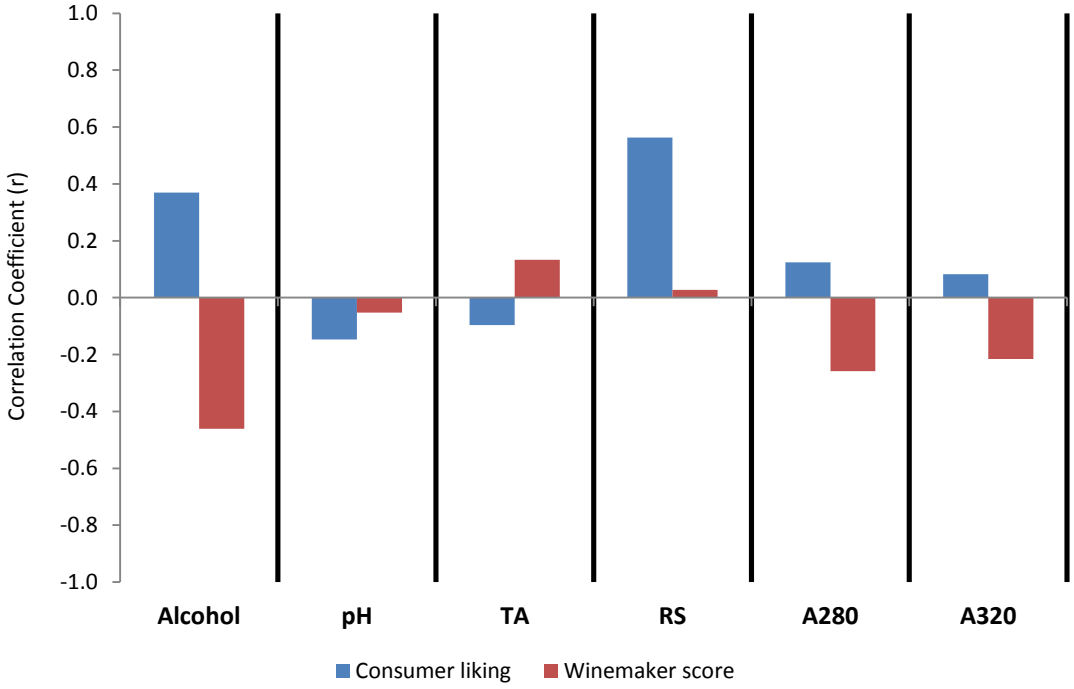



**Figure 4-2: Correlations between consumer liking, winemaker quality score and basic wine composition of 24 commercial white wines.**

## Relating Winemaker perception of quality to composition

The predictive power of the PLS model relating winemaking score to compositional variables was also not significant (p=0.395). This was either due to the exclusion of important compositional variables that influenced quality, or that the winemakers lacked specific criteria in assessing such a diverse range of wines. When only Riesling wines were considered, there was a substantial improvement in the predictive power of the PLS model using the same compositional variables (p=0.001). This suggests that winemaker assessment of quality was more reliable when they were able to apply a specific set of style based criteria.

Using alternative approaches: 1) the proportion of winemakers who thought that the wine tasted 'phenolic' was correlated against wine composition (Table 4-3), and 2) individual winemaker yes/no responses to 'phenolic taste' were modelled against the quality score that they assigned.

The binary logistic regression slope of winemaker quality score against the perceived existence of a 'phenolic character' was highly significant ($p<0.001$) with an odds ratio significantly less than 1 (odds ratio= 0.65: 95% confidence limit 0.56 - 0.75). This strongly suggests that the perceived presence of phenolic character in the 24 commercial wines was associated with lower quality ratings by these winemakers. This was confirmed by assessing average quality score against the proportion of winemakers who perceived a phenolic character in the wine ($r= -0.47$, $p<0.05$) (Figure 4-3 : Average winemaker quality score vs percentage of winemakers assessing wine as being 'phenolic'.

**Table 4-3 : Correlation coefficients between compositional variables and proportion of winemakers that perceived a 'phenolic' character in 24 commercial white wines.**

| Variable | r | Significance |
|---|---|---|
| Alcohol | -0.131 | ns |
| pH | -0.264 | ns |
| TA | 0.207 | ns |
| Residual Sugar (RS) | -0.039 | ns |
| $A_{280}$ | 0.172 | ns |
| $A_{320}$ | 0.348 | * |

* Denotes significant at 5%

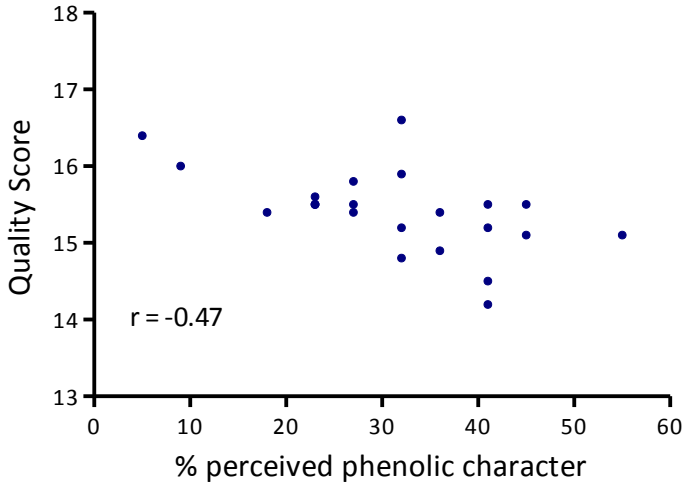



**Figure 4-3 : Average winemaker quality score vs percentage of winemakers assessing wine as being 'phenolic'**

# 5 The Effect of pH and Alcohol on the 'Phenolic Taste' in White Wine

## 5.1 Introduction

A solid body of work has established that pH and alcohol level greatly influence the perception of astringency and bitterness of phenolic compounds found in red wines (see General introduction for a review). Although white wine is relatively low in phenolics compared to red wine, the range in alcohol and pH levels of white wines is greater.

Work from this project (Chapter 3) established that alcohol concentration positively enhanced four major taste/textural attributes (astringency, viscosity, bitterness and hotness) in white wine, and that phenolics and alcohol contributed in an additive way to these attributes. Interestingly, research into stylistically different whole wines demonstrated that the astringency of Pinot Gris/Grigio wines was mostly associated with low pH.

In order to further explore the influence and interactions of phenolics with the key matrix elements of alcohol and pH on taste/textural attributes, an experiment was designed using a whole phenolics fraction isolated from Riesling. Two alcohol concentrations (11.4% and 12.6% v/v) and two pH levels (3.0 and 3.3) were used.

## 5.2 Methods

### 5.2.1 Tasting Panel

A panel of 13 volunteer assessors comprising four female and nine male employees of the Australian Wine Research Institute was convened. All had at least two years general wine tasting experience as part of their profession, but none had previously participated in tastings specifically related to white wine texture. All the panelists were experienced in using unstructured line scales to assess sensory intensities.

### 5.2.2 Taster Training

Training consisted of two, forty-minute sessions. In the first session, tasters assessed and discussed the texture of six white wines that had previously been deemed by an experienced wine tasting panel to vary in texture. The wines selected were made from both low phenolic (an Australian Riesling and unwooded Chardonnay) and high phenolic styles (an Australian Fiano, Alsatian Gewurztraminer and Italian

Vermentino and Verdicchio wines). The discussions were focused on the textural characteristics of astringency, bitterness and hotness. During the second training session, tasters assessed and discussed these attributes in the context of a low phenolic Riesling wine to which 1% v/v ethanol, 15 mg/L quinine sulfate (for bitterness), and 200 mg/L of white wine tannin (for astringency) (Tannin Galacool, FR) had been added.

### 5.2.3 Formal Assessment

A one year old commercial South Australian Riesling was selected as a base wine as it was low in alcohol (11.4 % v/v), and had a moderate pH (3.3). A whole phenolic extract was taken from the same wine by capturing them on Amberlite FPX-66 resin, followed by elution with 96% v/v ethanol (Tarac Technologies, SA). The ethanol was removed under vacuum, milliQ water added and the sample freeze dried. The whole phenolics extract was kept at -80°C until use. The added phenolic treatment was produced by adding half of the whole dried phenolic extract to the same volume of base wine that the phenolics had been extracted from. The proportion of the wine's total phenolics that was used was estimated in two ways. Firstly, the $A_{280}$ (Somers and Ziemelis 1985) of the original wine was compared to that which passed through the column. Secondly, an estimate of the the residual phenolics not captured by the column was made by comparing the $A_{280}$ of the pool the phenolics collected after three sequential reintroductions and elutions of the flow-through,to that of the first elution. The extraction efficiency of the column was estimated to be approximately 60% using both methods.Therefore, the phenolic treatment was estimated to comprise a 30% increase in total phenolic content over the control.

Tasting samples were made up on the day of tasting by adding the equivalent of 50% by weight of the total phenolic mass extracted from the original wine and added back to the same wine that had been 1) fortified with 1.2% alcohol and 2) had its pH reduced to 3.0 by the addition of sulfuric acid. These additions resulted in 8 treatment combinations - two ethanol levels (11.3% and 12.6%), two pH levels (3.0 and 3.3) and either no or 30% phenolic addition. The phenolic level was chosen by four experienced white wine assessors on the basis that it had a clearly perceptible impact on the taste and texture of the base wine. Later work (Chapter 8) confirmed that the phenolic addition was within the range that could be encountered when applying white wine production processes. The alcohol and pH levels were selected to represent a range found in light bodied dry white wines.

The formal assessment was conducted in tasting booths with 30 mL of each sample being presented in ISO wine tasting glasses at room temperature (22°C ± 1°C) under amber lighting. Three replicates were presented over two tasting sessions conducted a week apart. An entire set of treatment combinations were randomly presented each week, with the third replicate being split across the two tasting sessions. Perceived astringency, bitterness and hotness were rated on a 15 cm unstructured line scale with the word anchors "low" and "high" at the 10% and 90% points respectively. The ballots were served to tasters by the FIZZ v 1.30 sensory data acquisition software (Biosystèmes, Couternon).

### 5.2.4 Statistical Analysis

A three way ANOVA (Factors: phenolics, pH, alcohol) with assessors as a blocking variable was conducted using MINITAB v14. Means separation was determined with Fisher's Least Significant Difference.

## 5.3 Results and Discussion

The addition of 30% more phenolics to the base wine significantly increased the perception of astringency (p=0.046) (Table 5–1). However the strength of the effect depended on pH. At a low pH of 3.0, astringency was perceived to be high, and the addition of phenolics had little further effect. However, at a more realistic pH level of 3.3, the addition of whole phenolics increased astringency to the same level as the low pH condition (Figure 5–1A). This suggests that for this particular wine, the 0.3 unit difference in pH had the same effect on astringency perception as did an additional 30% phenolics (or vice versa). An estimate of the impact of phenolic content on astringency can be made in a real-world winemaking context following the winemaking trials conducted in 2010 (Chapter 8). White wines were made from three varieties using both conventional and non-conventional winemaking processes designed to maximise the differences in total phenolic concentration in the finished wines. Of the winemaking treatments selected, a 30% increase in phenolics over those in free run controls (as used in this model study) was achieved in wines that were made from heavy pressings (measured by total HPLC peak area at 280 nm (Figure 8–10)). While more work is required to quantify the relative importance of pH with total phenolic content, it would appear that pH as a factor in white wine astringency has been underestimated.

The base wine was a commercial Riesling so it contained phenolics. Therefore, the increased astringency resulting from pH reduction could either be the result of accentuation of the astringency elicited by the native phenolics, or that elicited by pH itself. Both effects have been extensively reported. The astringency of gallic acid (Guinard *et al.* 1986), catechin (Kallithraka *et al.* 1997), tannic acid (Peleg *et al.* 1998), flavanol oligomers extracted from grape seed (Fontoin *et al.* 2008), and whole grape seed tannin (Fia *et al.* 2008) all increased with decreasing pH. Both organic and inorganic acids have also been shown to be astringent like in the absence of phenolics (Hartwig and McDaniel 1995). The maximum level of astringency produced by three organic acids declined as pH increased (Lawless *et al.* 1996) and was also shown to be a function of pH rather than the acid concentration or anion species present.

While the addition of alcohol did not have a significant effect on astringency perception (p=0.524), higher alcohol tended to increase astringency particularly at lower pH (Figure 5-1 (A)). This trend is contrary to other findings whereby the astringency of red wine (Gawel *et al.* 2007) and model wines with added oligomeric proanthocyanidins (Fontoin *et al.* 2008) decreased with increasing alcohol. Ethanol is thought to weaken the interaction between salivary protein acceptor sites and the polyphenol hydroxyl groups by disrupting hydrophobic interactions (Pascal *et al.* 2008). Indeed, fewer monomeric phenolics were

precipitated by a salivary like protein when more alcohol was present (Pascal *et al.* 2008). To date, all the studies investigating the role of alcohol on astringency perception have used, or contained, polymerised phenolics that did not constitute a significant proportion of the phenolic fraction used in this study which was dominated by the presence of the small molecular weight phenolics particularly hydroxycinnamates. This may explain why no significant effect of alcohol was observed here.

While adding 30% more phenolics did not significantly increase bitterness ($p=0.217$), a consistently higher bitterness under all combinations of alcohol and pH was observed (Figure 5-1 (B)). Perceived bitterness decreased with the addition of alcohol ($p=0.079$). The enhancing effect of alcohol on the bitterness of the phenolics was at odds with Fontoin *et al.* (2008) when adding oligomeric flavanols (mean degree of polymerisation of 2.1) to model wine and Fischer and Noble (1994) when adding the monomeric flavanol catechin to de-alcoholised wine. The increase in perceived bitterness of the phenolic compounds at lower pH was clear ($p=0.001$). Fontoin *et al.* (2008) found no effect, while Fischer and Noble (1994) reported a small increase over a similar pH range to that studied here. The reason for the differences in effects resulting from alcohol and pH is unclear but may relate to the different phenolics used in the respective studies. Some tasters find alcohol sweet (Skinska *et al.* 2000). The effect of adding alcohol on bitterness perception was purely subtractive (phenolic x alcohol $p=0.972$) which lends weight to the possibility that the alcohol sweetness masked the bitterness of the phenolics in this case. We also used a food grade alcohol distilled from grapes, while the other studies used analytical grade ethanol. So this may also have been a factor. The effect of pH on bitterness perception was also strongly additive (pH $p=0.001$, phenolics x pH $p=0.770$) with lower pH resulting in greater bitterness. Fontoin *et al.* (2008) found no effect of pH on the bitterness of oligomeric flavanols, and using catechin Fischer and Noble (1994) found a small increase with decreasing pH over the range used in this study, but an inconsistent trend over a wider pH range. In the earlier studies, pH was increased by titrating with NaOH. Our use of a real wine as a base required the reduction in pH using a strong inorganic acid. The different counterions that necessarily are included by such practices may explain the differences across studies. However, it could be expected that the method used here to modify pH was less intrusive than the earlier approaches as the base used here was a commercial wine that required little adjustment.

Not surprisingly, adding alcohol to the base wine made it taste 'hotter' ($p<0.001$, Figure 5-1(C)). However, so did the addition of phenolics ($p<0.001$). The strength of the effect of phenolic addition on hotness depended on the alcohol level. At a higher alcohol level, the addition of phenolics had less influence on hotness than at the lower alcohol level. Tellingly, the addition of phenolics increased the 'hotness' of the low alcohol wine to around that of the high alcohol wine without phenolics, suggesting that the role of phenolic load on palate 'hotness' in this very low alcohol (11.4% v/v) wine was not inconsequential. The previous studies on phenolic-ethanol interactions (Fontoin *et al.* 2008; Fischer and Noble 1994) did not consider the possibility that phenolics themselves may contribute to the taste/textural properties elicited by the action of the wine matrix. The effect of adding phenolics on perceived hotness decreased as alcohol content increased. A similar modulation of the effect of possible phenolic induced hotness on commercial white wines following ethanol addition was observed in another study (Chapter

3). The highest alcohol used in this study was 12.6% v/v which is representative of light bodied white wines. The effect of adding 30% more phenolics on the perceived hotness of a 12.6% v/v alcohol wine was relatively small. So while it was demonstrated that phenolics have the capacity to contribute to hotness in white wine, it seems likely that in fuller bodied styles that are typically higher in alcohol than 12.6% v/v, the impact of phenolics on hotness is likely to be small – and it is in these higher alcohol styles where winemakers are more likely to employ methods that increase phenolics (i.e. skin contact, pressing addition). Later, it was shown that the hotness displayed by wines with an average 13.2% v/v alcohol but made using winemaking methods that increased their total phenolic levels by up to 70% did not differ in perceived hotness (Chapter 8).

Lowering the pH also resulted in an increase in perceived 'hotness' at both alcohol levels (p=0.004). The reason for this is unclear, but the lack of interaction with phenolics (p=0.384) and alcohol (p=0.984) suggests that the increased hotness was a direct effect of pH.

Perceived acidity elicited by the Riesling base wine appeared to be accentuated by phenolic addition but only at the lower alcohol level (p=0.057, Figure 5-1(D)). The wine that the phenolics was extracted from was characterised by HPLC as having a relatively high level of hydroxycinnamic acids (data not shown) - which is typical of Riesling wines made using reductive winemaking processes. While hydroxycinnamic acids are weak acids, in combination they may contribute to the acid taste of the wine. However the role of other phenolic compounds cannot be ruled out as more defined phenolic fractions that contained a smaller number of hydroxycinnamic acids did not increase the wine acidity (Chapter 6).

**Table 5-1: Significance (p values) of effect of composition on sensory.**

| | Astringency | Hotness | Bitterness | Acidity |
|---|---|---|---|---|
| Phenolics | **0.046** | **0.000** | 0.217 | 0.278 |
| Alcohol | 0.524 | **0.000** | **0.079** | **0.009** |
| pH | **0.074** | **0.004** | **0.001** | 0.130 |
| Alcohol x Phenolics | 0.819 | **0.012** | 0.972 | 0.164 |
| Alcohol x pH | 0.302 | 0.984 | 0.183 | 0.057 |
| pH x Phenolics | 0.095 | 0.384 | 0.770 | 0.957 |
| Alcohol x pH x phenolics | 0.916 | **0.021** | 0.144 | 0.355 |

Bold highlight indicates statistical significance (p<0.10)

These results suggest that phenolics can impact on the perception of astringency, hotness and possibly bitterness in white wine, but their influence on these attributes are dependent on both pH and alcohol levels. While both the pH and alcohol levels employed in this study were at the lower end of those typically found in white table wine, it did appear that the tastes and textures elicited by the presence of phenolics are more pronounced at lower alcohol levels and at moderate pH levels. This also suggests that low pH levels may elicit a considerable level of astringency in their own right, as well as enhancing bitterness.

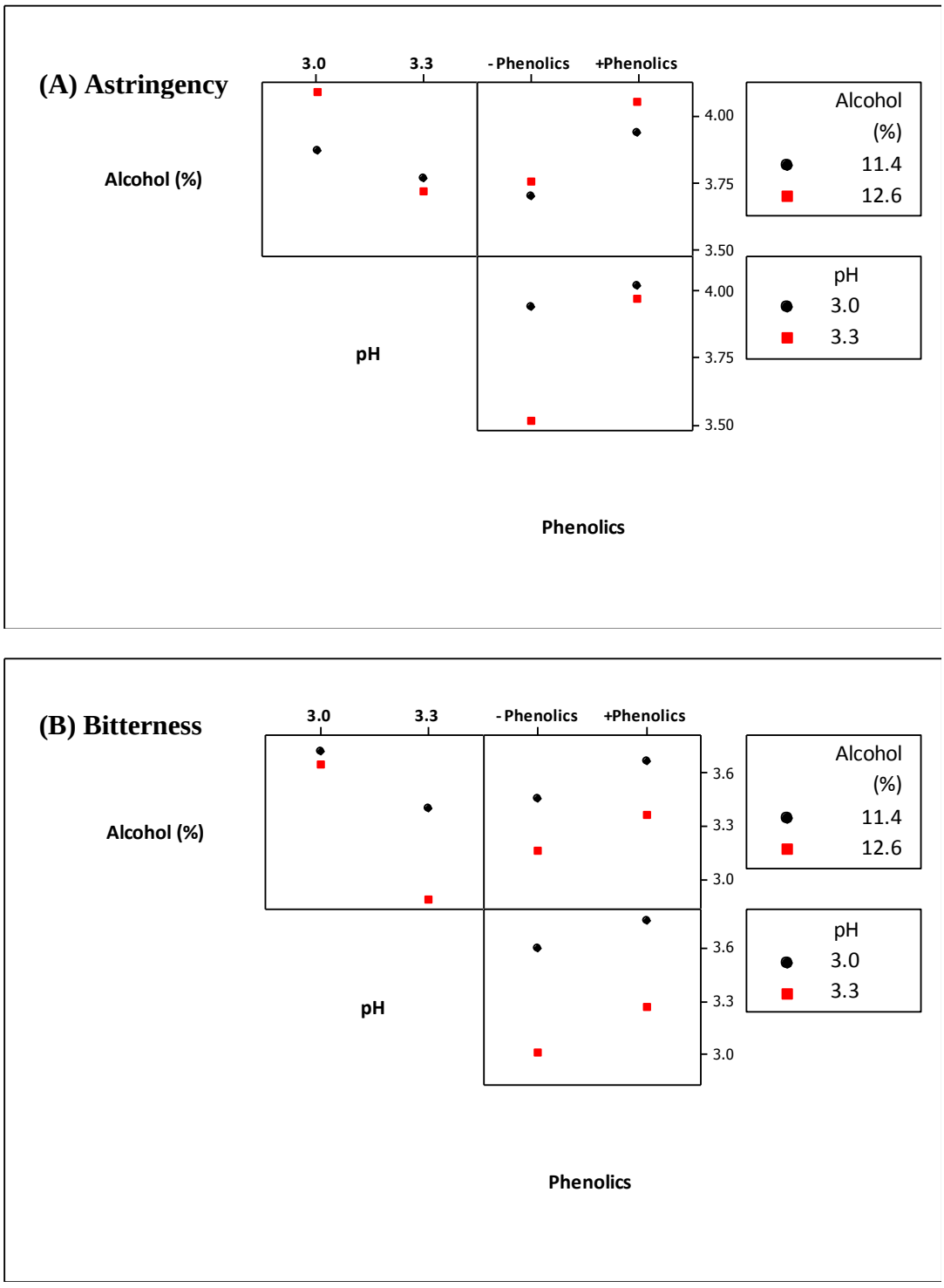



**Figure 5-1: Interaction Plots: Astringency (A), Bitterness (B)**

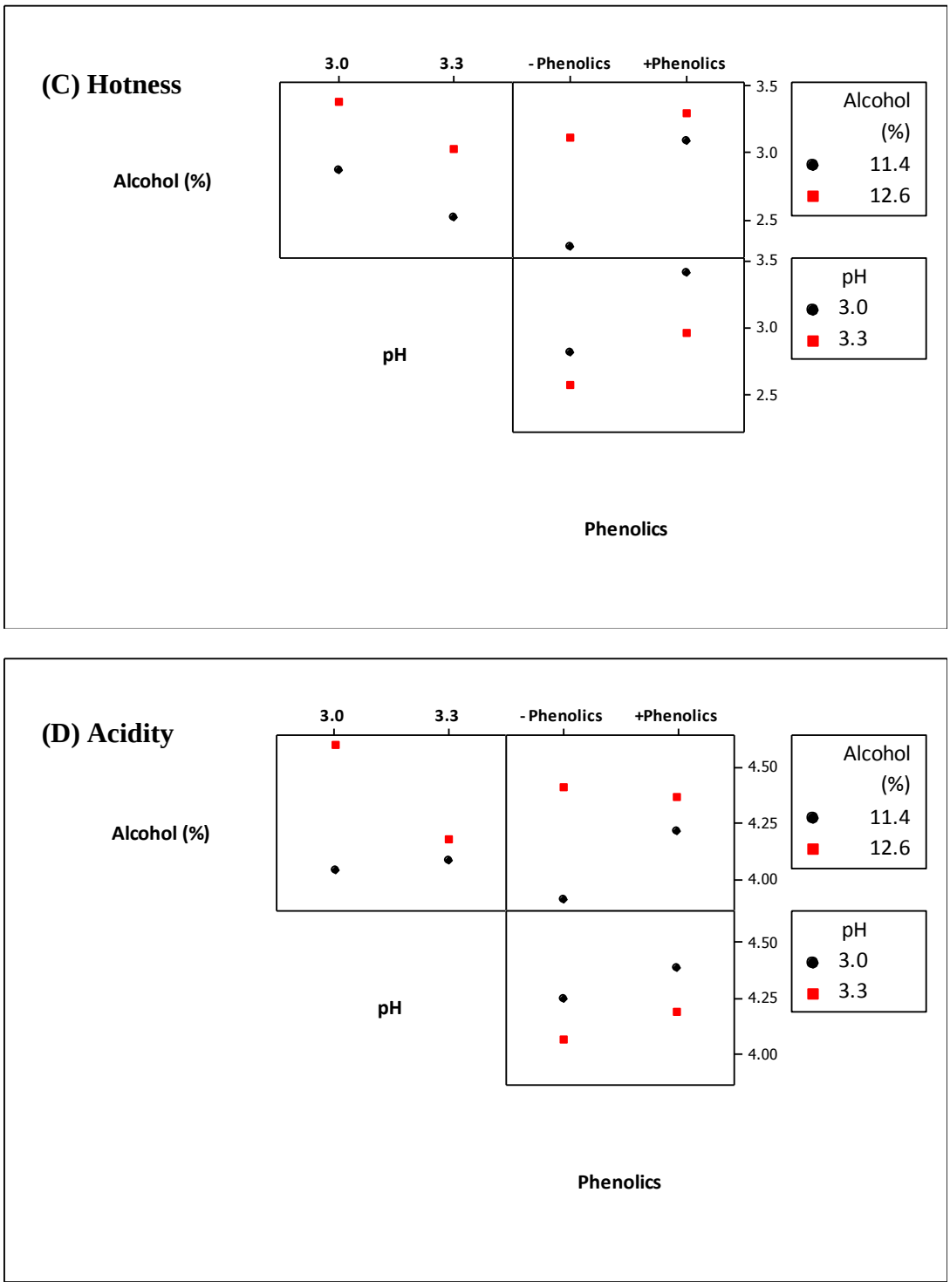



**Figure 5-1: Interaction Plots: Hotness (C) and Acidity (D)**

# 6 Sensory Impact of Fractions Taken from Three Commercial White Wines

## 6.1 Introduction

Following the addition of total phenolics taken from stylistically diverse wines and manipulation of the matrix, we demonstrated that variation in white wine tastes and textures could be attributed to both phenolic composition and the interaction with the wine matrix. While these were important steps towards increased understanding the molecular basis of textural perception in white wine, the identity of the phenolic molecules (or groups of molecules) that cause these differences in tastes and textures remains unclear. To begin to address this, phenolics from three wines were separated into four fractions and tasted by a descriptive panel. Two of the three wines that were fractionated were the same as those from which whole phenolic fractions were isolated and tasted in Chapter 3. A base was constructed using the same Chardonnay wine used in the previous study, except in this study it was extensively stripped of its phenolics prior to use so as to accentuate the taste effects of the fractions while still maintaining a realistic wine like matrix.

## 6.2 Methods

### 6.2.1 Fractionation

Three wines were fractionated – a 2009 McLaren Vale Fiano, a 2008 Alsatian Gewurtztraminer and a 2009 Eden Valley Riesling made from hard pressings. The first two were those used in the study described in Chapter 3.

The ethanol from 2.25 L of each wine was removed from under vacuum at 40°C. $K_2HPO_4$ (0.1M), EDTA (5 mM) and PMS (50 ppm) was added to the dealcoholised wine before being filtered (0.22 µm membrane) and loaded onto a 25 mm ID, 300 mm chromatography column packed with Oasis HLB resin. Once the loading finished, the column was eluted with water and the flow through (FT) was collected until the UV reading dropped and stabilized (recorded at 280, 320 and 370 nm). Thereafter, the effluent was switched to 15% v/v acetonitrile (producing fraction F1), 45% v/v acetonitrile (F2) and 30% v/v acetonitrile, 68% v/v methanol, 2% v/v formic acid (F3). The solvent change was conducted when UV readings fell and stabilised. The FT fraction was loaded onto an Amberlite FPX-66 column to retain phenolics. The column was then exhaustively eluted with 96% ethanol and collected. Fractions were dried off solvents under vacuum at less than 40°C, water added and freeze dried.

### 6.2.2 Tasting Panel

Nine tasters (five males and 4 females) were trained for this study. They were drawn from a group of people with considerable experience in profiling wine aroma, flavour and the taste sensations of bitterness and acidity. While some tasters were experienced in assessing the astringency of red wines, none had previously assessed phenolic tastes in white wine.

Tasters had been previously accepted for panel membership on the basis of their general sensory acuity including sensitivity to the bitter tasting quinine sulfate (15 mg/L) (ISO 8586). Tasters were further screened just prior to the trial for their sensitivity to bitterness elicited by 3.2 mM 6-*n*-propylthiouracil (PROP). An aqueous solution of the compound was rated for bitterness using a labeled magnitude estimation scale. As this scale has been shown to exhibit ratio properties, the data showed that the tasters differed around three-fold in their perception of bitterness. Despite this expected variation in bitterness sensitivity, the initial screening using quinine sulfate suggested that all of the tasters involved in the study were adequately able to detect bitterness.

### 6.2.3 Taster Training

A two hour training session was used to arrive at descriptors and definitions for the textural sensations associated with the fractions. The training examples that were tasted and discussed were 1) a commercial unwooded McLaren Vale Chardonnay that had been extensively stripped of its phenolics using Amberlite FPX-66 resin (the base wine), 2) the base wine with its whole phenolics added back, and 3) the base wine plus the whole phenolics extracted from an Adelaide Hills Chardonnay. The base wine was the same as that used in the formal assessment of the phenolic fractions.

The attributes, astringency, bitterness, viscosity, burning, acidity (in mouth) and acidity aftertaste (AT) were agreed upon by the tasters as adequately representing the samples presented during training. The agreed definition of the terms is given in Appendix B, Table B-3.

### 6.2.4 Formal Assessment

Tastings were conducted in triplicate, one week apart. During each session, all 13 treatment combinations including the control were presented in a randomised order to each assessor. The fractions were added to a one year old unwooded McLaren Vale Chardonnay (the same wine used in Chapter 3) that had been extensively stripped of its phenolics by the Amberlite FPX-66 resin. 30 mL of sample were tasted from ISO wine tasting glasses at room temperature and under amber lighting. Tasters assessed the intensity of astringency, bitterness, acidity, viscosity and 'burning' using a 15 cm partially structured line scale as described previously. Assessors were asked to rinse their mouth with water and were required to wait two minutes before tasting the following sample, and to rest for 15 minutes after every fourth sample. The ballots were served to tasters by the FIZZ v 2.46 sensory data acquisition software (Biosystèmes, Couternon).

### 6.2.5 Analysis of Fractions

The fractions were analysed using the HPLC method described in section A.3. Peak areas were used to evaluate the relative contribution of different phenolic species to the fraction. $A_{280}$, $A_{320}$ and $A_{370}$ of the sensory samples were taken at the conclusion of each session.

### 6.2.6 Statistical Analysis

A two way analysis of variance with assessors and fraction addition as factors was conducted by wine from which the fractions were extracted. Judges were treated as random factors. While the fractions were collected in the same way from each wine, HPLC analysis clearly indicated that they varied in at least one component. Therefore it was necessary to analyse each set of fractions collected from each of the three wines separately. A Dunnett's test was conducted to compare the sensory effects of each fraction addition on that of the control wine. A Partial Least Squares analysis was also conducted with the sensory attributes as dependent variables and $A_{280}$, $A_{320}$ and $A_{370}$ as dependent variables.

## 6.3 Results and Discussion

### 6.3.1 Fraction Composition

The Oasis HLB resin has both hydrophilic and hydrophobic groups and as such it works in a mixed mode. As a result, few fractions contained phenolic compounds that were confined to a particular phenolic class. However, there were distinct compositional patterns; Figure 6-1 depicts the HPLC traces of each fraction while Table 6-1 gives the relative contributions of compounds classed by their $A_{280}$, $A_{320}$ and $A_{370}$.

*Flow Through (FT) fractions* were complex, but primarily consisted of the esterified hydroxycinnamic acids and grape reaction products. But even these differed. Notably, the fraction taken from the Gewurztraminer was distinguished by a cysteine GRP derivative not found in the other FT fractions.

*F1 fractions* contained tyrosol and mostly free hydroxycinnamic acids, and in the case of the two Australian wines, a syringic acid like compound. The F1 fractions taken from the two Australian wines also contained quercetin-3-glucuronide.

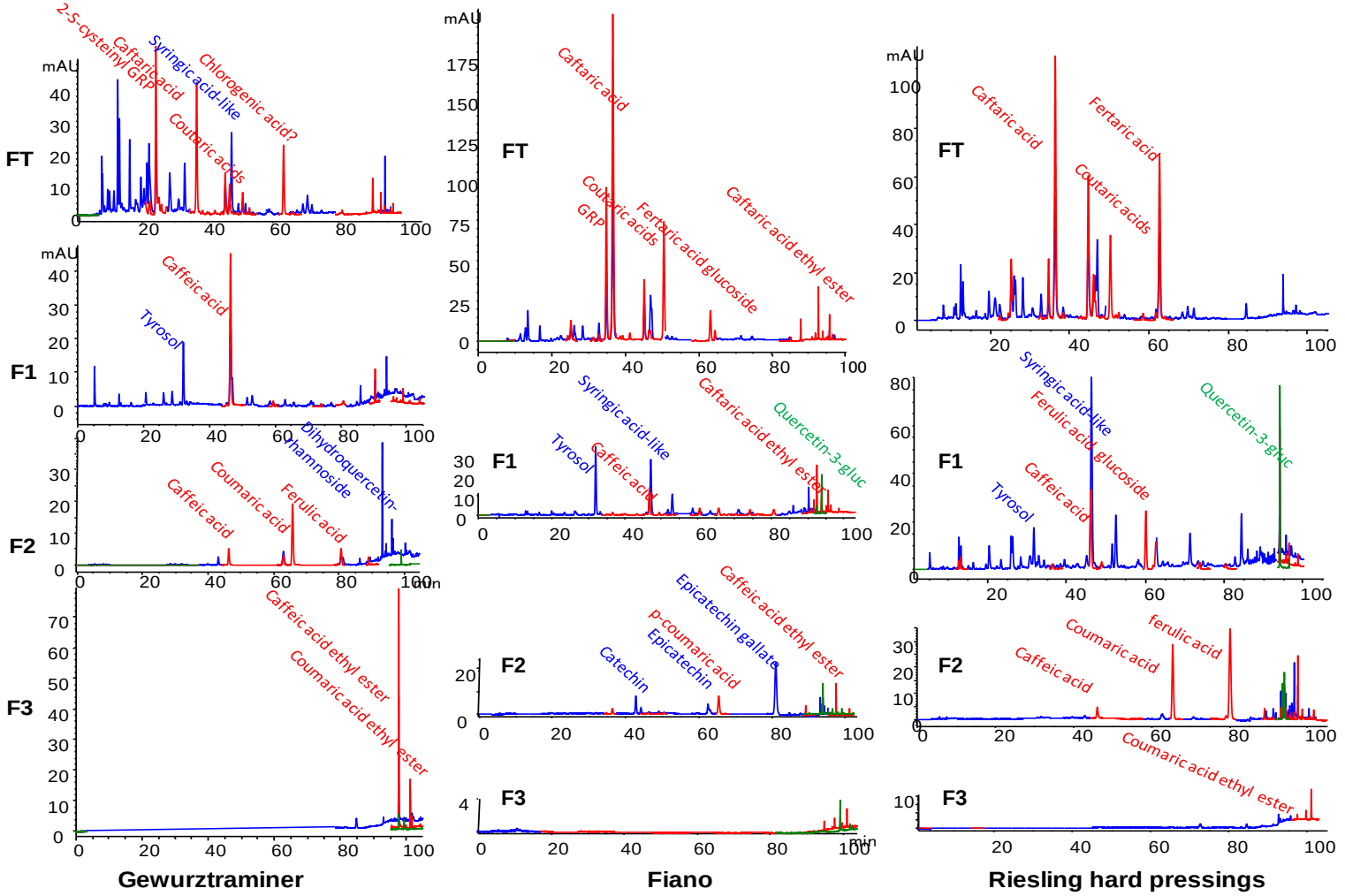



**Figure 6-1 : HPLC-DAD traces of phenolic fractions separated from three commerical wines on an HLB chromatography column.** **Blue = A280 nm, Red = A320nm, Green = A370nm**

**Table 6-1: Summary of major compounds found in each of the fractions tasted.**

| Var | Frac | 280 nm active compounds | 320 nm active compounds | 370 nm active compounds |
|---|---|---|---|---|
| Gewurztraminer | FT | syringic acid like (7%)<br>epicatechin galate (4%) | cysteinyl caftaric acid GRP (24%)<br>caftaric acid (22%)<br>coutaric acids (13%)<br>unknown (12%) | - |
| | F1 | tyrosol (18%) | Caffeic acid (31%) | - |
| | F2 | dihydroquercetin-rhamnoside (22%) | coumaric acid (48%)<br>ferulic acid (14%)<br>caffeic acid (11%) | - |
| | F3 | - | caffeic acid ethyl ester (81%)<br>coumaric acid ethyl ester (16%) | - |
| Fiano | FT | - | caftaric acid (41%)<br>coutaric acids (22%)<br>GRP (15%) | quercetin-3-glucuronide (90%) |
| | F1 | syringic acid like (31%)<br>tyrosol (22%)<br>unknown (7%) | caffeic acid (20%)<br>caffeic acid ethyl ester (11%) | quercetin-3-glucuronide (90%) |
| | F2 | epicatechin galate (48%)<br>catechin/epicatechin (20%) | coumaric acid (36%)<br>caffeic acid ethyl ester (17%) | - |
| | F3 | - | - | - |
| Riesling HP | FT | - | caftaric acid (29%)<br>coutaric acids (23%)<br>ferulic acid (19%) | - |
| | F1 | syringic acid-like (25%)<br>unknown (7%)<br>tyrosol (4%) | caffeic acid (29%)<br>ferulic acid glucoside (19%) | quercetin-3-glucuronide (90%) |
| | F2 | - | ferulic acid (40%)<br>coumaric acid (31%) | - |
| | F3 | - | coumaric acid ethyl ester (64%) | - |

**Green indicates < 25%, orange 26-49%** and **red > 50%** of HPLC peak area**.**

*F2 fractions* varied greatly amongst wines. The Gewurztraminer contained free hydroxycinnamic acids, but was distinguished by dihydroquercetin-rhamnoside content. The Fiano fraction primarily consisted of flavan-3-ols particularly epicatechin gallate but also catechin and epicatechin. The Riesling F2 fraction was most like the Gewurztraminer F2 as it was also dominated by free hydroxycinnamic acids. However it did not contain any significant proportion of dihydroquercetin-rhamnoside.

*F3 fractions* contained mostly ethyl esters. However the concentration of these was very low.

Only qualitative and tentative assignments of phenolic influences on tastes and textures can be made as there as only a relatively small number of fractions were assessed, and some of these had overlapping phenolic profiles.

**Table 6-2 : Significance (p values) of ANOVA factors between sensory attributes and wine fraction**

| | Astringency | Viscosity | Bitter | Burning | Acidity | Acid AT |
|---|---|---|---|---|---|---|
| **Fiano** | | | | | | |
| Fraction | 0.621 | 0.812 | 0.571 | **0.024** | 0.462 | 0.282 |
| Assessor x Fraction | 0.982 | **0.068** | 0.797 | 0.974 | 0.778 | 0.563 |
| **Gewurztraminer** | | | | | | |
| Fraction | 0.230 | 0.112 | 0.406 | **0.032** | 0.173 | **0.029** |
| Assessor x Fraction | 0.827 | **0.050** | 0.672 | 0.999 | 0.785 | 0.348 |
| **Riesling Hard Pressings** | | | | | | |
| Fraction | 0.158 | 0.985 | 0.127 | 0.534 | 0.892 | 0.645 |
| Assessor x Fraction | 0.973 | 0.128 | 0.100 | 0.669 | 0.258 | 0.130 |

Bold indicates significance effect ($p<0.1$). AT= Aftertaste.

Figure 6-3 shows that adding a diverse set of phenolic fractions to a base wine stripped extensively of its phenolics did not increase its astringency or viscosity. In fact, perceived viscosity was consistently suppressed by adding phenolics. The strongest suppression occurred when the coumaric and caffeic acid ethyl ester rich fraction taken from the Alsatian Gerwurztraminer F3 (Figure 6-1) was added ($p=0.112$). Astringency was barely altered when phenolic fractions were added, regardless of the type or origin of the fraction. The largest increase in astringency occurred from the F1 fraction extracted from the hard pressings Riesling wine. The increase was only 0.2 sensory units. The same fraction increased bitterness to a level unequalled by any fraction for any taste attribute – a result which puts the relative importance of phenolics to astringency and bitterness into perspective (at least in the context of the phenolics found in these three wines).

In contrast to astringency and viscosity, bitterness increased in eight of twelve instances where phenolic fractions were added. A three-fold variation in bitterness perception across tasters was recorded following PROP screening. This could explain why a statistically significant difference in bitterness was not seen despite some relatively large increases in mean bitterness ratings. Most of the bitter tasting fractions varied substantially in composition which made comparisons difficult. However, in one case, two fractions of similar composition differed substantially in bitterness - the bitter F1 Riesling fraction, and the non-bitter tasting F1 Fiano fraction (Figure 6-1). They differed compositionally in one major aspect - the bitter tasting F1 Riesling fraction contained more ferulic acid glucoside than the non-bitter F1 Fiano fraction.

Two non flow-through fractions also contained quercetin-3-glucuronide as a significant proportion of the phenolic compounds that absorb maximally at 370 nm. Various flavonol glycosides (of which quercetin-3-glucuronide is a member) have been reported to elicit astringency at very low concentrations (Scharbert *et al.* 2004; Brock and Hofmann 2008). However, of the two fractions that contained quercetin-3-glucuronide (F1 Fiano and F1 Riesling), only one was perceived to be more astringent than the low phenolic control, and only marginally so. Therefore quercetin-3-glucuronide does not seem to contribute to the astringency of these fractions.

Adding phenolic fractions (regardless of source or type) resulted in a consistent reduction in perceived burning sensation and acid aftertaste. At this stage of the project, tasters were trained to understand the notion of hotness resulting from alcohol, but had not been introduced to the possibility that other forms of back-palate hotness might exist. The base wine had been extensively stripped of its phenolics, so it could be reasonably assumed that any hotness arising from the base wine was alcohol derived. In this context, every phenolic fraction, regardless of its source or type reduced the perception of burning sensation (presumably resulting from alcohol). Of all the fractions, the F3 fraction from the Gerwurztraminer was the most effective in reducing the burning aftertaste ($p=0.0074$). It contained a higher proportion of caffeic and coumaric ethyl esters that the other fractions amounting to 97% of the compounds active at 320 nm.

Phenolic addition generally resulted in a reduction in the perception of acid aftertaste. The three fractions that resulted in the greatest reduction in acid aftertaste had a common feature in that they were all rich in free hydroxycinnamic acids. However, F2 from the Gerwurztraminer was also abundant in these, but its addition only marginally decreased the acid aftertaste.

For the first time we show that phenolic addition has little effect on acidity when perceived in mouth, but significantly affects the intensity of acidity once the wine has been expectorated. The reason for this is unclear, but due to its implications on style perception this result should be pursued.

The Fiano F2 fraction differed from the others in that it was rich in monomeric flavan-3-ols (epicatechin gallate, catechin and epicatechin). It was also perceived to be more bitter than the control than any other fraction. Epicatechin gallate has recently been shown to react most strongly amongst the common monomeric flavanols found in wine with the human bitter taste receptor hTAS2R39 (Narukawa *et al.* 2011). It also was deemed to be bitter and astringent in human taste tests, and it elicited a taste that was the least preferred amongst the common monomeric flavanols; epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate (Narukawa *et al.* 2010). The other two flavanols found in this fraction, catechin and epicatechin are also known to contribute to bitterness (Kielhorn and Thorngate 1999; Peleg *et al.* 1999). Interestingly, all the above-mentioned authors have reported that the monomeric flavanols elicit astringency as well as bitterness. Hufnagel and Hofmann (2008) concluded that flavanols did not contribute to astringency in red wine, a conclusion supported by the data here. The Fiano F2 fraction whilst being slightly more bitter than the control was not more astringent. Hufnagel and Hofmann (2008) also suggested that the major bitter compounds in red wine were ethyl esters of caffeic, vanillic

and syringic acid rather than the flavan-3-ols. Here, of the two fractions that contained reasonable quantities of caffeic acid ethyl ester, one was more bitter than the control (Fiano F2) and one was less bitter (Fiano F1). Furthermore, the interpretation is confounded by the fact that Fiano F2 also contained significant quantities of monomeric flavanols as well as hydroxycinnamic ethyl esters.

In a previous trial (Chapter 3), adding whole phenolics extracted from wines and added to commercial base wines were shown to result in increases in most sensory attributes related to phenolic taste. However in this study where phenolic fractions were added, we mainly saw a suppression of phenolic tastes. In previous work we have used wines with a reasonable amount of phenolics as the base, while in this study we have utilised a base wine that had been stripped of most of its phenolics. While the possibility that phenolic compounds may synergise to accentuate phenolic tastes cannot be ruled out, to our knowledge this has not previously been reported.

### 6.3.2 Modelling Taste and Textures on Absorbance Measures at 280, 320 and 370 nm

PLS modelled the burning sensation on $A_{280}$, $A_{320}$ and $A_{370}$ very well (p model fit = 0.048, Figure 6.2). The burning sensation was heavily negatively weighted on $A_{320}$ (i.e. total hydroxycinnamates), and strongly positively weighted on $A_{370}$. HPLC showed that quercetin-3-glucuronide made up 90% of the active compounds at this wavelength in most of the fractions, which suggests that this compound may play a role in the perception of this sensation. However, the relationship between the total absorbance at a particular wavelength and the relationship between that and specific compounds is tentative.

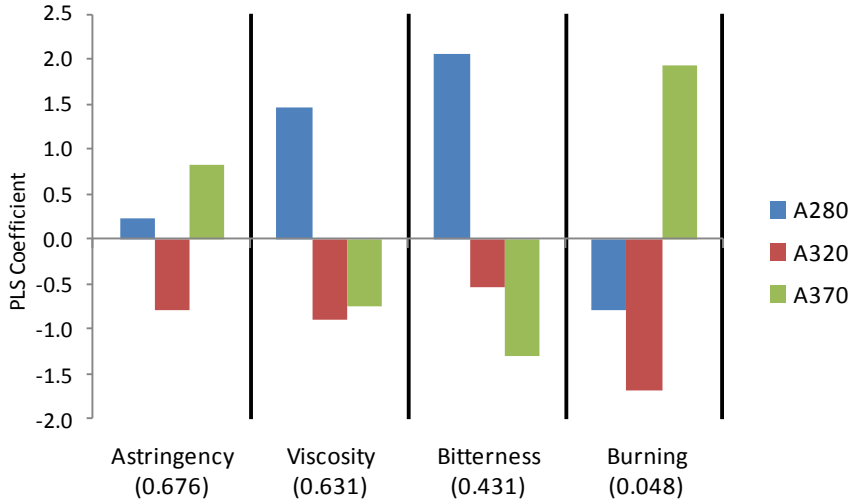



**Figure 6-2: PLS regression coefficients modelling sensory attribute ratings on analytical parameters. Significance of model fit given in parenthesis.**

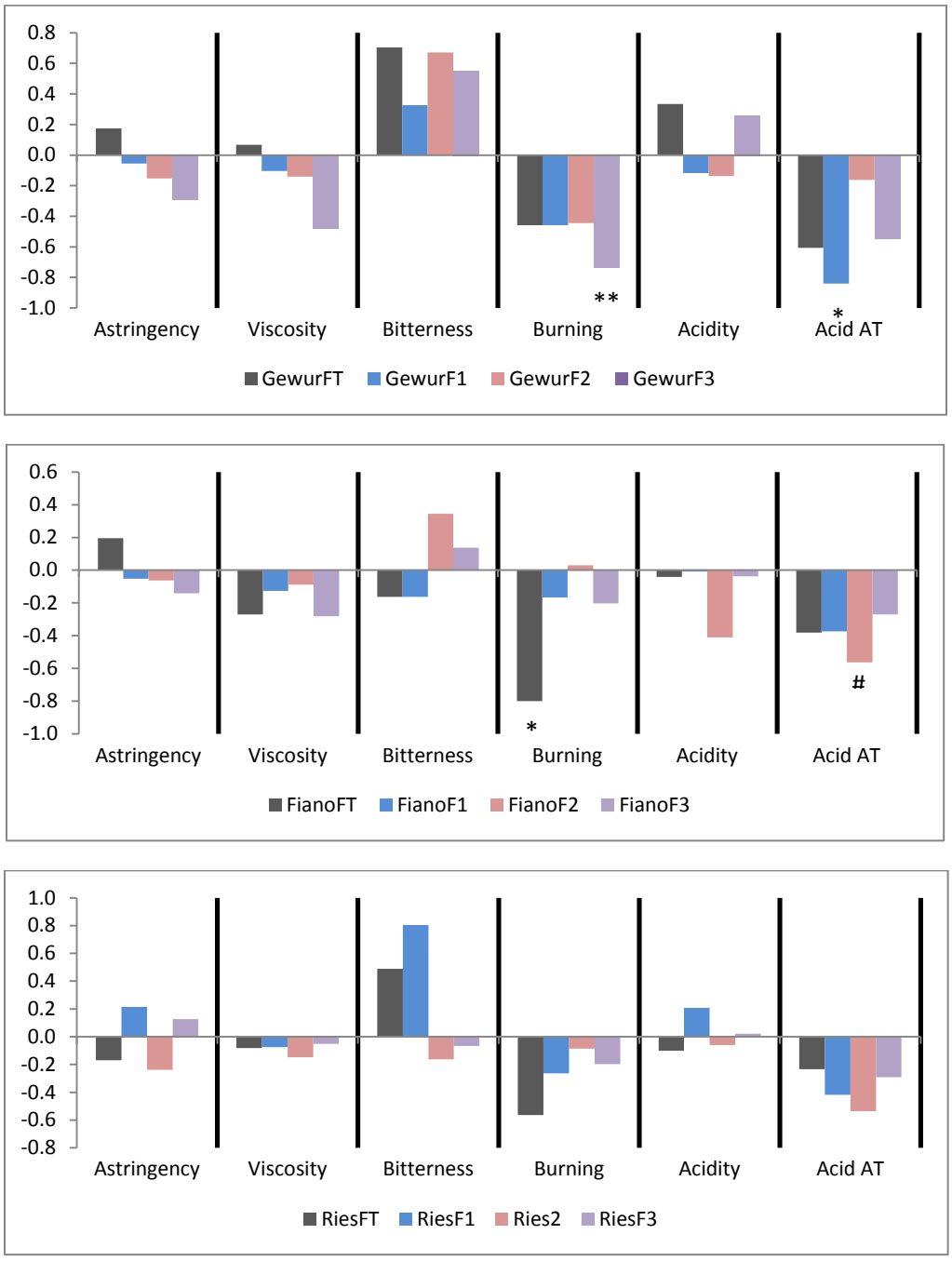



**Figure 6-3: Mean difference of sensory ratings of phenolic fractions extracted from three commercial wines from the sensory ratings of a base wine. #,*,** indicate difference at 10, 5 and 1% respectively.**

# 7 The Effect of Caftaric Acid and Grape Reaction Product on the Sensory Character of Model Wine

## 7.1 Introduction

The tartaric acid ester of caffeic acid, known as caftaric acid and its derivative 2-S-glutathionyl caftaric acid, better known as Grape Reaction Product or GRP, are among the most dominant phenolics in Australian white wines. GRP forms when polyphenol oxidase enzymes oxidise caftaric acid to the quinone, which then non-enzymatically reacts with the grape peptide glutathione. The relative amount of GRP to caftaric acid in wine can be increased by utilising winemaking practices that involve oxidative juice handling. Their abundance and ability to be influenced by winemaking made caftaric acid and GRP key molecules of interest. As such, they were isolated and their taste properties quantified.

## 7.2 Methods

### 7.2.1 Isolation of Caftaric Acid and GRP for Sensory Analysis

GRP and caftaric acid were extracted from 36 L of 2010 vintage wines made as part of this project and described in Chapter 8. The wines used were made from the light and heavy pressings of Chardonnay (18 L), Viognier (4.5 L) and Riesling (13.5 L).

Whole phenolics were extracted from each of the wines by acidifying them with 0.1M HCl and loading them onto a 500 mL chromatography column packed with Amberlite FPX66 resin (Dow, Camberwell, Australia). The column content was washed with 2 L water before eluting the phenolics with 2 L of 96% v/v ethanol. The eluate was chilled at 4°C overnight before being dried under vacuum at 40°C.

A Quattro Mk II high speed counter-current chromatography (HSCCC) device (AECS-QuikPrep Ltd, Bridgend, S. Wales, UK) equipped with a 500 mL stainless steel preparative scale coil (2.16 mm i.d tubing) was used to fractionate the whole phenolics and collect GRP and caftaric acid. The device was used with a Waters 600E controlled pump and two single wavelength UV detectors (UPC-900 Amersham Biosciences for $A_{280}$and a GBC LC 1210 for $A_{320}$) and a fraction collector (Amersham Pharmacia Biotec).

A two stage separation was used. Full details of the methods development are given in Appendix A.2. Firstly, GRP was isolated from the whole phenolic extract using a highly polar butanol : water (1:1) system. For each of six runs, whole phenolics from 6 L of wine were dissolved in 50 mL of a mixture

of the mobile and stationary phase and injected. The system was run at 800 rpm, 2 mL/min flow rate with the aqueous layer as the mobile phase for 250 minutes, during which the early-eluting GRP fraction was collected and retained. The stationary phase was recovered and dried under vacuum at 30°C to recover the remaining phenolics. These were re-dissolved in 20 mL of a mixture of mobile and stationary phase of the HEMBWAT (3:7:3:0:7) system. The sample was injected and fractionated under the same conditions as the previous step for 120 minutes, whereby a fraction containing caftaric acid was obtained. The phenolic identity of the fractions were assessed by HPLC using the method described in Appendix A.3 before being dried under vacuum, freeze dried and stored at -80°C. The presence of macromolecules such as proteins and polysaccharides were assessed using size exclusion chromatography (Phenomenex Biosep Sec-S-2000 column, 1 mL/min $NaNO_3$, 40°C, 5 mg/mL, 25µL injection).

## 7.2.2 Preparation of Sensory Samples

A comparison of the reported extinction coefficient of GRP (Cheynier *et al.* 1986) with that of the GRP isolate suggested the presence of impurities. Size exclusion chromatography showed that they were not phenolic (as they did not absorb at 280nm), being less than 1Kda in molecular weight were far too small to be either polysaccharides or proteins (compounds that have the potential to affect texture (Jones *et al.* 2008)). The retention time of the major impurity coincided with that of potassium hydrogen tartrate, a ubiquitous wine compound not known to contribute to either the phenolic taste or texture of wine.

The caftaric and GRP isolates were added to the model wine (10% v/v ethanol, pH 3.5) on the basis of their reported extinction coefficients to 30 and 60 mg/L. These levels were selected after a review of the literature and following a tasting by an experienced taste panel. The tasting samples were prepared two hours prior to tasting.

## 7.2.3 Sensory Assessment

### Tasters

Fourteen tasters (eight females and six females) were trained for this study. All had previous experience in the texture profiling of white wines, and most had previously evaluated the in-mouth texture of phenolic fractions extracted from white wines.

### Taster Training

Taster training was conducted over six sessions. Four commercial wines are presented in the first session. These were a Verdiccio from Marche, Italy, a McLaren Vale Fiano, a Pinot Grigio from East Gippsland Victoria, and a Gewuztraminer from Alsace, France. These wines were selected by an experienced white wine tasting panel to represent examples of bitterness, oiliness, astringency and hotness.

A'phenolic hotness' standard was needed. A workshop on white wine phenolics was conducted at the 13$^{th}$ Australian Wine Industry Technical Conference. Some participants identified a character that

they called 'phenolic hotness' that they described as being a burning sensation at the back of the throat that was unrelated to the whole mouth sensation of alcohol warmth. Informal tastings of phenolic wines stripped of most of their alcohol under vacuum by an experienced panel supported the existence of a back of the throat burning sensation. In the absence of a known chemical standard for this attribute, a fresh Australian extra virgin olive oil that was described by judges at the 2010 Australian National Extra Virgin Olive Oil Show as being pungent was presented as a training example. Pungency is an officially recognised term used by olive oil assessors to describe the throat burning sensation elicited by the olive oil phenolic oleocanthal. The sensation was thought to be a good proxy to illustrate a burning character as it was of known phenolic origin. Aqueous solutions of 0.5 g/L aluminium potassium sulfate, quinine sulfate (15 mg/L) and carboxymethycellulose (3 g/L) were also presented to demonstrate astringency, bitterness and viscosity respectively.

Two or three examples of model wines (pH 3.5, 10% v/v alcohol) containing either high (60 mg/L) or low (30 mg/L) concentrations of Grape reaction product (GRP) and caftaric acid or their mixtures, were presented over the following three training sessions in order to generate and define taste and texture attributes associated with the samples. The final two training sessions were used to familiarise the tasters with the use of the 15 cm partially structured tasting scale and to identify any attribute redundancies. Four samples (model wine, high and low GRP and high caftaric acid) were presented in duplicate over consecutive days for this purpose.

### 7.2.4 Formal Assessment

The test samples were assessed in triplicate. The fifteen test samples were presented over three sessions in a randomised order. 40 mL of sample was presented in black tasting glasses at room temperature (23°C ±1°C). A two minute rest was enforced between samples, and an additional eight minute rest was enforced after the third sample. The list of selected attributes and their definitions are provided in Appendix B, Table B-3. The intensity of these was assessed using the 15 cm partially structured line scale described previously.

### 7.2.5 Statistical Analysis

Where zero ratings for a particular attribute were given to all samples on all tasting occasions by an assessor, their ratings were excluded from analysis as they were considered either insensitive to the attribute or did not understand it. The ratings from one assessor were excluded for both the 'astringency' and 'metallic' attributes, two were excluded for 'oiliness', and three for the 'prickly' character. The ratings from at least eight assessors remained for each attribute which is considered acceptable for descriptive analysis. Assessor consistency across tasting sessions was assessed using Kendall's coefficient of concordance (Siegel 1956). A three way analysis of variance of each tasting attribute was conducted with assessors, caftaric acid concentration and GRP concentration as treatment effects, with assessors being treated as random effects. Means separation was conducted using Fishers Least Significant Difference. The relationship between attributes was also investigated using Pearson's correlation coefficients.

## 7.3 Results and Discussion

Typical HSCCC separation runs measured at $A_{320}$ are shown in Figure 7-1 and Figure 7-2. The composition of the fractions obtained from individual runs was assessed by HPLC. Their identity was initially determined by their UV-spectra and later confirmed by mass spectroscopy. The fractions that exclusively contained high levels of GRP or caftaric acid (as per those shown in the inserts of Figure 7-1 and) were pooled and used for sensory analysis.

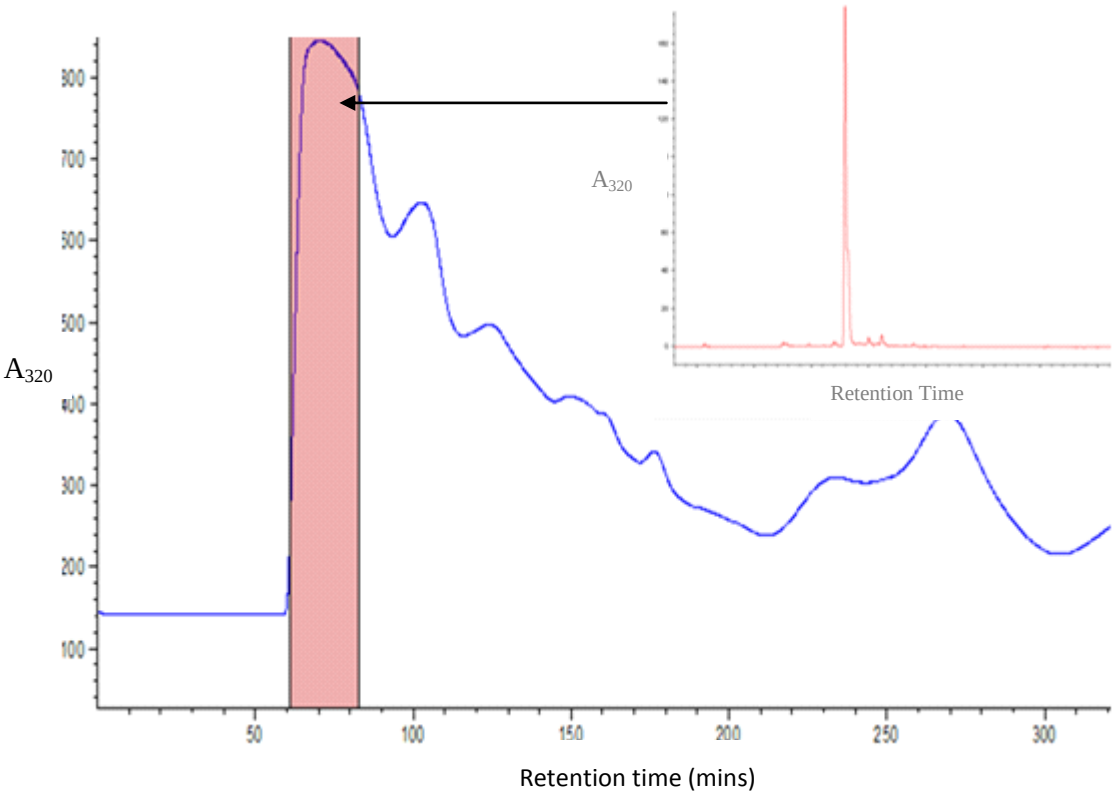



**Figure 7-1: HSCCC Chromatogram: Butanol : Water 1:1 solvent system: absorbance intensities at 320nm: Infill shows fraction cut. Insert: HPLC chromatogram at 320 nm showing GRP peak.**

### 7.3.1 Sensory Outcomes

The ability of assessors to agree with themselves across three tasting samples was poor to moderate, with correlations ranging from 0.04 to a maximum of 0.56, with an average of 0.32. Concordance across the entire tasting panel was predictably poorer than within individuals, as in general, individuals would be expected to be in better agreement with themselves than with other tasters. A significant degree of panel concordance ($p<0.05$) was observed for both the 'astringent' and 'oily' taste attributes (Appendix B4 – Table B-1).

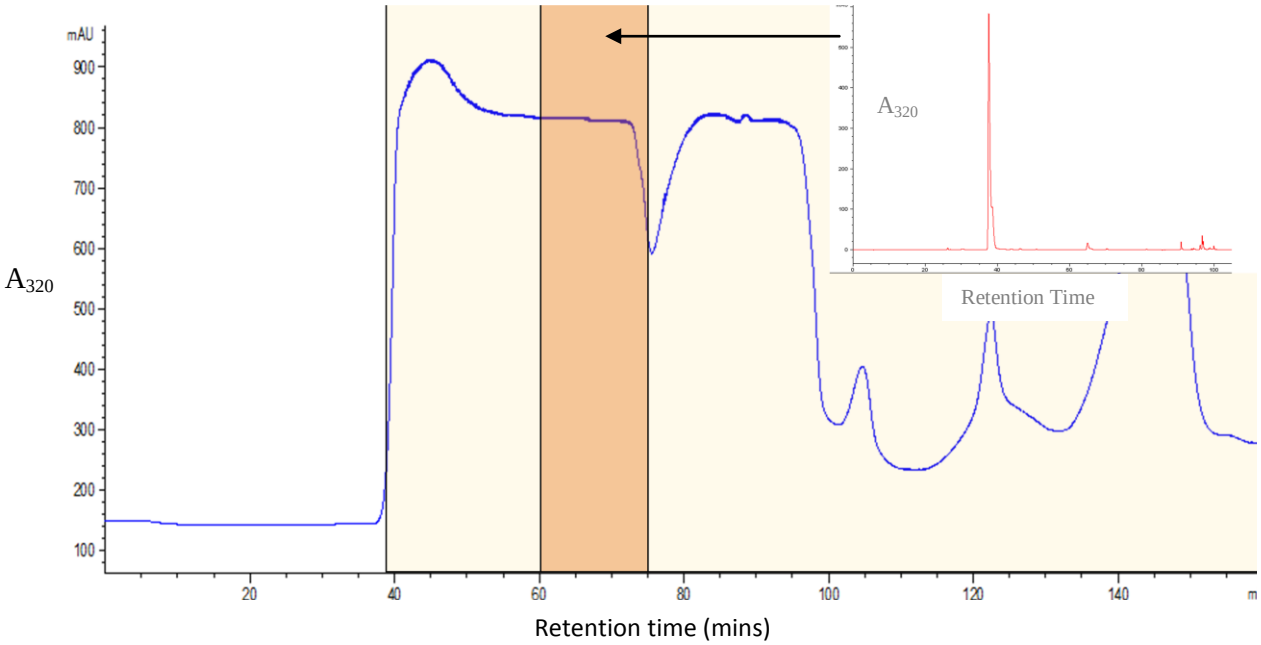



**Figure 7-2: HSCCC Chromatogram: Hexane: Ethyl Acetate: Methanol:Water 3:7:3:7 solvent system. Absorbance intensities at 320nm: Infill shows fraction cut: Insert: HPLC chromatogram at 320 nm showing caftaric acid peak.**

Despite this general poor agreement across the tasting panel, both caftaric acid concentration and GRP concentration was observed to significantly affect 'astringency' ($p<0.1$, Figure 7-3). The mean astringency intensity was lower for most combinations of caftaric acid and GRP compared with the model wine (Figure 7-4).Caftaric acid concentration also had a significant effect on the intensity of the 'burning aftertaste' sensation ($p=0.031$), and GRP concentration affected the intensity of 'oily mouth-feel' ($p<0.1$). No other significant differences for any other sensory attribute were observed except for a weak interaction between caftaric acid and GRP for the viscous attribute ($p=0.089$). The strength of treatment effects and interactions for each taste attribute is given in Table 7-1.

The model wine was deemed to be astringent despite not containing phenolic compounds. Therefore, the astringent sensation was presumably caused by either acid or alcohol. It has been known for some time that acidity and alcohol cause mouth dryness which presumably is analogous to the dryness

caused when tasting phenolics. However, while the sensations might be similar, the physiological mechanism underlying them may differ. Astringency in its classic sense is thought to arise when phenolic compounds bind to salivary proteins or when they directly bind to the oral mucosa (Gawel, 1998; Payne *et al.* 2008). The model wine contained no phenolics so its astringency must have arisen from either alcohol, acidity or both.

**Table 7-1: Significance (p values) of ANOVA factors between sensory attribute and phenolic composition**

| | Astringency | Viscosity | Oily | Bitter | Metallic | Prickling | Hotness | Acidity | Alcohol | Acid AT | Hotness AT | Burning AT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caftaric | **0.093** | 0.421 | 0.193 | 0.506 | 0.622 | 0.369 | 0.959 | 0.288 | 0.582 | 0.189 | 0.467 | **0.031** |
| GRP | **0.100** | 0.380 | **0.096** | 0.396 | 0.655 | 0.712 | 0.861 | 0.328 | 0.903 | 0.757 | 0.717 | 0.657 |
| Interaction | 0.314 | **0.089** | 0.206 | 0.143 | 0.760 | 0.272 | 0.998 | 0.616 | 0.855 | 0.306 | 0.876 | 0.782 |

Bold indicates significant effect ($p<0.1$)

Using a similar hydroxycinnamic acid to that of caftaric acid, Naish *et al.* (1993) reported that the intensity and persistence of astringency elicited by high concentrations of chlorogenic acid in water decreased with concentration. Chlorogenic acid has a similar molecular weight and structure to caffeic acid in that chlorogenic acid is the quinic acid ester of caffeic acid while caftaric acid is its tartaric acid ester. Importantly both chlorogenic acid and caftaric acid are small molecules with one phenol ring that has a single 1,2-dihydroxyphenyl residue. Recently, Rawel *et al.* (2006) could not find evidence of binding of chlorogenic acid to biologically important proteins with varying structures. While they did not test proteins that have been implicated in astringency perception, the fact that chlorogenic acid did not bind to any of the diverse proteins they tested is notable. A possible explanation to the lack of binding of chlorogenic acid to proteins is that it does not have a sufficient number of phenol rings or hydroxyl groups necessary for robust binding with salivary proteins. The lack of any increase in astringency resulting from caftaric acid and its derivative GRP are consistent with these previous studies.

The effect of caftaric acid and GRP concentration on 'burning aftertaste' is shown in Figure 7-5. Increasing caftaric acid levels clearly reduced the 'burning aftertaste' in these samples ($p=0.031$) regardless of GRP level. This figure also suggests that while GRP may increase 'burning aftertaste', the intensity of the character may be modulated by caftaric acid, with higher levels having a greater proportional effect.

The exact nature of the back of the throat burning sensation is unknown. 'Phenolic hotness' was identified by winemakers attending a workshop on white wine phenolics at the 13$^{th}$ Australian Wine Industry Technical Conference as being part of the vocabulary they use to describe phenolic character in white wine. Small molecular weight phenolics that interact with the receptors in the throat to

produce irritation exist. Oleocanthal is one such compound. It is responsible for the peppery, back of the throat burning sensation that typifies extra virgin olive oil. Recently, oleocanthal receptors located in the human pharynx have been identifed (Peyrot des Gachons *et al.* 2011).

The 'burning aftertaste' perceived by the assessors was negatively associated with both in-mouth hotness (r= -0.331) and hot aftertaste (r= -0.395), suggesting that 'burning aftertaste' represents a sensory character that is not totally related to alcohol content. However, the model wine (that contained alcohol but not phenolics) was rated as having a burning aftertaste, suggesting that alcohol induced hot aftertaste and a similarly perceived phenolic induced character were not totally differentiated by the tasters.

Adding caftaric acid to the model wine suppressed the perception of burning aftertaste, while adding GRP tended to increase burning sensation. This suggests that GRP produces a burning sensation in its own right, while the presence of caftaric acid interferes with the burning aftertaste induced by alcohol. Therefore two mechanisms are likely to be in play.

GRP was found to have an enhancing effect on 'oily mouth-feel' (Figure 7-7, p=0.096). The relationship between perceived viscosity and oiliness is less clear as they were moderately correlated (r= 0.53, p=0.141). However, by keeping both 'oily mouth-feel' and 'viscosity' in their list of relevant attributes, they were seen as distinct characters worthy of inclusion by the tasting panel.

What is new here is the notion that small molecular weight phenolics from white wines may suppress astringent and 'burning aftertaste' sensations arising from acidity or alcohol rather than causing them as has previously been presumed. The mechanisms underlying this are unclear but warrant further investigation. While there is evidence that GRP contributes to a burning aftertaste, in general, GRP and caftaric acid when tasted at wine like concentrations have either no effect or a suppressive effect on 'phenolic tastes' in white wine.

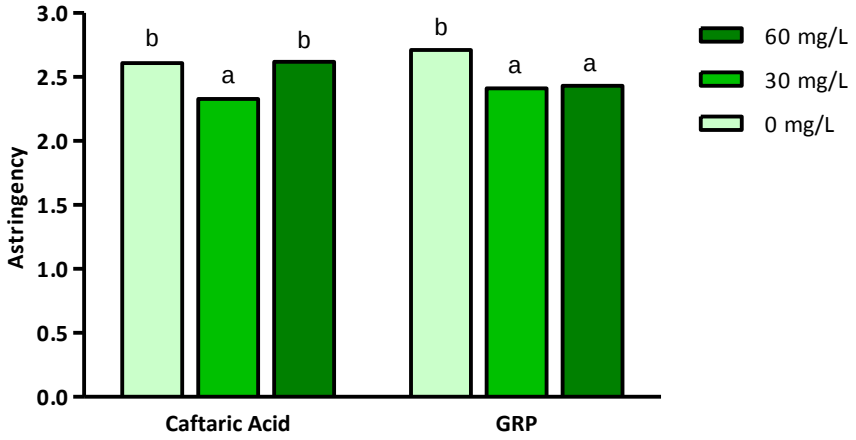



**Figure 7-3: Mean 'astringency' intensity. Means with different subscripts are significantly different (p<0.1)**

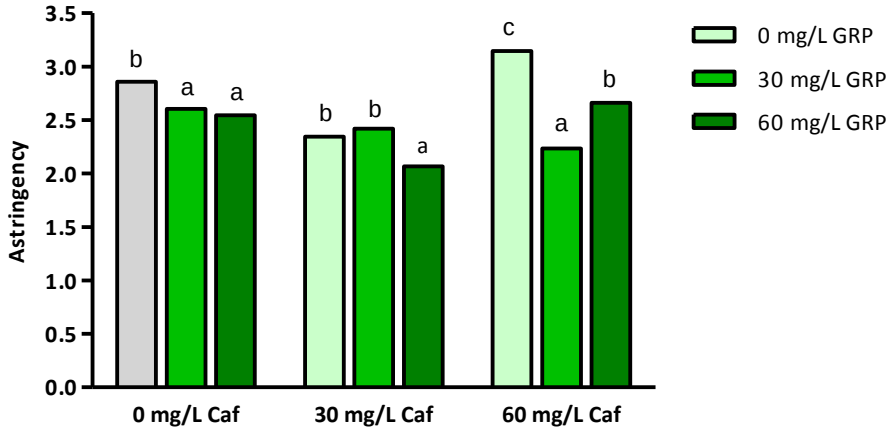



**Figure 7-4: Mean 'astringency' intensity by caftaric acid and GRP concentration. Grey bar represents the model wine. Means with different subscripts are significantly different (p<0.1).**

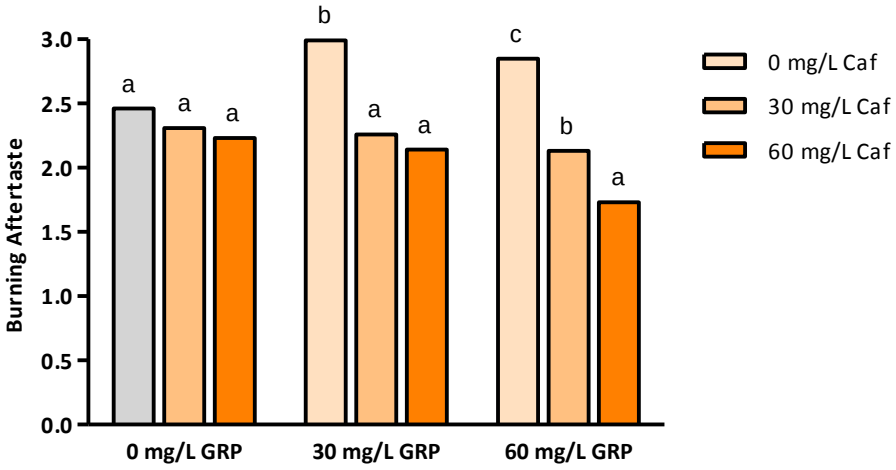



**Figure 7-5: Mean 'burning aftertaste' intensity by caftaric acid and GRP concentration. Grey bar represents model wine. Means with different subscripts are significantly different (p<0.1).**

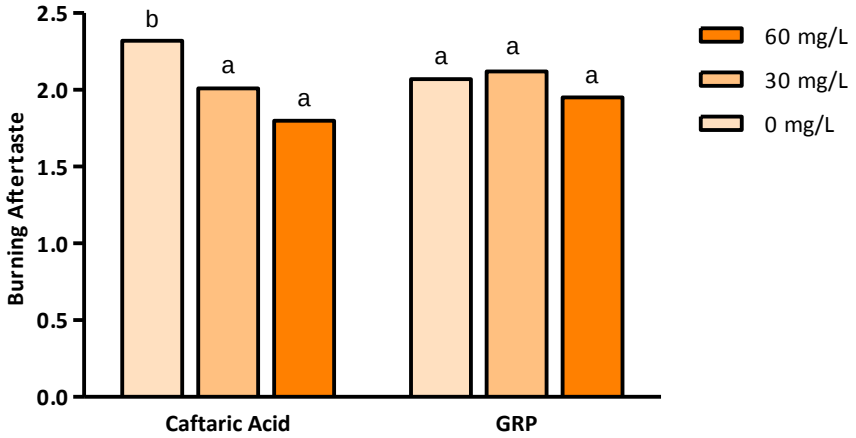



**Figure 7-6: Mean 'burning aftertaste' intensity. Means with different subscripts are significantly different (p<0.1).**

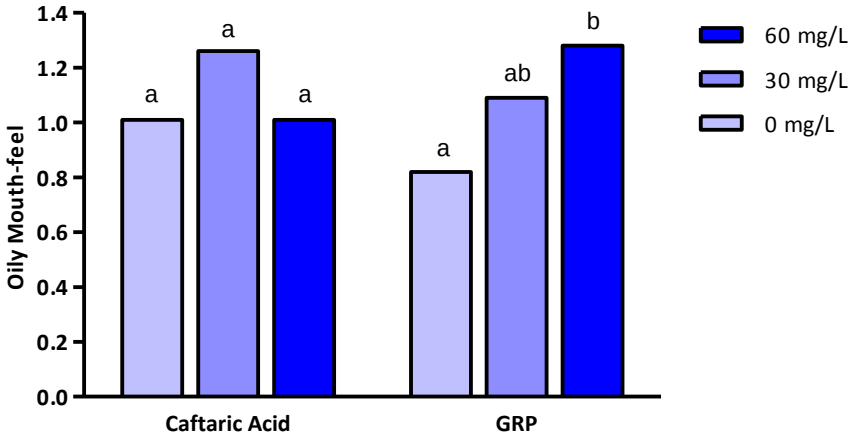



**Figure 7-7: Mean 'oily mouth-feel' intensity. Means with different subscripts are significantly different (p<0.1).**

# 8 Sensory Characteristics of Different Phenolic Composition Brought About by Variations in Winemaking

## 8.1 Introduction

We aimed to produce a set of white wines that varied enough in phenolic composition to elicit measurable differences in both taste and texture. A mix of both conventional and less practiced white wine making techniques were used over two vintages. Hyperoxidation and skin contact were two processes conducted pre-fermentation. Wines were also made from musts that were high in solids, consisted of light and heavy press fractions, or included a small proportion of whole berries.

Most winemaking treatments were repeated over two years (2010 and 2011) using the same varieties, and in the case of Riesling and Chardonnay the fruit was sourced from the same vineyards. Therefore, in addition to providing wines of varying phenolic profile that could be used to identify those responsible for 'phenolic taste', the work had the potential to provide preliminary data on the effect of maceration and other aspects of juice handling on the phenolic profile and tastes/textures in white wine. However, it should be noted that while fermentations were conducted in duplicate to ensure an adequate set of sound wines with similar basic analysis, the winemaking treatments were not replicated within each vintage.

## 8.2 Methods

### 8.2.1 Winemaking

#### Treatments

Musts were vinified over two vintages (2010 and 2011) from both low and high phenolic varieties to create a set of wines with divergent phenolic composition. The treatments applied are given in Table 8-1.

#### Grape Sources

The Riesling and Chardonnay grapes were sourced from the Orlando St Helga vineyard (Eden Valley, South Australia) and the Jacob's Creek Lyndoch vineyard (Barossa Valley, South Australia) respectively. Viognier grapes destined for commercial production were sourced from the Adelaide Hills in 2010 and the Barossa Valley in 2011. All fruit was handpicked in order to avoid uncontrolled maceration prior to winemaking, and chilled overnight.

**Table 8-1: Summary of vinification treatments over two vintages**

| Treatment | Code | 2010 | 2011 |
|---|---|---|---|
| Whole bunch pressed | WBP | ✓ | ✓ |
| Free run | FR | ✓ | ✓ |
| Hyperoxidised free run | HOX-FR | ✓ | ✓ |
| Free run with high solids | SOL | | ✓ |
| Free run with 10% skin ferment | SKI | | ✓ |
| Light pressings | LP | ✓ | ✓ |
| Hyperoxidised light+heavy pressings | HOX-LHP | ✓ | |
| Heavy pressings | HP | ✓ | ✓ |
| Hyperoxidised heavy pressings | HOX-HP | | ✓ |
| Macerated and pressed | MAC | ✓ | ✓ |

## Juice Preparation

Grape processing was carried out in the HRWSL using a destemmer-crusher (Diemme 4 tonne/ hr), crusher-destemmer (Demoisy 7EP) and membrane press (Willmes "Merlin", 1 t whole bunch and 2.5 t crushed capacity). Figure 8-1 and Figure 8-2 provide a schematic of the treatments.

Specifically, the winemaking treatments were (in italics):

*Whole-bunch pressing (WBP):* the press was loaded with 500 kg of whole bunches and pressed from 1.0 to 2 bar. Juice yield was typically 450 L/ tonne.

The press was loaded with 1750 kg of destemmed and crushed fruit and dry ice was added to the press and the receival tray. The must was pressed using a manual press cycle to yield three fractions:

- *Free run (FR),* press pressure < 0.5 bar pressure
- *Light pressing (LP),* 0.5 – 1.0 bar pressure
- *Heavy pressing (HP*), 1.0–2.0 bar pressure

*Hyperoxidation (HOX-LHP):* 100 L of the FR, HP or 50:50 mix of FR and HP juices were immediately sparged with 100% oxygen at 8-10 L/min until a dissolved oxygen value of approximately 40 mg/L was reached, measured using a Nomasense PSt3 optrode.

*Cold soak/maceration (MAC*): destemmed and crushed must from 750 kg was held in either the Willmes press (2010), or a 1,000L (2011) open fermenter sealed with shrink wrap film, with 100 mg/L $SO_2$ as PMS solution added for 60 hours at around 5°C. It then followed a standard press cycle with combination of pressings and free run.

*Fermentation on skins (SKI):* 2.5 kg of crushed grapes were added to 25 kg of FR juice.

*Solids fermentation (SOL):* FR juice was not cold settled with pectolytic enzyme prior to fermentation.

## Fermentation, Fining and Bottling

The juices were pH adjusted using tartaric acid or potassium carbonate, and (excepting the solids ferment treatment) were cold settled with the aid of pectolytic enzymes for 24-48 hours. They were then divided into 2 x 30 L fermentation vessels and inoculated with *S. cereviseae* strain EC1118. Bentonite (Sodium) was added (1 g/l) to every vessel in order to achieve protein stability. The ferment proceeded at 0.5 to 1.0 °Bé per day until dryness. Thereafter, 60 mg/L $SO_2$ (as PMS) was added before storing the wine at 0°C.

20 L vessels were completely filled with the finished wines under dry ice protection. Tartrate stabilisation was carried out by seeding with 4 g/L KHT at 4°C once any necessary final pH/TA adjustments were made. If required, further bentonite was added for protein stabilisation at the level determined by the 80°C/2 hour test with the sample passing if <2 NTU. The wines were sparged with nitrogen, argon or both in order to attain a dissolved oxygen level of less than 0.6 ppm. The finished wine was bottled in 375 ml antique green bottles under Sarin-tin ROTE screw caps.

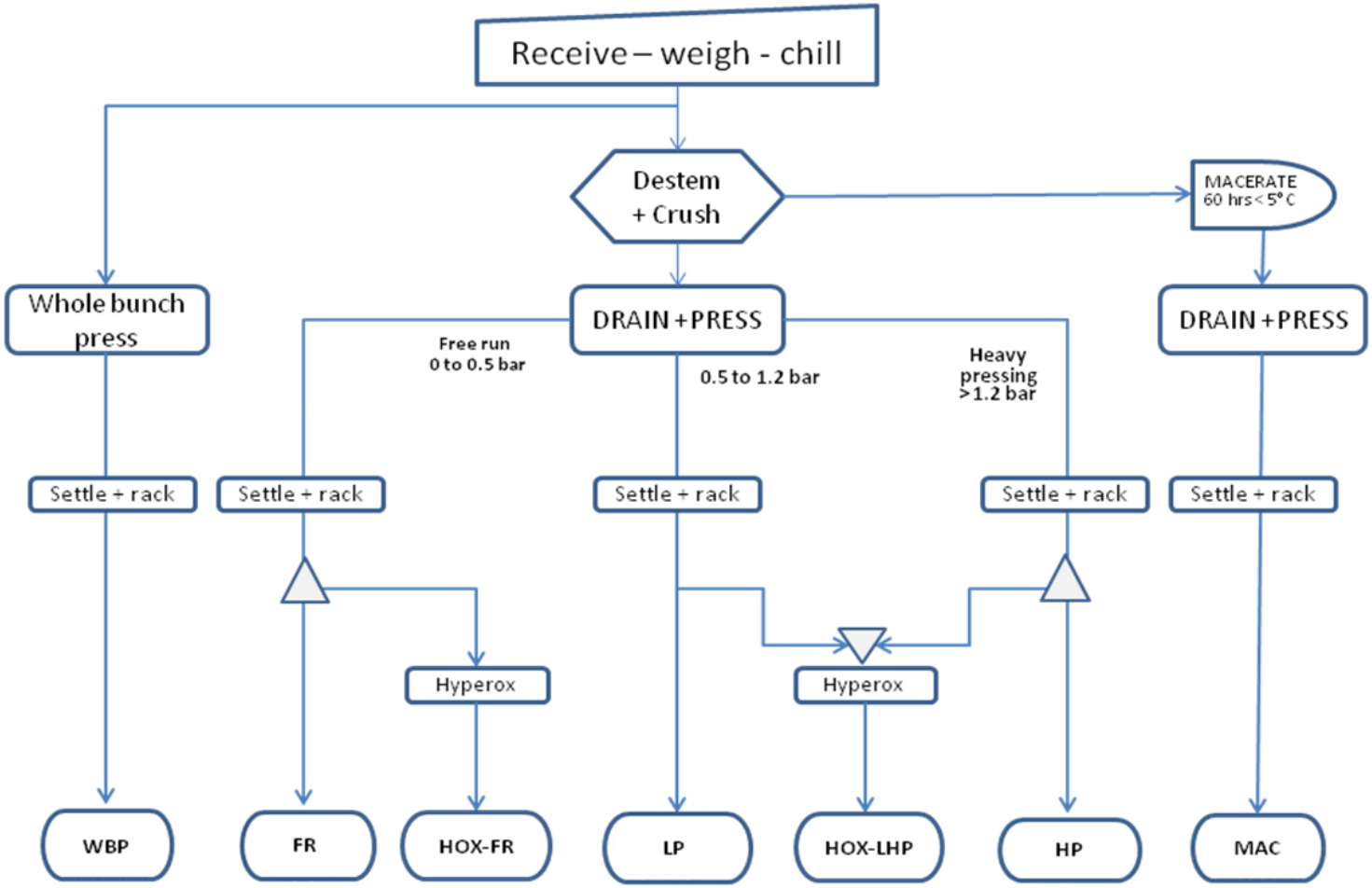



**Figure 8-1: Flow chart of different pressing treatments to obtain white wine of different phenolic levels (2010 Vintage)**

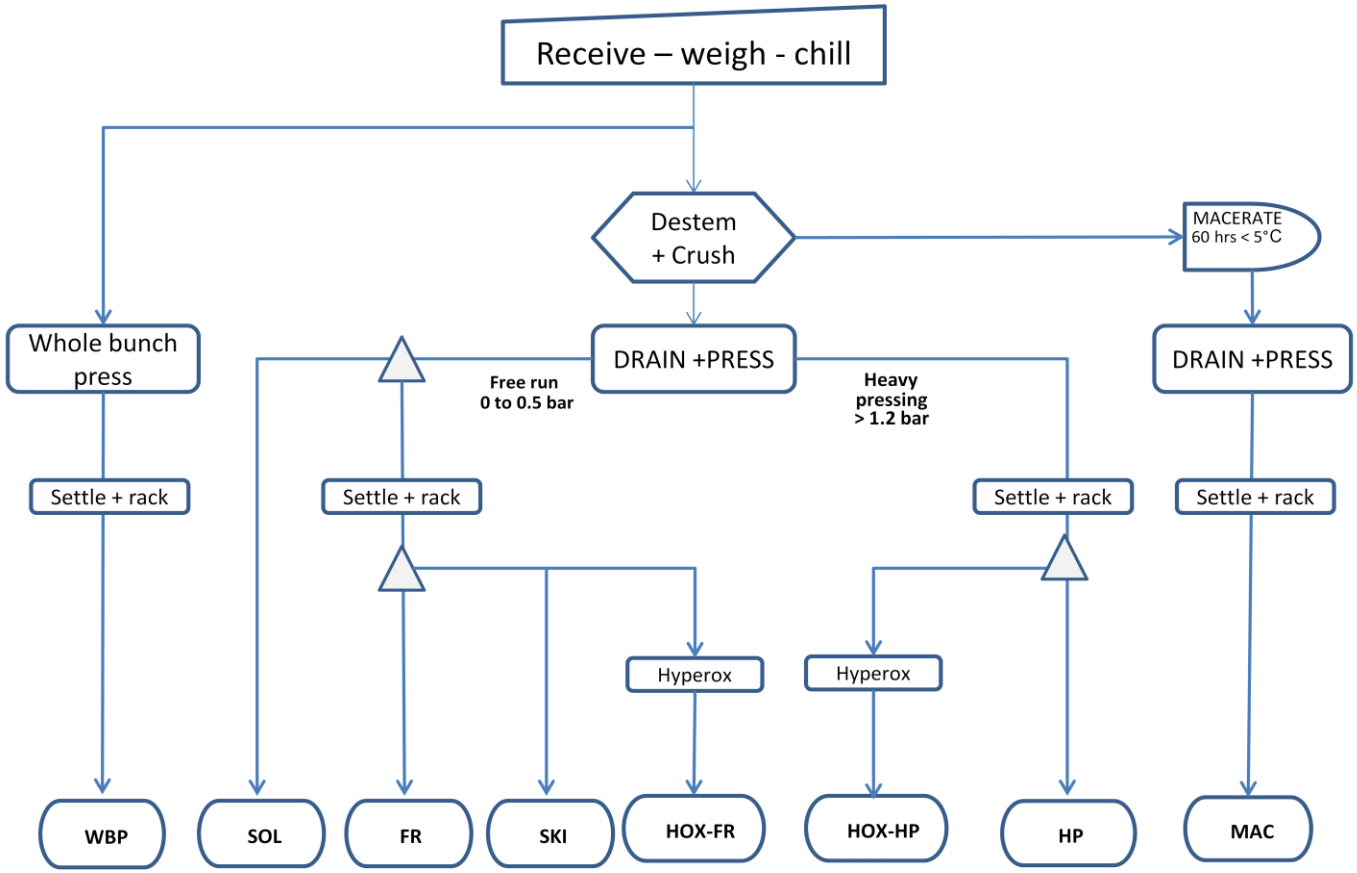



**Figure 8-2. Flow chart of different pressing treatments to obtain white wine of different phenolic levels (2011 Vintage)**

# 8.3 Analytical Methods

## 8.3.1 UV Spectra and Somers Measures (2010 and 2011 wines)

Analyses of the finished wines were carried out following bottling. The UV spectra were recorded in 2 mm matched quartz cuvettes using a Varian Cary 300 UV-Vis spectrometer. Total phenolics, total hydroxycinnamates and flavonoid extractibles were calculated as per Somers and Ziemelis (1985) (Appendix A.1).

## 8.3.2 Phenolic Composition by HPLC (2010 wines only)

A detailed analysis of the phenolic composition of the 2010 wines was determined using HPLC-DAD analysis following evaporation of the alcohol and a four-fold concentration under reduced vacuum (Section A.3). Peak identification was carried out by comparison of UV spectra derived from a sample that had been analysed by HPLC-MS/MS. Peak area was extracted from the chromatograms and expressed in equivalent mg/L of a related parent phenolic compound. All hydroxyphenolics/benzoic acids/flavanol were expressed in gallic acid equivalents (GAE), all hydroxycinnamates and grape reaction products (GRP) derivates as ferulic acid equivalents (FAE) and flavonols as quercetin-3-glucoside equivalents (Q3GE).

# 8.4 Sensory Methods

## 8.4.1 Wines from 2010 vintage

### Tasters

Thirteen tasters (five males and eight females) were trained for this study. All were experienced in the descriptive analysis of aroma and flavour, and the palate sensations of bitterness, astringency and acidity. Ten of the tasters had previously been involved in taste and texture profiling of phenolic fractions taken from commercial white wines.

### Taster Training

Training was conducted over six sessions. The first three sessions involved attribute generation, attribute definition and refinement, with the latter also involving identifying attribute redundancies. During these sessions, two wines made from whole bunch pressed and free run juices (low phenolics) were tasted and compared with two wines made from either hard pressing juices or those that had undergone extensive skin contact before fermentation (high phenolics). Standards for astringency, viscosity and bitterness (Appendix B, Table B-4) were also presented. A commercial white wine made with extensive pre- and

post fermentation skin contact and fermented on skins was also presented as an example of a wine that most likely displayed many of the characters associated with phenolic taste.

Tasters were then familiarised with the use of the partially structured 15 cm line rating scale (described previously) that was to be used. This was achieved by them rating 15 of the test wines on the previously accepted attributes (Appendix B, Table B-3) in duplicate. Significant discrimination between the test samples was achieved for viscosity, astringency and acidity ($p<0.001$). Hotness ($p=0.964$) and bitterness ($p=0.175$) discrimination were not significant.

### Formal Assessment

The test wines were assessed in triplicate, twenty wines were tasted per day over six sessions that extended over a two week period. Tasters assessed the attributes (astringency, bitterness, acidity, hotness and viscosity) using the 10cm partially structured line scale described previously. 30mls of wine were tasted at room temperature (22°C ± 1°C) in clear ISO tasting glasses under natural lighting. One minute of rest was forced upon tasters between samples, and tasters were encouraged to rest between every fourth wine.

## 8.4.2 Wines from 2011 vintage

### Tasters

A panel of fourteen assessors (five male, nine female) was convened for this study, all of whom are part of the AWRI trained descriptive analysis panel. Some of the tasters had previously been involved in taste and texture profiling of experimental wines made in a similar way to those of this study, and of phenolic fractions taken from other white wines.

### Taster Training

Panellists attended three initial training sessions to determine and refine appropriate descriptors for rating in the formal sessions. Wines were assessed by palate only. During the practice sessions the panellists assessed wines from the study which represented the full range of sensory properties, as well as three young Italian commercial white wines that were identified by four experienced wine-tasters to be examples that could well demonstrate phenolic tastes in wines (Tuscan Vermentino, Marche Verdicchio and Alto Adige Pinot Blanco).

Standards for a number of attributes were presented during the training period (Appendix B, Table B-3).

Assessors then attended two practice sessions in booths to rate the wines for twelve palate attributes determined during training. Following examination of the data from this session, a further tasting and discussion session was completed to further refine the terms. A final list of twelve palate terms was assessed in the formal sessions. Details of the attributes used and their definitions are shown in Table B-3. This information was available to panellists during the formal sessions.

### Formal Assessment

Samples were presented to tasters in 30 mL aliquots in 3-digit-coded, covered, ISO standard wine glasses at 22–24°C, in isolated booths under amber lighting. The entire set of wines was presented in a randomised order. The assessors were presented with four trays of four wines per tray. They were forced to rest for 90 second between samples, and ten minutes between trays. During the ten minute break assessors were requested to leave the booths. Samples were assessed over four days of formal sessions. Forty-eight wines were assessed in triplicate, therefore 144 samples were presented in total.

The intensity of each attribute was rated using an unstructured 15 cm line scale from 0 to 10, with indented anchor points of "low" and "high" placed at 10% and 90% respectively. Data was acquired using FIZZ v 2.46 sensory data acquisition software (Biosystèmes, Couternon).

Panel performance was assessed using Fizz, Senstools (OP&P, The Netherlands) and PanelCheck (Matforsk) software, and included analysis of variance for the effect of sample, judge and presentation replicate and their interactions, degree of agreement with the panel mean and degree of discrimination across samples (data not shown). All judges were found to be performing to an acceptable standard.

# 8.5 Results and Discussion

## 8.5.1 Standard Chemical Analyses

The experimental winemaking protocol used in both years produced uniform wines within fermentation replicates and with analytical parameters that were within the ranges generally expected of commercial white wines (Table 8-2). The wines were also technically sound, with less than 1.7 g/L residual sugar, medium alcohol levels and low volatile acidity. In 2010 the wines made from different varieties were also uniform with the exception of pH. Consequently, in 2011 the wines were carefully managed during vinification and adjusted prior to bottling to achieve a pH value of 3.2 for all wines. Data for individual wines are in Appendix B, Table B-5 and Table B-9.

**Table 8-2: Summary of pre-bottling standard oenological data for 2010 and 2011 wines**

| Variety | | Glu+Fru (g/L) | pH | TA (g/L) | Free $SO_2$ (ppm) | Total $SO_2$ (ppm) | Alcohol (% v/v) | Acetic acid (g/L) |
|---|---|---|---|---|---|---|---|---|
| 2010 Chardonnay | Min. | 0.42 | 3.10 | 6.5 | 30 | 106 | 13.1 | 0.32 |
| | Max. | 0.96 | 3.22 | 8.3 | 37 | 163 | 13.7 | 0.36 |
| | **Average** | **0.60** | **3.18** | **7.1** | **33** | **130** | **13.4** | **0.34** |
| | SD | 0.14 | 0.04 | 0.6 | 2 | 17 | 0.19 | 0.01 |
| 2010 Riesling | Min. | 0.19 | 2.94 | 6.5 | 30 | 81 | 12.4 | 0.29 |
| | Max. | 0.88 | 3.17 | 8.0 | 37 | 118 | 13.1 | 0.37 |
| | **Average** | **0.46** | **3.07** | **7.1** | **32** | **94** | **12.8** | **0.34** |
| | SD | 0.21 | 0.08 | 0.5 | 3 | 12 | 0.20 | 0.02 |
| 2010 Viognier | Min. | 0.43 | 2.89 | 7.1 | 30 | 114 | 13.2 | 0.35 |
| | Max. | 1.65 | 3.34 | 8.4 | 43 | 155 | 13.6 | 0.39 |
| | **Average** | **0.84** | **3.10** | **7.9** | **33** | **130** | **13.3** | **0.37** |
| | SD | 0.33 | 0.13 | 0.4 | 3 | 13 | 0.12 | 0.01 |
| 2010 All varieties | Min. | 0.19 | 2.89 | 6.5 | 30 | 81 | 12.4 | 0.29 |
| | Max. | 1.65 | 3.34 | 8.4 | 43 | 163 | 13.7 | 0.39 |
| | **Average** | **0.64** | **3.12** | **7.4** | **33** | **119** | **13.2** | **0.35** |
| | SD | 0.28 | 0.10 | 0.6 | 3 | 22 | 0.3 | 0.02 |
| 2011 Chardonnay | Min. | 0.2 | 3.13 | 6.2 | 32 | 123 | 13.3 | 0.23 |
| | Max. | 1.20 | 3.28 | 7.3 | 40 | 176 | 13.7 | 0.38 |
| | **Average** | **0.79** | **3.20** | **6.8** | **36** | **142** | **13.5** | **0.31** |
| | SD | 0.33 | 0.05 | 0.3 | 2 | 14 | 0.12 | 0.04 |
| 2011 Riesling | Min. | 0.50 | 3.07 | 7.4 | 34 | 88 | 12.4 | 0.31 |
| | Max. | 1.50 | 3.28 | 8.0 | 37 | 130 | 13 | 0.42 |
| | **Average** | **1.00** | **3.15** | **7.7** | **36** | **109** | **12.8** | **0.38** |
| | SD | 0.34 | 0.07 | 0.2 | 1 | 12 | 0.2 | 0.03 |
| 2011 Viognier | Min. | 0.40 | 3.10 | 5.8 | 34 | 90 | 12.7 | 0.18 |
| | Max. | 1.10 | 3.25 | 6.9 | 40 | 137 | 13.0 | 0.29 |
| | **Average** | **0.78** | **3.17** | **6.4** | **36** | **114** | **12.8** | **0.24** |
| | SD | 0.22 | 0.05 | 0.4 | 2 | 13 | 0.13 | 0.03 |
| 2011 All varieties | Min. | 0.20 | 3.07 | 5.8 | 32 | 88 | 12.4 | 0.18 |
| | Max. | 1.50 | 3.28 | 8.0 | 40 | 176 | 13.7 | 0.42 |
| | **Average** | **0.86** | **3.17** | **6.9** | **36** | **122** | **13.0** | **0.31** |
| | SD | 0.31 | 0.06 | 0.6 | 2 | 20 | 0.4 | 0.07 |

## 8.5.2 Phenolic Measures

Absorbances in the 230 to 430 nm range spectra of the 2010 wines are shown in Figure 8-3. To simplify the figures, only duplicate wines with the lowest and highest phenolic composition are shown. They are typical of white wines in having local maxima at 265–280 nm, representing phenolic acids as well as flavanols, and at around 325 nm representing hydroxycinnamic acids (HCA's) and grape reaction products (2-S-glutathionyl caftaric acids and some other analogues). The absorbance patterns of wines produced in the subsequent vintage were similar (Figure 8-4). Individual values of Somers' measurements are listed in Appendix B, Table B-6 and Table B-10 and are shown in Figure 8-5 (2010 wines) and Figure 8-6 (2011 wines).

The free run treatment represents a logical standard to which to compare others as it is the most widely used method of white wine production.. A percentage deviation from the free run wines for total Phenolics and total HCA are given in Figure 8-7. Whole bunch pressed wines from all three varieties had much less phenolic material compared to free run, and maceration resulted in much higher values. The HCA's of the light and heavy pressings are only slightly higher in Chardonnay and Riesling but the HCA of Viognier was 20% lower.

The hyperoxidised wines represented a probable extreme. Here, the musts were subjected to an excess of oxygen resulting in the consumption of most of the phenolic substrates. In typical commercial practice of hyperoxidation, not as much oxygen is used as in this experimental trial and this may result in residual phenolics.

Naturally occurring glutathione in grapes acts as an antioxidant by reacting with *o*-quinones to form GRP, thus preventing further oxidation. Hyperoxidation of both free run and pressings juices reduced total wine phenolics and total wine HCA's of all three varieties. The relative decreases in total phenolics and total HCA of wine following juice hyperoxidation compared with before treatment are given in Figure 8-8.

Hyperoxidation of free run juice reduced the total phenolics of Viognier wines the most and Chardonnay wines the least. On the other hand, the decrease in total phenolics after hyperoxidation of pressings was less pronounced for Viognier than the other two varieties.

The decrease in HCA of hard pressings is very similar for all varieties suggesting that natural preservatives such as glutathione have already been pressed from the grape flesh and do not provide any protection to press juice.

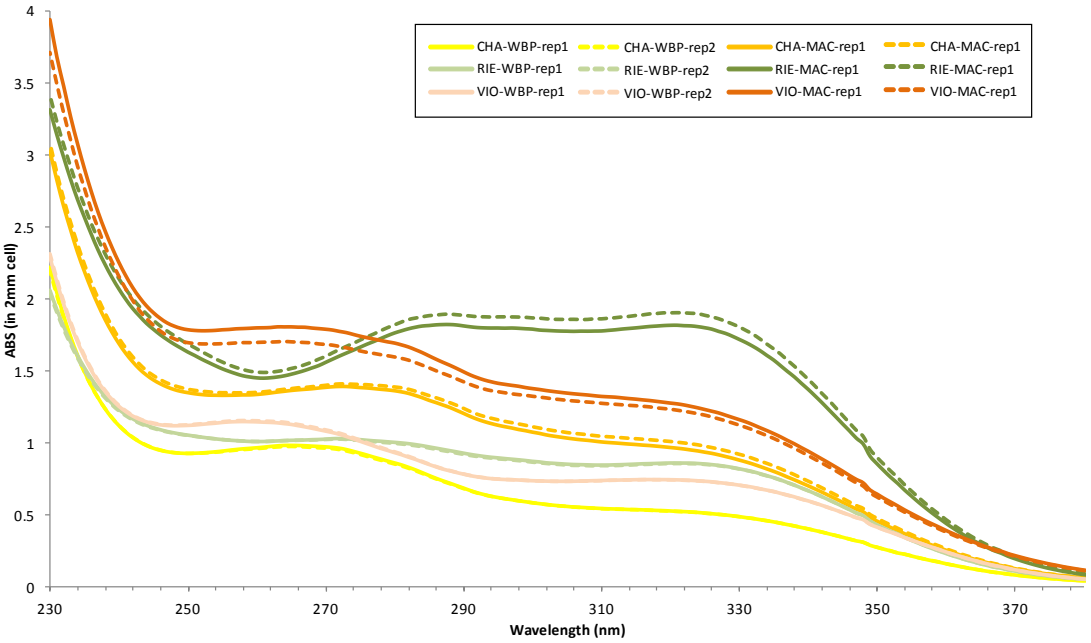



**Figure 8-3: UV spectra of the highest and lowest phenolic 2010 wines**

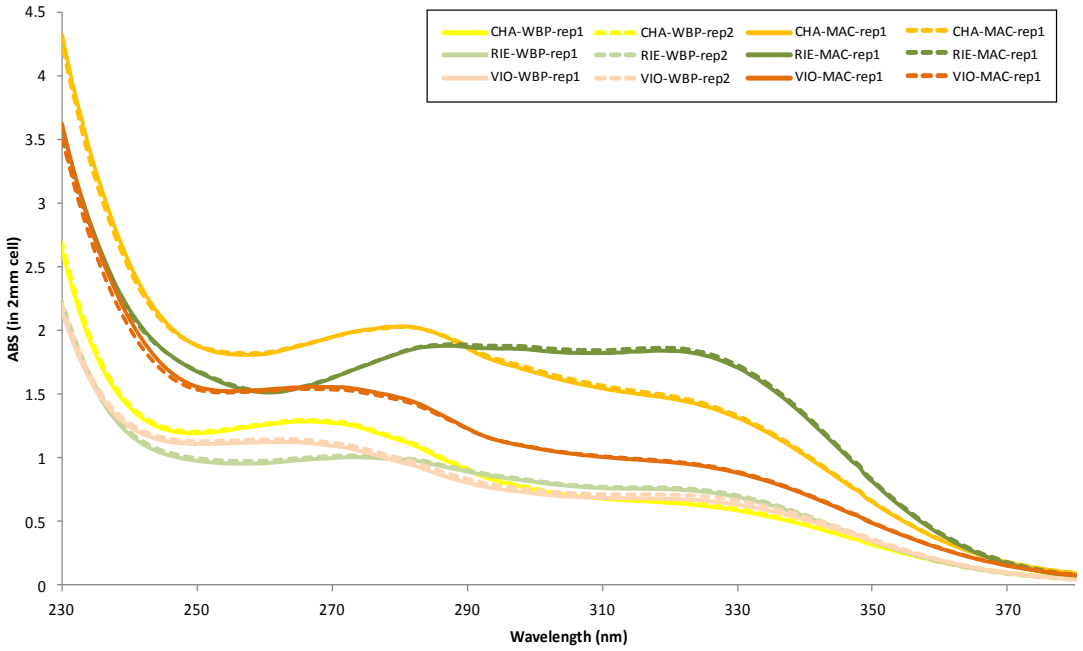



**Figure 8-4: UV spectra of the highest and lowest phenolic 2011 wines**

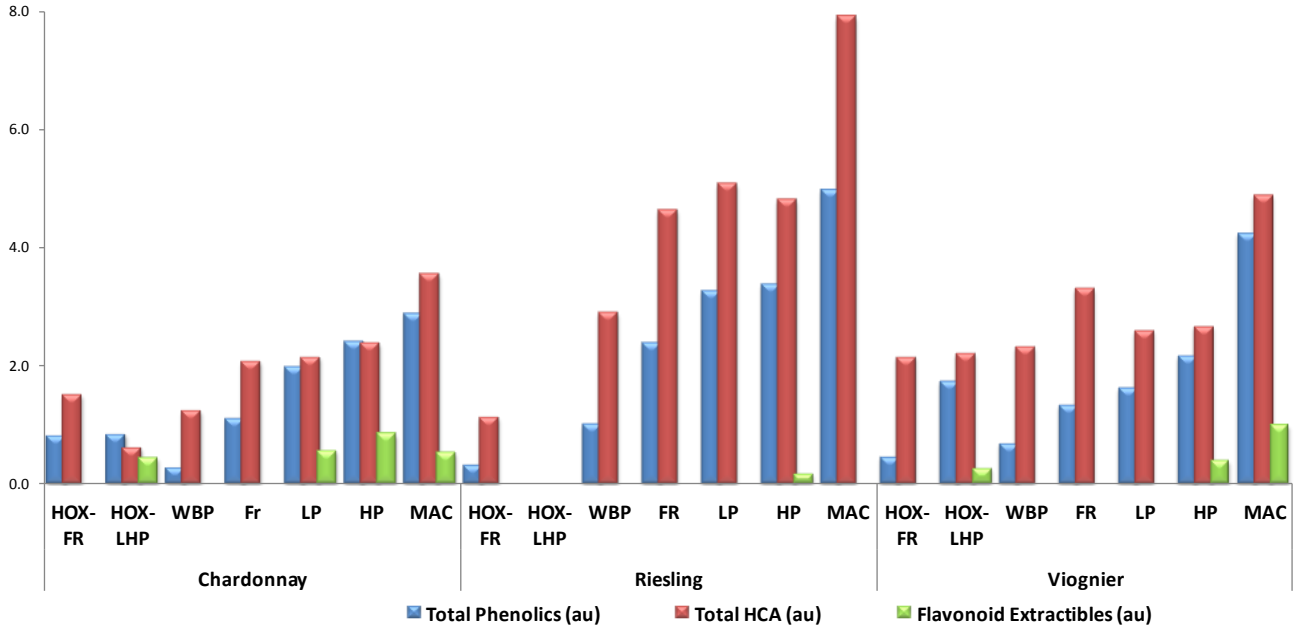



Figure 8-5: Somers' measurements for 2010 wines

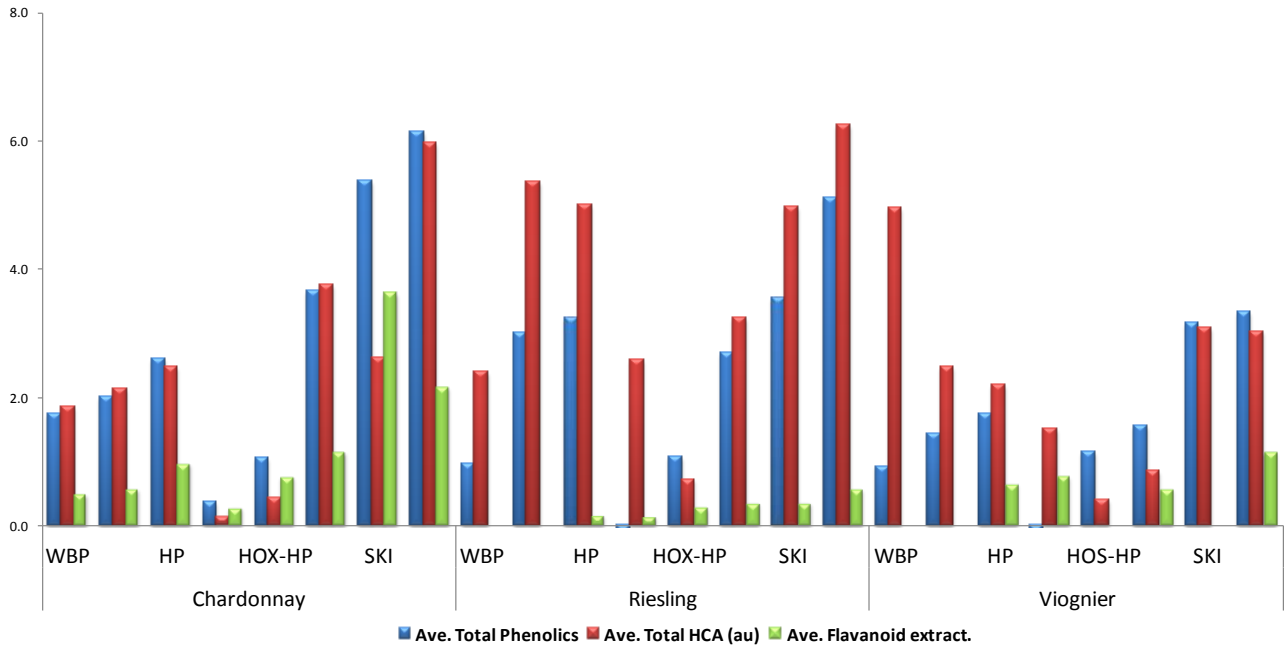



Figure 8-6: Somers' measurements for 2011 wines

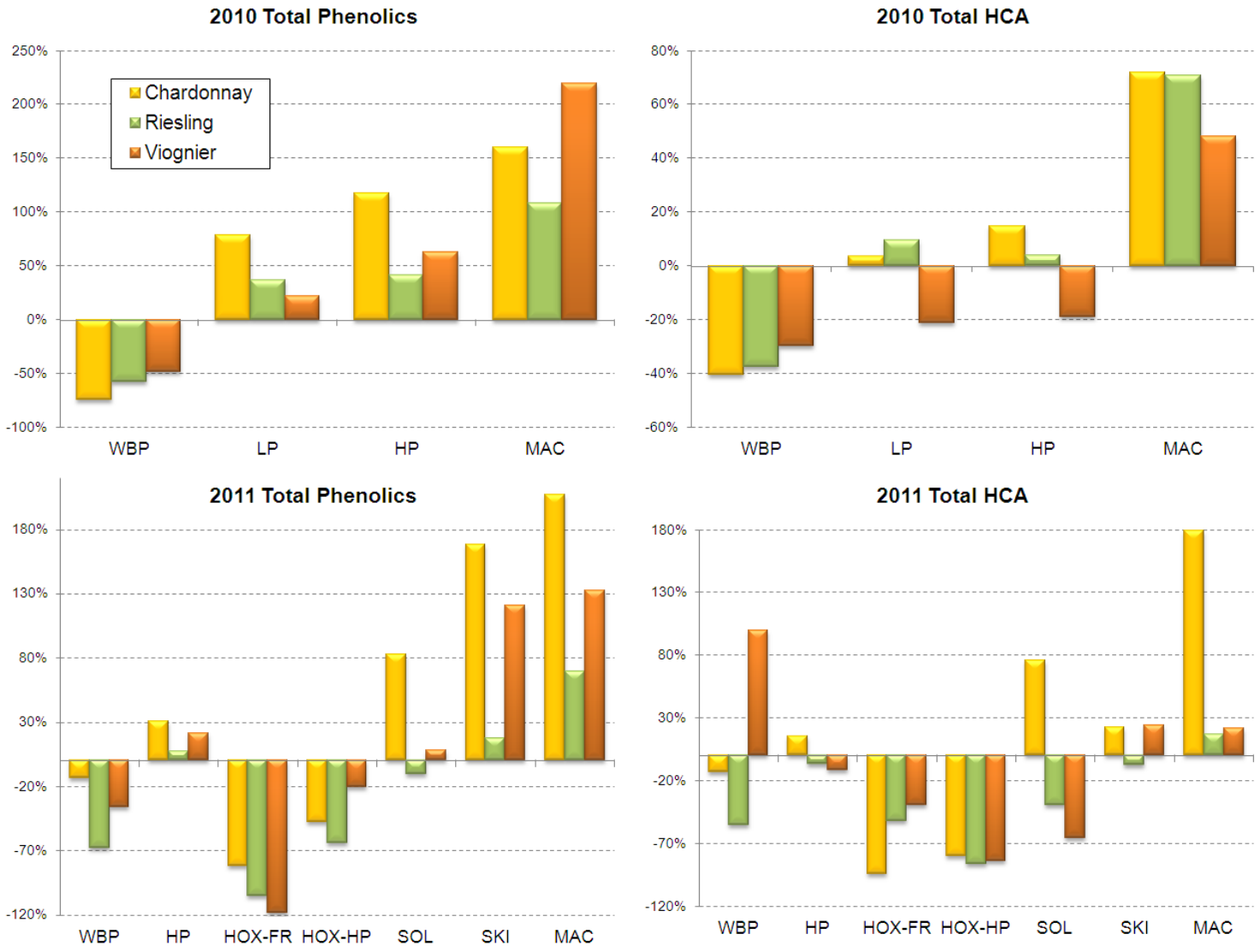



**Figure 8-7: Variation of total phenolics and total hydroxycinnamic acids in difference winemaking techniques expressed as a % change compared to free run**

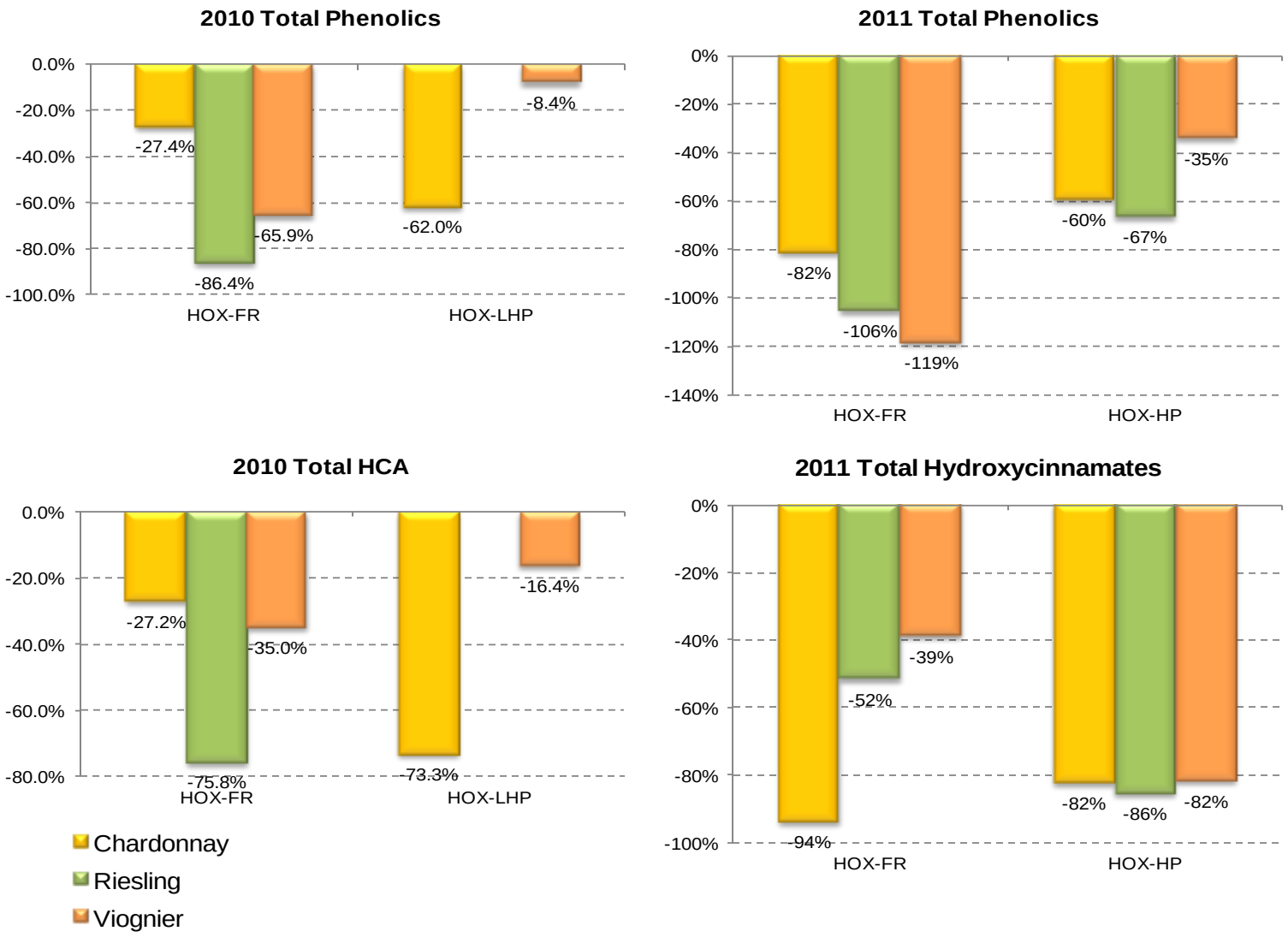



**Figure 8-8; % change in total phenolics and total hydroxycinnamates of wines made from hyperoxidised free run (FR) and pressing juices (LHP and HP) compared with wines made with non-hyperoxidised juices**

### 8.5.3 Phenolic Measures (HPLC) – 2010 wines

Over 50 phenolic compounds were detected, putatively identified and quantified in this study and they can be classified in several ways: in a broad, polyphenolic class (hydroxybenzoic acids, hydroxycinnamic acids, flavan-3-ols, flavonols, GRP-conjugates etc.), or with these subdivided into ethyl esters (EE) or glycoconjugates. A list of the identified compounds, their classifications groups and the equivalent units used for quantification is given in Appendix B, Table B-7. These data are presented alongside average literature values collected by the INRA Montpellier as part of the Phenols Explorer project (Neveu et al. 2010). The total phenolics were determined by summing unidentified early eluting compounds (expressed as mg/L GAE) and all the named compounds. Mean values are shown for each variety in Figure 8-9 and sorted by treatment of all three varieties in Figure 8-10.

The concentrations of the broad polyphenolic classes averaged across all varieties and treatments are given in Figure 8-11, by variety (Figure 8-12), and by winemaking treatment (Figure 8-13). Some classes such as the flavonols, flavanones, phenols, and cinnamic acids and GRP conjugates vary widely between varieties. Riesling tends to show higher cinnamic acids, flavanones and flavanol-type compounds, while Viognier has higher GRP analogues and flavonols (Figure 8-12). The wines from macerated juices had the highest concentrations in flavonols, flavanonols and cinnamic acids (Figure 8-13) and would suggest that these compounds are located in skins and increase in concentrations during cold soaking. However, benzoic acids, other phenols and flavan-3-ols show fairly constant concentrations across the different treatments and potentially coming only from grape flesh are not influenced by maceration.Figure 8-14 shows a finer sub-division of some of the broad classes into ethyl esters (EE) and glycoconjugates averaged across all varieties and treatments. The variations described earlier can be more precisely ascribed to free hydroxycinnamoyl tartaric acids (caftaric acid, coutaric acid and fertaric acid) and flavonol glycoconjugates (mainly quercetin-3-O-glucuronide).

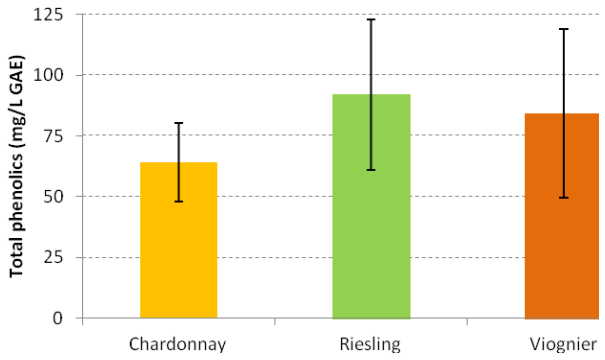



**Figure 8-9: Total phenolics (mg/L GAE) by HPLC per variety. Error bars represent ± 1 SD**

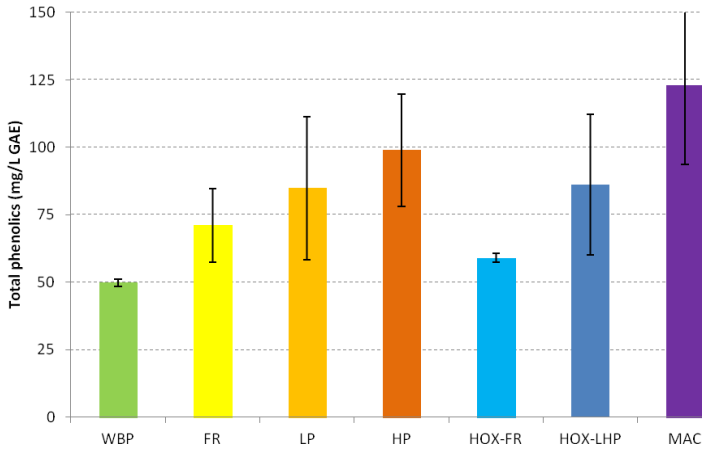



**Figure 8-10: Total phenolics (mg/L GAE) by HPLC per treatment. Error bars represent ± 1 SD**

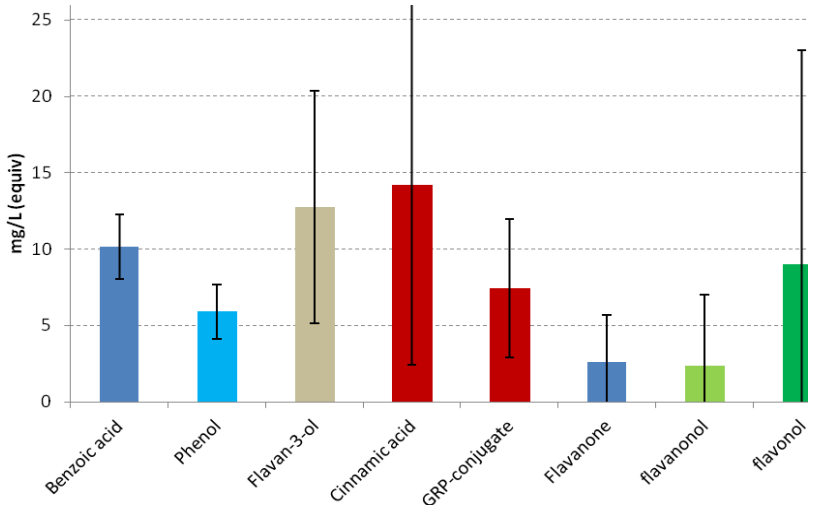



**Figure 8-11: Variation of broad phenolic classes. All 2010 treatments and varieties. Error bars represent ± 1 SD**

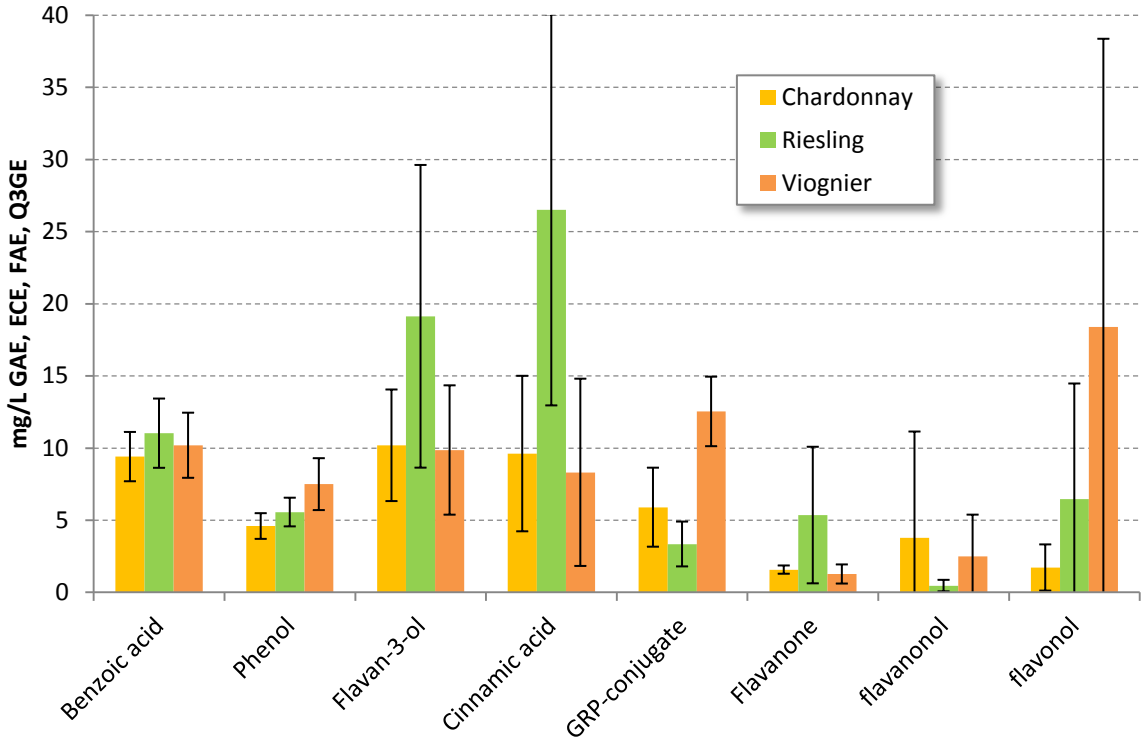



**Figure 8-12 : Concentrations of broad phenolic classes of all 2010 wines by variety. Error bars represent ± 1 standard deviation**

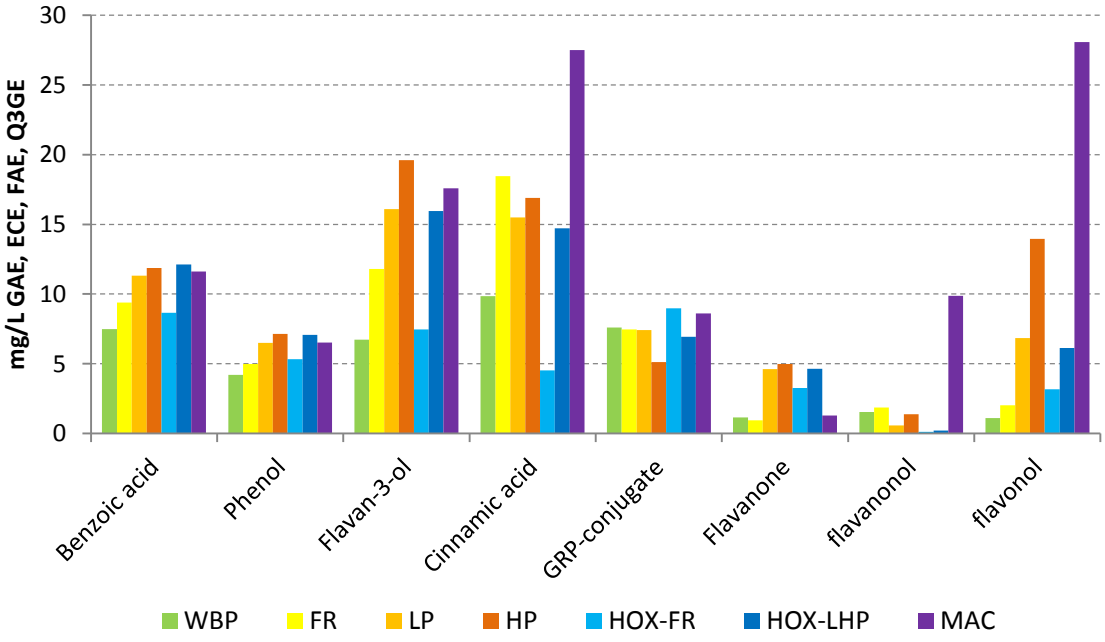



**Figure 8-13: Concentrations of broad phenolic classes of all 2010 wines by treatment**

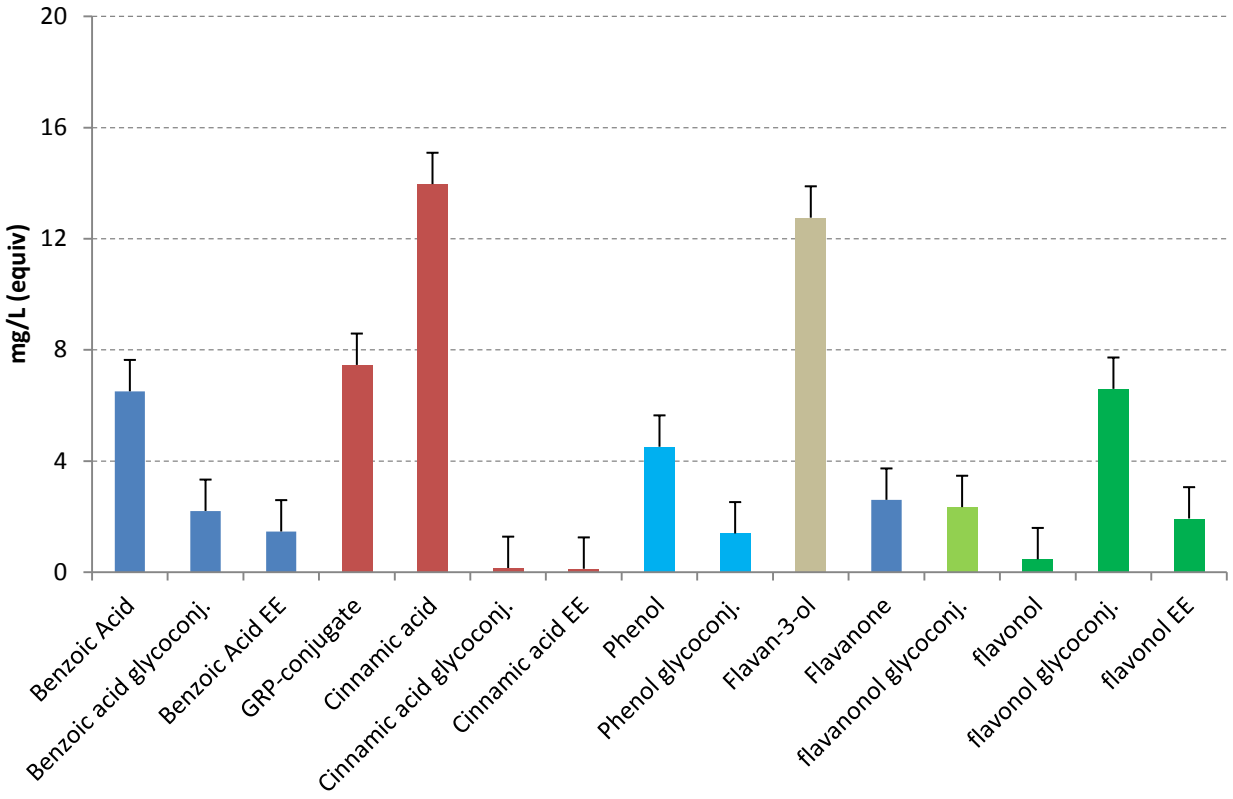



**Figure 8-14: Variation of detailed phenolic classes. All 2010 wines. Error bars represent ± 1 SD. EE = ethyl ester.**

## 8.5.4 Effect of Winemaking on 'Phenolic Tastes'

The mean attribute intensity ratings given to the 2010 wines are given in Figure 8-15. The order that the treatments are presented is based on the total phenolic content across the three varieties (Figure 8-10) which corresponds to the level of maceration and hyperoxidation. The astringency of the Riesling and Viognier wines generally decreased with the level of phenolics, while viscosity increased. There was no consistent change in the astringency or viscosity of the Chardonnay wines with phenolic level (Figure 8-15). Astringency of the Riesling and Viognier wines were very strongly negatively correlated with pH (-0.87 and -0.86 respectively) and the viscosity was strongly positively correlated with pH (0.6 and 0.89 respectively). The correlation between the astringency and viscosity of the Chardonnay wines with pH (-0.16 and 0.44) were not significant probably as a result of a narrow pH range in the Chardonnay wines. However, across the three varieties the correlation between astringency and pH was -0.87 ($p<0.001$, Figure 8-16), and the correlation between viscosity and pH was 0.70, ($p<0.001$) (Figure 8-17). The increase in pH with maceration level possibly occurred as a result of the diffusion of potassium ions from the skins into the must.

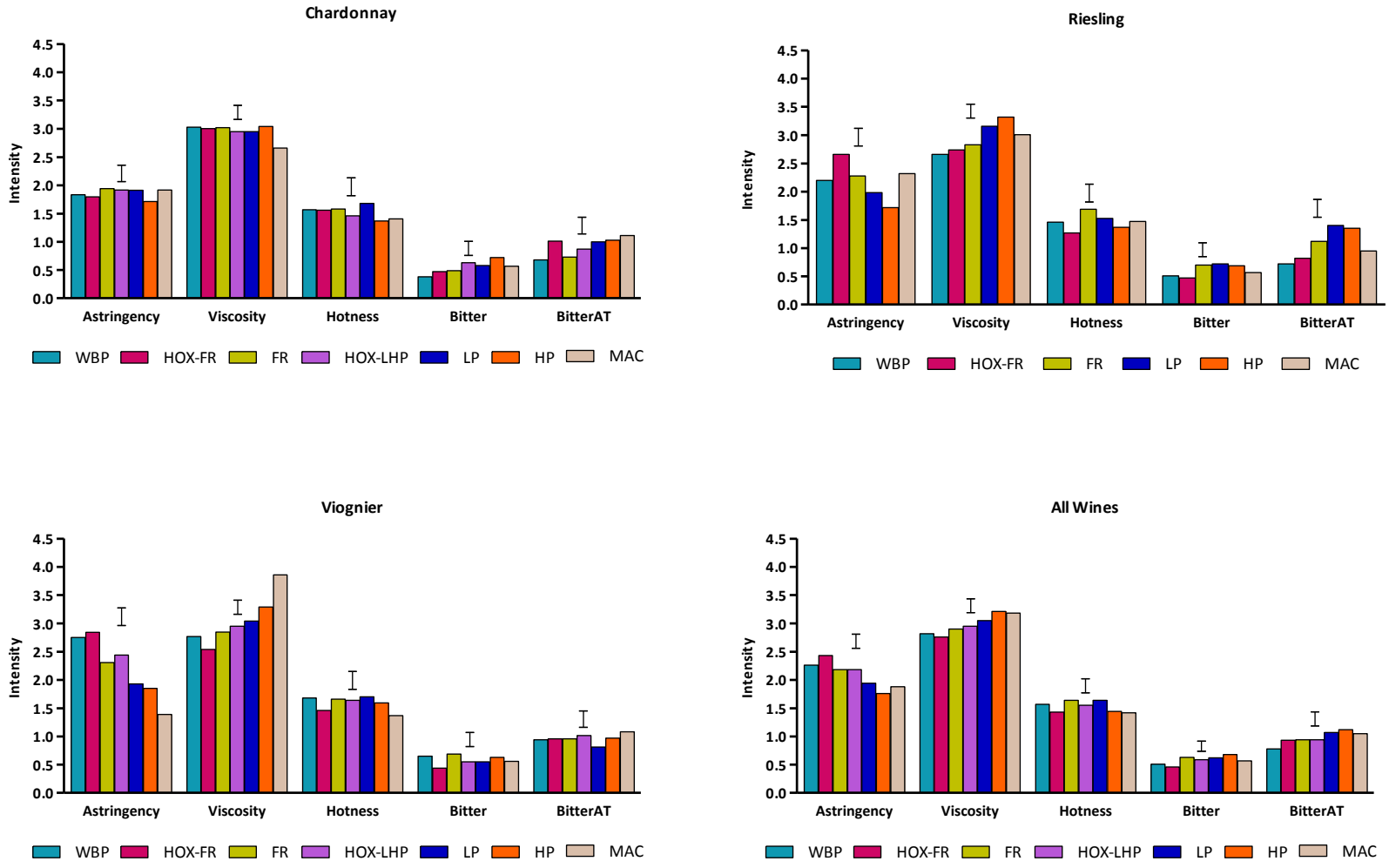



**Figure 8-15: Mean sensory attribute ratings by winemaking treatment: 2010 wines.**

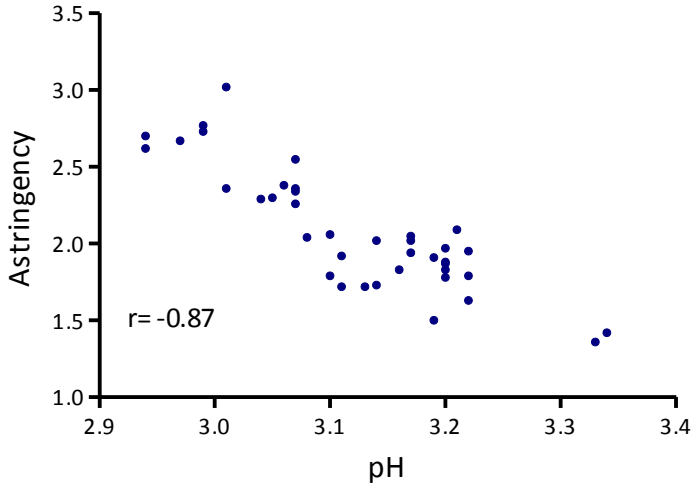



**Figure 8-16: Perceived astringency vs. pH – all 2010 wines**

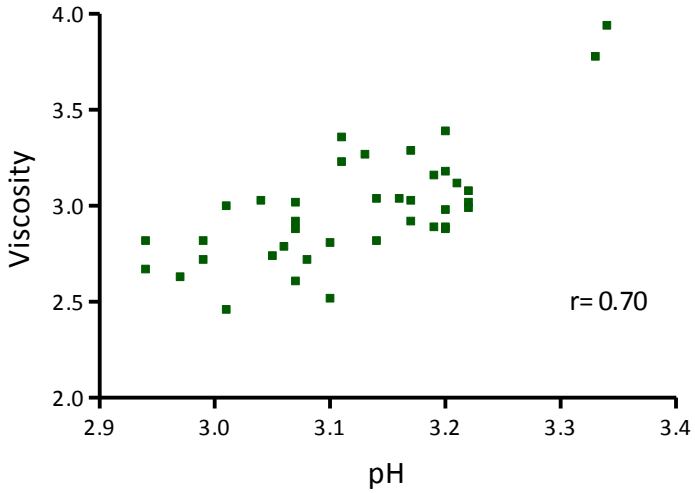



**Figure 8-17: Perceived viscosity vs. pH – all 2010 wines**

It would be expected that under the salivary protein-phenolic model of astringency, that the higher phenolic wines should be more astringent rather than less, which was observed here. Acids are known to elicit astringency in their own right, with astringency being most strongly correlated with pH (Lawless *et al.* 1996). Therefore, it would appear that the variations in astringency in the Riesling and Viognier wines

were the result of pH rather than phenolic level. The observed relationship between viscosity and pH has not been reported before. Anecdotally, the mouth-feel of high pH wines has sometimes been described as 'soapy', which may relate to viscosity. However, while this explanation is speculative, the very strong relationship between pH and viscosity suggests that the effect of pH on viscosity may be a direct one.

In order to assess the role of other factors other than pH on the astringency of these wines, the strong linear effect of pH was subtracted from the total observed effect to obtain a 'residual' effect attributable to other factors including phenolics. The same strategy was applied to the viscosity ratings. A significant difference in astringency following the removal of the pH effect was observed (p=0.058), suggesting that other factors including phenolic content contributed to the astringency of these wines. There was no overall effect of winemaking treatments on difference in pH adjusted viscosity as the observed effects were variety dependent.

There were no significant differences in hotness resulting from the winemaking treatments (p=0.349), (Table 8-3). There was a significant difference in bitterness between samples (p=0.13), with bitterness increasing in the order of increased maceration and total phenolics (Figure 8-15). While bitter aftertaste did not differ statistically (p=0.239) it increased in a parallel fashion to in-mouth bitterness.

**Table 8-3: Significance (p values) for experimental effects 2010 wines**

| | **Astringency adj for pH** | **Viscosity adj for pH** | **Bitter** | **Bitter AT** | **Hotness** |
|---|---|---|---|---|---|
| Variety | 0.787 | 0.589 | 0.497 | 0.492 | 0.542 |
| Treatment | **0.058** | 0.392 | **0.013** | 0.239 | 0.349 |
| Variety*Treatment | 0.550 | **0.055** | 0.833 | 0.455 | 0.841 |
| Ferment(Treatment) | 0.918 | 0.559 | 0.975 | 0.668 | 0.442 |

Bold indicates statistical difference (p<0.1)

In 2011, the wines were made with the aim of minimising differences in pH in order to reduce the direct effect of pH on taste and to better assess the impact of phenolics. This was partially achieved with the range in pH of the 2011 wines being less than half that of the 2010 wines, and the average pH being only 0.05 units higher in the 2011 wines.

In order to further differentiate the wines in phenolic content, two additional winemaking treatments were trialled in 2011 – fermenting in the presence of 10% skins and fermenting juices that were higher in solids. This was achieved, with skin fermentation resulting in the highest average total phenolics and solids fermentation in a low average total hydroxycinnamics for Viognier (Figure 8-6).

The sensory assessment in 2011 also quantified oily, metallic and burning aftertaste characters in addition to the characters assessed in 2010.

Figure 8-18 (individual varieties in Figure 8-19) gives the mean sensory attribute ratings of the 2011 wines by treatment and variety, and Table 8-4 the significance of treatment effects. Significant effects were seen for astringency, viscosity (including when adjusted for pH effects) and bitterness. There was also weak evidence for winemaking treatment on metallic and oiliness. However, the effect of winemaking on the wines taste properties was strongly dependent on variety. When averaged across varieties, skin maceration resulted in the highest astringency and bitterness, while maceration produced the lowest intensity of both these attributes. These results were despite these two treatments having the same high $A_{280,}$ suggesting that the wines had equivalent total phenolic content (Figure 8-6). Therefore either the differences in the respective wine matrices are affecting perception, or the differences are due to variations in phenolic profile. The high overall average bitterness of the low phenolic hyperoxidised free run wines is also noteworthy as higher bitterness is normally associated with higher phenolics.

The solids ferment wines consistently displayed higher level of metallic character across the three varieties, and were on average one of the most oily, hot and burning of the treatments. With the exception of an early study by Singleton *et al.* (1975), the textural properties of wine resulting from the relatively common practice of fermenting juice containing grape solids has been not been reported. The early work found that fermenting turbid juices did not cause an increase in total wine phenolics, but it did result in consistently more bitter and astringent wines. We found that solids fermentation impacted on total phenolics, total hydroxycinnamates and flavanoid extract, but the effect was variety dependent. Higher solids resulted in higher flavanoid extract in all three varieties compared with the free run wines. The total phenolics were the same for the Riesling and Viognier wines, but the Chardonnay wines fermented on solids had nearly twice the total phenolics as did the free run wines. The presence of grape particles in a must could provide a source of compartmentalised phenolics that are liberated during alcoholic fermentation. This may explain the unique textures displayed by the solids wines in this trial.

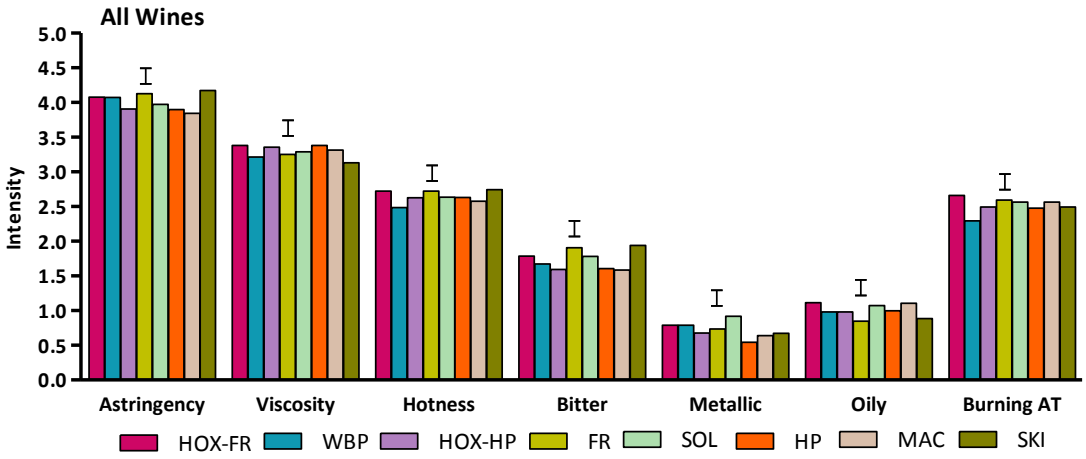



**Figure 8-18: Mean intensity ratings of attributes associated with 'phenolic taste'in 2011 wines. All wines. Treatments ordered from lowest to highest in average $A_{280,}$ Bars show 10% LSD.**

**Table 8-4: Significance (p values) for experimental effects 2011 wines**

| | Astringent | Viscosity | Astringency adj for pH | Viscosity adj for pH | Bitterness | Hotness | Metallic | Burning | Oily |
|---|---|---|---|---|---|---|---|---|---|
| Variety | 0.555 | **0.072** | **0.092** | **0.001** | **<0.001** | **<0.001** | 0.421 | **<0.001** | **<0.001** |
| Treat | **0.002** | **0.017** | **0.032** | **0.014** | **0.020** | 0.312 | 0.116 | 0.480 | 0.152 |
| Variety*Treat | 0.361 | 1.000 | **0.076** | 0.368 | 0.162 | 0.875 | 0.792 | 0.983 | 0.917 |
| Ferment(Treat) | 0.995 | 0.991 | 0.830 | 0.552 | 0.942 | 0.857 | 0.471 | 0.375 | 0.767 |

Bold indicates statistical difference ($p<0.1$)

The association between basic composition and absorbances with sensory ratings are given in Table 8-5 (2010 wines) and Table 8-6 (2011 wines). They show significant differences between vintages. Of note are:

1) the consistent negative associations between titratable acidity and all non-acid related attributes in 2011 but not in 2010,
2) the consistent positive associations with ethanol and all sensory attributes in 2011 while there were no strong associations with ethanol in 2010, and
3) for the 2010 wines, with the exception of astringency, most of the tastes and textures were positively associated with phenolic content ($A_{280}$, $A_{320}$ and $A_{370}$). In 2011 the relationships were generally weaker and were negatively associated with phenolics. The exception was bitterness which was positively associated with phenolics in both years.

The reason for these differences across vintages in unclear as the range and average value of alcohol content were almost identical in both years, and the TA's were slightly lower in 2011 where the negative effect of TA was more consistent. The reduced impact of pH on astringency and viscosity in the 2011 wines is evident by the significantly lower correlations. The standardisation of pH in the 2011 wines was achieved by acid adjustment using potassium carbonate. Its impact on mouth-feel perception is unknown, and may explain some of the differences between the 2010 and 2011 results. A general increase in the skill level of the taste panel over the period between studies is also a possibility. This is brought out by the observation that despite the alcohol levels being almost identical over the two vintages, the correlation between alcohol level and its classic sensory response 'hotness' was much higher in 2011 ($r= 0.75$, $p<0.001$) compared with 2010 ($r= 0.25$, $p>0.10$).

**Table 8-5: Correlation coefficients between absorbances/Somers measures and sensory attributes for 2010 wines. * indicates sensory attribute was adjusted for pH effect. Red indicates negative correlations, green positive correlations.**

| | Glu+Fru | pH | TA | Ethanol | A280 | A320 | A370 | Flavonoid Ext |
|---|---|---|---|---|---|---|---|---|
| Astringency | 0.29 | 0.87 | 0.53 | 0.21 | 0.39 | 0.09 | 0.15 | 0.55 |
| Viscosity | 0.20 | 0.70 | 0.34 | 0.02 | 0.55 | 0.32 | 0.53 | 0.49 |
| Astringency* | 0.18 | 0.26 | 0.20 | 0.04 | 0.23 | 0.11 | 0.09 | 0.18 |
| Viscosity* | 0.19 | 0.17 | 0.27 | 0.15 | 0.31 | 0.21 | 0.17 | 0.15 |
| Hotness | 0.07 | 0.03 | 0.07 | 0.25 | 0.11 | 0.06 | 0.02 | 0.21 |
| Acidity | 0.25 | 0.74 | 0.59 | 0.10 | 0.27 | 0.06 | 0.13 | 0.44 |
| Bitter | 0.35 | 0.17 | 0.10 | 0.15 | 0.37 | 0.28 | 0.19 | 0.23 |
| Acid AT | 0.18 | 0.73 | 0.67 | 0.11 | 0.24 | 0.09 | 0.13 | 0.36 |
| Bitter AT | 0.25 | 0.12 | 0.12 | 0.16 | 0.46 | 0.39 | 0.31 | 0.17 |

Correlations greater than 0.265, 0.313, 0.404 and 0.503 Significant at 10, 5, 1 and 0.1% respectively

**Table 8-6: Correlation coefficients between absorbances/Somers measures and sensory attributes for 2011 wines. * indicates sensory attribute was adjusted for pH effect. Red indicates negative correlations, green positive correlations.**

| | Glu+Fru | pH | TA | Ethanol | A280 | A320 | A370 | Flavonoid Ext |
|---|---|---|---|---|---|---|---|---|
| Astringency | 0.26 | 0.50 | 0.15 | 0.24 | 0.11 | 0.07 | 0.26 | 0.02 |
| Viscosity | 0.01 | 0.27 | 0.32 | 0.34 | 0.24 | 0.35 | 0.11 | 0.02 |
| Astringency* | 0.05 | 0.00 | 0.19 | 0.33 | 0.16 | 0.03 | 0.04 | 0.24 |
| Viscosity* | 0.15 | 0.00 | 0.33 | 0.32 | 0.39 | 0.42 | 0.24 | 0.15 |
| Hotness | 0.04 | 0.04 | 0.24 | 0.75 | 0.14 | 0.16 | 0.12 | 0.41 |
| Oily | 0.27 | 0.19 | 0.45 | 0.15 | 0.07 | 0.18 | 0.06 | 0.09 |
| Metallic | 0.06 | 0.26 | 0.18 | 0.24 | 0.18 | 0.09 | 0.12 | 0.15 |
| Acidity | 0.29 | 0.25 | 0.68 | 0.08 | 0.05 | 0.23 | 0.16 | 0.20 |
| Bitter | 0.29 | 0.13 | 0.40 | 0.46 | 0.19 | 0.04 | 0.03 | 0.38 |
| Acid AT | 0.30 | 0.28 | 0.75 | 0.07 | 0.09 | 0.28 | 0.12 | 0.18 |
| Burning AT | 0.17 | 0.21 | 0.39 | 0.80 | 0.14 | 0.19 | 0.31 | 0.44 |

Correlations greater than 0.243, 0.288, 0.373 and 0.465 Significant at 10, 5, 1 and 0.1% respectively

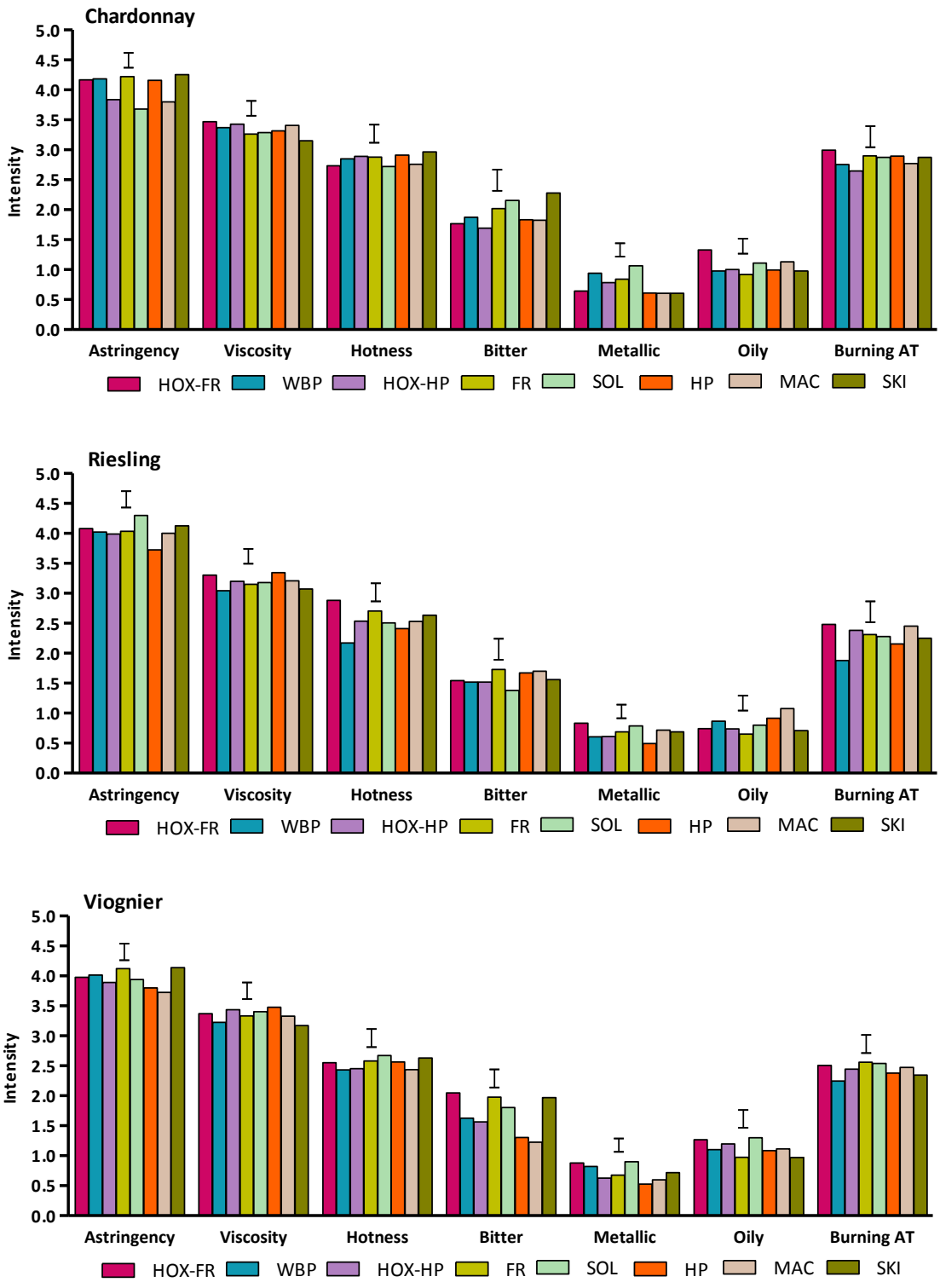



**Figure 8-19: Mean intensity ratings of attributes associated with 'phenolic taste': 2011 wines. Treatments ordered from lowest to highest in average $A_{280}$. Bars show 10% LSD.**

### 8.5.5 Sensory Properties of the 2010 and 2011 Wines Modelled on Basic Analysis and Absorbance Values

The PLS model coefficients for the sensory attributes modelled on basic chemical parameters and absorbances are given in Figure 8-20 and Figure 8-21 (for 2010 and 2011 respectively).

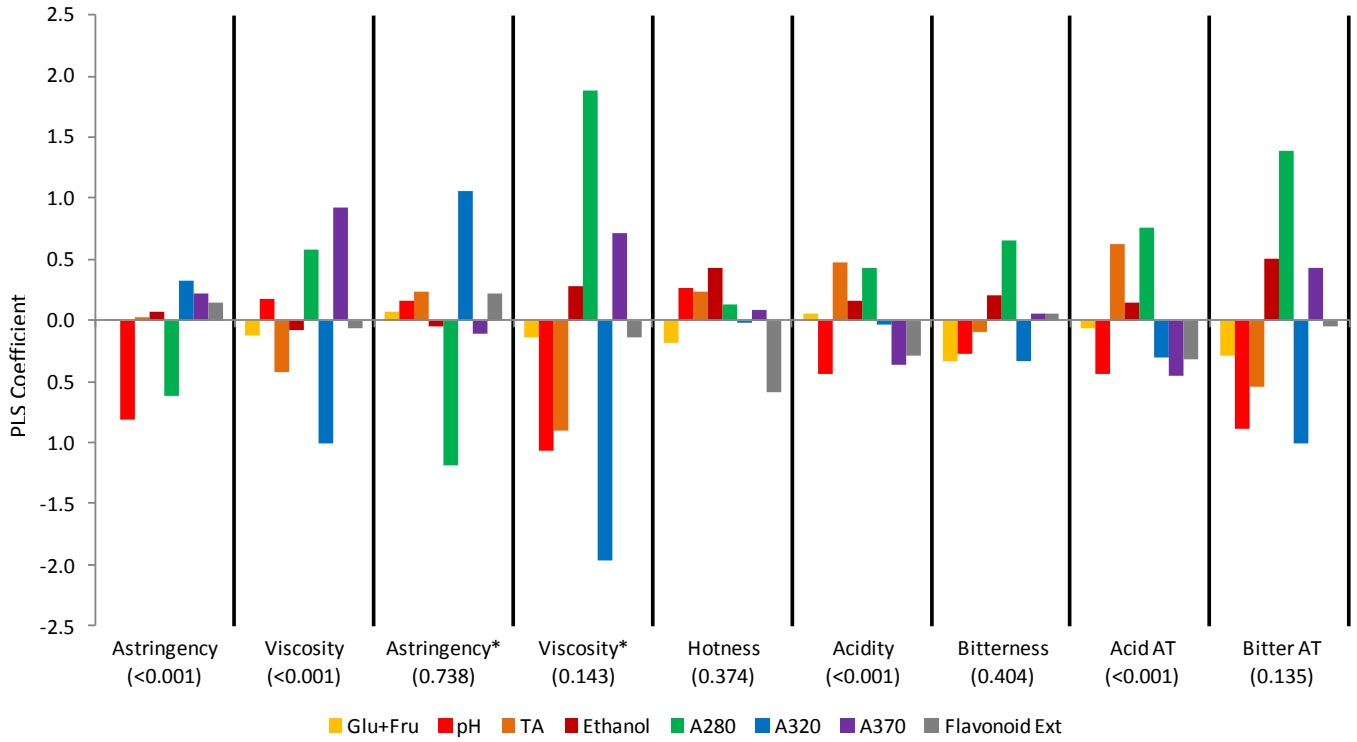



**Figure 8-20: PLS regression coefficients between wine composition and sensory attributes – All 2010 wines. * indicates sensory attribute was adjusted for pH effect. p model fit given in parenthesis.**

Conclusions regarding the influence of basic composition on taste properties have been drawn when:

1) the PLS model was shown to fit the data well and
2) the compositional variable influenced the sensory properties in the same way over two vintages.

Using these criteria, the average PLS coefficients are shown in Table 8-7.

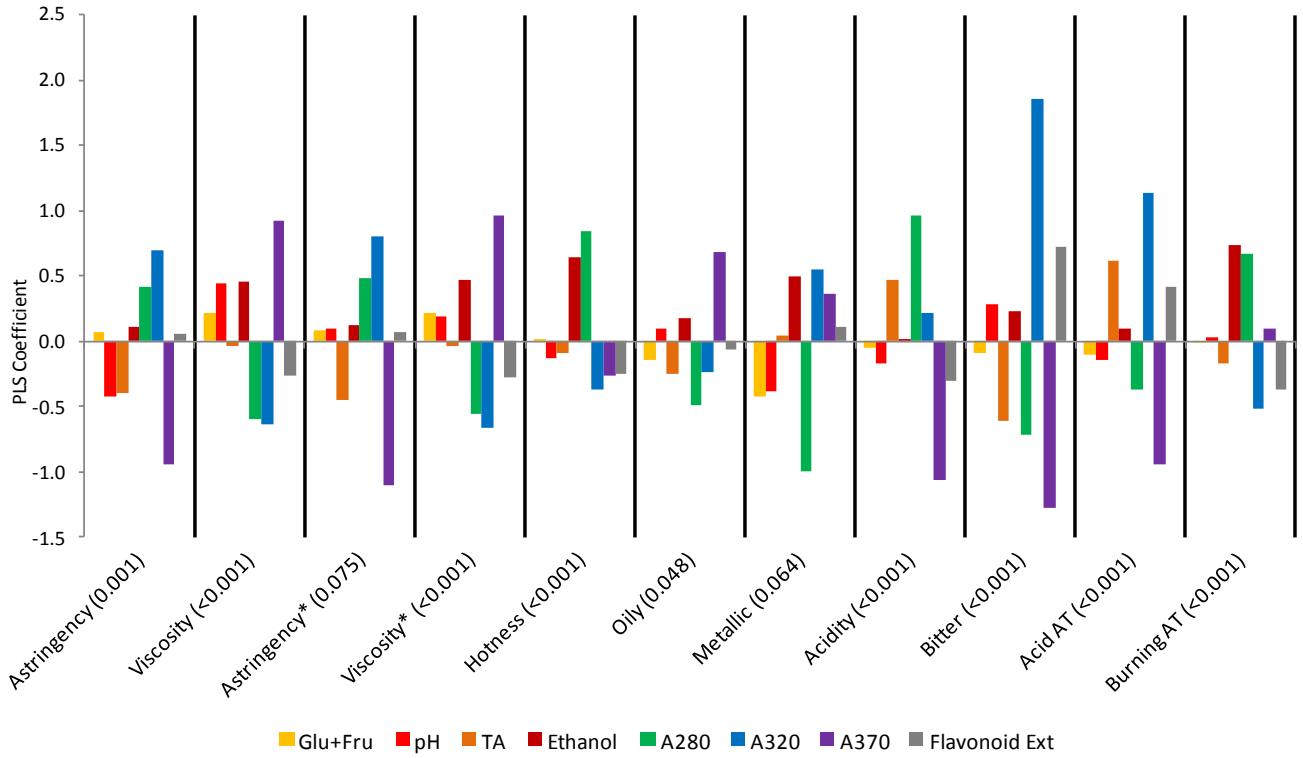



**Figure 8-21: PLS regression coefficients between wine composition and sensory attributes. All 2011 wines. * indicates sensory attribute was adjusted for pH effect. p model fit given in parenthesis.**

These averaged PLS coefficients should be regarded as an index (rather than an absolute) as they reflect both the predicted influence of the compositional variable (i.e. pH, $A_{280}$) and the consistency of its effect across both years. Lower pH was generally associated with higher astringency and acidity, and lower perceived viscosity. Higher TA was related to lower perception of astringency, viscosity and bitterness. The lower bitterness is probably the result of suppression by acidity (Pangborn *et al.* 1964). Higher ethanol was associated with greater bitterness and viscosity. Ethanol has a bitter-sweet taste (Scinska *et al.* 2000), and may also contribute to wine viscosity (Gawel *et al.* 2008).

**Table 8-7: Average PLS coefficients from 2010 and 2011 vintages***

| Attribute | Glu+Fru | pH | TA | Ethanol | A280 | A320 | A370 | Flavonoid Ext |
|---|---|---|---|---|---|---|---|---|
| Astringency | 0.04 | 0.62 | 0.19 | 0.09 | | 0.50 | | 0.10 |
| Viscosity | | 0.31 | 0.23 | 0.27 | | 0.82 | 0.92 | 0.16 |
| Viscosity* | | | 0.47 | 0.38 | | 1.32 | 0.84 | 0.20 |
| Acidity | | 0.30 | 0.47 | 0.08 | 0.70 | 0.09 | 0.72 | 0.30 |
| Acid AT | 0.08 | 0.29 | 0.62 | 0.13 | | | 0.69 | |
| Bitter/Bitter AT | 0.19 | | 0.58 | 0.36 | | | | 0.39 |

* data shown when both PLS regression model fit was good ($p<0.1$) and regression coefficient were (+) or (-) in both years.

However, perceived viscosity, both before and after being statistically adjusted for pH effects, was associated with higher $A_{370}$were also linked to lower acidity perception and higher viscosity.

## 8.5.6 Effect of Phenolic Classes on Sensory Properties

The 2010 wines were also characterised by HPLC and grouped into phenolic classes. Figure 8-22 shows that the concentration of most phenolic classes were positively correlated with bitter aftertaste and viscosity (after adjustment for pH effect), but were negatively correlated with hotness and astringency. Grape reaction products (GRP) are an abundant phenolic class in white wine. They were one of the few that were positively associated with astringency and hotness.

PLS regression analysis (Figure 8-23) elucidated these effects further. After adjusting the astringency ratings for pH effects, benzoic acids and GRP were the major contributors to astringency. However, it should be noted that astringency that was not related to pH was estimated to be in the order of 0.1 to 0.3 sensory units (on a 10 point scale).

The abundant hydroxycinnamic acids and GRP both were positively associated with viscosity as were the flavanones, flavonol glycosides and flavonol ethyl esters. The PLS analysis using basic absorbances is consistent with the role of the flavonols as they absorb strongly at 370 nm. However as both flavonol concentration and pH of wine generally increase as a result of skin maceration, assigning a cause to perceived viscosity is not possible.

Figure 8-23 shows that GRP and cinnamic acids contributed positively to pH adjusted viscosity ratings, which was consistent with the increases in 'oily' character seen when these compounds were added to model wine (Chapter 7). In 2010 the tasters were not specifically trained on oily character, so it is presumed that 'viscosity' was likely to be analogous with 'oilyness'.

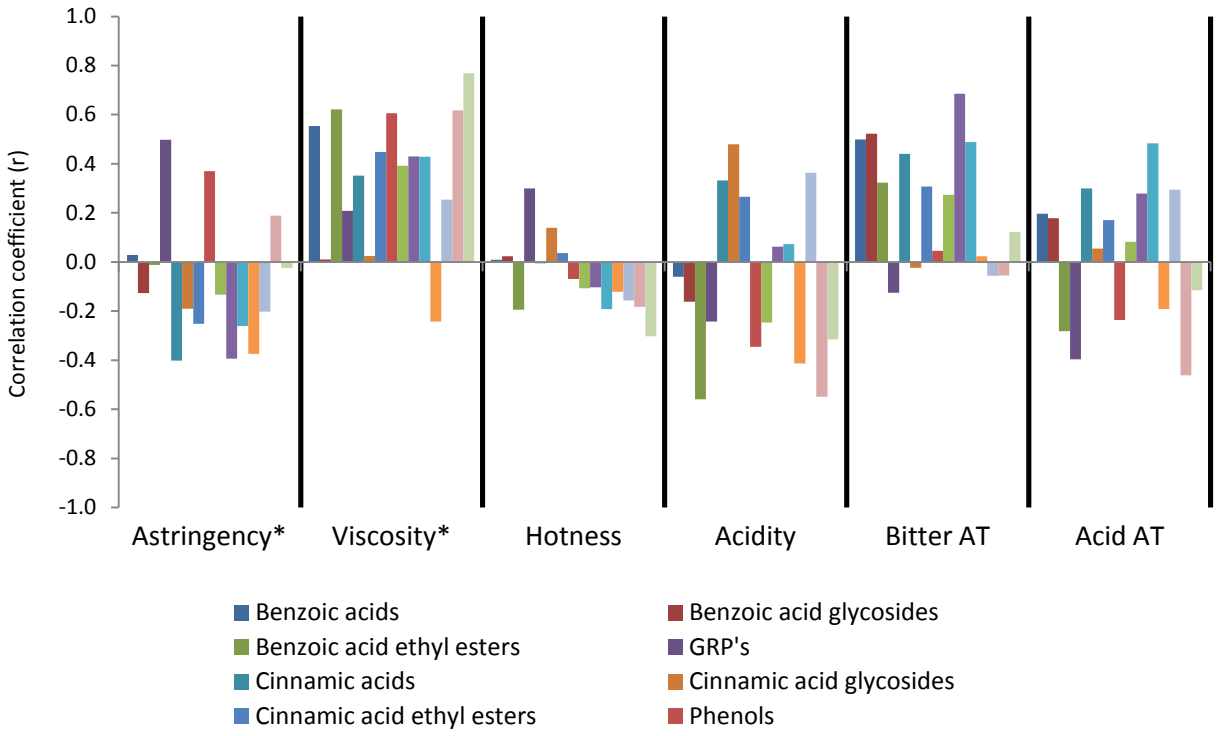



**Figure 8-22: Correlation coefficients between concentration of phenolic compound classes and sensory attributes. All 2010 wines. * indicates sensory attribute was adjusted for pH effect**

Good associations were seen between bitter and bitter aftertaste character and $A_{280}$ in 2010 (Table 8-5). The concentrations of many of the phenolic groups were associated with bitter aftertaste (Figure 8-22). PLS regression analysis showed that a significant contributor to the relationship were the flavan-3-ols, benzoic acids and to a lesser extent GRP and the cinnamic acids. Flavan-3-ols were found to be abundant in one of the few bitter fractions extracted from commercial wines (Chapter 6), and have been reported to be important bitter compounds in tea (Narukawa *et al.* 2010). Adding GRP and caftaric acid to model wine did not result in a consistent increase in bitterness (Chapter 7). PLS analysis suggested that GRP and caftaric acid have some influence on bitterness. However, it is reasonable to assume that the direct approach of assessing these compounds in isolation will be more robust than using correlative methods such as PLS.

Total peak area measured at 280 nm has been used as a proxy for total phenolics and was correlated against sensory ratings given to the 2010 wines (Figure 8-24). Astringency (adjusted for pH effect), hotness and acidity were negatively correlated with peak area, while viscosity (adjusted for pH effect) and bitter aftertastes were positively correlated with peak area.

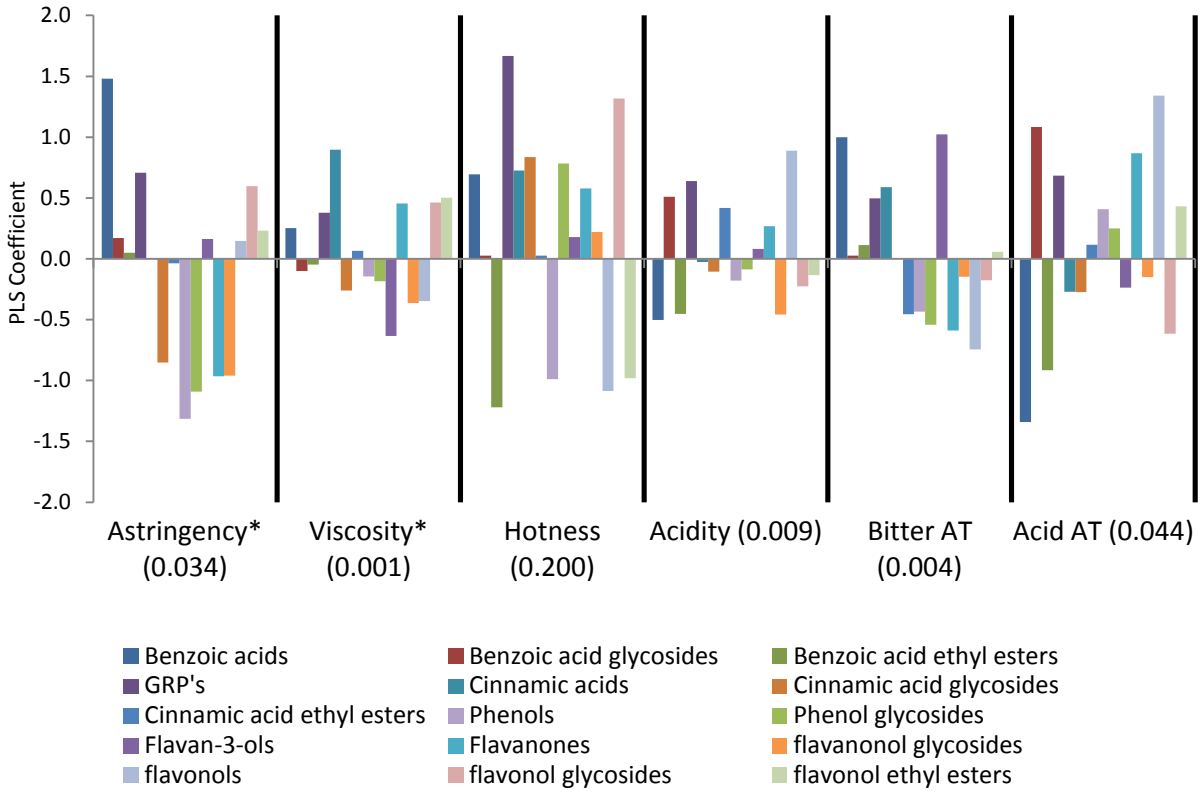



**Figure 8-23: PLS regression coefficients for sensory attributes modelled on concentration of phenolic classes. All 2010 wines. * indicates sensory attribute was adjusted for pH effect.**

These correlations were in close agreement with the correlations with spectrometric measures at 280 nm. The relationships between phenolic composition and phenolic tastes have only been examined in a single vintage, so the results should be considered as indicative.

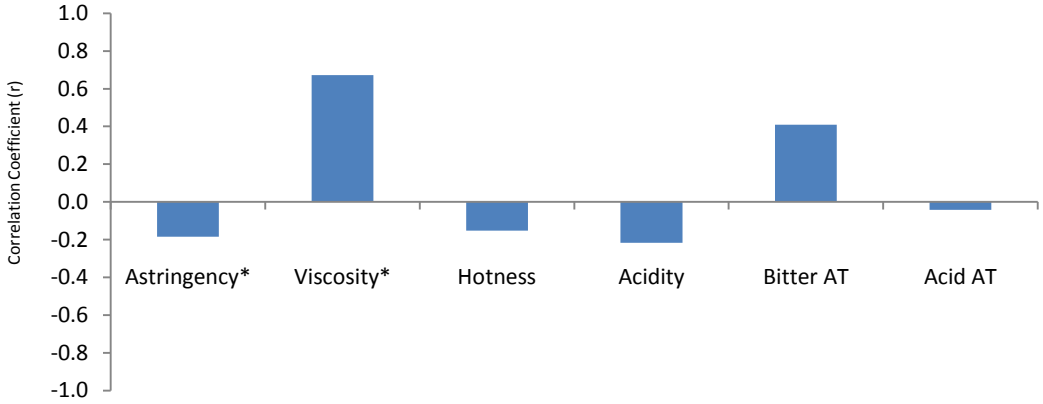



**Figure 8-24: Correlation coefficients (r) for sensory attributes and total phenolics measured by HPLC peak area. * indicates sensory attribute was adjusted for pH effect.**

# 9 Outcomes, Conclusions and Recommendations

Significant progress has been made in finding the molecular drivers of phenolic tastes in white wine. The task was, and remains, challenging as white wine contains over 100 different phenolic compounds spanning a dozen or more structural classes. While the direct effect of only some of the most abundant phenolic compounds were investigated, a further body of correlative work has allowed us to direct our focus on certain phenolic classes as candidates for either causing or suppressing phenolic tastes in white wine.

Using 'total phenolics' isolated from commercial wines we, for the first time, demonstrated that wines with different phenolic composition, when presented at wine like concentrations, can display different textures when tasted in the same matrix (alcohol, pH, TA etc). This suggests that differences in phenolic composition influences textural differences in white wines.

Phenolics were shown to be important in defining wine style. Total phenolics were one of the major factors that differentiated the two recognised styles of the commercially important variety, Pinot G. Lower total phenolics and a higher proportion of total hydroxycinnamates were shown to be strongly associated with the perception of quality in commercial Rieslings by Australian winemakers. On the other hand the acceptability of commercial white wines by Sydney wine consumers was less affected by phenolic levels and more by residual sugar or alcohol concentration.

We also established that alcohol concentration enhanced four major taste/textural attributes (astringency, viscosity, bitterness and hotness) in white wine, and that phenolics and alcohol contributed in an additive way to these attributes. Interestingly, research into stylistically different whole wines demonstrated that the astringency of Pinot Gris/Grigio wines was mostly associated with low pH. In order to further explore the influence and interactions of phenolics with the key matrix elements of alcohol and pH on taste/textural attributes, we demonstrated that variation in white wine tastes and textures could be attributed to both phenolic composition and their interaction with the wine matrix. These were important steps towards an understanding the molecular basis of textural perception in white wine; the identity of the phenolic molecules (or groups of molecules) that caused differences in tastes and textures remains unclear.

To tackle this, the two most dominant phenolic molecules in Australian white wines were isolated and then tasted. The first was caftaric acid. It is usually the most abundant hydroxycinnamate in both juice and wine. The second was 2-S-glutathionyl caftaric acid (better known as Grape Reaction Product or GRP). It is a derivative of caftaric acid and its levels can be increased in white wine by using oxidative

juice handling, but only at the expense of caftaric acid. Caftaric acid was shown to reduce the burning hotness from alcohol and GRP was shown to increase palate oiliness.

The discovery that these two phenolics decrease the dry mouth-feel produced by alcohol and acidity, and caftaric acid suppressed alcohol hotness was unforeseen. It has long been assumed that most 'phenolic taste' elements came from phenolics and that the wine matrix (e.g. acid, alcohol) modulated these perceptions, so the finding that some principal phenolic taste characteristics come from the matrix and are then modulated by some phenolics was unexpected. This has significant practical implications because these two compounds can be varied in winemaking through oxygen exposure and so variations in their concentrations, and that of similar compounds, may allow modulation of the significant sensory effects from alcohol and pH.

An advanced HPLC method has been developed and now allows separation of > 80 identified (40 of which can currently be quantified) phenolics in white juices and wines. This is a notable advancement on the previous methods available, in particular because it requires minimal sample preparation. This was a challenging aspect to the project that was critical to analysing the winemaking experiments described next.

We produced a set of white wines that varied greatly in phenolic composition, so as to elicit measurable differences in both taste and texture. A mix of both conventional and less practiced white wine making techniques were used over three vintages and most winemaking treatments were repeated over two of the years (2010 and 2011). The winemaking experiments, together with the outcomes of the previous experiments in the project, allow insight into the molecular basis of 'phenolic' taste which is summarised below.

'Astringency' ratings in white wines were found to be strongly negatively correlated with pH (i.e. lower pH gives higher astringency). Aggregate measures of phenolics, various complex mixtures of phenolic compounds, and the two major individual phenolics (caftaric acid and GRP) were, in general, not particularly associated with astringency. A striking example of this is the minimal difference in astringency between low phenolic hyper-oxidised wines and the high phenolic macerated and skin contact wines made in 2011. These are among several findings of significance that contradict the widely held assumption that phenolics are the main cause of astringency in white wines.

'Viscosity' ratings in white wines were found to be strongly positively correlated with pH (i.e. lower pH gives lower viscosity). This new discovery further emphasises the importance of this matrix effect on the perception of mouth-feel in white wines.

'Hotness' and 'burning after taste' are most highly associated with alcohol concentration, not aggregated phenolic measurements or caftaric acid. Phenolics have anecdotally been implicated in these sensory characteristics, but mostly phenolics seem not to be positively related to these heat attributes, with the exception of some small effects from GRP and GRP-like compounds. In a further discovery, these

measures of heat are indeed generally suppressed by the presence of phenolics, including caftaric acid. This shows that, in the absence of variations to matrix composition, phenolics may allow winemakers to 'dial down' white wine hotness in some circumstances.

Bitterness was, however, generally shown to be positively associated with phenolics. However, the two major phenolics in Australian white wines (GRP and caftaric acid) do not contribute to bitterness. This means some other phenolic or phenolic class in white wine does, but their identity remains unknown. However, various correlative studies consistently implicated flavanol and glycosylated flavonols as candidates for these bitter tasting compounds.

Observations from winemaking treatments in 2010 show that, as anticipated, increased skin contact increases phenolics in the wines. However, somewhat unexpectedly astringency decreased and viscosity increased and this is mostly due to the pH increase (caused by potassium extraction from skins). Therefore, the desired phenolic outcome of any given winemaking treatment needs to be carefully considered from the perspective of the concomitant effect of pH on astringency and viscosity. Observations from winemaking treatments in 2011 (which were adjusted to similar pH's) show that skin contact before and during fermentation did not generally differ much in 'phenolic taste' compared to wines made from whole bunch pressed, free run or hyper-oxidised wines despite large differences in phenolics.

Overall, the notion that all phenolic compounds necessarily contribute to phenolic tastes should be re-examined in the light of our findings that some either do not affect tastes normally associated with the presence of phenolics (i.e. bitterness), or indeed caused decreases in others (i.e. astringency). Furthermore, pH, acidity, and alcohol levels impact strongly on how phenolics manifest themselves in taste and texture. At low alcohol levels typical of lighter bodied white wines, phenolics contributed to these tastes and textures, but at higher alcohol levels their impact was less apparent. Therefore, interpretation of the possible sensory impact of phenolics in lighter bodied white wines requires consideration of the underlying matrix, particularly pH and alcohol levels.

## Implications for broader industry practices

The Australian wine industry has been receptive of new varieties and the uptake of novel processes in the pursuit of new wine styles. The recent surge in the planting and production of Pinot G to the point where it is now a commercially important variety in the Australian market is testimony to this. Pinot G. and other emerging varieties present a new challenge for Australian winemakers, as arguably the phenolics derived from the grape play a major role in defining their overall structure and style.

Relatively high levels of phenolics (equivalent to the total phenolics encountered in wines made from hard pressings) when added to a light bodied wine were shown to increase its astringency, bitterness and viscosity. However, the effect of the phenolics was dependent on the matrix. Adding phenolics to a low

pH wine failed to substantially increase its astringency. Phenolics also only significantly increased hotness when the wine was at the lower end of alcohol in dry white wines (11.4%). At 12.6% alcohol, the effects of phenolics on hotness were far less pronounced. Overall, low pH levels produced wines that were more astringent, hot and bitter, and increased alcohol resulted in wines that were more hot and bitter. Therefore, these two fundamental aspects of pH and alcohol in the wine matrix were shown to have an overarching effect on those tastes normally associated with phenolics – often greater than the phenolics themselves.

In moderately alcoholic wines made using a range of commercial practices, perceived astringency and viscosity were more strongly influenced by pH than by phenolic level. As the degree of skin contact/maceration increased, so did total phenolics and pH. The higher pH (and phenolic) wines were perceived to be less astringent, more viscous and more bitter.

To date, wineries have generally measured phenolics as a total 'pool' using simple spectrophotometric methods i.e. absorbance at 280 and 320 nm. Any conclusions drawn from these measures must necessarily assume that all phenolic species that absorb at these wavelength have a similar taste impact. This is not likely to be the case. While adding whole phenolics to wine generally increases phenolic tastes, the presence of some important phenolic species that absorb at these wavelengths appear to have a suppressive effect also. Therefore, in light of this finding, better rapid analytical methods for measuring key phenolic subcomponents or classes associated with sensory impacts are required.

Finally, the knowledge that different phenolics or phenolic classes either add or suppress different tastes arising from phenolics themselves or from the influence of pH or alcohol, could warrant investment in the development of fining agents that are tailored to modify the tastes and textures of white wine through removal of key phenolics, or alternatively, may lead to a more effective use of existing fining agents.

## Future Research Recommendations and Opportunities

Further opportunities for research into the molecular basis for phenolic taste exist. Of particular relevance is bitterness, which was one of the few sensory attributes clearly attributable to phenolics but the molecular drivers remain unknown. Suppressive effects on alcohol hotness and increases in oiliness from GRP-like compounds may also present opportunities.

While investigating ways of fractionating caftaric acid and GRP from wines using counter-current chromatography we identified potential new ways of fractionating phenolic compounds identified in our other studies as potential drivers of the negatively perceived characters of bitterness and metallic taste. The new HPLC method developed as part of this project can be used to assess the composition and purity of any resultant fractions submitted to sensory assessment.

The sensory properties of only two phenolic compounds have been extensively studied to date. Further research should be conducted to ascertain whether other hydroxycinnamates and their derivatives (which make up the bulk of the total phenolics in white wines) affect phenolic tastes in a similar way to caftaric acid and GRP. An answer to this question would allow simple absorbance measures at 320nm to be better interpreted in light of expected sensory outcomes.

Few sensory interactions are balanced. Usually one sensory characteristic tends to have a disproportionate effect on the other. An assessment of the strength of the suppressive effect of caftaric acid (at various concentrations) on alcohol hotness is required in order to put this positive effect of phenolics into practical perspective.

The taste and textures of the 2011 wines made using different winemaking treatments have been characterised but their detailed phenolic profiles are yet to be quantified. This task is currently underway. The wines made from high solids and from 10% skin fermentation showed distinct taste profiles from the other wines. In particular, the solids wines showed negative taste characters (such as metallic) not seen in any of the wines studied to date. Further investigation into the possible causes of taste and textural characters with the potential to detract from consumer acceptability is warranted.

# A Preparative and analytical laboratory Methodologies

## A.1 Measurements of Total Phenolics by Spectrometry: the Rationale Behind Somers Measures

Somers and Ziemelis (1985) proposed the simple measurements of total phenolics, total hydroxycinnamates and the calculation of the flavonoid extract to assess musts and pressings. The clarified juice or wine is measured directly in a one or 2mm quartz cuvette (with the result multiplied by 10 or five to give the 10mm equivalent reading of absorbance at 280nm ($A_{280}$) and 320nm ($A_{320}$) or 0.5mL diluted into 10mL 3% aqueous acetic acid, and read in a 10mm quartz cuvette depending on the sample.

**Total phenolics = $A_{280}$-4** absorbance units (au)

**Total hydroxycinnamates** = **$A_{320}$-1.4** au

**Flavonoid extract = ($A_{280}$-4) – 2/3($A_{320}$-1.4)** au (A=10 mm equivalent)

The correction factors of 4 and 1.4 are estimates to allow for the small contribution to absorbance from non-phenolics (Somers and Pocock 1991). Besides phenolic compounds, some other types of compounds absorb in the UV-Vis region (200-600 nm), and contribute in a small way to the absorbance. For example, tannin-protein complexes, nucleic acids, and other compounds such as $SO_2$ contribute to $A_{280}$ (Myers and Singleton 1979). Sorbic acid absorbs at 280 nm and can influence the result significantly, so wines with added sorbic acid are not suitable for these UV-Vis measurements.

$A_{280}$ is a measure of total phenolics and includes all the different classes of phenolics including flavonoids and non-flavonoids. $A_{320}$ is a reasonably specific measure of hydroxycinnamates. The flavonoid extract includes flavanols, condensed tannins, and flavonols (aglycones and glycones), that are located in the solid parts of the grape and, therefore, are extracted into the wine or juice with skin contact and pressing. The flavonoid extract can account for 0-80% of $A_{280}$, related to the degree of extraction from the grape solids (Somers and Pocock 1991). Measuring the hydroxycinnamates by $A_{320}$, total phenolics by $A_{280}$ and calculating the flavonoid extract is, therefore, a simple method to characterise the broad phenolic profile of a sample.

# A.2 High speed Counter-current Chromatography

## A.2.1 Introduction

High speed counter-current chromatography (HSCCC) is a partition chromatography process that separates analytes through high speed mixing and separation between two immiscible liquid phases. The process offers numerous advantages over conventional preparative chromatographic methods such as good sample recovery both in terms of purity and yield, zero irreversible adsorption and high loading capacity.

HSCCC is particularly suited to the isolation and fractionation of phenolic compounds as their varied sizes and degree of hydroxylation affects their hydrophobicity and therefore their relative affinities for the (generally) polar mobile phase and non-polar stationary phase. Indeed, HSCCC has previously been used to fractionate flavanols (Wang *et al.* 2008), flavonol glycosides (Zhang *et al.* 2007), flavonoids (Costa and Leitao, 2011) and hydroxycinnamic acids (Maier *et al.* 2006), but only on an analytical scale.

HSCCC systems have the potential to achieve higher loading capacities than do traditional preparative chromatographic approaches that utilise solid supports with finite binding capacities. Therefore HSCCC could allow the relatively large amount of phenolic material required to undertake replicated sensory testing to be isolated in a relatively small number of runs.

The effectiveness of any HSCCC solvent system in separating analytes from a complex mixture depends on the analytes having different partition coefficients between the two immiscible layers that form when the solvents are mixed. If the partition coefficients are the same then the compounds will co-elute. On the other hand, if a compound solely partitions into one phase or the other then it will either elute close to the solvent front, or alternatively it will be retained in the stationary phase. In the former case, the compound of interest will usually will co-elute with other compounds with similarly extreme partition coefficients, or in the latter case, it will never elute. Therefore the most important step of HSCCC optimisation involves finding the optimal mix of solvent components that best differentiate the compounds to be separated on the basis of their partition coefficients.

To this end, a white wine HSCCC method was developed that provided enough caftaric acid and Grape Reaction Product to allow their sensory properties to be formally assessed.

## A.2.2 Selection of Solvent System

Two HSCCC solvent systems (mixtures of solvents that partition into two phases when mixed) were selected for screening and optimisation following a review of the literature. The systems trialled were 1) hexane, ethyl acetate, methanol and water (HEMBWAT system) and 2) tert-butyl methyl ether, acetontrile and water (ETAWAT system). Both had previously been used to successfully fractionate phenolic compounds from natural product extracts, including those from wine.

The partitioning of compounds representing most of the major phenolic classes in white wine were assessed using the HEMBWAT and ETANWAT solvent systems listed in Costa and Leitao (2011), ordered by their theoretical ability to resolve mixtures of polar compounds through to mixtures of non-polar compounds. The phenolics assessed were catechin (representing flavan-3-ols), caffeic acid (hydroxycinnamic acids), chlorogenic acid (hydroxycinnamic acids esterified to an organic acid), quercetin (flavonoid) and rutin (flavonoid glycoside). The partitioning trials suggested that the HEMBWAT system in the ratio (3:7:0:3:7), and the ETAWAT system in the ratio (2:2:3) could be used to separate these phenolic classes.

## A.2.3 Semi-preparative Scale-up

A semi-preparative scale-up was performed using these two selected systems to assess whether HSCCC could be used to adequately separate different phenolic types, both in a model system and from a phenolic extract of white wine. 20 mg of each of the phenolic test compounds were injected and run individually to determine their retention times. The whole phenolics were extracted from 375 mL of a McLaren Vale unwooded Chardonnay using the method described previously was also subjected to HSCCC.

A two phase solvent system was prepared by thoroughly mixing the solvents in the ratios stated above in a separating funnel and allowing it to equilibrate at room temperature. The upper layer was separated from the lower layer and used immediately.

HSCCC was performed using a Quattro Mk II (AECS-QuikPrep Ltd, Bridgend, S. Wales, UK) equipped with a 100 mL coil. For the HEMBWAT (3:7:3:0:7) system, the coil was filled with the upper organic rich phase, and the lower aqueous phase was pumped into the head end of the column at a flow rate of 1 mL/min with the coil rotating at 800 rpm. For testing the ETANWAT (2:2:3) system, the coil was filled with the lower phase, and the upper phase was pumped into the tail end of the column. After the system reached a temperature of 30$^{o}$C and hydrodynamic equilibrium had been attained, the sample was dissolved in 5 mL of the mobile phase and injected. Two single wavelength UV detectors (UPC-900 Amersham Biosciences for 280 nm, Pharmacia Biotech Superfrac GBC LC 1210 for 320 nm) were used to detect the presence of phenolics in the system effluent. Fractions eluting from the whole phenolics were collected every two minutes, and their composition was assessed by HPLC using the method described in Section A.3.

### A.2.4 Preparative Scale Up

To obtain sufficient quantities of caftaric acid and grape reaction product for formal sensory assessment, the system was scaled up in an attempt to fractionate the phenolics from 2.25 L of wine using a 500 mL coil. However, it was found that this scale up resulted in a substantial loss of resolution between caftaric acid and grape reaction product due to overloading (data not shown), and although the problem could be resolved, it meant loading smaller amounts of sample in combination with excessively long and impractical run times. To overcome this limitation, a preliminary separation stage was applied, and is described below.

### A.2.5 Compound Extraction and Purification

Both solvent systems provided good resolution of the compounds tested. While the ETANWAT (2:2:3) system was shown to be equally as effective in separating the phenolic test compounds as the HEMBWAT (3:7:3:0:7) system, the latter was chosen for further testing as its solvent components could be found in food grade form.

# A.3 Development of a Separation and Identification Strategy for Phenolic Compounds in White Wine by HPLC-DAD and HPLC-QTOF

## A.3.1 Introduction

In order to identify and quantify the diverse 'phenolic' compounds found in white wine, an HPLC technique suitable for migration to mass spectrometry was developed. With the express intention of not discriminating against possible species present that might elicit taste sensations characterized by the broad term 'phenolic', a SPE sample clean-up step was not considered appropriate. These decisions meant the chosen method had to be highly selective and discriminatory. Sample preparation was thus based only on removal of alcohol by rotary evaporation and centrifugation replaced filtration to avoid adsorption onto filtration fibres.

Method development followed a path of column selection – based on separating the maximum number of separate peaks – and then mobile phase optimization.

## A.3.2 Material and Methods

### Chemicals

All chromatographic solvents were HPLC grade and chemicals were analytical grade unless otherwise stated. Water was obtained from a Milli-Q Academic (Millipore, France) purification system using a "Quantum" cartridge. methanol, acetonitrile (LiChrosolv, Merck, Germany), formic acid (98% v/v Emsure, Merck, Germany) and trifluoroacetic acid (100% v/v Thermo, IL) were purchased from Rowe Scientific (Lonsdale, SA, Australia). Polyphenolic standards were purchased from Sigma-Aldrich or Extrasynthèse (France).

### Wine Samples

Experimental wine was made at the Hickinbotham-Roseworthy Wine Science Laboratory using established protocols. For the purposes of method development, a 2009 Riesling and a 2009 Chardonnay wine, made from the hard pressings juice fractions, was used throughout.

### Preparation of Wine Samples

White wine samples were prepared by first removing alcohol by rotary evaporation (Heildorf, Germany) with a water bath temperature <40°C, completing with Milli-Q water, internal standard and starting mobile phase constituents (see text). The sample was centrifuged at 15,000 rpm for 3 minutes in a Thermo Electron IEX Micromax (Biolab, Mulgrave, Vic, Australia) centrifuge in 1.5 mL Eppendorf tubes and not filtered to avoid possible adsorption onto the filter material. All HPLC analyses were carried out on an Agilent 1100 instrument (Agilent Forest Hill, VIC, Australia) equipped with a quaternary or binary pump and a diode array detector (DAD). Data acquisition and analysis was performed using ChemStation LC-3D software (version B.03.01).

### Selection of Column

A number of HPLC columns with different separations chemistries were screened using the 2009 Chardonnay or Riesling hard pressings wine. The choice of column for further optimization was determined by the maximum number of resolved peaks (obtained using standardized integration parameters) at 280 nm (all phenyl-containing compounds) and the peaks in which the absorbance was higher at 320 nm than at 280 nm (hydroxycinnamate-containing compounds). The initial screening looked at different separation chemistries or column presentations: UDC Cholesterol (Cogent); Synergi Polar RP, Synergi Hydro RP, Gemini C6-Phenyl, Kintex PFP (Phenomenex); Supelco Ascentis Phenyl (Sigma) Spherisorb Phenyl, XBridge (Waters).

Throughout the experiments, 20 µL of the same wine sample (2009 Riesling hard pressings wine), stored at -18°C was injected. Standardised integration parameters were used to assess the number of peaks resulting for the different columns or operating conditions: slope sensitivity = 1; peak width = 0.2 min; area reject = 50 mAu; height reject = 5 mAu. The total number of peaks at 280 nm was recorded as was the number of peaks where the absorbance at 320 nm was greater than that at 280 nm which represented the hydroxycinnamates.

## A.3.3 Results and Discussion

The success of column selection was measured in terms of the total number of separate peaks observed at 280 nm and peaks arising from hydroxycinnamoyl-containing compounds. Over 100 separate peaks arising from phenolic compounds were detected. Of those columns studied, the Gemini C6-Phenyl and Kintex PFP columns appeared to provide the highest degree of separation of phenolic compounds (Table A-1). As further planned research will have require up-scaling for semi-preparative separations for sensory analysis, the former column was chosen as more packing options were available at the time of this work.

### Optimization of Chromatography Using Gemini C6-Phenyl Column.

Further optimization was carried out on the formic acid concentration and effect of TFA concentration. In order to improve the resolution of early-eluting compounds, the pH was modified to be potentially below the $pK_a$ of some hydroxybenzoic or cinnamic acids so that they were predominantly in the unionized form. This was carried out by including trifluoroacetic acid into both mobile phases and also modulating the concentration of formic acid. In view of the complexity of the chromatogram with the number of peaks eluting, all dead volumes were minimized by fitting the shortest possible lengths of 0.12 mm i.d. stainless steel tubing from injector seat to detector and using a semi-micro flow cell. A binary pump was installed instead of the quaternary to minimize pressure fluctuations. An injection volume of 10 µL was also adopted as this slightly improved resolution of peaks. The samples were matched to the starting chromatography conditions by making up samples to volume with 2% formic and 0.01% TFA. Both of these measures improved the retention time variability. A typical HPLC-DAD chromatogram is shown in Figure A-1 with some of the major peaks identified. The trace arising largely from benzoic acids, flavan-3-ols and other phenol- ring containing compounds can be seen when $A_{280}$ being the largest.

## Table A-1 : Resolving power of phenolic species in chardonnay and Riesling hard pressings wine (2009) using standardized integration parameters[1]

| Column details | Conditions used | No. peaks 280 nm | No. peaks A320 ≥ A280 | No. peaks 320 nm |
|---|---|---|---|---|
| Synergi Polar RP[2] (Phenomenex) 4.6 x 250 mm; 4µm | Start: 1% ACN in water/1% formic acid/ 0.1% MeOH; ramp to 80% ACN /2% formic acid/10% MeOH from 5' to 50'; hold for 5' return over 5'; 1 mL/min | 67 | 6 | 39 |
| Synergi Hydro RP[2] (Phenomenex) 2.1 x 150 mm; 4µm | Start: 1% MeCN in water/1% formic acid; ramp to 80% MeCN /2% formic acid from 5' to 50'; hold for 5' return over 5'; 1 mL/min | 49 | 7 | 41 |
| UDC Cholesterol (Cogent) 4.6 x 250 mm; 4µm | Start: 1% ACN in water/1% formic acid/ 0.1% MeOH; ramp to 80% ACN /2% formic acid/10% MeOH from 5' to 50'; hold for 5' return over 5'; 1 mL/min | 49 | 8 | 41 |
| Spherisorb Phenyl (Waters) 4.6 x 250 mm; 5µm | Start: 1% MeOH in water/1% formic acid; ramp to 80% MeOH /2% formic acid from 5' to 50'; hold for 5' return over 5'; 0.2 mL/min; 45°C | 41[2] | 12 | 38 |
| Ascentis Phenyl (Supelco Sigma) 2.1 x 150 mm; 3µm | 2% formic acid constant; ramp to 40% MeOH from 0' to 50'; ramp to 100% for 5' return over 2'; 0.3 mL/min; 45°C | 76 | 9 | 51 |
| Gemini C6-Phenyl (Phenomenex) 2.1 x 150 mm; 3µm | 2% formic acid constant; ramp to 40% MeOH from 0' to 50'; ramp to 100% for 5' return over 2'; 0.2 mL/min; 45°C | 108 | 12 | 86 |
| XBridge (Waters) 2.1 x 150 mm; 3.5µm | 2% formic acid constant; ramp to 45% MeOH from 0' to 50'; ramp to 90% for 5' return over 2'; 0.3 mL/min; 45°C | 82 | 9 | 65 |
| Kintex PFP[3] (Phenomenex) 2.1 x 150 mm; 3µm | 2% formic acid constant; ramp to 40% MeOH from 0' to 50'; ramp to 100% for 5' return over 2'; 0.2 mL/min; 50°C | 106 | 13 | 75 |

[1] *standardized integration parameters: slope sensitivity = 1; peak width = 0.2 min; area reject = 50 mAu*blns*; height reject = 5 mAu*

[2] *RP: reversed phase*

[3] *PFP: pentafluorophenyl*

The cinnamic acids and GRP-analogues are represented where the $A_{320}$ trace is the biggest and flavonols and their glyco-conjugates when $A_{370}$ is biggest. In the example shown, the acids caftaric, coutaric, fertaric and ferulic (red $A_{320}$ trace) are the most dominant and quercetin-3-glucuronide is the major flavonol glyco-conjugate. Note all peaks elute within 70 minutes.

Coupling two column together in series can give an even better separation was possible if two columns were connected. It was necessary to subsequently reduce the flow rate to 0.16 mL/min to

keep back pressure under 300 bar and reduce the flow rate to 0.13 mL/min during the high methanol phase. With two in series the time taken to elute all peaks has now increased to just over 100 minutes. To ensure post-run column equilibration was attained, a wait time of 20 minutes was incorporated into the method program, meaning that a complete run lasts 155 minutes (Figure A-1). Using two columns increased the resolution by a factor of two and allowed caffeic acid and one of the coutaric acid isomers to be separated. This improvement in resolution also allows the separation of 2-S-glutathionyl adduct of *trans*-caftaric acid (GRP) from the free acid. These are depicted Figure A-2. Unfortunately the resolution between quercetin-3-O-glucoside and quercetin-3-O-glucuronide is reduced in the two-column configuration. Mass spectrometry highlights the elution of UV-inactive material eluting 30 minutes after the last phenolic peak. This is seen in Figure A-3 where there is still a total ion count (TIC) trace after 110 minutes but no UV absorbance.

| | **$R_S$ (1x column)** | **$R_S$ (2x columns)** |
|---|---|---|
| *trans*-caftaric acid + 2-S-glutathionyl *trans*-caftaric acid (GRP) | 1.0 | 2.3 |
| *cis* and *trans* isomers of coutaric acid | 4.75 | 8.16 |

*Resolution, $R_S = (t_{R1} - t_{R2}) / 0.5(t_{W1} + t_{W2})$ where $t_R$ is elution time and $t_W$ is tangent baseline width*

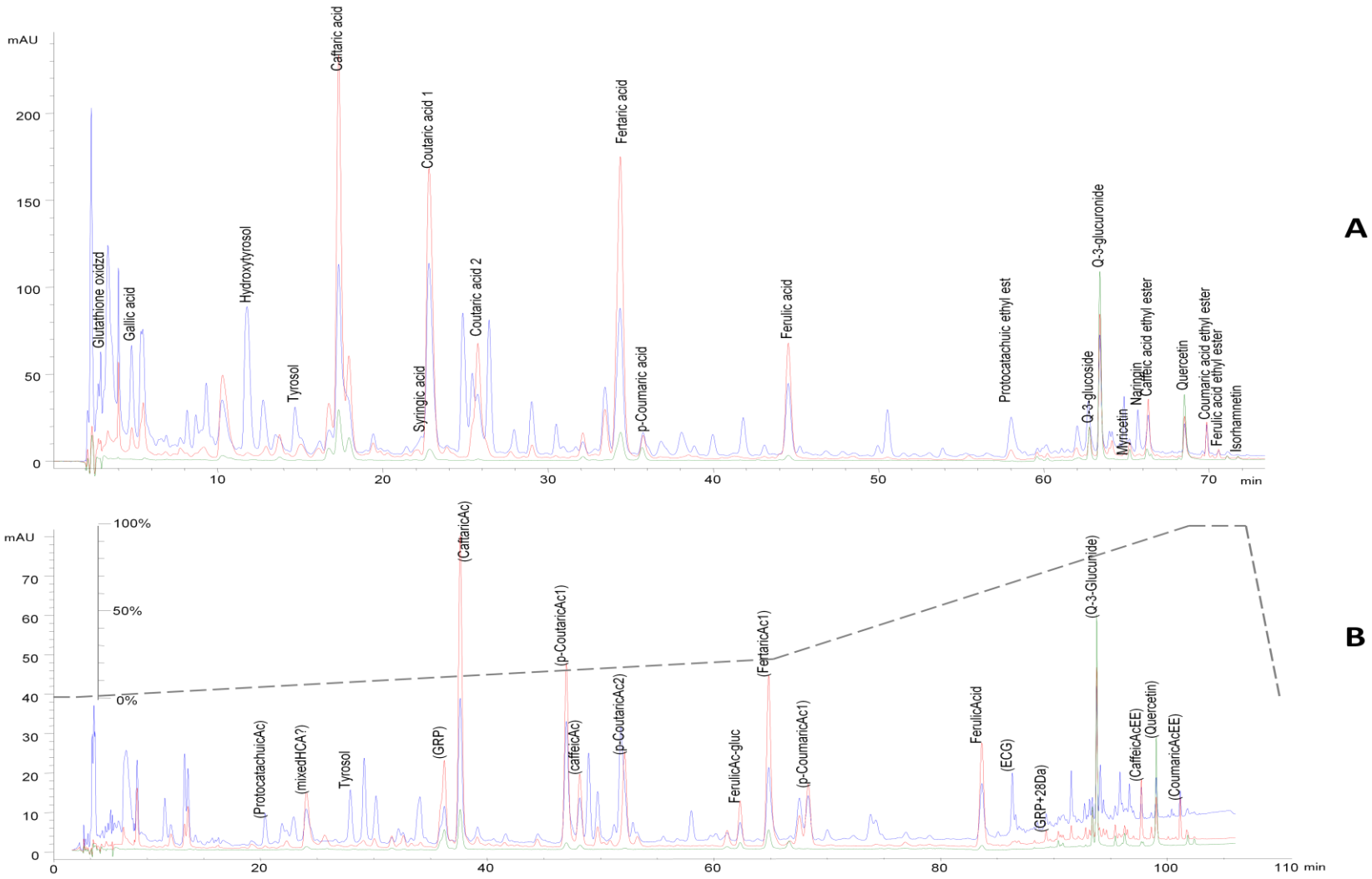



**Figure A-1 : HPLC-DAD chromatogram of 2009 Riesling hard pressings with (A) one Gemini C6-phenyl column and (B) two columns in series [Legend: — $A_{280}$ — $A_{320}$ — $A_{370}$ - - - MeOH gradient]**

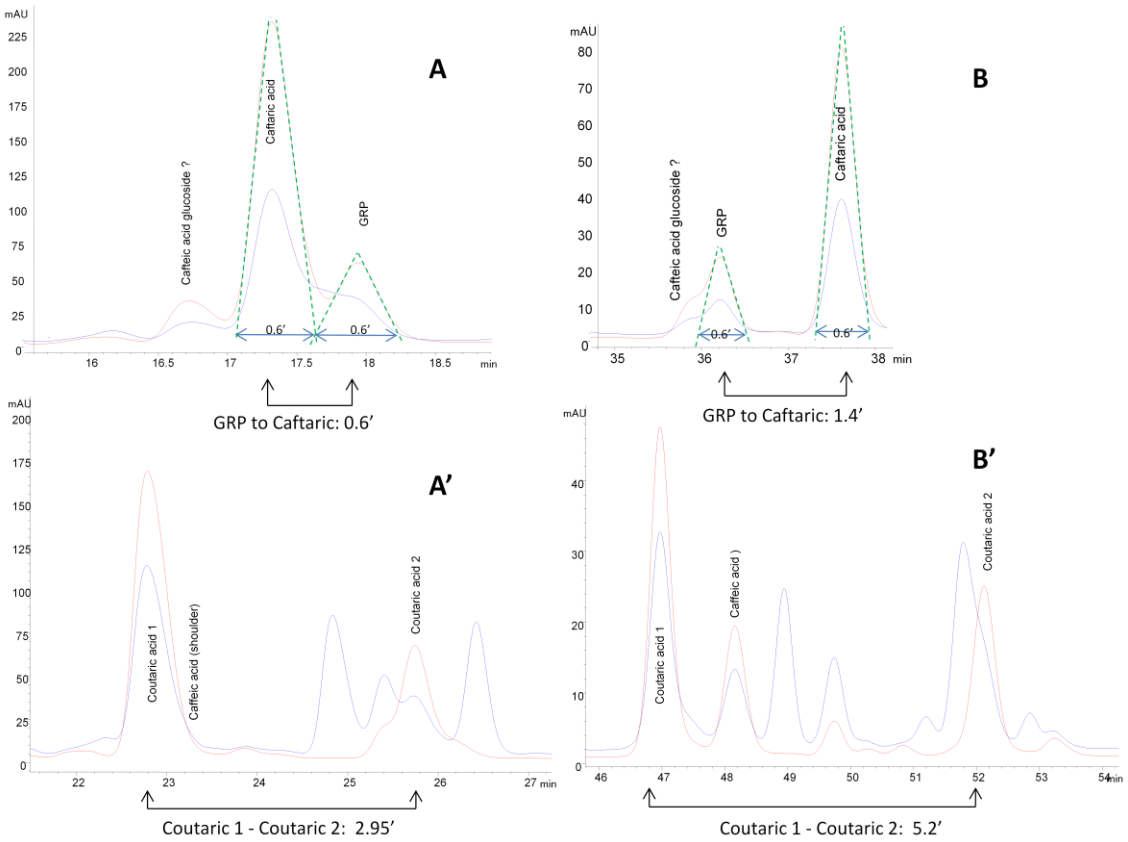



**Figure A-2 : Depiction of improvement in resolution for cis and trans-coutaric acid isomers and caffeic acid using single column (A) and dual in-line columns (B)**

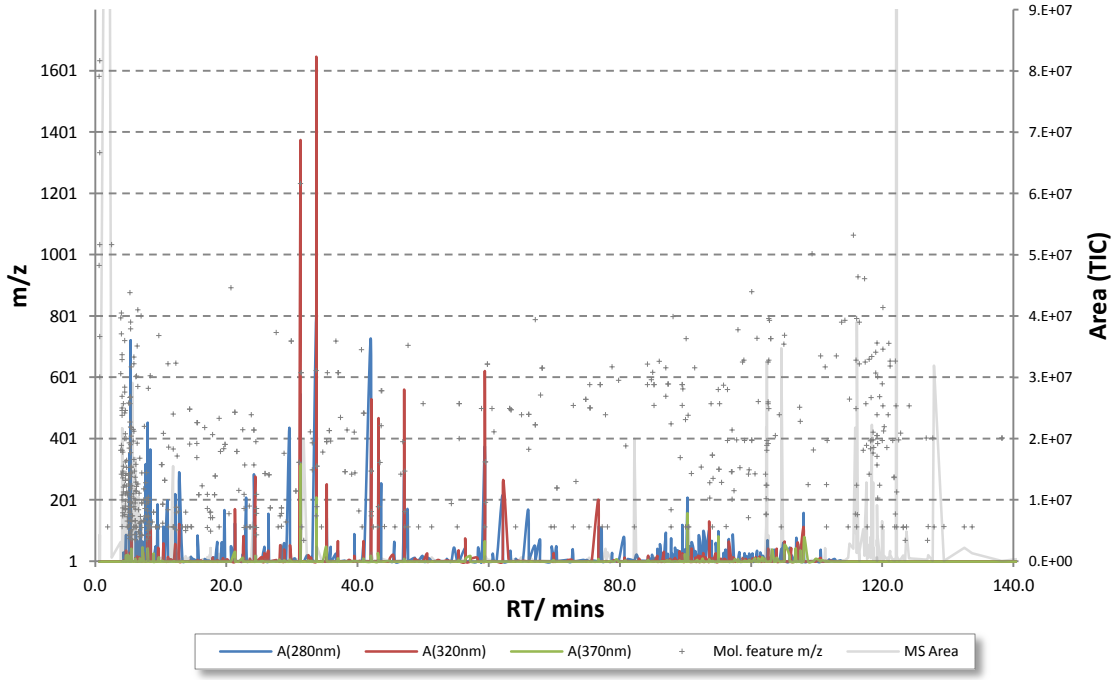



**Figure A-3: Reconstruction of HPLC-QTOF-MS trace (UV absorbances, and m/z and intensities of molecular feature) of 4x concentrated macerated Riesling**

## Table A-2: Chromatographic and spectral characteristics of pure reference phenolic compounds analysed by HPLC-DAD (listed by elution order on C6-Phenyl column)

| Compound name | Phenolic group | RT (min) | $\lambda_{max}$ v = valley, sh = shoulder, bln=baseline end | Response factor |
|---|---|---|---|---|
| Gallic acid | Benzoic acid | 12.26 | 271, 325bln | 1.15E-02 |
| p-Hydroxybenzoic acid | Benzoic acid | 32.90 | 256, 241v, 293bln | 1.48E-02 |
| Tyrosol | Benzoic acid | 33.84 | 276, 243v, 295bln | 2.90E-02 |
| Gentisic acid | Benzoic acid | 36.50 | 328, 268v, 375bln | 2.24E-02 |
| Caftaric acid | Hydroxycinnamic acid | 39.40 | 328, 296sh, 264v, 386bln | 1.36E-02 |
| Catechin | Flavanol | 45.65 | 279, 251v, 301bln | 4.28E-02 |
| Vanillic acid | Benzoic acid | 48.60 | 260, 292, 281v, 320bln | 1.30E-02 |
| Caffeic acid | Hydroxycinnamic acid | 49.18 | 323, 295sh, 263v, 379bln | 5.82E-03 |
| Chlorogenic acid | Hydroxycinnamic acid | 57.02 | 325, 301sh, 269v, 382bln | 1.21E-02 |
| Syringic acid | Benzoic acid | 61.00 | 274, 241v, 323bln | 7.37E-03 |
| Procyanidin B2 dimer | Flavanol | 62.40 | 279, 255v, 298bln | 1.29E-01 |
| Epicatechin | Flavanol | 66.10 | 278, 251v, 301bln | 5.58E-02 |
| Ethyl gallate | Benzoic acid | 66.57 | 272, 240v, 325bln | 9.67E-03 |
| p-Coumaric acid | Hydroxycinnamic acid | 69.23 | 310, 248v, 362bln | 4.57E-03 |
| EGC | Flavanol | 72.19 | 274, 248v, 327bln | 3.27E-01 |
| EGCG | Flavanol | 72.74 | 274, 248v, 335bln | 4.97E-02 |
| Ferulic acid | Hydroxycinnamic acid | 84.04 | 323, 296sh, 261v, 377bln | 2.93E-03 |
| Dihydroquercetin | Flavononol | 87.40 | 289, 250v | 1.23E-02 |
| Sinapic acid | Hydroxycinnamic acid | 89.00 | 324, 263v, 380bln | 5.89E-03 |
| ECG | Flavanol | 90.11 | 278, 246v, 328bln | 2.12E-02 |
| Protocatachuic acid ethyl ester | Benzoic acid | 91.81 | 261, 295, 281v, 324bln | 1.38E-02 |
| Q-3-galactoside | Flavonol | 96.08 | 256, 357, 301sh, 281v, 400bln | 1.19E-02 |
| Q-3-glucoside | Flavonol | 96.45 | 256, 356, 307sh, 282v, 400bln | 2.93E-02 |
| Quercetin-O-3-rutinoside | Flavonol | 96.50 | 256, 356, 304sh, 282v, 400bln | 1.62E-02 |
| Q-3-glucuronide | Flavonol | 96.60 | 256, 356, 304sh, 282v, 400bln | 2.04E-02 |
| Resveratrol | Stilbene | 97.45 | 306+318, 256v, 356bln | 1.07E-02 |
| Naringin | Flavone | 98.23 | 284,330sh, 245v, 374bln | 1.51E-02 |
| myricetin | Flavonol | 98.51 | 374, 254, 310sh, 286v, 405bln | 1.88E-02 |
| Fisetin | Flavonol | 99.71 | 361, 249, 326sh, 280v, 400bln | 5.19E-02 |
| Syringetin-O-3-glucoside | Flavonol | 99.85 | 253, 359, 311sh, 284v, 400bln | 1.21E-02 |
| Caffeic acid ethyl ester | Hydroxycinnamic acid | 101.11 | 327, 302sh, 264v, 380bln | 2.44E-03 |
| Quercetin (dihydrate) | Flavonol | 103.20 | 372, 255, 313sh, 285v, 405bln | 3.61E-03 |
| Laricitrin | Flavonol | 104.09 | 375, 253, 206sh, 286v, 407bln | 2.97E-03 |
| Luteolin | Flavone | 104.81 | 350, 254, 282v, 400bln | 5.88E-03 |
| Naringenin | Flavone | 105.07 | 290, 330sh, 249v, 370bln | 7.15E-03 |
| Ferulic acid ethyl ester | Hydroxycinnamic acid | 106.37 | 325, 299sh, 263, 376bln | 4.79E-03 |
| Kaempferol | Flavonol | 106.78 | 367, 265, 322sh,282v, 400bln | 6.98E-03 |
| Syringetin | Flavonol | 107.11 | 375, 253, 308sh, 286v, 408bln | 4.35E-03 |
| Isorhamnetin | Flavonol | 107.61 | 371, 255, 286v, 410bln | 6.49E-02 |
| Apigenin | Flavone | 107.84 | 339, 267, 281v, 397bln | 3.64E-03 |

## Identification of Chromatography Peaks

Identification was carried out initially by analysing commercially-produced HPLC-grade reference compounds by HPLC-DAD. Table A-2 lists obtained retention times (RT), UV detector response factors and spectral characteristics ($\lambda_{max}$ listed first) in the order they elute. A composite sample of Chardonnay, Riesling and Viognier wine made from whole bunch pressings, hard pressings and juice macerated for 60 hours was concentrated (wine freeze dried and reconstituted in 2% formic acid to yield a 10x concentration factor) was also analysed at the same time and this chromatogram and Figure A-4 : Reference compound HPLC-DAD chromatograms (graphically normalised) overlayed on a chromatogram of a mastermix of CHA, RIE, VIO whole bunch-pressed and high phenolic wines represents this chromatogram positioned under the peaks for the reference compounds. Comparing the RT of peaks in an unknown wine samples with this database of RTs and spectral characteristics it is possible to tentatively identify compounds in the unknown. However, there are potentially 100 phenolic compounds in white wine and the reference compounds cannot reflect all of these potential components.

The only way to identify the larger majority of these compounds is to use an accurate mass detection system instead of the diode array UV detector. The technique of HPLC-ESI-QTOF-MS/MS (Electrospray ionization quadrupole time-of-flight mass spectrometry) allows the effluent of the chromatographic column to be sprayed into a mass spectrometer where the mass of eluting compounds is determined with an accuracy of four decimal places. By comparing the mass of the majority ion of each compound and the breakdown products against all possible combinations of carbon, hydrogen, oxygen and sulfur it is possible to identify chromatographic peaks and most importantly for those which no commercial reference compounds are available. The difference between the measured mass and the theoretical mass of a tentatively identified compounds is Δ, given in mDa; this must be < 3 for a reliable fit of structure. All of the hydroxycinnamic acids have potentially two positional isomers (trans or cis) which cannot be assigned by mass spectrometry, only by running reference compounds or using NMR can these configurations be assigned and in the case of the tartaric esters and GRP conjugates these are not commerically available and therefore requiring lengthy and complicated synthesis. The same sample composite sample and reference compounds were analysed under the same chromatographic conditions as above. Trifluroacetic acid (TFA) was not included in the mobile phase to minimise ionisation suppression. This only had a marginal effect on peak shape for a small number of compounds. Major peaks were identified by comparing the exact mass of extracted molecular features against the MassBank database. With these new compounds identified, the UV spectra of the hitherto unknown peaks were added to the UV library potentially allowing better identification when using only the HPLC-DAD technique.

## A.3.4 Conclusion

This new analytical method using two inline Gemini C6-phenyl HPLC columns now makes it possible to analyse greater than 80 identified phenolic components. This degree of separation has been achieved with only very minimal sample handling (removal of alcohol). Such degree of separation has only been achieved to date with extensive time-consuming extraction/isolation (Baderschneider &

Winterhalter, 2000, 2001). A large proportion of these compounds are identified as glycosidically bound as well as methyl and ethyl esters of many of the phenolic acids.

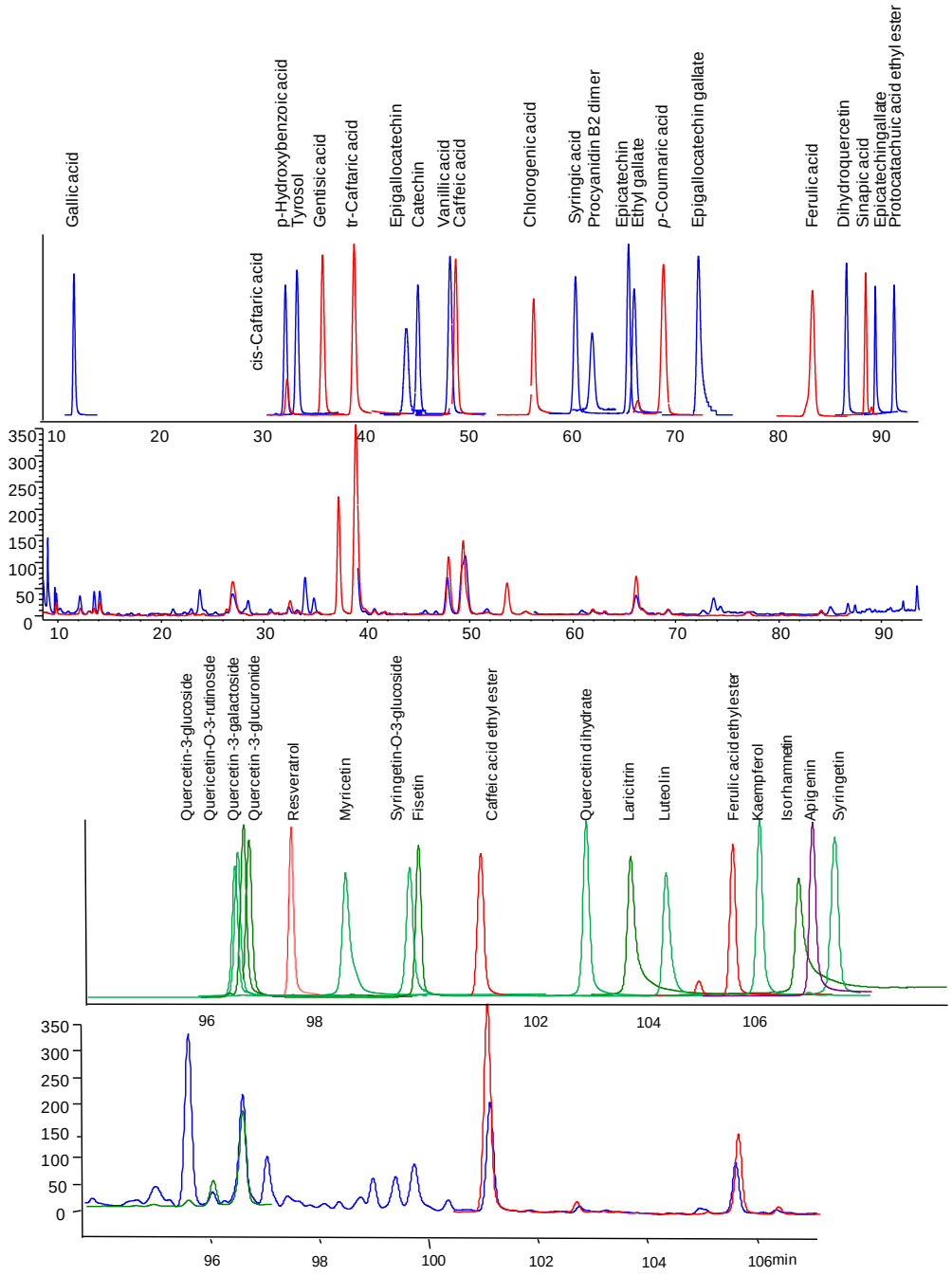



**Figure A-4 : Reference compound HPLC-DAD chromatograms (graphically normalised) overlayed on a chromatogram of a mastermix of CHA, RIE, VIO whole bunch-pressed and high phenolic wines**

## Table A-3. Identification of phenolic compounds in white wine determined by HPLC-ESI-QTOF-MS /MS

| RT (min) | $[M-H]^-$ | Δ (mDa) | Molecular Formula | Product ions (m/z) | Proposed compounds | Reference |
|---|---|---|---|---|---|---|
| 11.8 | 169.0149 | 1.7 | C7H6O5 | 125.024 | Gallic acid | MB#: KO000889, 9 |
| 20.5 | 153.0203 | 2.1 | C7H6O4 | 109.030 | Protocatechuric acid | 9 |
| 22.8 | 331.0669 | 1.0 | C13H16O10 | 169.015, 151.005, 125.025 | Gallic acid glucoside | |
| 23.6 | 487.0643 | -2.1 | C18H20N2O12S | 311.070, 211.007, 167.018, 149.010 | 2-S-(glycinylcysteinyl) caftaric acid | 8 |
| 23.8 | 305.0672 | 0.5 | C15H14O7 | 179.036, 167.036, 165.021, 139.040, 137.025, 125.025 | Epigallocatechin | |
| 25.2 | 430.0437 | -1.3 | C16H17NO11S | 298.040, 211.008, 167.018, 149.010 | 2-S-Cysteinyl caftaric acid | 8 |
| 26.0 | 315.0734 | 1.2 | C13H16O9 | 153.019, 152.013, 109.029, 108.020 | Protocatechuric acid glucoside | |
| 27.6 | 315.1077 | -0.8 | C14H20O8 | 153.057, 123.046 | Vanillyl alcohol glucoside | |
| 32.6 | 311.0415 | 0.6 | C13H12O9 | 179.036, 149.010, 135.046 | Caftaric acid | |
| 32.7 | 353.0880 | 0.2 | C16H18O9 | 191.057, 179.036, 173.047,135.046 | Chlorogenic acid | MB#: KO000468, 9 |
| 33.0 | 559.0858 | -0.7 | C21H24N2O14S | | 2-S-(γ-Glutamylcysteinyl) caftaric acid | |
| 34.6 | 616.1081 | -0.9 | C23H27N3O15S | 484.102, 466.092, 440.112, 272.089, 211.007, 167.017, 149.010 | 2-S-Glutathionyl-caftaric acid | 8 |
| 36.3 | 341.0884 | 1.7 | C15H18O9 | 179.037, 161.026 | Caffeic acid glucoside | |
| *36.4* | *921.1736* | *-2.5* | *C33H42O21N6S2* | | *2,5-di-Glutathionyl-caftaric acid* | *10* |
| 37.5 | 311.0413 | 0.4 | C13H12O9 | 179.036, 149.011, 135.046 | Caftaric acid | 8 |
| 38.8 | 325.0930 | 1.2 | C15H18O8 | 163.041 | p-Coumaric acid glucoside | |
| 40.1 | 616.1084 | -0.6 | C23H27N3O15S | 484.102, 466.091, 440.112, 272.089, 211.007, 167.018, 149.010 | 2-S-Glutathionyl-caftaric acid | |
| 41.0 | 616.1065 | -2.5 | C23H27N3O15S | 484.101, 466.090, 440.111, 272.086, 211.007, 167.018, 149.010 | 2-S-Glutathionyl-caftaric acid | |
| 42.7 | 299.0772 | 1.1 | C13H16O8 | 137.025 | Hydroxybenzoic acid glucoside | |

| RT (min) | [M-H]⁻ | Δ (mDa) | Molecular Formula | Product ions (m/z) | Proposed compounds | Reference |
|---|---|---|---|---|---|---|
| 43.0 | 577.1327 | -1.4 | C30H26O12 | | Procyanidin dimer | |
| 43.7 | 289.0720 | 0.2 | C15H14O6 | 245.082, 205.050, 203.071, 179.035, 151.040, 137.024, 125.024 | Catechin | |
| 44.0 | 577.1332 | -0.9 | C30H26O12 | 451.103, 425.087, 407.076, 289.071, 125.024 | Procyanidin dimer | |
| 44.2 | 285.0440 | 3.5 | C15H10O6 | 163.041, 120.997, 119.050 | Unknown Kaempferol like | MB#: PR100641 but not Kaempferol |
| 44.9 | 329.0879 | 1.2 | C14H18O9 | 167.036 | Vanillic acid glucoside (later than vinillyl alcohol glucoside?) | |
| 46.3 | 465.1024 | -0.4 | C21H22O12 | 303.051 (303.050) | Dihydroquercetin galactoside/glucoside (Rt is too early) | |
| 47.6 | 295.0462 | 0.3 | C13H12O8 | 163.041, 149.010, 119.050 | p-Coutaric acid | |
| 47.9 | 179.0364 | 1.4 | C9H8O4 | 135.046 | Caffeic acid | |
| 49.1 | 325.0934 | 0.5 | C15H18O8 | 163.041, 119.050 | p-Coumaric acid glucoside | |
| *50.3* | *325.0938* | *0.9* | *C15H18O8* | | *Coumaric acid glucoside* | |
| 52.4 | 295.0462 | 0.3 | C13H12O8 | 163.041, 149.010, 119.050 | p-Coutaric acid | |
| 55.9 | 865.1954 | -2.0 | C45H38O18 | 577.131 | Procyanidin trimer | |
| 58.9 | 481.0963 | -1.4 | C19H22O14 | | Dihydromyrcetin glucoside | |
| 60.1 | 577.1320 | -2.1 | C30H26O12 | | Procyanidin dimer | |
| *60.8* | *165.0559* | *1.3* | *C9H10O3* | | *Hydroxybenzoic acid ethyl ester?* | |
| 61.5 | 355.1026 | -0.9 | C16H20O9 | 193.051, 176.044, 175.041 | Ferulic acid glucoside | |
| 61.7 | 325.0550 | -1.5 | C14H14O9 | 193.051, 149.010, 134.037 | Fertaric acid | |
| *63.9* | *355.1038* | *0.3* | *C16H20O9* | | *Ferulic acid glucoside* | |
| 64.2 | 289.0714 | -0.4 | C15H14O8 | 245, 205, 203, 179, 151, 125, 109 | epicatechin | |
| 64.7 | 644.1381 | -1.6 | C25H31N3O15S | 512.131, 469.144, 468.143, 300.119, 211.006, 167.017, 149.010 | 2-S-Glutathionylcaftaric acid ethyl ester | 8 |

| RT (min) | [M-H]⁻ | Δ (mDa) | Molecular Formula | Product ions (m/z) | Proposed compounds | Reference |
|---|---|---|---|---|---|---|
| 65.1 | 325.0559 | -0.6 | C14H14O9 | 193.051, 149.061, 134.037 | Fertaric acid | |
| 66.3 | 389.1229 | -1.3 | C20H22O8 | 269.081, 241.086 | Resveratrol--C- glucoside | 11 |
| 66.4 | 197.0454 | -0.1 | C9H10O5 | 169.015, 162.840, 160.842, 125.024 | Gallic acid ethyl ester | |
| 68.0 | 389.1230 | -1.2 | C20H22O8 | | Resveratrol-glucoside | |
| 73.6 | 366.1183 | 0.0 | C17H21NO8 | 201.067, 186.056, 142.066 | Indolelactic acid glucoside | Fragment ions are reasonable |
| 76.9 | 167.0355 | 0.5 | C8H8O4 | | Protocatechuric acid methyl ester | |
| 78.3 | 465.1019 | -0.9 | C21H22O12 | | Dihydroquercetin glucoside | |
| 79.7 | 577.1326 | -1.5 | C30H26O12 | | Procyanidin dimer | |
| 82.0 | 521.1999 | -1.8 | C26H34O11 | 359.148 | Lignan 26, 28 or 30a | 3 |
| 84.2 | 644.1383 | -1.4 | C25H31N3O15S | | 2-S-Glutathionylcaftaric acid ethyl ester | 8 |
| 84.7 | 403.1593 | -0.57 | C18H28O10 | 241.108, 197.118 | Unknown (glucoside) | |
| 85.4 | 303.0512 | 0.2 | C15H12O7 | 285.0471, 178.999, 177.021, 151.003, 153.01925, 125.024 | Dihydroquercetin | |
| 85.8 | 465.1014 | -1.4 | C21H22O12 | 303.050, 285.039, 259.060, 178.999, 151.004, 125.023 | Dihydroquercetin glucoside | 3 |
| 86.5 | 435.0928 | 0.6 | C20H20O11 | 303.049, 285.039, 241.050, 178.997, 151.004, 125.023 | Dihydroquercetin Xyloside | 3 |
| 86.4 | 644.1380 | -1.7 | C25H31N3O15S | 466.090, 371.041, 272.087, 211.006, 192.996, 177.040 | 2-S-Glutathionylcaftaric acid (ethyl tartarate) | 8 |
| 87.7 | 521.2002 | -1.5 | C26H34O11 | | Lignan 26, 28 or 30a | |
| 87.8 | 389.1236 | -0.6 | C20H22O8 | 227.071 | Resveratrol-*O*-glucoside | 5,6 |
| 88.7 | 479.0809 | -1.1 | C21H20O13 | | *Myricetin glucoside* | |
| *89.0* | *493.0632* | *1.9* | *C21H18O14* | | *Myricetin glucuronide* | |
| 89.8 | 181.0519 | 1.3 | C9H10O4 | 153.020, 152.012,109.029, 108.021 | Gentistic acid/Protocatechuric acid ethyl ester | |

| RT (min) | [M-H]⁻ | Δ (mDa) | Molecular Formula | Product ions (m/z) | Proposed compounds | Reference |
|---|---|---|---|---|---|---|
| 90.4 | 305.0301 | 1.0 | C14H10O8 | 169.015, 151.005, 125.025, 107.013 | 2-(3,4-Dihydroxybenzoyloxy)-4,6-dihydroxybenzoate | (Ring-open quercetin) |
| 91.2 | 339.0728 | 0.6 | C15H16O9 | 179.038, 177.041, 161.025, 159.031, 103.003 | Caftaric acid ethyl ester | 3 |
| 92.0 | 523.2134 | -3.9 | C26H36O11 | | Lignan 31a | |
| 92.0 | 339.0728 | 0.6 | C15H16O9 | 179.038, 177.041, 161.025, 159.031, 103.003 | Caftaric acid ethyl ester | 3 |
| 92.3 | 449.1073 | *-0.5* | C21H22O11 | 303.050, 285.0340, 269.138, 151.0043 | Dihydroquercetin rhamnoside | 5, |
| 92.5 | 287.0549 | *-0.1* | C15H12O6 | 259.061, 243.066, 201.054, 178.998, 125.024 | Dihydrokaempferol | |
| 92.9 | 463.0861 | -0.1 | C21H20O12 | 301.034, 300.026, 161.045 | Quercetin galactoside/glucoside | 5,7 |
| 92.9 | 463.0863 | 0.1 | C21H20O12 | 301.034, 300.026, 178.999, 161.045, 151.004 | Quercetin glucoside/galactoside | 5,7 |
| 93.1 | 477.0664 | -1.1 | C21H18O13 | 301.035, 178.999,151.005, 113.024 | Quercetin glucuronide | MB#: PR100978, 5 |
| 93.4 | 323.0778 | 1.7 | C15H16O9 | 177.042, 159.031, 145.030, 130.999, 103.002 | Coutaric acid ethyl ester | |
| 93.7 | 389.1240 | 0.9 | C20H22O8 | 227.072 | Resveratrol-*O*-glucoside | 5,6 |
| *94.9* | *435.1275* | -1.0 | *C21H24O10* | *289.071, 273.076, 225.055, 167.035, 125.020* | *3-deoxyleucocyanidin rhamnoside* | |
| 95.4 | 447.0921 | -0.1 | C21H20O11 | 301.034, 300.027 | Quercetin rhamnoside | |
| 95.5 | 323.0778 | 1.7 | C15H16O8 | 177.046, 163.046, 159.031, 151.006, 145.032 | Coutaric acid ethyl ester | 3, reported only caffeoyl ethyl tartrate |
| 95.6 | 447.0924 | 0.2 | C21H20O11 | 285.041, 284.033 | Kaempferol glucoside | 3 |
| 95.7 | 433.1136 | -0.4 | C21H22O10 | 287.055, 286.0472, 269.045, 259.061,180.007, 178.999, 151.004 | Dihydrokaempferol rhamnoside | 3 |
| 95.9 | 353.0876 | 0.9 | C16H18O9 | 177.042 | Fertaric acid ethyl ester | |
| *96.0* | *461.0717* | *0.2* | *C21H18O12* | | *Kaempferol glucuronide?* | |
| 96.3 | 353.0870 | 0.3 | C16H18O9 | 207.067, 193.051, 177.043, 159.031, 115.040 | Fertaric acid ethyl ester | 3, reported only caffeoyl ethyl tartrate |
| 96.7 | 227.0718 | 1.5 | C14H12O3 | 185.060, 143.048 | Resveratrol | MB#: PB003661, 4, 5 |

| RT (min) | [M-H]⁻ | Δ (mDa) | Molecular Formula | Product ions (m/z) | Proposed compounds | Reference |
|---|---|---|---|---|---|---|
| 97.6 | 207.0669 | 0.6 | C11H12O4 | 179.036, 161.026, 135.046 | Caffeic acid ethyl ester | 2 |
| 99.1 | 301.0363 | 0.9 | C15H10O7 | 273.041, 180.004, 179.000, 169.016, 152.008, 151.005, 121.030 | Quercetin | 1 |
| 101.6 | 191.0724 | 2.1 | C17H12O3 | | Coumaric acid ethyl ester | |
| 102.6 | 285.0416 | 1.1 | C15H10O6 | 257.016, 151.005 | Kaempferol | MB#: PR040026, 1 |
| 103.3 | 315.0512 | 0.2 | C16H12O7 | 300.028, 271.062, 227.079, 150.997 | Isorhamnetin | MB#: PR040022 |

MB# = MassBank Record number

**References in table:**


1. Fabre *et al.* (2001)
2. Frega *et al.* (2006)
3. Baderschneider & Winterhalter (2001)
4. Careri (2004)
5. Monagas *et al.* (2005)
6. Baderschneider & Winterhalter (2000)
7. Hvattum (2002)
8. Cejudo-Bastante *et al.* (2010)
9. Sun *et al.* (2007)
10. Salgues *et al.* (1986)
11. Püssa *et al.* (2006)

# B Administrative Appendices

## B.1Communication

A critical aspect of this project has been ongoing dialogue with a broad range of wine industry stakeholders to confirm the relevance and help to set the direction of the project for the Australian wine industry.

An Industry Reference Group Meeting was held in July 2009 to present a summary of the project (UA 06/03) that preceded this one (AWRI 0901) and discussions and feedback on project directions and winemaker views were sourced.

Two articles in Industry publications that are relevant, but precede the timeframe of this project, are:

- Gawel, R.; Dimanin, P. A.-G.; Francis, I. L.; Waters, E. J.; Herderich, M. J.; S., P. Coarseness in white table wine. Aust NZ Wine Ind J. 2008, 23, 19-22.
- Parker, M.; Mercurio, M.; Jeffery, D.; Herderich, M.; Holt, H.; Smith, P. A. An overview of the phenolic chemistry of white juice and wine production. Aust. N.Z. Grapegrower Winemaker 2007, 509a, 74-80.

Project outcomes and a workshop was presented at 14 AWITC in July 2010 and project outcomes have been developed into AWRI road show material for delivery to regional groups across Australia

A half day workshop entitled "White Wine Phenolics" was presented at the Australian Wine Industry Technical Conference; convened by Richard Gawel, with speakers Martin Day, Steve Van Sluyter, Richard Gawel (AWRI), Hildegarde Heymann (UC Davis) and Peter Leske (SAWIA, Industry practitioner, *inter alia*). Informal but unprompted feedback from attendees suggested that the workshop was very well received. The workshop was fully subscribed with delegates from Australia, New Zealand, Spain, South Africa and California. The workshop explored the concept of phenolic taste in white wines, and the desirability or otherwise of building phenolic structure into white wines. The 2010 project wines were presented for discussion, as were phenolic fractions taken from Fiano and Gewurztraminer commercial wines. Twelve international commercial wines thought to display phenolic character were also tasted and discussed in light of their measured phenolic and polysaccharide content.

A presentation titled 'Putting the texture back into white wines. The role of phenolics, polysaccharides, alcohol and pH in white wine structure and style' was delivered by Richard Gawel at the 63rd American Society for Enology and Viticulture National Conference in June 2012 in Portland, Oregon.

A presentation titled 'The role of phenolic composition, pH, polysaccharides and alcohol level on the in-mouth texture of white wine' was delivered by Richard Gawel at the 'Crush 2011' conference in September 2011 on the Waite Campus, Adelaide.

An AWRI road-show presentation titled 'Putting the texture back into white wine – the role of white wine phenolics' has been developed and made available for AWRI road-shows. This presentation has been presented in Southern Victoria (Gippsland, Mornington Peninsula and Yarra Valley) in November 2010 and Coonawarra in November 2011.

In April 2011 an article titled 'Towards better use of White wine phenolics' was included in the GWRDC 'R&D at work' newsletter that is published in Australian Grapegrower and Winemaker.

Submission of several peer-reviewed publications is anticipated in 2012, including manuscripts titled;

- The role of phenolics in white wine style, winemaker perception of quality and consumer acceptance.
- The effect of pH and alcohol on the phenolic character in white wine.
- The effect of the major white wine phenolics Caftaric acid and Grape Reaction Product on the phenolic taste of model wine.
- A comprehensive HPLC method development for analysing phenolics in white juices and wines.

Submission to an Industry publication of a manuscript titled 'Putting the texture back into white wines' is anticipated in 2012.

The AWRI roadshow 'Putting the texture back into white wine – the role of white wine phenolics' continues to be available to regions for selection as a presentation in the coming AWRI roadshow calendar.

## B.2 Intellectual Property

Project intellectual property includes analytical methods for isolation and quantification of molecules and knowledge about which molecules are likely lead candidates for investigation in relation to impacts on sensory properties. None of the intellectual property is anticipated to require patenting or is subject to other forms of IP protection. It is anticipated that the majority of the findings will be published.

# B.3References

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# B.4 Supplementary Sensory and Analytical Data

**Table B-1: Taster Concordance Values by Attribute – 2011 wines (p values < 0.56 significant at 10%)**

| Judge | Astringency | Bitter | Metallic | Oily | Viscous | Prickly | Burning | Hotness | Acid | Acid AT |
|---|---|---|---|---|---|---|---|---|---|---|
| 595 | n/a | 0.44 | 0.28 | n/a | 0.34 | 0.37 | 0.04 | 0.09 | 0.33 | 0.27 |
| 601 | 0.35 | 0.46 | 0.24 | 0.26 | 0.24 | n/a | 0.33 | 0.43 | 0.29 | 0.40 |
| 602 | 0.44 | 0.18 | 0.24 | 0.21 | 0.11 | 0.16 | 0.27 | 0.36 | 0.25 | 0.22 |
| 603 | 0.53 | 0.21 | n/a | 0.08 | 0.20 | n/a | 0.43 | 0.16 | 0.18 | 0.45 |
| 604 | 0.51 | 0.26 | 0.23 | 0.25 | 0.34 | n/a | 0.41 | 0.20 | 0.79 | 0.34 |
| 605 | 0.32 | 0.22 | 0.34 | 0.12 | 0.25 | 0.16 | 0.58 | 0.27 | 0.51 | 0.55 |
| 608 | 0.44 | 0.35 | 0.34 | 0.19 | 0.45 | 0.43 | 0.44 | 0.39 | 0.34 | 0.47 |
| 609 | 0.49 | 0.16 | 0.13 | 0.17 | 0.38 | 0.08 | 0.15 | 0.44 | 0.23 | 0.28 |
| 615 | 0.22 | 0.13 | 0.18 | 0.22 | 0.17 | 0.23 | 0.48 | 0.60 | 0.30 | 0.31 |
| 618 | 0.37 | 0.47 | 0.35 | 0.49 | 0.38 | 0.35 | 0.29 | 0.56 | 0.26 | 0.40 |
| 702 | 0.15 | 0.34 | 0.08 | 0.46 | 0.56 | 0.43 | 0.14 | 0.08 | 0.52 | 0.58 |
| **Overall** | **0.202** | **0.148** | **0.100** | **0.236** | **0.109** | **0.178** | **0.072** | **0.035** | **0.121** | **0.120** |
| **p** | **0.037** | **0.111** | **0.368** | **0.014** | **0.295** | **0.180** | **0.610** | **0.929** | **0.222** | **0.220** |

## Table B-2: Basic analyses of commercial wines used for investigation of style and perceived quality.

| Wine | Variety/style | Year | Alcohol % v/v | pH | TA (g/L) | G + F (g/L) | Acetic acid (g/L) | Free $SO_2$ (mg/L) | Total $SO_2$ (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Nepenthe | Unwooded Chardonnay | 2007 | 12.9 | 3.3 | 6.8 | 0.5 | 0.2 | 22.6 | 124.0 |
| Goundrey Homestead | Unwooded Chardonnay | 2006 | 13.2 | 3.4 | 5.9 | 3.0 | 0.4 | 16.3 | 139.7 |
| Yalumba Y Series | Unwooded Chardonnay | 2007 | 13.2 | 3.4 | 6.3 | 3.6 | 0.3 | 24.4 | 132.0 |
| Capel Vale | Unwooded Chardonnay | 2007 | 13.7 | 3.6 | 5.7 | 1.0 | 0.5 | 27.6 | 123.3 |
| Chapel Hill | Unwooded Chardonnay | 2007 | 13.6 | 3.3 | 6.9 | 1.8 | 0.4 | 20.4 | 127.0 |
| Saltram Makers Table | Unwooded Chardonnay | 2007 | 13.5 | 3.5 | 5.8 | 4.4 | 0.4 | 25.8 | 138.3 |
| Cape Jaffa | Unwooded Chardonnay | 2007 | 13.3 | 3.3 | 6.6 | 3.6 | 0.3 | 14.7 | 107.3 |
| Orlando St. Helga | Riesling | 2007 | 12.4 | 3.2 | 6.8 | 0.9 | 0.2 | 16.3 | 105.3 |
| Knappstein Handpicked | Riesling | 2007 | 13.3 | 3.1 | 7.3 | 3.1 | 0.4 | 18.8 | 176.0 |
| Jacob's Creek Reserve | Riesling | 2007 | 13.3 | 3.1 | 6.8 | 1.4 | 0.2 | 16.8 | 108.0 |
| Hardys Siegersdorf | Riesling | 2006 | 12.5 | 3.0 | 7.1 | 3.1 | 0.3 | 24.0 | 128.3 |
| Leo Buring Clare Valley | Riesling | 2007 | 12.6 | 3.1 | 7.2 | 2.7 | 0.3 | 21.8 | 125.7 |
| Pewsey Vale | Riesling | 2007 | 12.9 | 3.1 | 6.4 | 3.6 | 0.3 | 25.0 | 142.7 |
| Pikes | Riesling | 2007 | 12.4 | 3.0 | 7.4 | 3.3 | 0.4 | 27.8 | 132.7 |
| Skillogallee | Riesling | 2007 | 12.8 | 3.0 | 7.5 | 2.2 | 0.4 | 18.2 | 125.7 |
| Wirra Wirra The Lost Watch | Riesling | 2007 | 12.2 | 3.1 | 6.5 | 0.6 | 0.2 | 21.2 | 98.9 |
| Wynns Coonawarra Estate | Riesling | 2007 | 12.2 | 3.1 | 6.7 | 1.2 | 0.3 | 19.7 | 118.0 |
| Richmond Grove | Pinot Grigio | 2007 | 12.5 | 3.3 | 6.3 | 1.0 | 0.3 | 15.0 | 86.4 |
| Yalumba Y Series | Pinot Grigio | 2007 | 12.7 | 3.3 | 5.7 | 2.8 | 0.2 | 28.6 | 97.0 |
| Jacob's Creek | Chardonnay | 2007 | 13.0 | 3.4 | 5.8 | 4.0 | 0.4 | 27.9 | 120.0 |
| Brookland Valley Verse 1 | Chardonnay | 2006 | 13.3 | 3.4 | 6.1 | 2.5 | 0.4 | 17.8 | 99.8 |
| Jamieson's Run | Chardonnay | 2007 | 13.6 | 3.6 | 5.4 | 3.5 | 0.4 | 29.6 | 133.0 |
| Queen Adelaide | Chardonnay | 2007 | 13.5 | 3.5 | 5.7 | 4.3 | 0.4 | 26.7 | 136.7 |
| Richmond Grove French Cask | Chardonnay | 2007 | 13.1 | 3.4 | 5.9 | 2.5 | 0.4 | 25.3 | 119.7 |

## Table B-3: List of sensory attributes and their definitions

| | | |
|---|---|---|
| **Chapter 3** | **Attribute** | **Definition** |
| | Astringency | The drying and mouth puckering sensation. |
| | Viscosity | The perception of body, weight or thickness of the wine in the mouth. |
| | Hotness | The level of hotness or warmth in the mouth after expectorating. |
| | Bitter | The intensity of bitter taste. |
| | Acid Aftertaste | The persistence of acidity after expectorating. |
| **Chapter 7** | **Attribute** | **Definition** |
| | Astringency | The drying and mouth puckering sensation in the mouth or after expectorating includes furry, chalky, grippy. |
| | Viscosity | The body, weight or thickness of the wine in the mouth. |
| | Hotness | The level of warmth or heat. |
| | Bitter | The bitter taste and or after taste. |
| | Oiliness | The feeling of oil in the mouth, mouth coating, a lingering oily feel. |
| | Acid Aftertaste | The acid/sourness tasted after expectorating. |
| | Acid | The sour/ acid taste, tangy, tart, the taste of lemon. |
| | Metallic | The metallic taste, spoon in the mouth, steely taste. |
| | Prickly | The prickly sensation on the tongue and after expectorating. |
| | Hotness Aftertaste | The warmth or heat after expectorating. |
| | Burning Aftertaste | The chilli like burning sensation in the mouth, different from hotness. |
| **Chapter 8-2010 wines** | **Attribute** | **Definition** |
| | Astringency | The drying and mouth puckering sensation. |
| | Viscosity | The perception of body, weight or thickness of the wine in the mouth. |
| | Hotness | The level of hotness or warmth perceived after expectorating. |
| | Bitter Aftertaste | The persistence of bitterness perceived in the mouth after expectorating. |
| | Acid Aftertaste | The persistence of acid perceived in the mouth after expectorating. |
| | Acid | Intensity of sour/acid taste. Tangy, tart in mouth. |
| **Chapter 8 – 2011 wines** | **Attribute** | **Definition** |
| | Astringency | The drying and mouth puckering sensation. Includes astringent aftertaste and chalky. |
| | Viscosity | The perception of the body, weight, thickness of wine in the mouth. |
| | Hotness | Hotness or warmth related to ethanol perceived in the mouth & after expectorating. |
| | Metallic | The 'tinny' flavour associated with metals and blood. |
| | Bitterness | The intensity of bitter taste perceived in the mouth and after expectorating. |
| | Oily | Intensity of mouthcoating buttery feel, oiliness on the surface of the mouth, including lips, both while in the mouth and after expectorating. |
| | Acid Aftertaste | Intensity of sour/acid taste after expectorating. |
| | Acid | Intensity of sour/acid taste. |
| | Burning aftertaste | Chemical burning, sensation of lips, tongue and/or throat associated with peppery olive oil, chilli or black pepper burn, includes prickly and tingly. |

**Table B-4: Standards used to Illustrate Sensory Attributes (unless specified otherwise in the text)**

| Attribute | Standards Presented |
|---|---|
| Sour | 1 g/L tartaric acid in RO water |
| Metallic | 0.016 g/L $FeSO_4$ in RO water |
| Bitter | 0.015 g/L quinine sulfate |
| Astringent (1) | 0.5 g/L aluminium potassium sulfate |
| Astringent (2) | Green banana – 2 cm cubes |
| Viscosity | 3 g/L carboxymethyl cellulose in RO water |
| Hotness | 20% ethanol in RO water |
| Burning (1) | Extra virgin olive oil |
| Burning (2) | Rocket leaves |
| Spritz | Sparkling mineral water |

## Table B-5. Chemical analysis of 2010 project wines

| Full Wine Code | Glu + Fru (g/L) | pH | TA (g/L) | Free $SO_2$ (ppm) | Total $SO_2$ (ppm) | % Alcohol (v/v) | Acetic acid (g/L) |
|---|---|---|---|---|---|---|---|
| CHA-WBP-rep1 | 0.96 | 3.14 | 6.7 | 34 | 144 | 13.2 | 0.32 |
| CHA-WBP-rep2 | 0.62 | 3.11 | 6.8 | 32 | 144 | 13.2 | 0.34 |
| CHA-FR-rep1 | 0.72 | 3.20 | 6.8 | 32 | 128 | 13.5 | 0.36 |
| CHA-FR-rep2 | 0.64 | 3.19 | 6.7 | 32 | 130 | 13.7 | 0.33 |
| CHA-LP-rep1 | 0.60 | 3.20 | 7.2 | 34 | 127 | 13.5 | 0.32 |
| CHA-LP-rep2 | 0.57 | 3.17 | 7.2 | 30 | 123 | 13.4 | 0.34 |
| CHA-HP-rep1 | 0.52 | 3.22 | 7.0 | 35 | 163 | 13.5 | 0.32 |
| CHA-HP-rep2 | 0.59 | 3.22 | 7.2 | 34 | 157 | 13.5 | 0.36 |
| CHA-HOX-FR-rep1 | 0.75 | 3.21 | 6.5 | 37 | 128 | 13.6 | 0.35 |
| CHA-HOX-FR-rep2 | 0.58 | 3.19 | 6.5 | 34 | 127 | 13.6 | 0.35 |
| CHA-HOX-LHP-rep1 | 0.43 | 3.20 | 6.8 | 37 | 125 | 13.1 | 0.34 |
| CHA-HOX-LHP-rep2 | 0.42 | 3.22 | 6.8 | 32 | 115 | 13.2 | 0.34 |
| CHA-MAC-rep1 | 0.49 | 3.10 | 8.3 | 32 | 106 | 13.2 | 0.35 |
| CHA-MAC-rep2 | 0.54 | 3.10 | 8.2 | 32 | 106 | 13.3 | 0.33 |
| RIE-WBP-rep1 | 0.45 | 3.08 | 7.1 | 30 | 83 | 12.9 | 0.37 |
| RIE-WBP-rep2 | 0.36 | 3.07 | 7.4 | 30 | 86 | 12.9 | 0.35 |
| RIE-FR-rep1 | 0.39 | 3.07 | 7.1 | 37 | 96 | 13.0 | 0.34 |
| RIE-FR-rep2 | 0.88 | 3.05 | 6.8 | 30 | 84 | 13.1 | 0.29 |
| RIE-LP-rep1 | 0.20 | 3.17 | 6.5 | 31 | 81 | 12.8 | 0.36 |
| RIE-LP-rep2 | 0.19 | 3.17 | 6.5 | 30 | 84 | 12.7 | 0.36 |
| RIE-HP-rep1 | 0.32 | 3.13 | 6.8 | 30 | 118 | 12.8 | 0.36 |
| RIE--HP-rep2 | 0.30 | 3.11 | 6.7 | 32 | 117 | 12.8 | 0.34 |
| RIE-HOX-FR-rep1 | 0.64 | 2.94 | 8.0 | 37 | 96 | 12.7 | 0.34 |
| RIE--HOX-FR-rep2 | 0.66 | 2.94 | 7.8 | 34 | 93 | 12.9 | 0.32 |
| RIE-MAC-rep1 | 0.66 | 3.04 | 7.6 | 32 | 94 | 12.5 | 0.36 |
| RIE-MAC-rep2 | 0.51 | 3.01 | 7.3 | 32 | 93 | 12.4 | 0.33 |
| VIO-WBP-rep1 | 0.47 | 2.99 | 8.1 | 34 | 118 | 13.3 | 0.38 |
| VIO-WBP-rep2 | 0.43 | 2.99 | 8.2 | 32 | 149 | 13.2 | 0.37 |
| VIO-FR-rep1 | 1.65 | 2.89 | 8.4 | 32 | 117 | 13.2 | 0.36 |
| VIO-FR-rep2 | 1.05 | 3.06 | 8.1 | 35 | 136 | 13.6 | 0.35 |
| VIO-LP-rep1 | 0.54 | 3.14 | 7.9 | 32 | 118 | 13.4 | 0.35 |
| VIO-LP-rep2 | 0.62 | 3.16 | 7.8 | 35 | 125 | 13.4 | 0.36 |
| VIO-HP-rep1 | 0.64 | 3.20 | 7.9 | 30 | 123 | 13.4 | 0.37 |
| VIO-HP-rep2 | 0.93 | 3.20 | 7.9 | 37 | 130 | 13.4 | 0.38 |
| VIO-HOX-FR-rep1 | 0.96 | 2.97 | 7.9 | 43 | 128 | 13.2 | 0.35 |
| VIO-HOX-FR-rep2 | 1.03 | 3.01 | 7.9 | 32 | 114 | 13.3 | 0.36 |
| VIO-HOX-LHP-rep1 | 1.05 | 3.07 | 8.3 | 32 | 125 | 13.5 | 0.39 |
| VIO-HOX-LHP-rep2 | 1.05 | 3.07 | 8.4 | 32 | 130 | 13.4 | 0.39 |
| VIO-MAC-rep1 | 0.57 | 3.34 | 7.1 | 32 | 155 | 13.2 | 0.37 |
| VIO-MAC-rep2 | 0.71 | 3.33 | 7.3 | 30 | 152 | 13.3 | 0.38 |

*CHA: Chardonnay, RIE: Riesling, VIO: Viognier; WBP: whole bunch pressed, FR: free run (<0.5 bar), LP: light pressings (< 1.5 bar), HP: heavy pressings ( up to 2 bar); HOX: hyperoxidised juice*

## Table B-6. Somers' and other spectral data for 2010 project wines

| Full Wine Code | $A^{10}_{320}$ (2mm)* | $A^{10}_{280}$ (2mm) | $A^{10}_{420}$ (10mm) | $A^{10}_{370}$ (10mm) | Total Phenolics (au) | Total HCA (au) | Flavonoid Extract (au) |
|---|---|---|---|---|---|---|---|
| CHA-WBP-rep1 | 0.525 | 0.859 | 0.057 | 0.415 | 0.29 | 1.22 | 0 |
| CHA-WBP-rep2 | 0.528 | 0.855 | 0.050 | 0.404 | 0.28 | 1.24 | 0 |
| CHA-FR-rep1 | 0.691 | 1.023 | 0.057 | 0.472 | 1.12 | 2.06 | 0 |
| CHA-FR-rep2 | 0.696 | 1.019 | 0.055 | 0.478 | 1.10 | 2.08 | 0 |
| CHA-LP-rep1 | 0.708 | 1.191 | 0.081 | 0.531 | 1.96 | 2.14 | 0.53 |
| CHA-LP-rep2 | 0.708 | 1.199 | 0.088 | 0.546 | 1.99 | 2.14 | 0.57 |
| CHA-HP-rep1 | 0.747 | 1.276 | 0.085 | 0.548 | 2.38 | 2.34 | 0.82 |
| CHA-HP-rep2 | 0.763 | 1.288 | 0.086 | 0.555 | 2.44 | 2.41 | 0.83 |
| CHA-HOX-FR-rep1 | 0.591 | 0.974 | 0.064 | 0.516 | 0.87 | 1.55 | 0 |
| CHA-HOX-FR-rep2 | 0.571 | 0.948 | 0.060 | 0.503 | 0.74 | 1.46 | 0 |
| CHA-HOX-LHP-rep1 | 0.400 | 0.967 | 0.072 | 0.375 | 0.84 | 0.60 | 0.43 |
| CHA-HOX-LHP-rep2 | 0.401 | 0.966 | 0.072 | 0.376 | 0.83 | 0.60 | 0.43 |
| CHA-MAC-rep1 | 0.968 | 1.362 | 0.073 | 0.636 | 2.81 | 3.44 | 0.52 |
| CHA-MAC-rep2 | 1.012 | 1.391 | 0.073 | 0.659 | 2.96 | 3.66 | 0.52 |
| RIE-WBP-rep1 | 0.861 | 1.005 | 0.056 | 0.541 | 1.02 | 2.91 | 0 |
| RIE-WBP-rep2 | 0.856 | 0.997 | 0.054 | 0.543 | 0.99 | 2.88 | 0 |
| RIE-FR-rep1 | 1.218 | 1.325 | 0.066 | 0.719 | 2.63 | 4.69 | 0 |
| RIE-FR-rep2 | 1.195 | 1.229 | 0.061 | 0.693 | 2.14 | 4.58 | 0 |
| RIE-LP-rep1 | 1.292 | 1.453 | 0.077 | 0.721 | 3.26 | 5.06 | 0 |
| RIE-LP-rep2 | 1.299 | 1.449 | 0.076 | 0.726 | 3.24 | 5.10 | 0 |
| RIE-HP-rep1 | 1.253 | 1.483 | 0.074 | 0.721 | 3.41 | 4.87 | 0.17 |
| RIE--HP-rep2 | 1.231 | 1.463 | 0.073 | 0.715 | 3.32 | 4.75 | 0.15 |
| RIE-HOX-FR-rep1 | 0.536 | 0.885 | 0.057 | 0.413 | 0.42 | 1.28 | 0 |
| RIE--HOX-FR-rep2 | 0.473 | 0.845 | 0.057 | 0.383 | 0.22 | 0.96 | 0 |
| RIE-MAC-rep1 | 1.819 | 1.766 | 0.066 | 0.983 | 4.83 | 7.70 | 0 |
| RIE-MAC-rep2 | 1.905 | 1.822 | 0.071 | 1.051 | 5.11 | 8.13 | 0 |
| VIO-WBP-rep1 | 0.744 | 0.933 | 0.048 | 0.591 | 0.66 | 2.32 | 0 |
| VIO-WBP-rep2 | 0.743 | 0.938 | 0.048 | 0.584 | 0.69 | 2.31 | 0 |
| VIO-FR-rep1 | 0.960 | 1.086 | 0.065 | 0.640 | 1.43 | 3.40 | 0 |

| Full Wine Code | $A^{10}_{320}$ (2mm)* | $A^{10}_{280}$ (2mm) | $A^{10}_{420}$ (10mm) | $A^{10}_{370}$ (10mm) | Total Phenolics (au) | Total HCA (au) | Flavonoid Extract (au) |
|---|---|---|---|---|---|---|---|
| VIO-FR-rep2 | 0.920 | 1.043 | 0.053 | 0.613 | 1.22 | 3.20 | 0 |
| VIO-LP-rep1 | 0.806 | 1.122 | 0.069 | 0.773 | 1.61 | 2.63 | 0 |
| VIO-LP-rep2 | 0.790 | 1.123 | 0.069 | 0.775 | 1.61 | 2.55 | 0 |
| VIO-HP-rep1 | 0.811 | 1.227 | 0.079 | 0.878 | 2.13 | 2.66 | 0.37 |
| VIO-HP-rep2 | 0.815 | 1.235 | 0.078 | 0.878 | 2.18 | 2.67 | 0.39 |
| VIO-HOX-FR-rep1 | 0.699 | 0.885 | 0.049 | 0.676 | 0.42 | 2.09 | 0 |
| VIO-HOX-FR-rep2 | 0.720 | 0.896 | 0.051 | 0.687 | 0.48 | 2.20 | 0 |
| VIO-HOX-LHP-rep1 | 0.719 | 1.139 | 0.094 | 0.780 | 1.70 | 2.19 | 0.24 |
| VIO-HOX-LHP-rep2 | 0.721 | 1.151 | 0.095 | 0.788 | 1.75 | 2.20 | 0.29 |
| VIO-MAC-rep1 | 1.279 | 1.696 | 0.093 | 1.117 | 4.48 | 5.00 | 1.15 |
| VIO-MAC-rep2 | 1.234 | 1.597 | 0.075 | 1.071 | 3.99 | 4.77 | 0.81 |

Measured in a 2 mm but expressed as 10 mm

## Table B-7. Phenolic compounds identified in 2010 project wines and their classification groups

| Elution order | Compound ID | Poly phenol Group (PP) | PP/ +glycoconj/ +EE | Unit |
|---|---|---|---|---|
| 44 | Ethyl gallate-like | Benzoic Acid | Benzoic acid EE | mg/L GAE |
| 1 | Gallic acid | Benzoic Acid | Benzoic acid | mg/L GAE |
| 37 | Gallic acid ethyl ester | Benzoic Acid | Benzoic acid EE | mg/L GAE |
| 5 | Gallic acid glucoside | Benzoic Acid | Benzoic acid glycoconj | mg/L GAE |
| 4 | Protocatachuic acid | Benzoic Acid | Benzoic acid | mg/L GAE |
| 38 | Protocatachuic acid ethyl ester | Benzoic Acid | Benzoic acid EE | mg/L GAE |
| 27 | Syringic acid | Benzoic Acid | Benzoic acid | mg/L GAE |
| 31 | Unknown 280 (1) | Benzoic Acid | Benzoic acid | mg/L GAE |
| 2 | Unknown Flavanone-like | Benzoic Acid | Benzoic acid | mg/L GAE |
| 3 | Unknown Flavanonol-like | Benzoic Acid | Benzoic acid | mg/L GAE |
| 47 | Caffeic acid ethyl ester | Cinnamic acid | Cinnamic acid EE | mg/L FAE |
| 13 | Caftaric acid | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 39 | Caftaric acid ethyl ester | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 50 | Coumaric acid ethyl ester | Cinnamic acid | Cinnamic acid EE | mg/L FAE |
| 48 | Coutaric acid ethyl ester | Cinnamic acid | Cinnamic acid EE | mg/L FAE |
| 23 | Fertaric acid | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 21 | Ferulic ac-glucoside | Cinnamic acid | Cinnamic acid glycoconj | mg/L FAE |
| 32 | Ferulic acid | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 30 | Ferulic acid-like | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 8 | mixed HCA? | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 25 | p-Coumaric acid | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 18 | p-coutaric acid 1 | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 19 | p-coutaric acid 2 | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 29 | Sinapic acid-like? | Cinnamic acid | Cinnamic acid | mg/L FAE |
| 51 | Unknown HCA ethyl ester | Cinnamic acid | Cinnamic acid EE | mg/L FAE |
| 16 | Catechin | Flavan-3-ol | Flavan-3-ol | mg/L ECE |
| 34 | ECG | Flavan-3-ol | Flavan-3-ol | mg/L ECE |
| 28 | EGCG-like5 | Flavan-3-ol | Flavan-3-ol | mg/L ECE |
| 22 | Epicatechin | Flavan-3-ol | Flavan-3-ol | mg/L ECE |
| 33 | Lignan | Flavan-3-ol | Flavan-3-ol | mg/L ECE |
| 20 | Luteolin-like? | Flavanone | Flavanone | mg/L Q3GE |
| 45 | Dihydrokaempferol | flavanonol | flavanonol | mg/L GAE |
| 40 | Dihydroquercetin rhamnoside | flavanonol | flavanonol glycoconj | mg/L Q3GE |
| 43 | Flavanol-like-gluc or ethyl ester | flavonol | flavonol EE | mg/L Q3GE |
| 53 | Isorhamnetin | flavonol | flavonol | mg/L Q3GE |
| 52 | Kaempferol | flavonol | flavonol | mg/L Q3GE |
| 49 | Quercetin | flavonol | flavonol | mg/L Q3GE |
| 41 | Quercetin-3-glucoside | flavonol | flavonol glycoconj | mg/L Q3GE |
| 42 | Quercetin-3-glucuronide | flavonol | flavonol glycoconj | mg/L Q3GE |
| 7 | 2-S-cysteinylcaftaric acid | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 6 | 2-S-glycinylcaftaric acid | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 15 | cis2-S-gluthionylcaftaric acid? | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 12 | 2-S-glutathionylcaftaric acid 'GRP' | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 35 | GRP ethyl ester | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 10 | GRP-like | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 14 | GRP-like1 | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 17 | GRP-like2 | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 24 | GRP-like3 | GRP-conjugate | GRP-conjugate | mg/L FAE |
| 9 | Hydroxytyrosol-glucoside | Phenol | Phenol Glycoside | mg/L GAE |
| 36 | Resveratrol-O-Gluc | Phenol | Phenol Glycoside | mg/L GAE |
| 11 | Tyrosol | Phenol | Phenol | mg/L GAE |
| 26 | Unknown at 69.5' | Phenol | Phenol | mg/L GAE |

## Table B-8. Full phenolic composition of 2010 wines by HPLC-DAD arranged in decreasing order of concentration

| Compound | Equiv. Unit g/L | Mean | sd | Chardonnay | | | | | | | Riesling | | | | | | Viognier | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | WBP | FR | LP | HP | HOX-FR | HOX-LHP | MAC | WBP | FR | LP | HP | HOX-FR | MAC | WBP | FR | LP | HP | HOX-FR | HOX-LHP | MAC |
| Early eluters | GAE | 15 | 4 | 11 | 15 | 20 | 23 | 20 | 20 | 13 | 10 | 17 | 16 | 17 | 17 | 9 | 10 | 11 | 13 | 14 | 15 | 20 | 13 |
| 1st eluters | GAE | 12 | 2 | 13 | 13 | 14 | 16 | 13 | 13 | 12 | 11 | 12 | 12 | 12 | 10 | 9 | 13 | 8 | 13 | 13 | 11 | 11 | 15 |
| Caftaric acid | FAE | 8 | 8 | 3 | 6 | 6 | 8 | 1 | 0 | 9 | 11 | 18 | 19 | 17 | 3 | 31 | 4 | 13 | 1 | 2 | 0 | 0 | 4 |
| 2-S-glutathionyl caftaric acid | FAE | 6 | 4 | 5 | 6 | 5 | 3 | 9 | 1 | 4 | 3 | 4 | 2 | 1 | 2 | 3 | 10 | 7 | 12 | 9 | 13 | 10 | 11 |
| Epicatechin | EpiCE | 5 | 6 | 2 | 3 | 2 | 10 | 2 | 6 | 3 | 0 | 0 | 21 | 21 | 0 | 1 | 0 | 1 | 6 | 6 | 4 | 5 | 2 |
| Quercetin -3-glucoside | Q3GE | 3 | 7 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 2 | 0 | 0 | 12 | 30 | 5 | 9 | 4 |
| Quercetin-3-glucuronide | Q3GE | 3 | 10 | 0 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 10 | 1 | 2 | 1 | 0 | 0 | 1 | 44 |
| Tyrosol | GAE | 2.8 | 0.4 | 2.5 | 2.4 | 2.7 | 3 | 2.5 | 2.4 | 2.6 | 2.7 | 3.6 | 3.2 | 2.8 | 3.5 | 2.6 | 2.2 | 2.9 | 2.4 | 2.7 | 2.7 | 3.2 | 3.4 |
| Catechin | EpiCE | 3 | 2 | 4 | 5 | 3 | 2 | 3 | 0 | 6 | 0 | 1 | 2 | 1 | 0 | 6 | 2 | 3 | 2 | 3 | 0 | 1 | 9 |
| Luteolin-like? | Q3GE | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 10 | 11 | 7 | 2 | 1 | 0 | 2 | 2 | 1 | 2 | 1 |
| Epicatechin gallate | EpiCE | 2 | 3 | 2 | 2 | 1 | 1 | 2 | 0 | 3 | 7 | 8 | 1 | 0 | 1 | 8 | 0 | 1 | 2 | 1 | 3 | 2 | 3 |
| Dihydroquercetin-rhamnoside | Q3GE | 2 | 5 | 3 | 3 | 0 | 0 | 0 | 0 | 20 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 4 | 0 | 1 | 9 |
| Gallic acid-glucoside | GAE | 2.2 | 0.6 | 1.2 | 1.7 | 2.9 | 2.9 | 2 | 3.1 | 2.6 | 1.9 | 2.6 | 3.2 | 2.9 | 2.1 | 1.5 | 1.6 | 1.2 | 2 | 2.3 | 1.9 | 2 | 2.4 |
| Unassined benzoic acid 2 | GAE | 2.1 | 0.5 | 1.4 | 2 | 2.2 | 1.9 | 1.9 | 1.7 | 1.9 | 1.2 | 2.5 | 2 | 2.2 | 2.5 | 1.6 | 1.3 | 2.8 | 2.2 | 2.5 | 1.9 | 3.6 | 2.4 |
| Lignan | EpiCE | 2 | 3 | 1 | 0 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 9 | 9 | 7 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| Flavanol-like-glucoside/ EE | Q3GE | 2 | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 2 | 1 | 1 | 3 | 3 | 2 | 5 | 1 | 1 | 2 | 4 | 1 | 2 | 8 |
| Unassined benzoic acid 1 | GAE | 1.8 | 0.5 | 1.2 | 1.4 | 1.5 | 1.5 | 1.5 | 1.6 | 1.9 | 1.7 | 2.3 | 2.2 | 2.1 | 2.1 | 2.7 | 1.1 | 1.3 | 1.6 | 2.2 | 1.5 | 2.5 | 1.7 |
| p-coutaric acid 2 | FAE | 1.8 | 1.7 | 0.6 | 1.4 | 1.2 | 1.4 | 0.4 | 0.5 | 2.6 | 1.2 | 2.7 | 3.9 | 4.2 | 0.6 | 6.8 | 0.9 | 1.9 | 0.4 | 0.7 | 0.1 | 0.1 | 3.6 |
| Unknown | GAE | 1.7 | 1.5 | 0 | 0.4 | 0.8 | 0.8 | 0.6 | 0.6 | 0.4 | 0.5 | 0.6 | 1.3 | 1.3 | 0.9 | 1.1 | 1.9 | 2.4 | 4.2 | 4.6 | 3.1 | 4.4 | 4 |
| p-coutaric acid1 | FAE | 1.6 | 1.1 | 0.4 | 0.7 | 1.8 | 2.6 | 0.9 | 1.1 | 3.2 | 0.6 | 0.9 | 2.1 | 3 | 0.6 | 3.1 | 0.7 | 0.9 | 1.7 | 3 | 0.7 | 0.5 | 4.1 |
| Fertaric acid | FAE | 1.6 | 1.2 | 1.1 | 1.1 | 1.3 | 1.4 | 1.1 | 1 | 1.3 | 2.8 | 3.2 | 3.6 | 3.6 | 2.9 | 3.9 | 0.6 | 0.7 | 0.7 | 0.8 | 0.1 | 0.7 | 0.9 |
| Syringic acid | GAE | 1.1 | 0.6 | 0.8 | 0.7 | 1.7 | 1.9 | 1 | 1.8 | 1.3 | 1 | 0.6 | 2.2 | 1.6 | 1.4 | 0.9 | 1.7 | 0.3 | 0.6 | 0.7 | 0.5 | 0.8 | 0.6 |
| Hydroxytyrosol-glucoside | GAE | 1 | 0.6 | 1.2 | 0.7 | 1.4 | 1.6 | 0.5 | 1.5 | 1 | 0.4 | 0.6 | 1.8 | 2.2 | 0.7 | 1 | 0.7 | 0.2 | 0.8 | 1.3 | 0 | 1.5 | 0.8 |
| Epigallocatechin gallate-like 5 | EpiCE | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 6 | 0 | 0 | 0 | 3 | 0 | 3 | 0 | 1 | 0 | 1 | 3 |
| Gallic acid | GAE | 0.8 | 0.2 | 1 | 1 | 1 | 1.1 | 1.1 | 0.9 | 1 | 0.4 | 0.7 | 0.7 | 0.7 | 0.5 | 1 | 0.5 | 0.6 | 0.7 | 0.6 | 0.6 | 0.5 | 1.2 |
| Mixed_HCA? | FAE | 0.6 | 0.7 | 0.6 | 0.2 | 0.3 | 0.2 | 0.2 | 0.1 | 1 | 0.5 | 0.8 | 0.9 | 0.4 | 0.2 | 0.5 | 0.7 | 0.8 | 0.7 | 0.6 | 0.4 | 0.4 | 3.3 |
| Unknown phenyl cmpd | GAE | 0.5 | 0.5 | 0 | 0 | 0.2 | 0 | 0.2 | 0.2 | 0 | 0 | 1.1 | 1 | 1.1 | 0.7 | 1.2 | 0.2 | 1.6 | 0.5 | 0.6 | 0.2 | 0.4 | 0.8 |
| Gallic acid EE | GAE | 0.5 | 0.4 | 0.7 | 0.7 | 0.3 | 0.3 | 0.2 | 0.2 | 0.4 | 0.4 | 0.3 | 0 | 0.6 | 0.2 | 0.1 | 0.4 | 0.6 | 1.1 | 1.5 | 0.6 | 1.1 | 0.2 |
| GRP-like 1 | FAE | 0.5 | 0.6 | 0.6 | 0.2 | 0.2 | 0.1 | 0.3 | 0 | 1 | 0.3 | 0.7 | 0.1 | 0.1 | 0 | 0.2 | 0.6 | 0.7 | 0.5 | 0.4 | 0.5 | 0.4 | 2.6 |
| Protocatachuic acid EE | GAE | 0.4 | 0.7 | 0 | 0 | 0.3 | 0.5 | 0.2 | 0.1 | 0 | 0 | 0 | 0.9 | 1 | 0.2 | 0.3 | 0.9 | 0.7 | 0 | 0 | 0 | 0 | 3 |
| Quercetin | Q3GE | 0.4 | 1.1 | 0 | 0 | 0 | 0 | 0 | 0 | 1.2 | 0 | 0.4 | 0.4 | 0.3 | 0 | 4.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0.6 |
| Resveratrol-O-glucoside | GAE | 0.4 | 0.4 | 0 | 0.2 | 0.7 | 0.6 | 0.7 | 0 | 0.1 | 0.2 | 0.4 | 0 | 0.2 | 0.4 | 1.2 | 0.3 | 0.6 | 0.2 | 0.3 | 0.1 | 0.2 | 1.3 |
| Protocatachuic acid | GAE | 0.3 | 0.1 | 0.4 | 0 | 0.2 | 0.3 | 0.2 | 0.2 | 0 | 0.2 | 0.2 | 0.3 | 0.4 | 0.2 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0 | 0.4 | 0.4 |
| 2-S-cysteinyl caftaric acid | FAE | 0.2 | 0.1 | 0.5 | 0.4 | 0.1 | 0.1 | 0.5 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.3 | 0.2 | 0.3 | 0.2 | 0.4 | 0.2 | 0.5 |
| Caftaric acid ethyl ester | FAE | 0.2 | 0.2 | 0 | 0.1 | 0.4 | 0.6 | 0.3 | 0.4 | 0.2 | 0.2 | 0.3 | 0.1 | 0.2 | 0.1 | 0.7 | 0.1 | 0.3 | 0.2 | 0.3 | 0.1 | 0.1 | 0 |
| Unassigned phenyl EE | GAE | 0.2 | 0.3 | 0 | 0 | 0.4 | 0 | 0.3 | 0.4 | 0 | 0 | 0 | 0.8 | 0.9 | 0.1 | 0 | 0 | 0 | 0.2 | 0.3 | 0.1 | 0.2 | 1 |

| Compound | Equiv. Unit g/L | Mean | sd | Chardonnay | | | | | | | Riesling | | | | | | Viognier | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | WBP | FR | LP | HP | HOX-FR | HOX-LHP | MAC | WBP | FR | LP | HP | HOX-FR | MAC | WBP | FR | LP | HP | HOX-FR | HOX-LHP | MAC |
| GRP-like 3 | FAE | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.3 | 0 | 0.2 | 0 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.4 | 0.4 | 0.4 | 0.3 | 0.5 | 0.4 | 0.2 |
| Gallate acid-like | GAE | 0.2 | 0.2 | 0 | 0 | 0.3 | 0.5 | 0.2 | 0.3 | 0.2 | 0.5 | 0.6 | 0 | 0 | 0 | 0.9 | 0.2 | 0.3 | 0.1 | 0 | 0 | 0.1 | 0.1 |
| Cis-GRP | FAE | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.3 | 0 | 0.1 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.4 | 0.3 | 0.4 | 0.2 | 0.5 | 0.4 | 0.5 |
| Ferulic acid-glucoside | FAE | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0 | 0.2 | 0.1 | 0.4 | 0.6 | 0.1 | 0 | 0 | 0.8 | 0.1 | 0.1 | 0 | 0 | 0.1 | 0 | 0.1 |
| GRP-like | FAE | 0.1 | 0.1 | 0.1 | 0.2 | 0 | 0 | 0 | 0 | 0.3 | 0.2 | 0.2 | 0 | 0 | 0 | 0.4 | 0.1 | 0.3 | 0.1 | 0 | 0.3 | 0.1 | 0.3 |
| Unassigned phenyl E 2 | GAE | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0 | 0 | 0.8 | 0 | 0 | 0.2 | 0.1 | 0 | 0.2 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0.3 |
| Sinapic acid-like | FAE | 0.08 | 0 | 0.09 | 0.09 | 0.11 | 0.15 | 0.1 | 0.12 | 0.16 | 0.04 | 0.04 | 0 | 0.1 | 0 | 0.07 | 0.07 | 0.06 | 0.08 | 0.11 | 0.07 | 0.09 | 0.1 |
| 2-S-glycinyl caftaric acid | FAE | 0.08 | 0.1 | 0.1 | 0.08 | 0 | 0 | 0.08 | 0 | 0.07 | 0 | 0.08 | 0 | 0 | 0 | 0.12 | 0.15 | 0.11 | 0.15 | 0.11 | 0.19 | 0.12 | 0.29 |
| Ferulic acid | FAE | 0.08 | 0.1 | 0 | 0 | 0.07 | 0.07 | 0.05 | 0.05 | 0 | 0.16 | 0.25 | 0.07 | 0.1 | 0.05 | 0.33 | 0.05 | 0 | 0.09 | 0.11 | 0 | 0.1 | 0 |
| p-Coumaric Ac | FAE | 0.07 | 0.1 | 0.07 | 0 | 0.09 | 0.09 | 0 | 0.1 | 0.1 | 0 | 0.13 | 0.14 | 0.09 | 0 | 0.17 | 0 | 0.11 | 0 | 0.07 | 0 | 0 | 0.19 |
| GRP ethyl ester | FAE | 0.06 | 0.1 | 0.07 | 0.08 | 0.03 | 0.02 | 0 | 0 | 0.08 | 0.05 | 0.08 | 0.12 | 0.13 | 0.03 | 0.06 | 0.19 | 0.16 | 0.04 | 0.06 | 0 | 0 | 0.08 |
| GRP-like2 | FAE | 0.06 | 0.1 | 0.12 | 0.13 | 0 | 0 | 0 | 0 | 0.08 | 0.05 | 0.09 | 0.07 | 0.07 | 0 | 0 | 0.26 | 0.2 | 0 | 0 | 0 | 0 | 0.18 |
| Kaempferol | Q3GE | 0.1 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3 |
| Caffeic acid EE | FAE | 0.04 | 0 | 0.03 | 0.04 | 0.03 | 0.04 | 0.03 | 0.02 | 0.03 | 0.05 | 0.08 | 0.07 | 0.07 | 0.05 | 0.05 | 0 | 0.12 | 0.05 | 0.04 | 0.03 | 0.04 | 0.03 |
| Coutaric acid EE | FAE | 0.04 | 0 | 0 | 0 | 0.02 | 0.05 | 0.03 | 0.01 | 0 | 0.05 | 0.06 | 0.05 | 0.12 | 0.06 | 0.1 | 0 | 0 | 0.03 | 0.09 | 0.06 | 0.11 | 0 |
| Isorhamnetin | Q3GE | 0.02 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unknown HCA EE | FAE | 0.02 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.08 | 0 | 0.03 | 0 | 0.01 | 0 | 0.04 | 0 | 0.24 | 0 | 0 | 0 | 0 | 0.03 |
| Coumaric acid EE | FAE | 0.01 | 0 | 0 | 0.03 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0.03 | 0 | 0 | 0 | 0.02 | 0 | 0.05 | 0 | 0 | 0 | 0 | 0.05 |
| Ferulic acid-like | FAE | 0.01 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.15 |
| **Total Phenolics** | GAE | 92 | 29 | 61 | 73 | 78 | 92 | 70 | 63 | 106 | 62 | 98 | 126 | 130 | 70 | 131 | 63 | 76 | 90 | 115 | 70 | 91 | 168 |
| **Total names cmpds** | GAE | 64 | 30 | 38 | 45 | 43 | 53 | 37 | 30 | 81 | 41 | 70 | 99 | 101 | 44 | 113 | 40 | 56 | 64 | 89 | 44 | 61 | 140 |
| **% named cmpds** | % | 68% | 0% | 62% | 62% | 56% | 58% | 53% | 48% | 76% | 67% | 71% | 78% | 78% | 62% | 86% | 63% | 74% | 72% | 77% | 63% | 67% | 83% |

EE = ethyl ester; GAE = gallic acid equiv; FAE = ferulic acid equiv; EpiCE = epicatechin equiv; Q3GE = quercetin-3-glcoside equiv

## Table B-9 . Chemical analysis of 2011 project wines

| Full Wine Code | Glu + Fru (g/L) | pH | TA (g/L) | Free $SO_2$ (ppm) | Total $SO_2$ (ppm) | % Alcohol (v/v) | Acetic acid (g/L) |
|---|---|---|---|---|---|---|---|
| CHA-WBP-rep1 | 1.2 | 3.13 | 6.8 | 34 | 123 | 13.6 | 0.35 |
| CHA-WBP-rep2 | 1.2 | 3.14 | 6.8 | 40 | 123 | 13.6 | 0.34 |
| CHA-FR-rep1 | 1.1 | 3.14 | 6.7 | 38 | 146 | 13.7 | 0.32 |
| CHA-FR-rep2 | 1.1 | 3.13 | 6.6 | 38 | 145 | 13.7 | 0.32 |
| CHA-HP-rep1 | 0.7 | 3.21 | 6.8 | 38 | 134 | 13.6 | 0.32 |
| CHA-HP-rep2 | 0.6 | 3.22 | 6.7 | 35 | 131 | 13.6 | 0.31 |
| CHA-HOX-FR-rep1 | 1.1 | 3.16 | 6.4 | 37 | 141 | 13.6 | 0.31 |
| CHA-HOX-FR-rep2 | 1.1 | 3.16 | 6.4 | 38 | 140 | 13.6 | 0.30 |
| CHA-HOX-HP-rep1 | 0.9 | 3.19 | 6.8 | 38 | 134 | 13.5 | 0.31 |
| CHA-HOX-HP-rep2 | 0.8 | 3.19 | 6.8 | 34 | 128 | 13.5 | 0.31 |
| CHA-SOL-rep1 | 0.2 | 3.26 | 7.1 | 35 | 155 | 13.4 | 0.25 |
| CHA-SOL-rep2 | 0.2 | 3.27 | 7.1 | 32 | 150 | 13.4 | 0.23 |
| CHA-SKI-rep1 | 0.5 | 3.20 | 6.2 | 32 | 163 | 13.6 | 0.28 |
| CHA-SKI-rep2 | 0.6 | 3.17 | 6.4 | 37 | 176 | 13.5 | 0.30 |
| CHA-MAC-rep1 | 0.7 | 3.27 | 7.2 | 34 | 141 | 13.3 | 0.37 |
| CHA-MAC-rep2 | 0.7 | 3.28 | 7.3 | 37 | 144 | 13.3 | 0.38 |
| RIE-WBP-rep1 | 1.1 | 3.10 | 8.0 | 35 | 94 | 12.4 | 0.38 |
| RIE-WBP-rep2 | 1.2 | 3.09 | 7.9 | 35 | 93 | 12.4 | 0.37 |
| RIE-FR-rep1 | 1.4 | 3.13 | 7.6 | 34 | 124 | 12.9 | 0.40 |
| RIE-FR-rep2 | 1.5 | 3.13 | 7.5 | 35 | 130 | 12.9 | 0.42 |
| RIE-HP-rep1 | 0.8 | 3.28 | 7.7 | 35 | 112 | 12.7 | 0.38 |
| RIE-HP-rep2 | 0.8 | 3.28 | 7.7 | 35 | 115 | 12.7 | 0.39 |
| RIE-HOX-FR-rep1 | 1.5 | 3.07 | 7.7 | 34 | 88 | 13.0 | 0.40 |
| RIE-HOX-FR-rep2 | 1.4 | 3.07 | 7.7 | 37 | 98 | 13.0 | 0.41 |
| RIE-HOX-HP-rep1 | 0.6 | 3.25 | 7.5 | 37 | 101 | 12.7 | 0.37 |
| RIE-HOX-HP-rep2 | 0.6 | 3.25 | 7.6 | 37 | 99 | 12.7 | 0.37 |
| RIE-SOL-rep1 | 1.1 | 3.12 | 7.4 | 37 | 114 | 13.0 | 0.35 |
| RIE-SOL-rep2 | 1.0 | 3.09 | 7.5 | 37 | 111 | 13.0 | 0.31 |
| RIE-SKI-rep1 | 0.5 | 3.12 | 7.5 | 35 | 118 | 12.8 | 0.33 |
| RIE-SKI-rep2 | 0.5 | 3.11 | 7.6 | 35 | 115 | 12.8 | 0.33 |
| RIE-MAC-rep1 | 1.0 | 3.19 | 7.9 | 34 | 110 | 12.6 | 0.39 |
| RIE-MAC-rep2 | 1.0 | 3.19 | 8.0 | 37 | 119 | 12.6 | 0.40 |
| VIO-WBP-rep1 | 1.0 | 3.10 | 6.5 | 34 | 111 | 12.7 | 0.28 |
| VIO-WBP-rep2 | 0.9 | 3.11 | 6.4 | 37 | 117 | 12.7 | 0.28 |
| VIO-FR-rep1 | 1.1 | 3.12 | 6.2 | 38 | 113 | 13.0 | 0.23 |
| VIO-FR-rep2 | 1.0 | 3.13 | 6.2 | 40 | 115 | 13.0 | 0.23 |
| VIO-HP-rep1 | 0.8 | 3.20 | 6.9 | 35 | 134 | 12.9 | 0.25 |
| VIO-HP-rep2 | 1.0 | 3.21 | 6.8 | 35 | 137 | 12.9 | 0.25 |
| VIO-HOX-FR-rep1 | 0.8 | 3.12 | 6.0 | 35 | 90 | 12.8 | 0.26 |
| VIO-HOX-FR-rep2 | 0.7 | 3.12 | 6.0 | 35 | 93 | 12.8 | 0.25 |
| VIO-HOX-HP-rep1 | 0.7 | 3.20 | 6.9 | 37 | 112 | 12.9 | 0.29 |
| VIO-HOX-HP-rep2 | 0.7 | 3.23 | 6.8 | 37 | 114 | 12.9 | 0.28 |
| VIO-SOL-rep1 | 0.4 | 3.16 | 6.1 | 34 | 102 | 13.0 | 0.21 |
| VIO-SOL-rep2 | 0.4 | 3.15 | 6.0 | 34 | 101 | 13.0 | 0.21 |
| VIO-SKI-rep1 | 0.6 | 3.24 | 5.8 | 37 | 114 | 12.7 | 0.18 |
| VIO-SKI-rep2 | 0.5 | 3.25 | 5.8 | 38 | 120 | 12.7 | 0.18 |
| VIO-MAC-rep1 | 0.9 | 3.19 | 6.7 | 35 | 128 | 12.7 | 0.24 |
| VIO-MAC-rep2 | 1.0 | 3.19 | 6.6 | 34 | 127 | 12.7 | 0.24 |

## Table B-10 . Somers' and other spectral data of 2011 project wines

| Full Wine Code | $A^{10}_{320}$ (2mm) | $A^{10}_{280}$ (2mm) | $A^{10}_{420}$ (10mm) | $A^{10}_{370}$ (10mm) | Total Phenolics (au) | Total HCA (au) | Flavonoid Extract (au) |
|---|---|---|---|---|---|---|---|
| CHA-WBP-rep1 | 0.646 | 1.138 | 0.086 | 0.466 | 1.69 | 1.83 | 0.47 |
| CHA-WBP-rep2 | 0.659 | 1.155 | 0.081 | 0.483 | 1.77 | 1.89 | 0.51 |
| CHA-FR-rep1 | 0.718 | 1.221 | 0.084 | 0.507 | 2.10 | 2.19 | 0.65 |
| CHA-FR-rep2 | 0.698 | 1.180 | 0.081 | 0.492 | 1.90 | 2.09 | 0.51 |
| CHA-HP-rep1 | 0.764 | 1.315 | 0.086 | 0.506 | 2.58 | 2.42 | 0.96 |
| CHA-HP-rep2 | 0.780 | 1.327 | 0.082 | 0.509 | 2.64 | 2.50 | 0.97 |
| CHA-HOX-FR-rep1 | 0.310 | 0.867 | 0.086 | 0.342 | 0.33 | 0.15 | 0.23 |
| CHA-HOX-FR-rep2 | 0.301 | 0.879 | 0.086 | 0.327 | 0.40 | 0.11 | 0.33 |
| CHA-HOX-HP-rep1 | 0.364 | 1.001 | 0.108 | 0.428 | 1.01 | 0.42 | 0.73 |
| CHA-HOX-HP-rep2 | 0.369 | 1.018 | 0.115 | 0.457 | 1.09 | 0.44 | 0.79 |
| CHA-SOL-rep1 | 1.031 | 1.532 | 0.111 | 0.686 | 3.66 | 3.76 | 1.16 |
| CHA-SOL-rep2 | 1.033 | 1.531 | 0.114 | 0.676 | 3.65 | 3.77 | 1.15 |
| CHA-SKI-rep1 | 0.816 | 1.849 | 0.095 | 0.598 | 5.24 | 2.68 | 3.46 |
| CHA-SKI-rep2 | 0.791 | 1.900 | 0.092 | 0.587 | 5.50 | 2.55 | 3.80 |
| CHA-MAC-rep1 | 1.464 | 2.029 | 0.114 | 0.900 | 6.14 | 5.92 | 2.20 |
| CHA-MAC-rep2 | 1.489 | 2.028 | 0.116 | 0.914 | 6.14 | 6.05 | 2.12 |
| RIE-WBP-rep1 | 0.750 | 0.985 | 0.052 | 0.427 | 0.93 | 2.35 | 0.00 |
| RIE-WBP-rep2 | 0.767 | 1.000 | 0.064 | 0.460 | 1.00 | 2.44 | 0.00 |
| RIE-FR-rep1 | 1.347 | 1.400 | 0.059 | 0.644 | 3.00 | 5.33 | 0.00 |
| RIE-FR-rep2 | 1.357 | 1.407 | 0.047 | 0.634 | 3.03 | 5.38 | 0.00 |
| RIE-HP-rep1 | 1.207 | 1.449 | 0.060 | 0.591 | 3.24 | 4.63 | 0.16 |
| RIE-HP-rep2 | 1.210 | 1.447 | 0.056 | 0.615 | 3.24 | 4.65 | 0.14 |
| RIE-HOX-FR-rep1 | 0.383 | 0.778 | 0.055 | 0.300 | -0.11 | 0.51 | 0.00 |
| RIE-HOX-FR-rep2 | 0.349 | 0.755 | 0.055 | 0.287 | -0.23 | 0.34 | 0.00 |
| RIE-HOX-HP-rep1 | 0.501 | 1.003 | 0.066 | 0.411 | 1.02 | 1.10 | 0.28 |
| RIE-HOX-HP-rep2 | 0.513 | 1.025 | 0.063 | 0.439 | 1.13 | 1.17 | 0.35 |
| RIE-SOL-rep1 | 1.343 | 1.352 | 0.064 | 0.704 | 2.76 | 5.31 | 0.00 |
| RIE-SOL-rep2 | 1.355 | 1.323 | 0.086 | 0.681 | 2.61 | 5.37 | 0.00 |
| RIE-SKI-rep1 | 1.191 | 1.478 | 0.093 | 0.589 | 3.39 | 4.56 | 0.36 |
| RIE-SKI-rep2 | 1.223 | 1.542 | 0.048 | 0.594 | 3.71 | 4.71 | 0.57 |
| RIE-MAC-rep1 | 1.838 | 1.822 | 0.049 | 0.866 | 5.11 | 7.79 | 0.00 |
| RIE-MAC-rep2 | 1.859 | 1.822 | 0.062 | 0.868 | 5.11 | 7.89 | 0.00 |
| VIO-WBP-rep1 | 0.679 | 0.968 | 0.061 | 0.478 | 0.84 | 2.00 | 0.00 |
| VIO-WBP-rep2 | 0.711 | 0.997 | 0.069 | 0.498 | 0.98 | 2.15 | 0.00 |
| VIO-FR-rep1 | 0.839 | 1.086 | 0.066 | 0.556 | 1.43 | 2.80 | 0.00 |
| VIO-FR-rep2 | 0.841 | 1.086 | 0.064 | 0.548 | 1.43 | 2.81 | 0.00 |
| VIO-HP-rep1 | 0.593 | 1.139 | 0.066 | 0.589 | 1.70 | 1.56 | 0.65 |
| VIO-HP-rep2 | 0.581 | 1.154 | 0.067 | 0.584 | 1.77 | 1.51 | 0.77 |
| VIO-HOX-FR-rep1 | 0.358 | 0.744 | 0.069 | 0.355 | -0.28 | 0.39 | 0.00 |
| VIO-HOX-FR-rep2 | 0.360 | 0.749 | 0.080 | 0.367 | -0.25 | 0.40 | 0.00 |
| VIO-HOX-HP-rep1 | 0.454 | 1.032 | 0.076 | 0.514 | 1.16 | 0.87 | 0.58 |
| VIO-HOX-HP-rep2 | 0.443 | 1.022 | 0.084 | 0.521 | 1.11 | 0.81 | 0.57 |
| VIO-SOL-rep1 | 0.887 | 1.109 | 0.071 | 0.629 | 1.54 | 3.03 | 0.00 |
| VIO-SOL-rep2 | 0.904 | 1.110 | 0.069 | 0.632 | 1.55 | 3.12 | 0.00 |
| VIO-SKI-rep1 | 0.886 | 1.385 | 0.077 | 0.699 | 2.93 | 3.03 | 0.91 |
| VIO-SKI-rep2 | 0.883 | 1.480 | 0.077 | 0.679 | 3.40 | 3.01 | 1.39 |
| VIO-MAC-rep1 | 0.969 | 1.474 | 0.082 | 0.807 | 3.37 | 3.44 | 1.08 |
| VIO-MAC-rep2 | 0.976 | 1.455 | 0.081 | 0.794 | 3.28 | 3.48 | 0.96 |

## Table B-11:Correlation coefficients of sensory parameters for 2011 wines

| | | Burning | Bitter | Metallic | AcidAT | Acidity | Astringent | Fruit | Sweet | Viscous | Oily AT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **All Wines** | **Hot** | 0.71 | 0.36 | 0.16 | 0.01 | 0.06 | 0.31 | 0.1 | 0.04 | 0.17 | 0.04 |
| | **Burning** | - | 0.42 | 0.13 | 0.24 | 0.17 | 0.07 | 0.08 | 0.12 | 0.32 | 0.36 |
| | **Bitter** | | - | 0.35 | 0.06 | 0.01 | 0.23 | 0.51 | 0.31 | 0.09 | 0.2 |
| | **Metallic** | | | - | 0.02 | 0.04 | 0.08 | 0.36 | 0.34 | 0.02 | 0.07 |
| | **Acid AT** | | | | - | 0.88 | 0.29 | 0.35 | 0.56 | 0.43 | 0.51 |
| | **Acidity** | | | | | - | 0.23 | 0.33 | 0.54 | 0.43 | 0.52 |
| | **Astringent** | | | | | | - | 0.2 | 0.26 | 0.26 | 0.13 |
| | **Fruit** | | | | | | | - | 0.79 | 0.49 | 0.31 |
| | **Sweet** | | | | | | | | - | 0.55 | 0.38 |
| | **Viscous** | | | | | | | | | - | 0.64 |

| | | Burning | Bitter | Metallic | AcidAT | Acidity | Astringent | Fruit | Sweet | Viscous | Oily AT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chardonnay** | **Hot** | 0.06 | 0.06 | 0.23 | 0.02 | 0.22 | 0.53 | 0.14 | 0.27 | 0.39 | 0.46 |
| | **Burning** | - | 0.17 | 0.17 | 0.16 | 0.37 | 0.17 | 0.24 | 0.18 | 0.19 | 0.29 |
| | **Bitter** | | - | 0.05 | 0.04 | 0.09 | 0.12 | 0.5 | 0.42 | 0.37 | 0.15 |
| | **Metallic** | | | - | 0.11 | 0.02 | 0.28 | 0.56 | 0.55 | 0.07 | 0.17 |
| | **Acid AT** | | | | - | 0.45 | 0.14 | 0.17 | 0.23 | 0.19 | 0.01 |
| | **Acidity** | | | | | - | 0.29 | 0.18 | 0.2 | 0.01 | 0.1 |
| | **Astringent** | | | | | | - | 0.25 | 0.33 | 0.2 | 0.06 |
| | **Fruit** | | | | | | | - | 0.81 | 0.6 | 0.13 |
| | **Sweet** | | | | | | | | - | 0.44 | 0.05 |
| | **Viscous** | | | | | | | | | - | 0.65 |

| | | Burning | Bitter | Metallic | AcidAT | Acidity | Astringent | Fruit | Sweet | Viscous | Oily AT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Riesling** | **Hot** | 0.72 | 0.16 | 0.25 | 0.24 | 0.05 | 0.28 | 0.35 | 0.35 | 0.22 | 0.09 |
| | **Burning** | - | 0.16 | 0.37 | 0.27 | 0.06 | 0.17 | 0.48 | 0.37 | 0.12 | 0.07 |
| | **Bitter** | | - | 0.06 | 0.02 | 0.37 | 0.34 | 0.03 | 0.04 | 0.37 | 0.01 |
| | **Metallic** | | | - | 0.41 | 0.14 | 0.46 | 0.06 | 0.27 | 0.11 | 0.53 |
| | **Acid AT** | | | | - | 0.52 | 0.27 | 0.75 | 0.8 | 0.38 | 0.3 |
| | **Acidity** | | | | | - | 0.08 | 0.59 | 0.68 | 0.29 | 0.34 |
| | **Astringent** | | | | | | - | 0.01 | 0.22 | 0.37 | 0.09 |
| | **Fruit** | | | | | | | - | 0.78 | 0.57 | 0.47 |
| | **Sweet** | | | | | | | | - | 0.55 | 0.49 |
| | **Viscous** | | | | | | | | | - | 0.33 |

| | | Burning | Bitter | Metallic | AcidAT | Acidity | Astringent | Fruit | Sweet | Viscous | Oily AT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Viognier** | **Hot** | 0.51 | 0.41 | 0.05 | 0.28 | 0.6 | 0.33 | 0.29 | 0.33 | 0.07 | 0.23 |
| | **Burning** | - | 0.08 | 0.32 | 0.09 | 0.28 | 0.18 | 0.03 | 0.2 | 0.28 | 0.5 |
| | **Bitter** | | - | 0.6 | 0.46 | 0.44 | 0.66 | 0.78 | 0.69 | 0.27 | 0.04 |
| | **Metallic** | | | - | 0.22 | 0.14 | 0.23 | 0.46 | 0.41 | 0.21 | 0.08 |
| | **Acid AT** | | | | - | 0.81 | 0.55 | 0.36 | 0.57 | 0.07 | 0.05 |
| | **Acidity** | | | | | - | 0.45 | 0.23 | 0.4 | 0.17 | 0.1 |
| | **Astringent** | | | | | | - | 0.6 | 0.82 | 0.23 | 0.12 |
| | **Fruit** | | | | | | | - | 0.82 | 0.57 | 0.28 |
| | **Sweet** | | | | | | | | - | 0.42 | 0.05 |
| | **Viscous** | | | | | | | | | - | 0.51 |

# B.5Project Staff

| Name | Position | Agency |
|---|---|---|
| Elizabeth Waters | Research Manager | AWRI |
| Paul Smith | Research Manager | AWRI |
| Yoji Hayasaka | Manager – Mass Spectrometry | AWRI |
| Richard Gawel | Research Scientist | AWRI |
| Martin Day | Research Scientist | AWRI |
| Steve Van Sluyter | Post Doctoral Research Fellow | AWRI |
| Alex Schulkin | Scientist | AWRI |
| Patrick Dimanin | Scientist | AWRI |
| David Jeffrey | Senior Research Scientist | AWRI |
| Mango Parker | Scientist | AWRI |
| Leigh Francis | Research Manager | AWRI |
| Belinda Bramley | Sensory Analyst | AWRI |
| Kate Lattey | R & D Manager | PRP |
| Inca Pearce | R & D Manager | PRP |
| Leon Deans | Technical Development Manager | PRP |
| Hylton McLean | White Wine Maker | PRP |
| Jai O'Toole | R & D Officer | PRP |

AWRI = The Australian Wine Research Institute;

PRP = Pernod-Ricard Pacific