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Factors affecting wine texture, taste, clarity, stability and production efficiency



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Project 3.1.4 – Factors affecting wine texture, taste, clarity, stability and production efficiency

Abstract

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence of haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment. Specifically, it covered molecular drivers of taste and texture, 'smart' surfaces for efficient production, predicting haze formation, understanding and preventing wine haze, effects of filtering red wines, solids management effects on white wine style and composition, impact of winemaking methods on wine macromolecules and texture, colour development/management and a practical method to determine extractable grape colour and tannin.

Executive summary

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence of haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment.

The compositional drivers for texture, hotness and bitterness were investigated in both red and white wines. Different white wine phenolic classes were shown to have different effects on mouth-feel (such as oiliness and viscosity) and bitterness of white wine, and could be manipulated by managing extraction from skins during white winemaking. This is to be contrasted with the limited effect of white wine phenolics on astringency demonstrated previously.

A significant discovery was made in relation to the presence of an indole conjugate which was found to statistically correlate with bitterness of white wine fractions. Sensory assessment showed that the indole derivative might be a new bitterant in white wine; this is a significant development as currently there are only few molecular targets known which cause bitterness in wine.

The perceived bitterness in white wine was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in a Chardonnay wine, which was consistent with a trend seen in a Viognier wine. Dissolved CO_2 did not significantly influence perceived viscosity or astringency. The perception of 'spritz' increased significantly with increasing dissolved CO_2 levels in both wines as expected, but in the case of the Viognier higher pH and higher ethanol content accentuated the perception of 'spritz' when dissolved CO_2 was high. The reasons for increased perceived sweetness and reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear, but warrant further investigation.

Tannin concentration drives most of the astringency perception in red wine, while pH and alcohol modulate it (as lower pH and lower alcohol increase astringency), but the mechanisms responsible remain unclear. Using red wine tannin, experimental results showed that across a wine-like range (10-15%), ethanol can influence the mechanisms of wine tannin-protein interactions and that the previously reported decrease in wine astringency with increasing alcohol may, in part, relate to a decrease in tannin-protein interaction strength. This is significant as it highlights a key element likely to influence the astringency perception mechanism that has not previously been highlighted.

The role of polysaccharides in wine sensory characteristics remains debated and efforts were directed towards improving the understanding of red wine polysaccharides. Sensory assessment of isolated red wine polysaccharide and three sub-fractions and their interactions with alcohol and pH showed a range of impacts. Astringency suppression was greater for low molecular weight rhamnogalacturonan-rich polysaccharides; low to medium molecular weight polysaccharides increased perceived viscosity; and the bitterness of the higher alcohol/higher pH wine was significantly reduced in the presence of medium molecular weight polysaccharides. Furthermore, perceived hotness from alcohol was reduced in the low alcohol (11.5% v/v) wines by medium molecular weight polysaccharides, consistent with work on white wines. Alcohol and pH most significantly impacted hotness and astringency respectively.

In a production environment, knowledge of macromolecular adsorption onto surfaces is critical for better understanding and control of processes such as filter fouling, binding to tanks and fittings and interactions with processing aids such as bentonite. To increase this knowledge, model surfaces with tailored surface properties (e.g. charge, polarity, chemical functionality, wettability) were developed to explore how wine constituents interact with them. The effect of surface chemical functionalities on the adsorption of white, rosé and red wine constituents was evaluated. The results may aid in the development of the next generation of low fouling membranes, tank materials, wine production surfaces and/or new sensing platforms that will reduce cost and improve productivity in wine and related industries.

Heat stability is an ongoing issue in the wine industry with cloudy wine having the potential to damage brands and reputations for wine quality. Understanding wine haze has been important to better predict and mitigate protein haze. For this project, the focus was on understanding the components that drive haze formation by investigating interactions between proteins and other wine matrix components in model wines and in real wines. Model wine investigations included analysis of interaction strength with isothermal titration calorimetry (ITC), protein stability with differential scanning fluorimetry (DSF) and particle size with nanoparticle tracking analysis (NTA). The components that were found to have the greatest impact on haze were analysed in a selection of real wines to assess the real-world impacts of matrix components on haze formation. However, no single factor had a statistically significant impact on haze formation in real wine, indicating a complex interdependency between matrix components and the haze formation process.

Predicting protein haze potential accurately is essential for determining the amount of bentonite required to prevent wines from developing a haze. Ideally, haze could be predicted easily from measuring different components of the heat test; however, trials undertaken to asses this possibility did not establish any obvious leads. The most widely used method in industry is a heat test method developed in the 1970s. This empirical method was revisited to improve reproducibility in industry and the new method has decreased the turnaround time for results from 24 hours to 5 hours without compromising accuracy.

The most widely used method for preventing wine protein haze is the addition of bentonite. However, issues with poor settling and selectivity have prompted much research into alternative

methods for removing proteins. These methods include new proteases for cleaving proteins, new adsorbents that act like bentonite but with better settling and selectivity properties and treatments such as heating grape must. The trials undertaken for this project further explored some potential natural proteases isolated from *Botrytis cinerea* and sunflowers, investigated a range of new protein-adsorbing material including coated magnetic nanoparticles, surface-engineered silica and macrosponges. Alternative treatments for grape must were explored including vortex fluidic device and flash pasteurisation parameters. Pasteurisation of juice (with or without aspergillopepsin enzymes) remains a viable option in many cases and magnetic nanoparticles show promise but require some further development.

Filtering red wines has long been a concern in industry, with the perception that the action of filtering removes some important colour and texture molecules. Laboratory-scale investigations have suggested that filter membranes can remove polysaccharides, tannins and anthocyanins; however, the impact of commercial-scale filtration on red wines was unknown. The results of experiments using industry-scale equipment showed that the particles removed during filtration had minimal impact on wine composition or texture. Further experiments with high solid juices demonstrated that typically white wine produced from high solids juices contained significantly higher concentrations of polysaccharides. In terms of sensory effects, fermentation of high solids juice generally increased 'fruity' aromas, viscosity and oiliness, with the magnitude of the effects varying somewhat between treatments and varieties. The results suggest that both textural and aromatic characters can be diversified through modified settling processes and the associated compositional changes. From a practical perspective they also suggest that the method of clarification will most likely not influence the total phenolic concentration of a wine.

A significant body of work was produced in relation to the use of winemaking methods to diversify wine macromolecules and texture. The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on wine tannin and polysaccharide. The magnitude of the effects can be large, but varies depending on the maturity of the grapes and the effect of the yeast, enzyme or maceration protocol on the mechanisms driving extraction and retention processes. The research also identified a potential new mechanism by which extracted grape tannin may be lost from red wine during vinification. Several experiments continued to demonstrate how various approaches to lowering alcohol (pre-ferment) affect tannin, polysaccharide and colour outcomes in red wine. Results indicate that to effectively lower alcohol while maximising wine macromolecule extraction and wine texture, must dilution treatments are likely to lead to more favourable outcomes than harvesting earlier.

The key finding from studies on colour development highlighted the important role that higher molecular weight tannins play in colour stabilisation. A simple extraction method was developed to determine 'wine extractable' tannin and anthocyanin in grapes. The 'wine-like' extraction method uses gently-crushed grapes, adjusted to 15% v/v ethanol, pH 3.4, in their own juice and provides a useful prediction of wine tannin and colour. A protocol is available on the AWRI website and a predictive spectral method to determine 'wine-like' extraction is available on the AWRI WineCloud analysis platform.

In conclusion, the project has successfully elucidated key compositional drivers of texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment.

Background

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence from haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on elucidating some of the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developing strategies to modulate them in a production-based environment. Key areas that were addressed in the research are:

- determining molecular drivers of texture and tastes to allow approaches to modulating these molecules that are practical and commercially viable
- characterising and understanding the roles of macromolecules and colloids in wine and their contribution to clarity, stability (protein and colour) and filterability
- the path from grapes to wine for macromolecules and the impacts of industry standard processing technologies such as flotation and filtration on their retention and form
- leveraging the increased understanding of influence of macromolecules to develop more consistent and reliable testing protocols for protein stability and to develop alternative processes to attain this stability.

Highlights

Compositional drivers for texture, hotness and bitterness

A non-targeted metabolomic study led to discovery of an indole conjugate that was found to correlate with the bitterness of white wine fractions. Sensory analysis suggests that this compound might be a new bitterant in white wine. This is a significant development as currently there are very few molecular targets for bitterness in wines.

In still wines, perceived bitterness was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in a Chardonnay wine, which was consistent with a trend seen in a Viognier wine. As such, it is recommended that dissolved CO_2 levels in still wines should be monitored by producers for their effect on the sensory characteristics of wines they produce.

Sensory assessment of isolated red wine polysaccharide and three sub-fractions and their interactions with alcohol and pH showed a range of impacts including suppression of astringency, hotness and bitterness but increased perceived viscosity. Low and medium molecular weight grape-derived polysaccharides contributed the most, more so than high molecular weight yeast-derived polysaccharides. This result was consistent with earlier work on white wine polysaccharides.

Improved tests for haze and alternatives to bentonite

The heat test remains the most useful method for predicting haze and determining bentonite addition rates. A shorter heat test has been developed that provides results with a turnaround time of only 5 hours instead of 24 hours. This research has demonstrated that controlling both the heating and cooling times for the heat test is critical for obtaining accurate and reproducible results.

A novel technology has been developed based on the use of coated magnetic nanoparticles to adsorb proteins. The method is very effective in removing pathogenesis-related proteins and the

nanoparticles can be simply separated from juice or wine with the use of an external magnet. This new technology has potential to become an alternative to bentonite treatment.

Understanding the effect of filtration on macromolecules that are important for wine texture
Filtering Cabernet Sauvignon and Shiraz wines using cross-flow filtration, followed by lenticular filters and then 0.65 µm and 0.45 µm membrane filters did not alter the concentration or composition of wine macromolecules (polysaccharides and tannins). Sensory analysis of samples from the trial suggested that filtration is unlikely to affect wine texture.

Lowering alcohol without losing tannin and texture
One of the ways of reducing alcohol in wine is to harvest grapes earlier but this can lead to less texture and colour in the wine. Through use of enzymes, a lower alcohol Shiraz wine was produced from earlier harvested grapes that had the same tannin concentration as a wine made from later harvested grapes. The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on wine tannin, polysaccharide and mouth-feel.

New tannin extraction method for grapes provides prediction of wine tannin
A new 'wine-like' extraction method for grape analysis of tannin and colour was evaluated against the standard extraction method. The 'wine-like' method was shown to better predict tannin content in the final wine than the standard method and allows producers to assess the likely extractability of their fruit.

New methods applied to understanding wine texture and stability
New methods were established to study colloids in wine; for example, aggregates of tannins, proteins and polysaccharides. Isothermal titration calorimetry (ITC), small angle x-ray scattering (SAXS) and dynamic light scattering (DLS) techniques are all now in use.

Objectives

This project focused on providing the required knowledge and tools to allow winemakers to more objectively manage texture, stability, clarity and filterability during winemaking. It achieved this through the improved understanding of precursor grape and wine compositional drivers and a clear understanding of the impact of winemaking processes on the macromolecules and colloids that are linked to these wine parameters.

Specifically, the project investigated:

- the compositional drivers for texture, hotness and bitterness;
- the role of macromolecules such as tannins, polysaccharides, proteins and their aggregate colloids in the expression of texture, stability, clarity and filterability;
- the impact of other wine matrix components on macromolecule function and expression;
- the source of these molecules or their precursors in grapes and yeast and the impact of winemaking processes such as clarification, flotation, vinification and filtration on their retention and/or transformation;
- the impact of filtration on macromolecules;
- strategies for modulation of specific compositional drivers through the use of grape-based fining agents;
- alternative strategies for achieving protein stability;
- practical methods for wineries to determine likely extractability of macromolecules during winemaking; and
- strategies for the stabilisation of colour independent of vintage effects.

The knowledge generated by the project provides a framework for the development of winemaking strategies and practical recommendations for managing colour (and colour stability), astringency, viscosity, hotness, bitterness, filtration processes and protein hazes.

Methods

Interactions between phenolics, alcohol and acidity in mouth-feel and bitterness perception in white wine

White wines made from the 2011 white wine phenolics project previously deemed to be bitter by a trained sensory panel were combined and their phenolics extracted using Amberlite FPX resin. The phenolics were then separated into fractions based on their hydrophobicity using high speed counter-current chromatography using a hexane: ethyl acetate: methanol: water system (described in Gawel et al. 2013). These fractions were analysed for phenolic content by HPLC (Gawel et al. 2014a) and assessed for mouth-feel by a trained sensory panel that had been screened for bitterness sensitivity in model wine at two pH (3.3 and 3.6) and two alcohol levels (11.5% and 13.5%).

Identifying compounds responsible for bitterness in white wine

The hydrophobic isolate from the study described above was sub-fractionated using preparative-scale C18 chromatography to obtain 27 fractions of varying hydrophobicity. These were then assessed for bitterness by a screened sensory panel by applying 1 mL aliquots to the back of the tongue using accepted sensory protocols.

The fractions were assessed by HPLC-MS using a Bruker micrOTOFII high resolution mass spectrometer (Metabolomics Australia) and bitterness ratings were correlated with compound features identified in the fractions to identify candidate compounds responsible for bitterness.

One of those identified was commercially available, but needed to be purified using preparative scale HPLC before being sensorially assessed for bitterness. As only small quantities of the purified compound were obtained, alternative sensory approaches to classical threshold testing were required. Therefore, a recently developed sensory method called 'napping' was used, whereby experienced wine tasters mapped their overall perception of the target compound presented at 100 mg/L and 200 mg/L in a sensory space defined by other compounds with accepted mouth-feel and taste properties, with an overall map being produced using a multi-dimensional scaling method.

Assessing the effect of dissolved carbon dioxide on the taste and texture of white wine

Commercially bottled Chardonnay and Viognier wines, each from the same bottling run, were directly sourced from the winery. The uniformity of dissolved CO_2 across bottles in each batch was assessed by in-bottle measurement using Orbisphere (Hach).

Wines with different levels of carbonation, pH and ethanol concentration required for sensory assessment were obtained as follows. A portion of wine was carbonated under pressure at 23^{o}C, and another was extensively sparged with nitrogen gas. These components and the original wine were mixed in-bottle to achieve four target carbonation levels ranging from low to very high by commercial standards (0.5, 1.0, 1.5 and 2.5 g/L). Wines were adjusted to pH 3.4 and 3.2 and to 12 and 14% v/v. The wines were immediately recapped and the sealed prior to immediate sensory assessment.

Samples of 150 mL of wine were poured into glasses typical of restaurant and domestic wine consumption (100 mm height, 80 mm bowl, 60 mm opening). The wines were poured in groups of four at 10-11^{o}C, and tasted within 10 minutes of pouring. Nine trained tasters rated the intensity of mouth-feel characteristics and overall aroma and flavour using standard descriptive protocols.

Dissolved CO_2 concentrations of the wines poured for the tasters were simultaneously sampled and measured directly from the glass using the Orbisphere system modified for *in situ* wine glass

sampling. This approach allowed variations in dissolved CO_2 arising from pouring and losses in dissolved CO_2 in the glass due to the elapsed time between pouring and tasting to be modelled.

Impact of polysaccharides on red wine sensory properties

Whole polysaccharides were extracted from a current vintage Shiraz wine, and fractionated by molecular weight (MW) using preparative scale size exclusion chromatography into high MW (> 93 kDa), medium MW (13-93 kDa) and low MW (5-12 kDa) fractions. These were characterised by their monosaccharide composition (Figure 2). 150 mg/L of each fraction were added to model red wines containing 0.5 g/L tannin (Tanin Galalcool, Laffort, Bordeaux, France) at pH 3.3 and 3.6, and 11.5 and 13.5% v/v alcohol. Their taste and mouth-feel attributes were profiled by a trained sensory panel using standard descriptive sensory methods (Gawel et al. 2016a).

Understanding the effect of juice solids management

In a small-scale scoping study, a Chardonnay juice was left unsettled and settled under gravity to two clarity levels using a pectolytic enzyme, bentonite, and without a clarifying agent. Controls for each solids level/clarification method combination were obtained by centrifugation to differentiate the influence of settling time from the effect of juice clarity.

In a larger scale study, wines from three white varieties were made from full solids juices and the equivalent low solids juices produced by whole bunch pressed, free run and hard pressing juices cold settled using a pectolytic enzyme.

Two further trials were conducted using juices obtained under commercial conditions. A Chardonnay and a Sauvignon Blanc juice were produced by medium-scale wineries using cold settling with pectolytic enzymes, and Chardonnay and Frontignac juices were produced by large-scale wineries using flotation.

With exception of the scoping study the wines were made in duplicate in 20 L fermenters using standard winemaking protocols and were sensorially profiled 12 to 18 months after bottling using standard descriptive techniques (both described in Gawel et al. 2014a,b). The scoping study was conducted using triplicate 500 mL fermenters of wine tank-like dimensions.

Polysaccharide concentrations were determined by either peak area using size exclusion chromatography (>10 kDa), or by phenol-sulfuric assay where proteins may be a significant confounding factor (see Gawel et al. 2016b). Monosaccharide composition was determined by the method described in Ruiz-Garcia et al. (2014). Total phenolics were determined by the Folin assay and specific phenolics by HPLC as previously described (Gawel et al. 2014a).

Surface characterisation

Plasma polymerisation was used to coat magnetic nanoparticles (Mierczynska-Vasilev et al. 2017) and to create surfaces of varied properties, such as polarity and charge (Mierczynska-Vasilev and Smith 2016a, b). Quartz crystal microbalance with dissipation (QCM-D) was used to study wine adsorption on plasma polymer coated surfaces (Mierczynska-Vasilev and Smith 2016a,b). X-ray photoelectron spectroscopy (XPS) analysis was used to determine the surface composition of various plasma polymer coated surfaces before and after wine adsorption (Mierczynska-Vasilev and Smith 2016a,b). Atomic force microscopy (AFM) was used to provide topographical images of wine constituents on polymer-coated surfaces (Mierczynska-Vasilev and Smith 2016a,b) and to determine the amount of hydration water within the wine layers (in combination with QCM-D) (Mierczynska-Vasilev and Smith 2016a). Scanning electron microscopy (SEM) was employed to determine the morphology of the magnetic nanoparticles (Mierczynska-Vasilev et al. 2017). The thickness of the deposited plasma polymers was determined using an ellipsometer (Mierczynska-Vasilev and Smith

2016b). Nanoparticle Tracking Analysis (NTA) was used for the assessment of wine macromolecules size and concentration (Mierczynska-Vasilev and Smith 2016a,b McRae et al. 2017, Mierczynska-Vasilev et al. 2017, Bekker et al. 2016, Bindon et al. 2016a). Wettability of various plasma polymer-coated surfaces was determined by measuring the contact angle (Mierczynska-Vasilev and Smith 2016a). The zeta potentials of the magnetic nanoparticles before and after plasma coating in aqueous suspensions were measured as a function of pH and proteins in wines were analysed by high-performance liquid chromatography (HPLC) (Mierczynska-Vasilev et al. 2017).

Phenolics, tannins, polysaccharides (soluble and insoluble) and cell walls

The methyl cellulose precipitable (MCP) tannin assay was used to measure the concentration of tannins in red wine and colour properties were assessed using the Somers colour measures for wine (Mercurio et al. 2007). Methods for isolating and fractionating tannins from wine included a solid phase extraction (SPE) method which afforded a comparatively uncomplicated technique for the consistent isolation and fractionation of tannin from wine (Jeffery et al. 2008), and a liquid-liquid fractionation, which allowed separation of isolated wine tannins into two fractions with distinctive properties, F2 and F3 (McRae et al. 2013). In addition to these methods, tannins were also isolated from grapes and wine using existing protocols for preparative-scale chromatography with Sephadex LH-20 (Bindon et al. 2010a, 2010b, 2011) or Toyopearl media (McRae et al. 2010). Multi-layer counter current chromatography (MLCCC) was used to isolate polyphenols from white wine (Gawel et al. 2013).

Tannin characterisation was achieved using established methods including gel permeation chromatography (GPC) for elucidating the average molecular mass of tannin (Kennedy and Taylor 2003) as well as acid-catalysed depolymerisation in the presence of phloroglucinol to determine the composition of the tannin subunits (Kennedy and Jones 2001). Also, isothermal titration calorimetry (ITC) was used to measure the protein-binding capacity (McRae et al. 2010).

The research on grape maturity and its impact on extractability of phenolics required isolation and characterisation of polysaccharides and cell wall material (fibre). Isolation of grape skin and flesh cell walls was achieved using a modified extraction with a buffered-phenol solution. Grape and wine soluble polysaccharides were obtained by precipitation in ethanol and dialysis. Semi-preparative fractionation of red wine soluble polysaccharides was based on size exclusion chromatography using a Sephacryl column. A colorimetric galacturonic acid assay was used for quantifying soluble polysaccharides and insoluble cell walls using a 96 well plate format. High-throughput monosaccharide analysis in polysaccharide hydrolysates was accomplished by using derivatisation with 1-phenyl-3-methyl-5-pyrazolone and analysis by HPLC. Cell wall linkage analysis was performed by methylation and analysis as partially methylated alditol acetates by GC-MS. Analysis of white and red wine soluble polysaccharide molecular mass distribution was achieved using size exclusion HPLC with a refractive index detector. Details of these methods can be found in Bindon et al. (2012) and Bindon and Smith (2013).

Best practice representative sampling, handling and processing of grape samples from viticultural trials was undertaken, for example as outlined in Holt et al. (2008). Red and white winemaking was undertaken on varying scales including 10 mL, 1 L, 2 L, 20 L, 80 L and 900 L ferments in rotary tanks, static tanks, coffee plungers, plastic containers, test tubes and other laboratory equipment. Sensory descriptive analysis of wines, and of isolated compounds reconstituted in either model wine solutions or real wines, and consumer preference testing were also performed following standard protocols.

A range of methods for detailed characterisation of phenolic compounds from grapes and wines were used including nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography mass spectrometry (LC-MS), ultra-violet to visible (UV-Vis) spectroscopy, mid infra-red (MIR) and

near infra-red (NIR) spectroscopy, high performance liquid chromatography (HPLC) including gel permeation chromatography (GPC), reverse phase, normal phase and other speciality columns.

Mechanisms of haze formation

To elucidate the mechanism of haze formation and obtain information required to trial new prevention strategies, proteins from wines had to be purified and characterised, and their aggregation behaviour analysed in reconstitution experiments and in combination with other wine components. Isolation of proteins from juice and wine was achieved using the two-step chromatographic method based on strong cation exchange (SCX) and hydrophobic interaction chromatography (HIC) (Van Sluyter et al. 2009). For protein characterisation, the purity of isolated proteins was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and rapid reverse phased-high performance liquid chromatography (RP-HPLC) (Culbert et al. 2017). Reconstitution experiments were also used, whereby key components likely to be involved in haze formation were purified and added back to wines or model wines. The aggregation behaviour upon heating of samples prepared with different combinations of purified proteins and other wine components was analysed by nephelometry (Pocock and Waters 2006), differential scanning fluorimetry, nanoparticle tracking analysis (McRae et al. 2017) and isothermal titration calorimetry (McRae et al. 2015).

Haze predictions

In order to develop a shorter method for haze prediction without compromising accuracy, wines were subjected to a heat test, bentonite fining trials and storage trials (Pocock and Waters 2006).

Viability of bentonite alternatives

In order to assess the viability of alternatives to bentonite, new processing aids were first trialled at laboratory-scale to define the optimal conditions. If successful, these conditions were scaled up in small-scale winemaking experiments. Experimental wines were prepared with different treatments (such as carrageenan, pectin, zirconia, flash pasteurisation with proctase, bentonite), and characterised through chemical and sensory analysis (Marangon et al. 2012, 2011).

Filtration effects on red wine

In order to assess the impacts of filtration on red wines, wines were first filtered through commercial-scale filters (cross-flow to 0.45 µm membranes) and assessed for particle size, macromolecule composition, chemical composition and sensory profiles (McRae et al. 2017).

Matrix effects on red wine astringency

Isothermal titration calorimetry was used to assess the strength of interactions between tannins and polyproline in different ethanol concentrations (McRae et al. 2015).

Results and discussion

Interactions between phenolics, alcohol and acidity in mouth-feel and bitterness perception in white wine

White wines made from the 2011 white wine phenolics project previously deemed to be bitter by a trained sensory panel were combined and their phenolics extracted using Amberlite FPX resin. The phenolics were then separated into fractions.

The more hydrophobic fraction contained a significantly higher proportion of flavonols (skin phenolics) and contributed to bitterness and perceived acidity of model wine at all pH and alcohol levels, and to hotness in lower alcohol model wines, while the less hydrophobic fraction increased bitterness of only the higher pH model wines (Gawel et al. 2016a). These results suggest that different phenolic classes impact differently on mouth-feel and bitterness of white wine, and therefore can be manipulated by managing extraction from skins during white winemaking.

Identifying compounds responsible for bitterness in white wine

The more hydrophobic isolate from the previous study was sub-fractionated using preparative-scale C18 chromatography to obtain 27 fractions of varying hydrophobicity. These were then assessed for bitterness by a screened sensory panel by applying 1 mL aliquots to the back of the tongue using accepted sensory protocols.

The bitter fraction isolated in the previous study was further fractionated into 24 sub-fractions to isolate the compound(s) responsible for its bitterness. These were found to vary substantially in perceived bitterness despite having the same phenolic concentration (Figure 1).

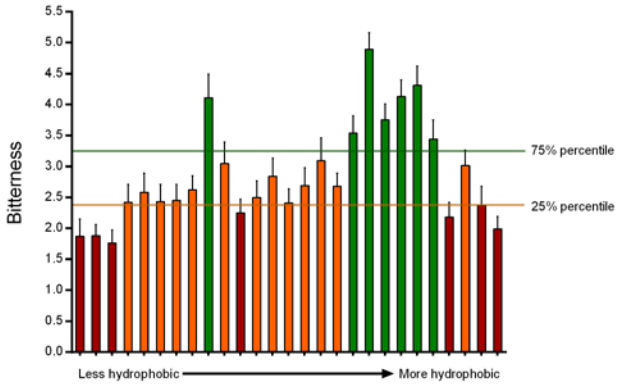


Figure 1. Perceived bitterness of compounds isolated from white wine

The presence of an indole conjugate was found to statistically correlate with the bitterness of the fractions. Sensory assessment showed that the characteristics of the target compound were perceptually more like those of the known bitter compounds epicatechin and quinine sulfate than the astringency of aluminium sulfate and grape skin tannin, hotness from ethanol, or acidity from malic acid. The results suggest that the indole derivative might be a new bitterant in white wine.

Indole compounds similar to that of the target compound have been reported previously. Further work should concentrate on determining the concentration range of this and related compounds in white wine, and establishing whether these bitterant(s) have the potential to work additively like other known bitterants (Keast et al. 2003). It could also explore how the concentration of these

compounds and the associated bitterness in wine are influenced by chemical and biochemical reactions.

The effect of dissolved carbon dioxide on the taste and texture of white wine

Still bottled white wines contain dissolved carbon dioxide (CO_2) as part of their bottling specification, with concentrations typically in the range of 0.5 to 1 g/L. While excessive levels of dissolved CO_2 in still white wines can impart in an obvious 'spritz' sensation which is inconsistent with consumer expectations, some dissolved CO_2 is known to enhance overall mouth-feel. However, its specific effects are unknown.

Commercially bottled Chardonnay and Viognier wines with different levels of carbonation, pH and ethanol required for sensory assessment were modified to four target carbonation levels ranging from low to very high by commercial standards (0.5, 1.0, 1.5, 2.5 g/L). Wines were adjusted to pH 3.4 and 3.2 and to 12 and 14% v/v. The wines were immediately recapped and sealed prior to immediate sensory assessment. The perceived bitterness in both the wines was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in the Chardonnay wine, a result which was consistent with a trend seen in the Viognier wine. Dissolved CO_2 did not significantly influence perceived viscosity or astringency. The perception of spritz increased significantly with increasing dissolved CO_2 levels in both wines as expected, but in the case of the Viognier wine, higher pH and higher ethanol content accentuated the perception of spritz when dissolved CO_2 was high.

It is believed that no previous studies have evaluated the effect of dissolved CO_2 at still white wine concentrations on mouth-feel perception. In the most analogous situation to white wine, 5 g/L of dissolved CO_2 increased the perceived astringency and sweetness of model apple cider containing polyphenols, but did not affect its bitterness (Symoneaux et al. 2015). These results contradict those of this study. The increased astringency may have resulted from lower pH levels resulting directly from the presence of carbonic acid formed by the dissociation of CO_2 in solution, or indirectly through an increased polyphenol-induced astringency resulting from the lower pH. In this study, the wines were equalised for pH after increasing CO_2 levels which may explain why astringency was unaffected by increased dissolved CO_2. The increase in perceived sweetness is consistent with the reduction in bitterness as they suppress each other, but the reasons for the reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear, but warrant further investigation.

Matrix effect of ethanol on tannin-protein interactions likely affects red wine astringency

The mechanism by which astringency is elicited within the oral cavity remains unknown, despite wide promulgation of the theory of saliva de-lubrication by tannins. Wines with lower ethanol concentrations are reportedly more astringent (at similar tannin concentrations) although the reasons for this are unclear. Isothermal titration calorimetry (ITC) was used to measure the binding strength between the model salivary protein, poly(L-proline), PLP, and a range of wine tannins (tannin fractions from a three- and a seven-year old Cabernet Sauvignon wine) across different ethanol concentrations (5, 10, 15 and 40% v/v). Tannin-PLP interactions were stronger at 5% ethanol than at 40% ethanol. The mechanism of interaction changed for most tannin samples across the wine-like ethanol range (10-15%) from a combination of hydrophobic and hydrogen-binding at 10% ethanol to only hydrogen binding at 15% ethanol. These results indicate that ethanol concentration can influence the mechanisms of wine tannin-protein interactions and that the previously reported decrease in wine astringency with increasing alcohol may, in part, relate to a decrease in tannin-protein interaction strength (McRae et al. 2015).

Effect of red wine polysaccharides on mouth-feel and taste

Whole polysaccharides (PS) were extracted from a current vintage Shiraz wine, and fractionated by molecular weight (MW) using preparative-scale size exclusion chromatography into high MW (> 93 kDa), medium MW (13-93 kDa) and low MW (5-12 kDa) fractions. These were characterised by their monosaccharide composition (Figure 2). Additions of 150 mg/L of each fraction were made to model red wines containing 0.5 g/L tannin (Tanin Galalcool, Laffort, Bordeaux, France) at pH 3.3 and 3.6, and 11.5 and 13.5% v/v alcohol. Their taste and mouth-feel attributes were profiled by a trained sensory panel using standard descriptive sensory methods (Gawel et al. 2016b).

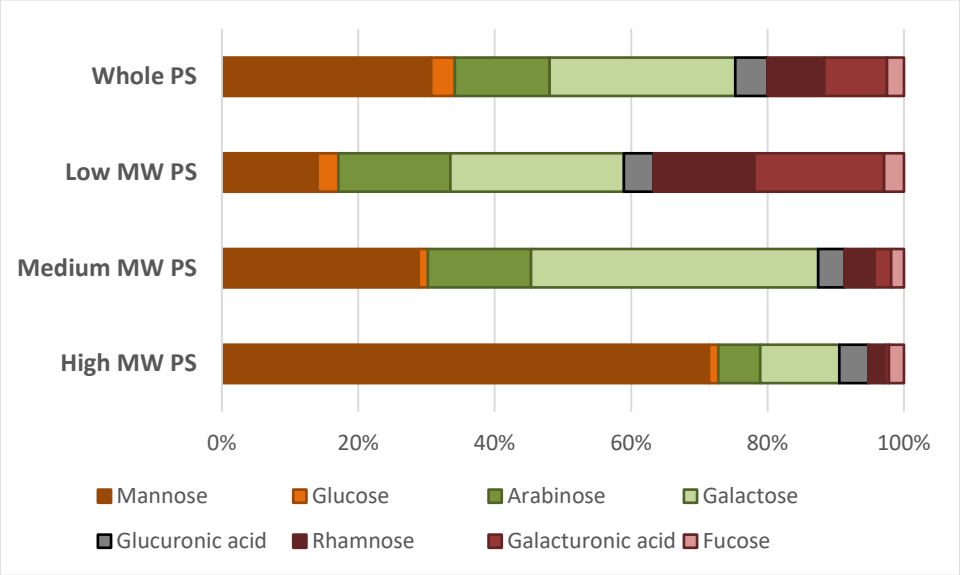


Figure 2. Monosaccharide composition of fractions derived from a red wine polysaccharide. Brown/orange is indicative of mannoproteins, green is indicative of arabinogalactan proteins, and red/pink/grey is indicative of rhamnogalacturonans

Overall astringency and hotness were more influenced by pH and alcohol (respectively) than by PS (Figure 1). However, consistent with work on white wine PS (Gawel et al. 2016), perceived hotness from alcohol was reduced in the low alcohol (11.5% v/v) wines by medium MW PS rich in arabinogalactans (Figure 2). All three PS fractions tended to reduce the astringency of the higher alcohol wines, but the greatest effect was from the low MW fraction consisting mainly of arabinogalactans and rhamnogalacturonans. Vidal et al. (2003) also observed suppression of astringent sub-qualities by purified rhamnogalacturonans. The reduced astringency by PS could be the result of 'shielding' of astringent polyphenols from salivary proteins due to the prior formation of molecular assemblies involving polyphenols and polysaccharides (Soares et al. 2012). The medium and low MW PS increased the perceived viscosity of the model wine most representative of red wine (13.5% v/v alcohol and 3.6 pH) (Vidal et al. 2004). However, in this study, whereby the fractions were also characterised by MW it was notable that a high MW fraction rich in mannoproteins, and typical of those released by yeast autolysis, did not increase perceived viscosity at any pH/alcohol combination. The bitterness of the higher alcohol, higher pH wine was significantly reduced in the presence of medium MW PS but the overall effect of PS on bitterness of the model wines was inconsistent (Figure 3).

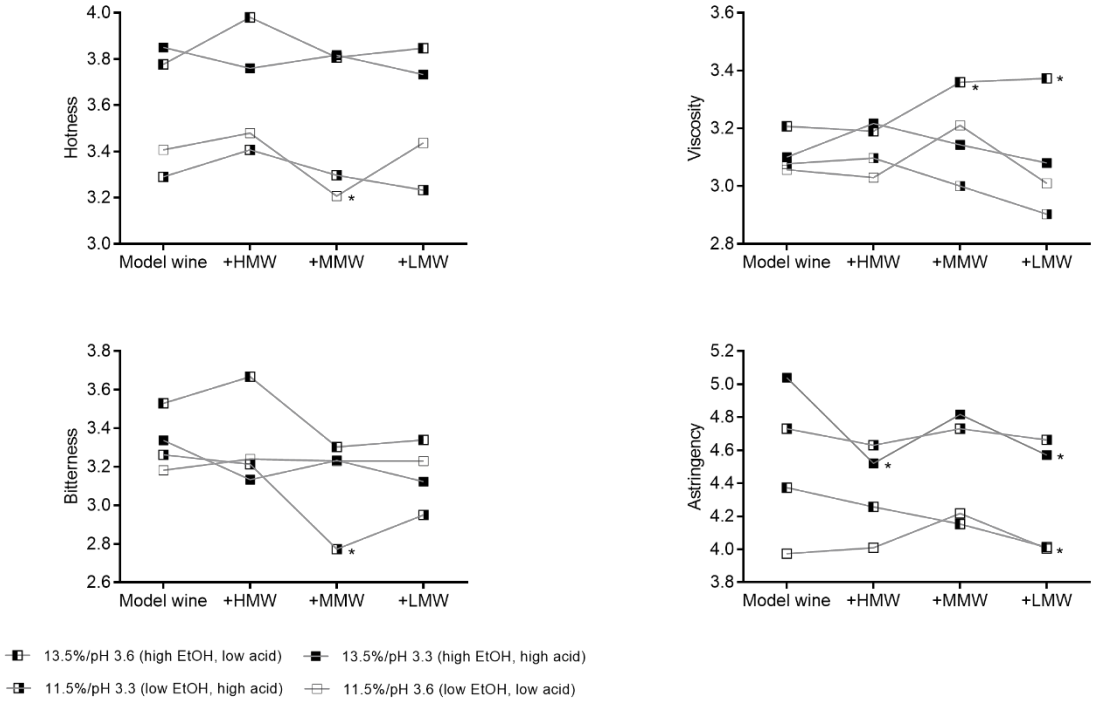


Figure 3. Effect of polysaccharides on the mouth-feel properties of model red wine. * indicates significant difference from respective control model wine ($p<0.1$).

Fundamental methods for understanding texture, taste, clarity, stability and filterability
Knowledge of macromolecular adsorption onto surfaces is critical for a better understanding and control of processes such as filter fouling, binding to tanks and fittings and interactions with processing aids such as bentonite. To improve this knowledge, model surfaces with tailored surface properties (e.g. charge, polarity, chemical functionality, wettability) were developed to explore how wine constituents interact with them. Surface analysis tools not traditionally used in wine science such as QCM-D, XPS and AFM were used to reveal the surface-binding characteristics of different types of colloids in wine without disturbing them through complex isolation and handling.

Surfaces were generated where the presence of surface functional groups such as amines, carboxyls, hydroxyls, formyls, methyls, sulfonate or ammonium could be used to bind wine constituents (Miercszynka-Vasilev and Smith 2016a). The goal was to explore the capacity of functional surfaces to promote or impede red wine constituents' adsorption. To study the adherence and viscoelastic properties of red wine before and after sterile filtration, a QCM-D technology was employed. QCM-D provides label-free measurements of molecular adsorption and/or interactions taking place on various surfaces. In addition to assessing adsorbed mass, measured as changes in oscillating frequency of the quartz crystal, the energy dissipation, which is the reduced energy per oscillation cycle, provides insights regarding structural properties of adsorbed layers. Furthermore, AFM of wine on different modified surfaces and wine morphology were correlated with the wine adherence and viscoelastic properties. The results showed that substrates modified with $-SO_3H$ and –COOH groups can adsorb more of the wine nitrogen-containing compounds whereas $-NH_2$ and $-NR_3$ groups encourage adsorption of carbon-containing compounds. Red wine constituents after filtration were adsorbed to a higher extent on $-NR_3$ and –CHO surfaces. The –OH modified surfaces had the lowest ability to absorb wine components. The results presented above demonstrate that modification of surface chemical states is a strategy to direct the adsorption of red wine constituents such as proteins, polysaccharides and polyphenols.

The effect of surface chemical functionalities on the adsorption of white, rosé and red wine constituents was also evaluated (Miercszynka-Vasilev and Smith 2016b). Allylamine, acrylic acid and ethanol were selected as precursors for plasma polymerization to generate coatings rich in amine, carboxyl and hydroxyl chemical groups, respectively. The results demonstrated that the amine and carboxyl modified surfaces encourage adsorption of constituents from white wine. The hydroxyl modified surfaces can preferentially adsorb rosé wine constituents, whereas red wine adsorbed to the highest extent on acrylic acid surface. Those results are meaningful for the development of the next generation low fouling membranes and/or new sensing platforms that will reduce cost and improve productivity in the wine and other relevant industries.

Improved measurement tools to support understanding of colloidal behaviour in wine
Complex phenomena associated with final macromolecular concentrations are related to colloidal stability and therefore particle size and charge (e.g. protein stability, tannin, polysaccharide extraction and loss). Colloidal systems are generally of a polydispersed nature, that is the molecules or particles in a particular sample vary in size. Typical methods for investigating the formation and properties of colloids in wine (i.e. macromolecular aggregates with particle sizes in the nanometre range) are quite complicated and have mainly been developed for applications in areas other than wine science. They are, however, fundamental to determining how colloids influence the outcomes of many processes in wine production including extraction, settling, clarification, filtration and stability.

To improve existing methods of colloidal characterisation and tailor them to applications in the wine industry, previous research focussed on application of the new techniques of isothermal titration calorimetry (ITC) (McRae et al. 2015), small angle x-ray scattering (SAXS) (McRae et al. 2014) and dynamic light scattering (DLS). Having established their utility, the current project applied a new method, nanoparticle tracking analysis (NTA) to accurately characterise particle size distributions in wine and monitor the zeta-potential of these particles. Zeta potential is a key indicator of the stability of colloidal dispersions and the magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion.

Many techniques are available for the specific analysis of particle size and particle size distribution such as dynamic light scattering (DLS), electron microscopy (EM) and atomic force microscopy (AFM), analytical ultracentrifugation and the recently developed nanoparticle tracking analysis (NTA) technique. Electron microscopy and AFM both offer direct observation of particle images with high resolution information about the size and morphology of the particles, but both techniques require time-consuming sample preparation. Analytical ultracentrifugation also provides high resolution information about particle size but the technique requires a degree of previous knowledge of the composition of the material and is time consuming. Ensemble methods based on light scattering like DLS are ideally suited for the analysis of monodispersed system but have a limited capability to analyse polydisperse systems. NTA has some clear advantages over DLS.

Nanoparticle tracking analysis (NTA) is an innovative system for sizing particles from about 10-30 nm to 1-2 µm, with the lower detection limit being dependent on the refractive index of the nanoparticles. NTA enables the visualisation of the sample, gives an approximate particle concentration and obtains size information based on the Brownian motion of individual particles. NTA is very accurate for sizing both monodisperse and polydisperse samples and has a substantially better peak resolution than other methods. The presence of a few large particles in a sample has little impact on NTA sizing accuracy. As such, the utility of the NTA was assessed in a range of applications to assess colloidal phenomena important to wine production.

The different macromolecular complexes that occur with different winemaking techniques are known to alter the extraction of tannin, and potentially polysaccharides and protein. While supporting a project on Pinot Noir winemaking techniques (investigating microwave and heating of musts) led by the Tasmanian Institute of Agriculture (TIA) there was an opportunity to apply this tool. By characterising the complexes formed in the different wines, the aim was to determine the proportion of tannin which became irreversibly bound in complexed forms, and to infer the compositional differences in macromolecules which drove this, if any. The role of altered macromolecule concentration and composition in the formation of complexes or colloids was studied in reconstituted wines using NTA. It was found that particle size increased when the precipitate was recombined with tannin, and was similar to that of the precipitate analysed alone (≅200 nm). Addition of a monomeric fraction increased the incidence and concentration of smaller particles (90 – 200 nm). There were only minor differences in particle size distribution between microwave treatments and standard maceration treatments. However, particle concentration was increased in response to the increased concentration of macromolecules in the microwave treatment. This suggests different colloidal behaviour may occur in these wines, although the functional impact of this remains to be established (Bindon et al. 2016a).

Haze formation in wines is a complex process involving interactions between wine proteins and between proteins and other wine matrix components including phenolics, ionic strength, sulfate ions and polysaccharides. The impact of matrix components on the stability of chitinase M1 protein was investigated using nanoparticle tracking analysis (NTA). The aim of this study was to examine, using reconstruction experiments, the aggregation behaviour of purified chitinase M1 protein and to measure the size and concentration of individual particles formed by this protein before and after heating in the presence or absence of wine phenolics and/or polysaccharides and/or sulfates using NTA. The role played by ionic strength and sulfate in the aggregation of chitinases was also assessed. The study confirmed that protein haze formation in white wine is a multifactorial process where ionic strength, sulfate and also wine molecules such as phenolic compounds and polysaccharides all modulate the white wine protein haze potential.

Macromolecule and colour extraction, stability and retention and influence on wine style and production practices

Tannin and polysaccharide concentration and composition are important in defining the texture of red wines, but can vary due to factors such as cultivar, region, grape ripeness, viticultural practices and winemaking techniques. Colour development in wine is dependent upon the extraction of sufficient quantities of tannin from the grapes together with anthocyanin, to ensure that stable non-bleachable (including polymeric) pigments form. The concentration and composition of these key macromolecules is dependent not only on grape tannin, anthocyanin and polysaccharide concentration and composition, but also their extractability and, in the case of polysaccharides, their formation by yeast.

Previous research at the AWRI has shown that the processes involved in the extraction of these macromolecules from grapes and their retention in wine are very complex. In particular, the isolation and characterisation of polysaccharides and cell wall material (fibre) has shown that grape cell wall material (CWM) can bind tannins and anthocyanins and modify the amount and type of macromolecules retained in wine. The effects of grape composition (including ripeness), macerating enzymes, yeast and certain winemaking treatments on this cell wall material can also profoundly influence the amount of tannin, colour and polysaccharide retained in wine (Holt et al. 2013, Smith et al. 2015). These recent advances present important new factors for consideration in grape selection and processing during winemaking which can allow winemakers to more rigorously control colour and mouth-feel in red wines. The ongoing AWRI research programme has aimed firstly to establish methods to predict tannin and colour extraction from grapes during primary fermentation,

and secondly to explore how diversifying grape composition and winemaking technique can result in a divergence from the predicted extractability values.

Establishing a technique to predict tannin and colour extractability from grapes

There are large differences in tannin and colour extractability and these may be due to the interaction between phenolic compounds and other macromolecules during fermentation. Insoluble cell wall material (fibre) can bind and thus trap tannin and anthocyanin during fermentation, and interactions between soluble polysaccharides and proteins may also result in a loss of otherwise soluble phenolics. These may be lost in marc or lees, or be sequestered in colloid complexes in red wines. Cell wall macromolecular composition may therefore influence anthocyanin as well as tannin extractability and retention. The AWRI's research has shown that biochemical changes occurring during grape ripening increase the extractability of grape skin tannins, but may reduce the extractability of seed tannins. Notably, the magnitude of this effect differs markedly between grape varieties.

To further address the role of cell wall composition in tannin release and back binding, a sequential fractionation technique was employed to produce cell wall fractions of defined polysaccharide composition. More than 54% of cell wall-bound tannin was found to be associated with pectic polysaccharides in the cell wall (Ruiz-Garcia et al. 2014). This observation was further corroborated by model adsorption experiments which confirmed that the removal of pectic polysaccharides most significantly reduced the adsorption capacity of cell walls for tannin. Follow-up experiments were designed using enzymes to depolymerise the pectic fraction of grape cell walls. This work showed that the enzyme initially increased polysaccharide extraction from CWM, but depolymerisation to galacturonic acid and arabinose sugars was ongoing, leading to reduced final polysaccharide concentration. Enzyme application reduced the ability of grape cell walls to adsorb tannin, but it was found that a prolonged period in buffer also resulted in a concurrent release of pathogenesis-related proteins from grape mesocarp (pulp) cell walls. This protein, although a minor component of the cell wall, could remove up to 50% of tannin from solution by the formation of unstable complexes. These complexes eventually precipitated, and were too large for an accurate size to be determined by NTA, but could be visualised by microscopy. This research identified a potential mechanism by which extracted grape tannin may be lost from wine during vinification, theoretically proceeding until all protein (or all tannin) is removed from solution. This might explain why enzyme application does not always result in significant increases in tannin concentration, and is dependent on the fruit composition.

These experiments indicated that to accurately predict extractable tannin (and colour) from grapes, a method would need to be developed which factored in potential variations in tannin, pectin and protein concentration in grapes and facilitated the multiple levels at which complexing of macromolecules could occur. Based on successful pilot studies, testing was conducted on the effectiveness of a simple extraction method to determine 'wine extractable' tannin and anthocyanin. Through collaboration with Accolade Wines, triplicate wines were made from 39 Shiraz and Cabernet Sauvignon grape samples sourced from different regions across South Australia. The 'wine-like' (EGT, extractable grape tannin) extraction method used gently crushed grapes, adjusted to 15% v/v ethanol, pH 3.4, in their own juice (Figure 3). To compare the effectiveness of the EGT extraction method against standard methods available to industry, the grapes were also homogenised and extracted in 50% v/v ethanol. Wines and extracts were analysed for tannin and anthocyanin, and colour density (wine only) (Bindon et al. 2014).

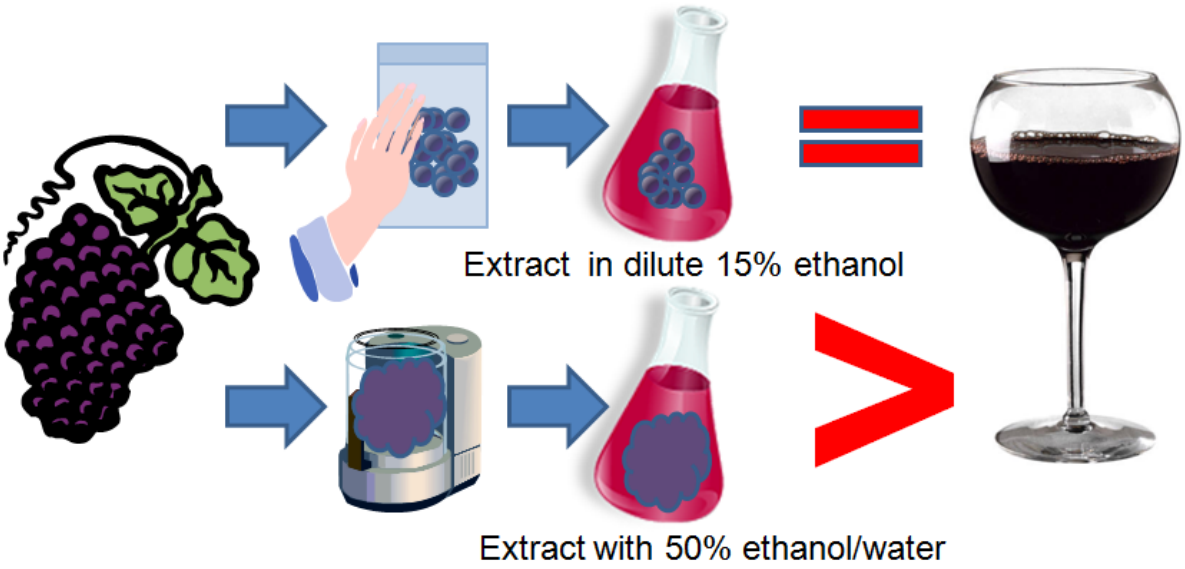


Figure 4. Comparison of 'wine-like' vs standard extraction protocols for tannin and colour analysis. The gentle 'wine-like' extraction in 15% ethanol (top) gives a good prediction of eventual wine tannin, while the standard protocol of grape homogenate extracted in 50% ethanol (bottom) overestimates the eventual wine tannin concentration.

The results were very encouraging, as it was found that the application of the EGT extraction method could provide a good indication of wine tannin (Figure 5) and anthocyanin concentrations extractable during fermentation, as well as predict wine colour density (Bindon et al. 2014). Strong regional differences were found for both grape varieties. When considered on a varietal basis, the 'total' tannin concentrations measured using the grape homogenate method also correlated with those measured in the wines, but were noticeably higher than the actual concentrations achieved through vinification. The overestimation of tannin concentrations in grape homogenate extracts was greater for Cabernet Sauvignon samples than for Shiraz. Correlations between the grape anthocyanin, and wine anthocyanin, as well as wine colour density were strong, and similar for both Cabernet Sauvignon and Shiraz and extraction method. In summary, the work demonstrated that the EGT extraction approach provides a useful prediction of wine tannin and colour.

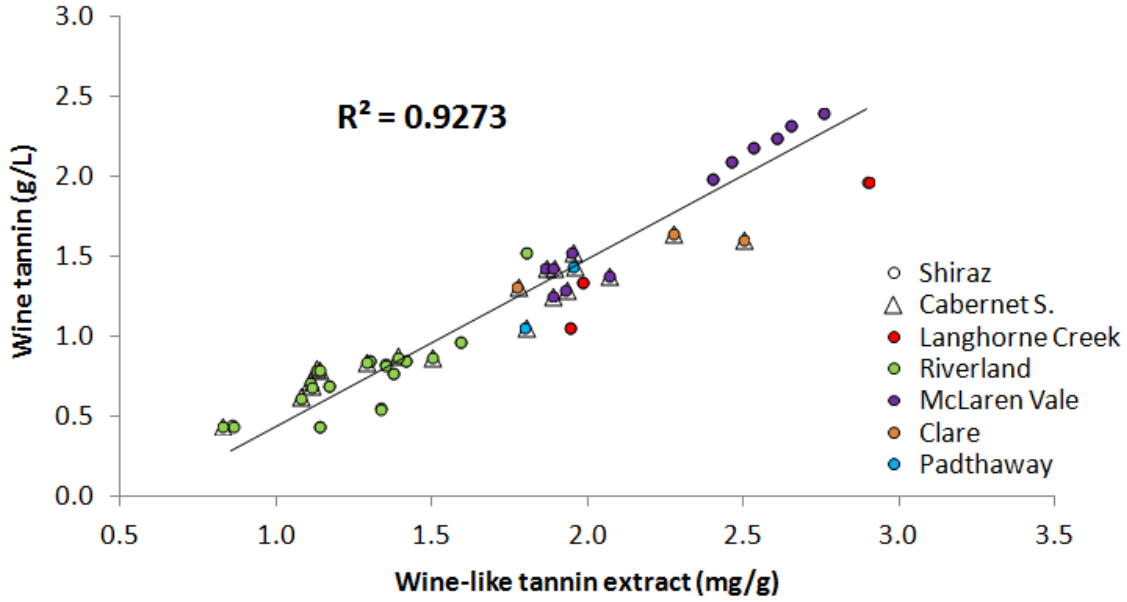


Figure 5. Correlation of grape tannin concentrations measured using the 'wine-like' extraction protocol with resulting wine tannin concentrations

Red winemaking tools to alter wine sensory properties by modulating macromolecule concentration

Once the EGT method was established to determine tannin extractability during primary ferment, ongoing research aimed to establish the extent to which winemaking interventions could influence the final tannin concentration from the predicted value. At a further level, the influence of winemaking treatments on polysaccharide concentration and composition was an area which is poorly understood. The experiments conducted focused on evaluating yeast strains and maceration processes during winemaking as tools to alter wine macromolecule concentration and composition.

In wine made in 2014 it was found that the choice of yeast strain (10 yeast strains were benchmarked) resulted in highly variable polysaccharide and tannin concentrations. At the end of primary fermentation, the two yeasts that yielded the highest wine tannin concentrations (1.5 g/L) resulted in wine with the lowest (0.45 g/L) and highest (0.66 g/L) polysaccharide concentrations respectively. It was found that the wine with the highest polysaccharide and tannin was associated with a transient release of pectic polysaccharides rich in galacturonic acid and arabinose from the grapes, suggesting pectolytic activity in yeast.

Analysis was conducted to compare the influence of yeast strain on wine tannin concentration compared with that predicted by the EGT method. In this instance, it was found that using yeast strain EC1118, that which was employed to establish the EGT method, the predicted value for tannin concentration varied by <5% from the actual value using this strain, confirming the accuracy of the EGT assay. However, the maximum tannin concentration (yeast 2323) was 33% higher than predicted (EC1118), and the range between the lowest (AWRI1503) and highest (2323) tannin concentrations in the wine was 64%, indicating that yeast strain alone can strongly influence the range of tannin outcomes for a given batch of grapes. The results for wine tannin were strongly expressed in wine sensory outcomes with astringency and opacity (colour) of the wines being major drivers of differences among yeasts. Wine opacity was strongly defined by wine colour after 24 months' ageing. Initially, wines which were produced using different yeast strains had very similar colour, however, after a period of 24 months it was observed that the colour density (and non-

bleachable pigments) of the wines made from the respective yeast strains was strongly correlated with wine tannin concentration. Since wine astringency was well correlated with wine tannin, this meant that wine astringency and opacity were also correlated. The strongest differences in wine astringency were shown for the low tannin strain AWRI1503 compared with high-tannin 2323 which also had the highest astringency.

Based on leads from this trial, in 2015 an experiment was performed to investigate the interactive effect of maceration time (7 vs 30 days), macerating enzyme and yeast strains ('high-tannin, 2323' vs 'low-tannin, AWRI1503' yeast) on wine macromolecules in 50 kg Shiraz ferments. A number of experiments on Shiraz grapes showed that macerating enzyme could increase tannin concentration up to 30% with minimal effects on colour. In the 2015 experiment it was found that at 7 days' maceration, enzyme treatment did not affect tannin concentration but yeast strain was a more important contributor. Similar to the preliminary study in 2014, yeast strain significantly affected tannin concentration, with wines made with 2323 having tannin concentrations 35% higher than those made with AWRI1503. Wine polysaccharides, on the other hand, were minimally affected by yeast strain at 7 days' maceration, unlike the earlier 2014 study where 2323 yeast had the highest polysaccharide concentrations. Investigation of wine monosaccharide concentrations showed that both 2323 and AWRI1503 had similar release of galacturonic acid independently of enzyme application, indicating that both yeast strains may in fact have endogenous polygalacturonase activity. As found for other experiments using macerating enzyme, polysaccharides in wines from this treatment were enriched in RGII (high rhamnose:galacturonic acid ratio) and had proportionally higher mannoprotein. The reason for mannoprotein enrichment is not known, but may be due to enhanced release of nutrients (sterols) following CWM breakdown. At 30 days post maceration, no effect of yeast strain on polysaccharide concentration or composition was observed, yet differences in tannin concentration were maintained with 2323 having 31% higher tannin than AWRI1503.

Maceration by itself significantly increased tannin and polysaccharide extraction independently of enzyme or yeast strain. Extending maceration facilitated increased extraction of tannin mainly from the seeds, which resulted in a large colour increase (non-bleachable pigment and colour density). When enzyme treatment was combined with extended maceration, differences between yeast strains were lost. The maximum tannin concentration achieved with the extended maceration and enzyme combination was 52% higher than the predicted value (using the EGT assay). The range in tannin concentration from the AWRI1503 control (lowest) to the extended maceration/enzyme treatment (highest) was 133%. In terms of wine polysaccharides, the type extracted depended upon whether enzyme was applied or not, but final concentrations were similar and independent of either yeast strain or enzyme.

In summary, the combined use of yeast strain and maceration techniques can have a marked effect on wine tannin, but 30 days of maceration achieved the most significant shift in tannin concentration and molecular mass. Interestingly, the effects of these treatments on wine sensory attributes were minimal. In terms of astringency, only wines made with the high-tannin yeast 2323 in combination with extended maceration and enzyme addition had higher astringency than the other treatments. This may point to the fact that polysaccharide increases in conjunction with extended maceration may ameliorate some of the astringency which might be expected with tannin concentration increases, and warrants ongoing study.

Retaining texture and colour in lower alcohol wines

A number of experiments continued to study how various approaches (pre-ferment) to lowering alcohol affect tannin, polysaccharide and colour outcomes in red wine, with the goal of understanding how these impact wine texture. A primary objective was to explore the possibility of harvesting grapes earlier to naturally lower wine alcohol, and to explore whether winemaking

techniques could be used to improve the texture of these wines. Changes to regulations in 2017 enabling the pre-fermentation addition of water to high sugar must (to a minimum of 13.5 Baume) warranted the inclusion of must dilution as a further objective of this research. For all the projects which studied grape maturity to modify wine alcohol, harvesting earlier consistently resulted in wines with reduced wine tannin, polysaccharide and colour. As expected, lowered levels of these key wine components in wines made from earlier harvests reduced astringency, palate fullness and colour, but also importantly, wine alcoholic hotness. To ameliorate the lack of texture and colour in the earlier-harvested wines a number of approaches were attempted, including macerating enzyme treatment, and additions of tannin, polysaccharide and marc.

An experiment compared a standard harvest (Shiraz) at 14% alcohol to a late harvest at 16.7% alcohol (Li et al. 2017). It was found that tannin was increased 28% from the early to late harvest with associated increases in wine colour. Later-harvest wines were found to have greater flavour intensity, 'dark fruit', 'jamminess', sweetness and hotness but importantly also greater wine palate fullness. While enzyme addition to the earlier-harvested fruit increased wine tannin concentration to the same concentration as that in the later-harvest wine, colour was not affected. The principal effect of enzyme addition was to increase tannin to the same concentration as found for the later-harvest wine, without effects on wine colour or polysaccharide. These enzyme-treated wines were found to have higher astringency, with the wines described as having greater surface coarseness. Enzyme-treated wines also lacked palate fullness compared with those made from riper grapes (Li et al. 2017) indicating a possible limitation in the use of macerating enzymes for this purpose. A further study also looked at early (11%, lower alcohol wine) and late-harvest (14.6%) Shiraz but with the addition of both enzyme and marc. Macerating enzyme addition most consistently increased tannin and colour in the wines made from less ripe grapes, but reduced wine polysaccharide. Marc addition (unfermented white grape marc) was also found to contribute additional tannin to early-harvest wines, but not to the same extent as enzyme addition.

As a further element to this work, dilution of high sugar must was explored in conjunction with earlier harvests as a means to lower wine alcohol. To do this, red grapes were harvested at various stages of maturity from early (unripe, low alcohol) to late (commercially ripe, overripe). Various approaches were explored, including water addition and addition of 'green harvest' (5% alcohol) wine. Two dilution approaches were implemented to trial this method of lowering alcohol: dilution and run-off/replace (saignée). Depending upon the condition of the grapes, must dilution produced varying results. A study by PhD student Olaf Schelezki from the University of Adelaide's ITTC Training Centre for Innovative Wine Production in collaboration with the AWRI showed that when grapes were overripe (17 Baume, shrivelled) dilution with either 'green harvest' wine or water had minimal effects on wine tannin, colour or polysaccharide and did not change any wine sensory attributes aside from reducing wine hotness (Schelezki et al. in press). In a study of commercially ripe (15.5 Baume, not shrivelled) grapes by visiting scientist, Dr Bo Teng, it was found that water addition lowered tannin and colour in wines independently of the mode of addition, showing that using a saignée type of approach to add water does not necessarily increase the retention of phenolics in the wine. Nevertheless, wine produced with dilution consistently had higher levels of tannin and colour than wines of the same alcohol concentration produced from earlier harvested grapes. These results indicate that to effectively lower alcohol whilst maximising wine macromolecule extraction and wine texture, dilution treatments are likely to lead to more favourable outcomes than early harvesting.

Understanding the type of tannin that contributes to stable colour formation

Earlier work at the AWRI established techniques to separate wine tannin (polymeric pigments) into small and large fractions, to determine the colour properties of these fractions. The aim of ongoing work in this project was to determine partitioning of colour to polymeric and non-polymeric wine

fractions and their relative contributions to wine colour. To do this, two approaches were followed. Firstly, grapes of very different colour properties were sourced and were made into wine using the same winemaking method. In this case Shiraz wines made from unique sites (terroirs) across the Barossa region were sourced and studied over three years of ageing. Secondly, wines produced using techniques with large colour differences (Pinot Noir) were sourced.

For the ageing study, Shiraz wines produced using a standard winemaking protocol from different locations in the Barossa Valley demonstrated large differences in colour properties, and differences were retained during three years of ageing. Strong correlations were observed between wine colour density and the following parameters: tannin concentration, tannin molecular mass, tannin % prodelphinidin (skin tannin) as well as increases in the presence of tannin and colour in two fractions of polymeric pigments F2 (small) and F3 (large). Total anthocyanin in wines was less important in determining colour and polymeric pigment formation (F2, F3 and total) than tannin. The distribution of colour as SO_2-resistant pigments changed only slightly with ageing, reaching a plateau at 18 months. Increases in the colour of F3 were greater than F2. These results showed that compositional differences in wine tannin (when anthocyanin is not limiting such as in Barossa Shiraz) are site-specific and critical to the formation of polymeric pigments and retention of colour in aged wines.

For the second aspect of the work, in collaboration with Dr Anna Carew from TIA, Pinot Noir wines were prepared using standard maceration, microwave treatment (Mwv) followed by standard maceration (+Sk) and, or microwave treatment followed by early press off (-Sk) (Bindon et al. 2016a). Colour properties were analysed at six months and one year. Wine colour density and non-bleachable pigments were higher in Mwv+Sk than in Mwv-Sk and the Control wine, and all underwent similar increases from 6 months to 1 year. Separation of wine pigments by solid phase extraction (using a HLB cartridge) into high (F3) and low (F2) molecular mass fractions was carried out. The contribution of F2 and F3 to wine colour density was similar for the wines, but the proportion of colour and non-bleachable pigments in F2 decreased with age and in F3, increased markedly with age. The change with ageing was similar between treatments. The proportion of colour and non-bleachable pigments in the non-polymeric fraction was initially 30% at 6 months, but was less than 14% at 1 year. This indicated that in Pinot Noir wines, the contribution of small derived pigments and anthocyanins to colour is negligible after one year.

The key finding from both studies on colour development was that the high molecular mass polymeric pigment fraction F3 was more important in colour development during wine ageing than the lower molecular mass fraction F2. Increases in non-bleachable pigment and wine colour density during ageing in F3 were greater than in F2. F3 was also found to form a greater contribution to total colour in aged wine. This points to the importance of the extraction and retention of higher molecular mass tannins in wine. The development of colour in these wines appeared to be somewhat independent of anthocyanin extraction. Rather, tannin, and importantly higher molecular mass tannin, played a greater role in the development of stable, non-bleachable polymeric pigments.

The impact of white juice clarification on wine composition and sensory properties

Fermenting juices with higher levels of solids is an option being taken up by some white winemakers with the view to adding textural complexity or mouth-feel, but this aspect, and what compositional factors may influence it are yet to be explored. As such, this project performed a series of related experiments to improve the understanding of these factors.

Mechanically crushing white grapes to release their fermentable juice always produces insoluble grape debris comprising pulp and skin cell wall fragments collectively known as ‘solids’. By weight, solids are mainly comprised of polysaccharides and lipids essential for yeast cell viability (Alexandre

et al. 1994, Casalta et al. 2012). Winemakers typically attempt to remove most solids from juice before fermentation as they are known to promote higher alcohol and reduce ester production by yeast leading to less fruity wines (Konitz et al. 2003). Solids removal is most frequently achieved by settling under gravity with settling agents including pectolytic enzymes and bentonite (Lambri et al. 2012), or by the efficient method of flotation whereby solids 'hitch a lift' on introduced gas bubbles to the top of the tank whereby they are removed.

In a small-scale scoping study (study 1), a Chardonnay juice was left unsettled and settled under gravity to two clarity levels using a pectolytic enzyme, bentonite, and without a clarifying agent. Controls for each solids level/clarification method combination were obtained by centrifugation to differentiate the influence of settling time from the effect of juice clarity.

In a larger scale study (study 2), wines from three white varieties were made from full solids juices and the equivalent low solids juices produced by whole bunch pressed, free run and hard pressing juices cold settled using a pectolytic enzyme.

Two further trials were conducted using juices obtained under commercial conditions. A Chardonnay and a Sauvignon Blanc juice were produced by medium-scale wineries using cold settling with pectolytic enzymes (study 3), and Chardonnay and Frontignac juices were produced by large-scale wineries using flotation (study 4).

A general observation was made that wines produced from high solids juices contained significantly higher concentrations of polysaccharides. More detail of results from individual experiments is provided below.

In study 2, high solids ferments produced from wines from all three cultivars were significantly higher in total polysaccharide concentration compared to low solids ferments produced from whole bunch pressed, free run and hard pressings juices following enzyme addition and cold settling. (Figure 6).

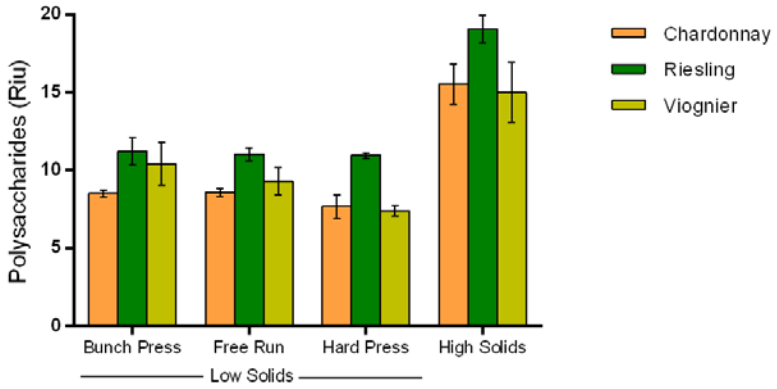


Figure 6. Polysaccharide content of wines made from high solids juice, and low solids juices produced using different extraction methods.

The results of the scoping study (Figure 7), and those of the studies involving commercial juices were consistent with this result.

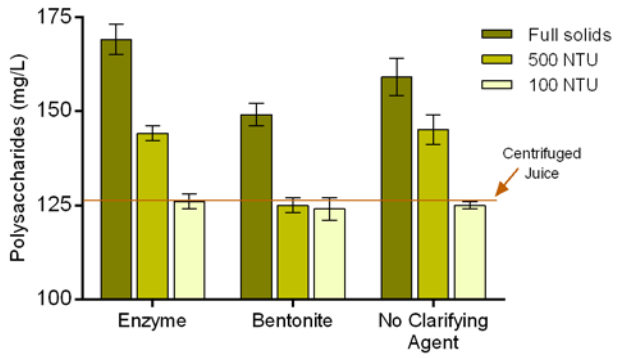


Figure 7. Effects of juice clarification on polysaccharides in white wine in initial scoping study (study 1)

Analysis of the monosaccharide profile in conjunction with the molecular weight distribution of the polysaccharides (study 1) revealed that for all clarification treatments, the polysaccharides in the full solids wines contained a significantly higher proportion of mannose – the diagnostic monosaccharide for yeast activity during fermentation and autolysis post-fermentation. Conversely the mannose content of the polysaccharides in wines produced from centrifuged juices were not consistently different. Full solids wines also had a significantly higher proportion of high molecular weight polysaccharides (>183 kDa), which previous work (Gawel et al. 2016b) has shown to be made up mainly (80%+) of mannoproteins. These results suggest that the increases in total polysaccharides in the full solids treatments were the result of increases in high molecular mannoproteins previously associated with yeast autolysis following fermentation.

The polysaccharides produced from lower solid juices, and therefore those that had experienced longer settling times, tended to have a higher proportion of monosaccharides mostly associated with rhamnoglacturonans (rhamnose, galacutonic and glucuronic acid), and (notably) its diagnostic sugar fucose. There were no consistent significant differences between wines made from high solids juices and those from low solids with respect to these sugars suggesting that rhamnogalacturans are extracted from solids during settling rather than from solids during fermentation.

Effect of juice clarification on white wine phenolics

In study 2, the total phenolics content of the solids wines did not differ from the low solids free run wines in two of the three varieties, with only Chardonnay showing higher phenolic content due to solids (Figure 8). Similarly, in study 1, there were no clear connections between solids level and total phenolics. Comparable studies have found that solids content in juice did not affect the total phenolic concentration of white wine (Singleton et al. 1975, Ollivier et al. 1987). However, HPLC analysis of phenolic composition in these wines showed that wines made from high solids juices possess a different phenolic profile from those made from low solids juices, with high solids wines being significantly higher in flavanol, flavanonol and caftaric acid concentrations, and lower in free hydroxycinnamic acids. Wines in study 1 made from different levels of solids could be clearly differentiated based on their non-polar metabolites, further suggesting that despite producing similar total phenolics, juice solids can influence the phenolic profile of wine.

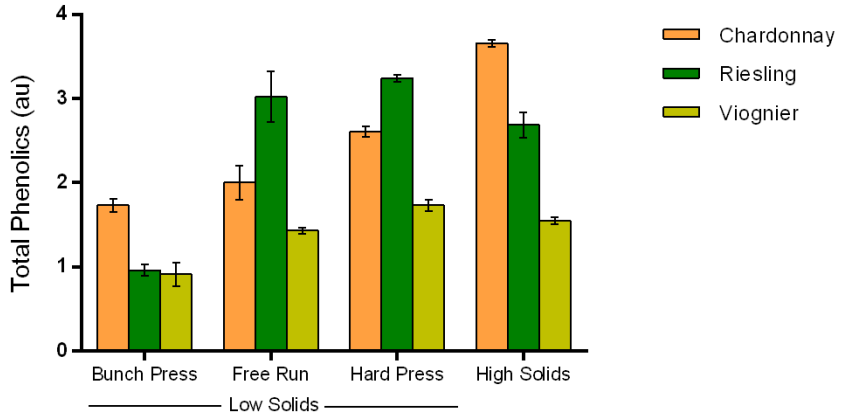


Figure 8. Total phenolic content of wines made from high solids juice, and low solids juices produced using different extraction methods (study 1).

Study 1 also found that the total phenolics levels from full solids ferments were lower with bentonite added than when either enzymes or no clarifying agent was used. This may be the result of low density grape particles distributing bentonite into the ferment via the action of carbon dioxide (Casalta et al. 2012), resulting in phenolic losses by absorption onto bentonite. However, while providing insight into the dynamics of phenolic extraction and loss from grape solids during fermentation, adding a clarifying agent and then simultaneously inoculating the ferment is not a typical commercial practice (Full solids, Bentonite and Enzyme treatments, Figure 7). From a practical perspective, if a winemaker wishes to conduct a low solids fermentation (e.g. 100 ntu) then these results suggest that the method of clarification will most likely not influence the total phenolic concentration of the wine.

The lack of difference in total phenolics between full solids on enzyme and full solids without clarifying agent suggests that there was little of the expected release of phenolics arising from any pectolytic breakdown of the grape particle (solids) cell walls.

Overall, the observation that total phenolic content is not linearly related to juice solid content suggests that a complex interplay between gains and losses by extraction and fining-like mechanisms by the grape solids both during the settling phase and during fermentation is likely.

Effect of juice clarification on white wine mouth-feel

'Oily' and 'metallic' characters were the only sensory attributes that significantly differentiated between low and high solids wines ($p<0.05$) (study 2) and these attributes were mostly associated with quercetin-glucuronide, ethanol and gentisic acid concentration, and with A420 which is a general indicator of phenolic compounds in white wine associated with browning (Figure 9).

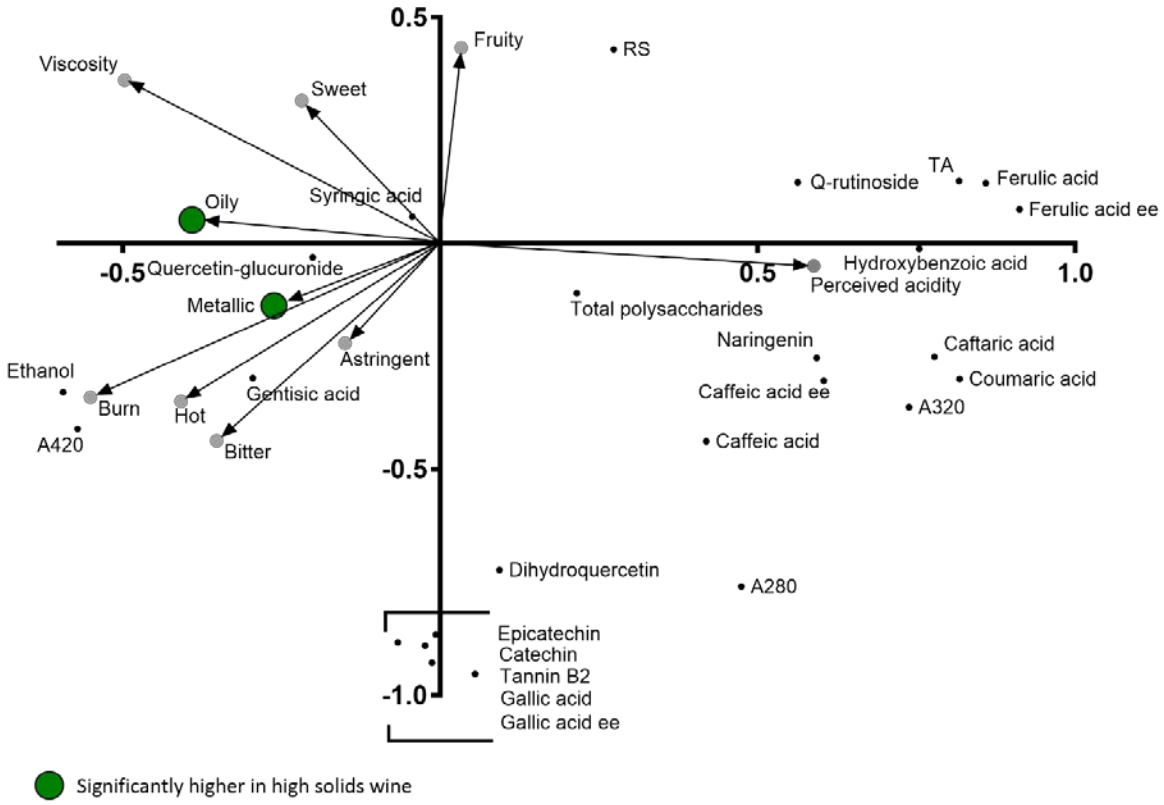


Figure 9. Total phenolic concentration of wines made from juices settled with different clarifying agents. Control indicates wines made from centrifuged juices (<40 ntu).

Using juices clarified in a medium-scale winery, higher juice solids resulted in wines that were perceived to be slightly more viscous and bitter in both varieties. Perceived viscosity was strongly associated with wine pH, alcohol, phenolic concentration and the concentrations of monosaccharides that mostly comprise rhamnogalacturonans. Increased perceived viscosity with increasing pH and phenolics (Gawel et al. 2013, 2014a), alcohol (Demiglio and Pickering 2008), and rhamnogalacturonans in model wine studies (Vidal et al. 2003) have been reported previously.

The most significant outcome related to volatile compounds and their impact on the flavour profile of these wines. Fermenting on solids resulted in both Chardonnay and Sauvignon Blanc wines having higher concentrations of esters, fatty acids, higher alcohols and thiols. In the case of Sauvignon Blanc, the four-fold increase in volatile thiol concentration in the wines produced by fermenting on solids resulted in the perception of significantly higher 'passionfruit' and 'flinty' characters.

Understanding protein haze in model wines

Individual proteins were isolated from juice for model wine studies of protein stability and aggregation. The influence of matrix parameters on protein stability was measured in multifactorial experiments conducted in collaboration with CSIRO. This involved exploring the influence of pH, ethanol, and salts on stability of four proteins. The results indicated that lower pH and higher salt concentration reduced protein stability and are likely to contribute to aggregation. This provides further evidence about the complexity of wine interactions and haze formation.

The strength of interactions between proteins and polysaccharides was assessed using isothermal titration calorimetry. When proteins were in their native state, there were limited interactions

between these macromolecules. However, when proteins were unfolded, such as after heat exposure, there were weak interactions between proteins and polysaccharides. Overall, this indicates that the protein-polysaccharide interaction is not likely to be a significant cause, or inhibitor, of protein haze formation.

Understanding protein haze in real wines

Grape protein classes, chitinases and thaumatin-like proteins, are the main wine components associated with haze in white wine; however greater protein concentration in wine does not necessarily indicate a greater haze potential (Van Sluyter et al. 2013). To better understand the variability surrounding haze formation, an analytical method was developed using high performance liquid chromatography (HPLC) to more rapidly identify and quantify specific proteins in white wine, reducing the analysis time from 60 minutes to 10 minutes per sample. To investigate the impact of haze formation on real wines, 60 protein-unstable wines were sourced from industry partners. The protein concentration and composition were measured using HPLC. Other components were also measured in each wine to assess the impact of the wine matrix on haze formation, including wine pH, sulfate, electrical conductivity, phenolics, polysaccharides, metal ions, and ethanol concentration. The only factors to have a statistically significant influence on protein haze in real wines were protein concentration and pH, however a highly accurate model for predicting haze based on these factors could not be developed due to the low correlation ($R^2 = 0.56$). It is likely that the other factors also play a role in haze formation that collectively has a great impact on haze; however, no other individual factor was shown to contribute significantly to haze formation.

Improving the efficiency of haze prediction

The number of different factors found to contribute to wine haze made the development of a predictive model based on wine matrix composition unfeasible. For this reason, a heat test was considered the best method for predicting haze and determining bentonite addition rates. However, the heat test can have issues with reproducibility and long turn-around times as well as suggestions that it can overpredict the amount of bentonite required. Further research was therefore warranted to improve reproducibility and potentially reduce turn-around time without compromising accuracy.

Preliminary trials assessed the impact of heating time and cooling time and temperature on haze formation. Heating wines at 80°C for 2 hours and cooling at 20°C for 3 hours produced haze ratings for wines that were similar to those reported by AWRI Commercial Services for the same wines (after heating 6 hours at 80°C and cooling for 20 mins at 20°C). The effect of bentonite fining and subsequent storage was also assessed on a range of unstable wines sourced from industry. A selection of wines were fined at bentonite dose rates predicted using the three methods below and stored for 12 months at 17°C and 28°C:

- 6 hours at 80°C, overnight cooling at 4°C, 1 hour at room temperature
- 2 hours at 80°C, 3 hours at 20°C in temperature-controlled bath
- 2 hours at 80°C, 2 hours at 0°C (ice bath), 1 hour at room temperature

Cooling temperature (20°C or 0°C) did influence the change in NTU, but did not influence the predicted bentonite dose rates. The results indicated that the amount of bentonite predicted from the 5-hour heat test (2 hours heat, 3 hours cool) was sufficient to stabilise wines for 12 months storage at 28°C (Figure 10). This suggests that the shorter heat test is a viable method for predicting haze.

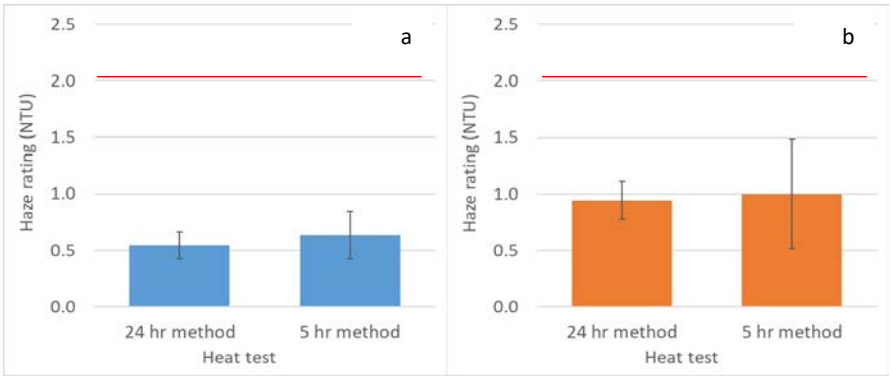


Figure 10. Turbidity of wines fined with a bentonite concentration predicted using the original heat test method (24-hour turnaround, 6 hours heating and 16 hours cooling) and the new heat test method (5-hour turnaround, 2 hours heating and 3 hours cooling) after 12 months storage at a) 17°C and b) 28°C. Results are shown as the average turbidity of seven wines in duplicate +/- one standard deviation. Wines less than 2.0 NTU are visually clear.

Preventing haze using alternatives to bentonite

Consumer and market expectations drive the push for clarity and absence of bottle deposits in white wines. Protein instability leading to haze in white wines is one of the most common non-microbial defects of commercial wine. Bentonite fining is currently used to remove haze-forming proteins from wine, but this has some major drawbacks including wine losses and waste disposal issues. The aim of this research was to develop rapid and selective methods for removing pathogenesis-related proteins from unfined white wines as alternatives to bentonite fining. These treatment options include new adsorbents for protein removal that are more selective, reusable and less wasteful, as well as protease enzymes with an ability to degrade proteins in wine.

New protein adsorbents trialled included surface-engineered silica, macrosponges and magnetic nanoparticles. Further investigations indicated that some types of surface-engineered silica are selective for proteins in wines and research should continue to evaluate these materials (Figure 11). Macrosponges were provided by collaborators and preliminary trials suggested that while some proteins can be removed by this material, a haze is formed from treated wine during a heat test.

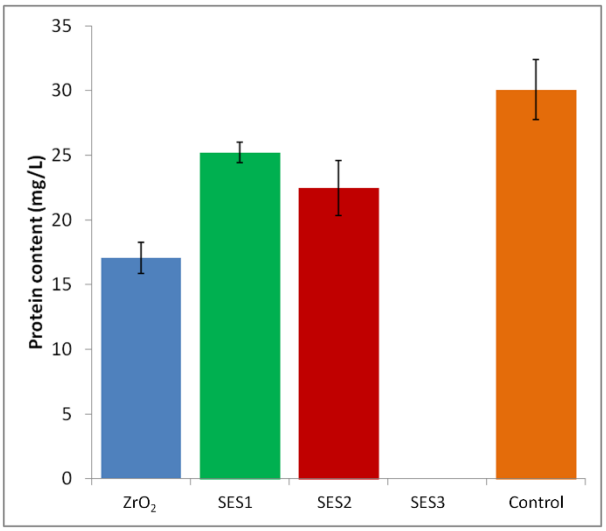


Figure 11. Protein concentration in Riesling wine after treatment with different surface-engineered silica (SES) at 10 g/L as compared with zirconium dioxide and the untreated control wine. SES3 removed all protein from the wine and therefore warrants further investigation.

A novel technology was also developed by the AWRI based on the use of acrylic acid (AcrA) plasma polymer coated magnetic nanoparticles (CMNPs) (Mierczynska-Vasilev et al. 2017). In the first step of the process, magnetic nanoparticles were coated by plasma deposition of gaseous acrylic acid to generate COOH-rich surfaces. The second step of the process involved conditioning of the acrylic acid-coated magnetic nanoparticles with white wine. The final step was a simple separation of coated magnetic nanoparticles from wine with the use of an external magnet.

The pathogenesis-related proteins in nine different white wines were selectively captured and removed by acrylic acid plasma-coated magnetic nanoparticles. Treated white wines were analysed for protein and phenolic content to assess the performance of the functionalised magnetic nanoparticles. The efficiency of the AcrA-coated magnetic nanoparticles to remove wine proteins was tested using different volume fractions of CMNPs. The effect of the coating conditions on the performance of the CMNPs in the removal of pathogenesis-related proteins from white wines was investigated in detail. It was found that using a plasma deposition power of 10 W, a plasma deposition time of 10 minutes and conditioning of CMNPs with wine for 10 minutes were the optimal conditions for the separation process. The new separation method is simple and very effective in removing both pathogenesis-related protein classes, TLPs and chitinases from white wines even when the wine contains an extremely high amount of proteins. Importantly, the phenolic content in the wines was not altered by the protein-removal treatment.

This new technology has potential to become an alternative to the conventional bentonite treatment. Furthermore, such rapid separation technology could benefit other areas such as water treatment, biotechnology and therapeutics.

As an alternative to removing proteins from finished wines by adsorption, proteases can potentially cleave haze-forming proteins. The AWRI has previously reported on success in using aspergillopepsin (AGP) enzymes to degrade wine proteins in combination with flash pasteurisation (Marangon et al. 2012). AGP has now been approved as a wine processing aid by Food Standards Australia New Zealand (FSANZ). Trials were also undertaken on other proteases to assess efficacy in grape juice without flash pasteurisation. Proteases isolated from *Botrytis cinerea* (BcAP8) and sunflowers (HaAEP1) were added to grape juice and the protein concentrations measured after storage at elevated temperatures and/or fermentation. BcAP8 decreased wine protein concentration by a small amount; however treated wines produced a haze in the heat test. HaAEP1 decreased the protein concentration of juice by around 50% after heating to 33°C for 7 days, warranting further research by directed evolution to produce an enzyme that can cleave haze-forming proteins in grape must without heating.

Trials of flash pasteurisation alone (without AGP enzyme) were also investigated as an alternative stabilisation strategy. Heating grape juice for one minute at 75°C was sufficient to produce stable Semillon and Sauvignon Blanc wines. The higher protein concentration of the Muscat Gordo must included in this study required three minutes of heating to produce stable wine (Figure 12). Therefore, pasteurisation for one to three minutes may be sufficient to stabilise wines and may be a viable alternative stabilisation strategy for the wine industry.

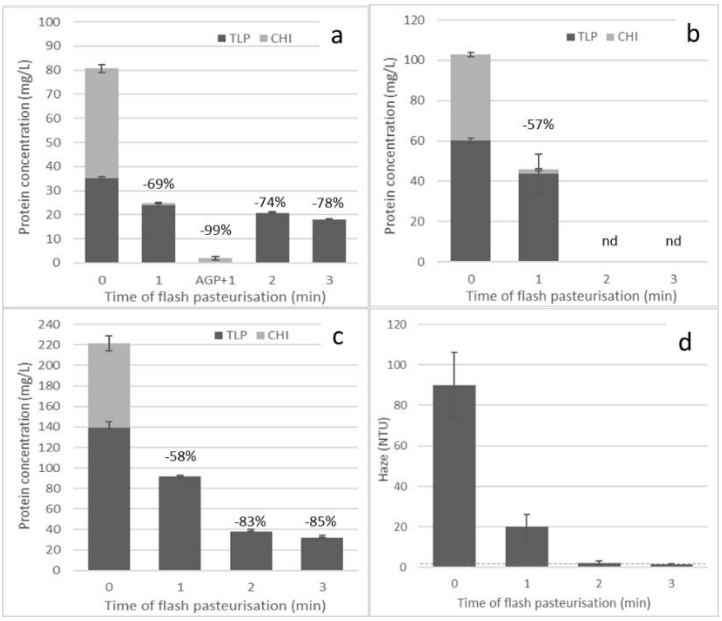


Figure 12. Total concentration of haze-forming proteins, including chitinases (CHI) and thaumatin-like proteins (TLP), in a) Semillon wines; b) Sauvignon Blanc wines and c) Muscat Gordo wines produced from must treated with flash pasteurisation (FP) at 75°C for 1-3 min. Numbers indicate the percent reduction in total concentration of haze-forming proteins compared to the control for significantly different results ($p < 0.5$); d) haze formation in the MG wines after FP for 1-3 min. The dashed line indicates the maximum haze for stable wines, nd = not determined for these samples and AGP+1 refers to aspergillopepsin I and II with 1 min FP. Results are given as the mean of triplicate ferments and error bars indicate +/- one standard deviation.

The use of flash pasteurisation in industry is not always possible and therefore alternative protein unfolding strategies were also investigated. A vortex fluidic device (VFD) uses shear force instead of elevated temperature to unfold proteins and has proven to be effective for unfolding and refolding pharmaceutical enzymes. However, applying this technique to grape juice was ineffective in unfolding haze-forming proteins. The limited availability of scale-up of the technology also suggested that flash pasteurisation would be a more effective technique to explore in future trials.

Sensory and macromolecule effects due to red wine filtration

Clarification and stabilisation, yeast and bacteria removal, final filtration for microbial stabilisation are all colloidal phenomena. Small-scale filtration investigations demonstrated the loss of colour and polysaccharides; however, the effect of commercial-scale filtration on red wines is unknown. For this reason, samples of four commercial wines (Cabernet Sauvignon and Shiraz from 2013 and 2014 vintages) were collected from two commercial bottling facilities before and after crossflow filtration and lenticular filtration, after 0.65 µm membrane filtration, and after 0.45 µm membrane filtration. Macromolecules and colour parameters were measured within a few weeks of bottling, after 9 months' ageing and after 18 months' ageing. Chemical analysis indicated no change in the tannin, colour, polysaccharide concentration or composition with any grade of filtration. The size of the particles in each wine at each filtration grade was assessed using NTA and particle size did decrease with crossflow filtration (Figure 13). This was likely due to the removal of residual microbes.

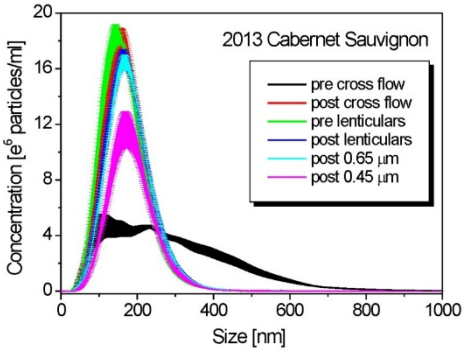


Figure 13. Measurement of particle size and concentration of 2013 Cabernet Sauvignon at various stages of filtration: cross-flow, followed by lenticular filtration and then 0.65 µm and 0.45µm membrane filtration.

After 18 months of ageing, the particle size of the 2013 wines increased in all wine samples at all filtration grades. This trend was not observed in the 2014 wines. Sensory analysis was undertaken on the pre-crossflow, post-crossflow and post-0.45 µm wines. There was no significant filtration-related difference in mouth-feel between samples (Figure 14). This suggested that winery-scale filtration does not significantly change wine macromolecules or mouth-feel but does remove the microbes associated with spoilage.

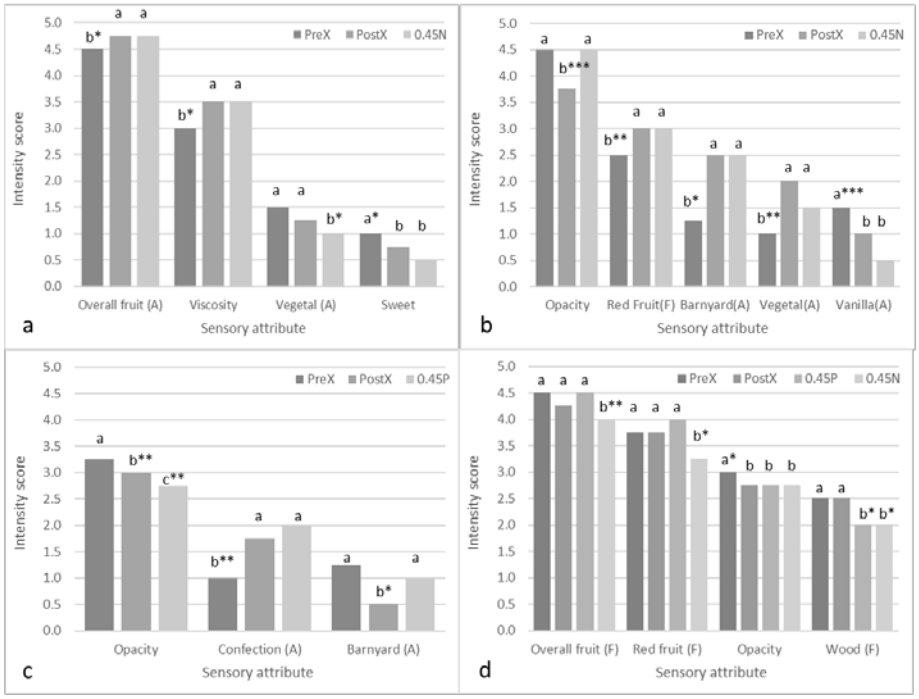


Figure 14. Intensity ratings of sensory attributes that showed significant differences between filtration grades for each wine: pre-cross flow (PreX), post-cross flow (PostX), 0.45 µm membrane of PES (0.45P) or nylon (0.45N). a) CAS13; b) SHZ13; c) SHZ14; d) CAS14. Different letters indicate significant differences between samples for each sensory attribute and asterisks indicate the significance level: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Outcome and conclusion

This project delivered improved knowledge and tools to allow winemakers to more objectively manage texture, stability, clarity and filterability during winemaking. It achieved this through the improved understanding of precursor grape and wine compositional drivers and a better understanding of the impact of winemaking processes on the macromolecules and colloids that are linked to these wine parameters.

Specifically, the project achieved the following objectives:

- compositional drivers for texture, hotness and bitterness were identified in red and white wines and include CO_2, tannin, polysaccharides and a new indole conjugate
- the role of macromolecules such as tannins, polysaccharides, proteins and their aggregate colloids in the expression of texture, stability, clarity and filterability have been investigated and shown to vary widely depending on treatment
- the impact of wine matrix components on macromolecule function and expression have been demonstrated
- some of the sources of these molecules or their precursors in grapes and yeast and the impact of winemaking processes such as clarification, flotation, vinification and filtration on their retention and/or transformation have been identified
- the impact of filtration on macromolecules has been demonstrated to be negligible
- alternative strategies for achieving protein stability have been developed and their efficacy demonstrated
- practical methods for wineries to determine likely extractability of macromolecules during winemaking were developed
- strategies for the stabilisation of colour independent of vintage effects were developed.

The knowledge generated by the project provides a framework for the development of winemaking strategies and practical recommendations for managing colour (and colour stability), astringency, viscosity, hotness, bitterness, filtration processes and protein hazes.

Recommendations

Molecular drivers of taste and texture

Bearing in mind that ethanol and pH most significantly impacted astringency and hotness respectively, low to medium molecular weight polysaccharides did cause modifications to a range of sensory characteristics including viscosity, hotness, astringency and bitterness. As such, it is recommended that more research into grape-derived polysaccharide fractions in wines is conducted to determine how significantly wine styles can be modified by manipulating them.

When deciding on ethanol concentrations in final wines, producers should be aware that across the wine-like range (10-15%), ethanol can influence astringency perception with lower levels of ethanol leading to higher astringency for a given amount of tannin. Decisions about the amount of tannin required for the desired wine style should be considered in the context of the target alcohol concentration.

A novel indole derivative has been identified as contributing to bitterness in white wine fractions. Further work should concentrate on determining the concentration range of this compound and compounds of the same class that might be found in white wine, establishing whether they have the potential to work additively like other known bitterants (Keast et al. 2003) to produce bitter tasting wines. It would also be beneficial to explore how the concentration of these compounds and the associated bitterness in wine are influenced by chemical and biochemical reactions.

The reasons for increased perceived sweetness and reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear and warrant further investigation. It is recommended that producers consider the CO_2 levels of their still wine products in the context of these potential sensory effects when deciding on wine style.

'Smart' surfaces for more efficient production

The evaluation of modified surfaces to reduce fouling should be pursued with collaborators to develop next generation low fouling membranes, tank materials, wine production surfaces and/or new sensing platforms that will reduce cost and improve productivity in wine and related industries.

Understanding wine haze

Wine haze is a complex process involving multiple factors that influence results. Higher haze is expected from wines with lower pH and greater concentrations of proteins, phenolics, sulfate, salts and metal ions. Due to the number of influencing factors, haze is best mitigated through protein removal and best predicted with haze-forming tests such as the newly proposed heat test rather than through wine component analysis.

Predicting haze formation

The new heat test method involves filtering wines at 0.45 μm, heating to 80°C for 2 hours, cooling at 20°C for 3 hours and measuring the turbidity before and after heating. It is recommended that wineries undertake their own cross-validation of this method with their current heat test method to provide additional confidence in the shorter method. This cross validation is currently underway at the AWRI and both the 2-hour heat method and the 6-hour heat method will be available for samples sent to AWRI Commercial Services in coming months.

Preventing wine haze

Accurate predictions of bentonite dose, the selection of the type of bentonite, partial addition during fermentation and post-addition treatment such as centrifugation can help minimise any adverse effects of bentonite additions. For many producers, bentonite addition currently remains

the most effective method for preventing wine haze; however new bentonite alternatives are still being developed.

The use of aspergillopepsin (AGP) enzymes in conjunction with flash pasteurisation has been demonstrated as a potential alternative for bentonite. These enzymes have now been approved for use in winemaking and several companies have expressed an interest in developing them for commercial use. Flash pasteurisation without enzyme addition is also a useful technique for reducing protein concentration and bentonite use and can be considered a viable alternative where flash pasteurisation facilities are available.

Solids management effects on white wine style and composition
Producers interested in diversifying some of the textural and aromatic properties of their white wines might consider settling processes that increase the level of solids in their juices, without needing to be overly concerned about increased phenolic extraction.

Matrix effects on red wine astringency
Lower alcohol wines may have a more 'grippy' or puckering astringency than wines with the same tannin concentration but higher alcohol content. This should be considered when developing wines with lower alcohol concentrations and processes to modulate textural properties in lower alcohol wines should be considered.

Impact of winemaking methods on wine macromolecules and texture
The use of a newly developed 'wine-like' extraction protocol for measurement of grape tannins is recommended to producers interested in understanding the likely extractability of fruit prior to fermentation and using the data to support winemaking decisions. Monitoring of individual blocks over multiple seasons or after viticultural interventions will lead to an increased knowledge of the factors that influence extractability.

The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on red wine tannin and polysaccharide. The magnitude of the effects varied depending on the maturity of the grapes and the effect of the particular yeast, enzyme or maceration protocol on the mechanisms driving extraction and retention processes. Nonetheless, experimentation by producers with these elements is likely to allow them to develop wines with a wide diversity of sensory and compositional profiles. Producers interested in effectively lowering alcohol whilst maximising wine macromolecule extraction and wine texture can consider must dilution treatments which may lead to more favourable outcomes than earlier harvesting.

Polysaccharides and red wine texture
Red wines produced using macerating enzymes had elevated levels of galacturonic acid and RGII, and may be associated with undesirable levels of astringency. However, early work into the sensory impacts of RGII on modulating tannin astringency showed that this was a key molecule which reduced tannin-derived astringency. Future research should seek to establish whether addition of purified RGII to wine and/or reducing galacturonic acid in wine can positively modify mouth-feel. Efficacy of RGII addition to wine should be tested in comparison to AGPs and mannoproteins as existing commercially available wine additives. Treating grape marc with enzyme can allow a high quantity of RGII to be extracted and grape marc could therefore be explored further as a significant source of RGII.

Red wine extractability and protein
The work on cell wall fractionation showed that pectic polysaccharides are a significant sink for tannin removal during winemaking, and that use of selected yeast/macerating enzymes can

depolymerise this material to the extent that tannin and colour may be increased in wines. However, cell wall fractionation studies also showed that mesocarp- and juice-derived proteins are potential precipitation (fining) agents early during fermentation. This opens up a number of possible future directions for research:

- The concentration of red wine protein pre- and post-fermentation in *V. vinifera* is poorly understood. The extent to which extracted tannin and colour are precipitated and removed in an active ferment is also unknown. A survey of red wine-grape protein concentrations with associated tannin removal impacts is required and further studies of winemaking practices which affect wine protein (bentonite fining, pasteurisation) and tannin-protein interaction is recommended.
- Viticultural factors (climate, water availability, disease pressure, elicitors, cultivar) may significantly impact upon red grape proteins in the same way that they do white grape cultivars. This may be an important contributor to tannin/colour removal during red winemaking, yet these impacts are unknown and warrant investigation.
- The use of natural grape proteins as a fining agent has recently been identified as an alternative to animal-based proteins, or protein sources from non-grape plants. Protein recovered from bentonite application in white wine might be another valuable source of fining agents.

Regional or vintage-related effects and targeted winemaking

The research into extractability has provided an indication of the range of levels of extractable tannin and colour which can be found for the two principal Australian red varieties Cabernet Sauvignon and Shiraz. This has highlighted that for certain warmer regions the development of tannin and colour in grapes is limited by the environment. In other regions, grape tannin may be at the upper limit of desirable concentrations, leading to excessive astringency/bitterness. Future research should seek to identify context-specific winemaking techniques to optimise wine phenolics based on the extractable grape tannin and colour measured. For example, in regions or vintages where colour and tannin phenolic potential may be limited, particular enzymes or thermal must processing methods such as flash-détente could be applied to maximise extraction while minimising tank time.

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