Stream 1.2: Phenolics and their contribution to wine composition and sensory properties

1. Abstract:

The knowledge generated by this stream has increased the wine industry's capacity for meeting wine specification and consumer expectations and improving profitability. In particular, this stream was undertaken to provide the Australian wine industry with an improved understanding of structures, measurement, formation and function of phenolic compounds in red and white grapes and wines. Compounds were targeted that play an important role in sensory attributes of wine including colour intensity and stability, mouth-feel, astringency and taste.

In red wine, binding of tannin by grape cell walls (i.e. fibre) was shown to limit tannin extraction during fermentation and to modify the amount and type of tannins retained in wine. This presents an important new factor for consideration in grape selection and processing during winemaking. Futhermore, it provides a basis to more objectively assess changes in the extractability of grape tannins during ripening.

Further research revealed that wine tannins of larger size and greater water-solubility were more astringent, but fractions with more colour and lower water solubility, which are characteristics associated with aged wine tannins, were less astringent. This means that future research targeting the creation of different amounts of these tannin might allow mouth-feel to be tailored.

The AWRI Tannin Portal was developed to harness the value provided by the analytical reference methods for quantifying tannins. This web-based interface provides industry practitioners with simple and unprecedented access to a range of phenolic parameter measurements and compositional trends including tannin and red wine colour that affect consumer preferences.

Results from research of white wine phenolics highlight opportunities to increase the desirable attributes of palate fullness; overall complexity by enhancing white wine phenolics; and to 'dial down' hotness in white wines by increasing their polysaccharide concentration.

2. Executive summary:

This stream was undertaken to provide the Australian wine industry with an improved understanding of structures, measurement, formation and function of phenolic compounds in red and white grapes and wines, and to develop the scientific framework for methodical improvements in red and white winemaking. Compounds were targeted that play an important role in sensory attributes of wine including colour intensity and stability, mouth-feel, astringency and taste. The structure, identity and relevance of many of the key compounds influencing wine quality, style and sensory properties needed to be established. This required the development of new methods for isolation, identification and quantification, as well as application of existing methods, to work out the complex chemical structures of tannins (responsible for colour stability and astringency) and other phenolics. Some analytical methods were developed through collaboration with others involved in the novel application of existing technologies for grape and wine phenolics research. Overall, the establishment of such knowledge was required to manage vineyards and winemaking to optimise phenolic grape and wine components, which in turn would translate into increased capacity for the Australian wine industry to meet wine specification, consumer expectations and profitability.

A number of key parameters important to wine colour, mouth-feel and taste in red and white wine have been identified and their impacts assessed. These include pH, ethanol, glycerol, polysaccharides, total phenolics, caftaric acid, the glutathione conjugate of caftaric acid known as grape reaction product (GRP) and tannins. This research proved that the fundamental wine components pH and alcohol have an overarching effect on those sensory attributes normally associated with phenolics – sometimes greater than the phenolics themselves. In addition, all phenolics do not necessarily increase phenolic tastes such as astringency, viscosity, bitterness and hotness. In a counter-intuitive result, it was proved that the two major phenolics (GRP and caftaric acid) in Australian white wines actually decreased the dry mouth-feel produced by alcohol and acidity (by caftaric acid) and increased palate oiliness (by GRP). These findings were unforeseen but have significant practical implications because these two phenolic compounds can be varied in winemaking through oxygen exposure, and so variations in their concentrations can allow modulation of texture. Also, at the outset of the project, polysaccharides were hypothesised to contribute to white wine viscosity and fullness. It has now been proved that they play a minimal role in white wine viscosity, but a new role they play in reducing hotness has been discovered. Overall, the results of this body of research highlight opportunities to increase the commercially desirable attributes of palate fullness and overall complexity in white wines by building in or retaining white wine phenolics. When considered with the overall project findings, this means that by managing both phenolics and polysaccharides, winemakers might be able to 'dial down' white wine hotness in some circumstances.

Through studies into the influence of grape maturity, winemaking and sensory impacts of red grape seed and skin tannins, this research has shown that the processes involved in the extraction of tannins from grapes and the retention of those tannins in wine are very complex. In particular, research into the isolation and characterisation of polysaccharides and cell wall material (fibre) has identified that grape cell wall material (CWM) can bind tannins and modify the amount and type of tannins retained in wine. This binding of tannin by cell walls can limit tannin extraction and retention during fermentation. It presents an important new factor for consideration in grape selection and processing during winemaking which can allow winemakers to more rigorously control colour and mouth-feel in red wines. It also presents an opportunity to better assess the extractability of tannins as their ability to bind changes during maturity, so that decisions about phenolic maturity can be supported with more objective measurements than are currently available. This will facilitate the development of industry-practicable methods to determine grape tannin extractability which is a pre-requisite to forecast wine tannin and astringency from grape data.

In red wine, new properties and chemical reactions involved with the formation and breakdown of polyphenols relevant to winemaking have been discovered. Tannin was confirmed to be important for capturing anthocyanins and giving long-term colour but it appears that other paths to pigmented polymer formation are occurring to quite variable levels. While low tannin concentration doesn't necessarily negate the formation of non bleachable pigments, higher tannin concentrations maximise the formation of long-term stable colour.

Astringency of red wine was demonstrated to depend on the type of tannin (i.e. composition) as well as the amount (i.e. concentration) and, in particular, research revealed that wine tannins with larger size and greater water-solubility were more astringent, but fractions with more colour, lower water solubility, which are characteristics associated with aged wine tannins, were less astringent. In the future, by targeting the creation and retention of the types of wine tannins identified in this project, it will be possible to craft wines that have the mouth-feel of an aged wine but at a much younger age.

Development of commercially practical, rapid analytical techniques that can be widely adopted by the wine industry for the measurement of both grape and wine tannins and other phenolic compounds has been achieved. Known as the AWRI Tannin Portal, a web-based graphical user interface created in conjunction with Streams 2.3 and 4.4, it allows simple access to measurement and contextual information of phenolics such as tannin. This new, rapid tool can be used to monitor key wine compositional trends which affect consumer preferences through impacting on red wine colour and astringency. It may also lead to cost savings in analytical testing, much improved turnaround times and better supported decision-making.

Finally, research in this stream has also highlighted the importance of understanding how macromolecules such as tannins and polysaccharides work together in wine. Further research is recommended into the additive and synergistic effects of macromolecular interactions on texture, filterability, colour and protein stability. In addition, development and implementation of a grape-to-wine extractability assay should be pursued in order to provide industry with an objective and practical solution for estimating the extractability and likely impact of key grape compounds. While most of this research has been conducted with industry partners and was communicated on an on-going basis, further extension and communication efforts would maximise the value from the results generated by this stream.

Affiliation	Area of support/contribution
Flinders University	Modelling astringency perception - Surface binding phenomena of tannins by SPR analysis
University of Queensland (now University of Sheffield, UK)	Thermodynamics of grape and wine tannin interaction with polyproline: implications for red wine astringency
ARC Centre of Excellence in Plant Cell Walls, School of Botany, University of Melbourne	Composition of cell walls that bind tannin
Australian Synchrotron, Melbourne	Size and shape of tannins in solution

3. Background:

This phenolics research program was developed to provide the Australian wine industry with an improved understanding of structures, measurement, formation and function of non-volatile phenolic compounds in red and white grapes and wines, and aimed to broaden the scientific framework for methodical improvements in red and white winemaking.

The research targeted compounds such as tannins, which play an important role in sensory attributes of wine including colour intensity and stability, mouth-feel, astringency and taste. These polyphenolics constitute a hugely heterogenic mixture in which the structure, identity and relevance of many of the individual key compounds influencing wine quality, style and sensory properties remain to be established. Also, separation, isolation and characterisation of pure compounds is enormously challenging, and a priority for this stream was the development of robust and selective methods for measurement of key compounds, such as seed and skin tannins in wine.

A fundamental increase in the understanding of the reactivity, structural and physicochemical properties of grape and wine phenolics and tannins was required to understand how inputs and processing factors such as extraction, fining, or filtration can affect wine composition and sensory properties. In addition, increasing the understanding of the transformations of grape seed and skin phenolic components aimed to broaden the existing knowledge base to enable targeted optimisation of viticultural and winemaking practices, such as grape harvesting, crushing and pressing, fermentation and fining.

Ultimately, the reward for establishing such knowledge will be an enhanced capacity to manage vineyards and winemaking to optimise phenolic grape and wine components, which will in turn translate to increased capacity in the Australian wine industry to meet wine specification, consumer expectations and profitability.

The expected outcomes or benefit from this phenolics research program to the Australian wine industry included:

- The ability to measure phenolic grape and wine components relevant for wine colour, mouth-feel, astringency and taste on a molecular level.
- The development of a more detailed insight into the winemaking-related transformations of phenolic compounds and the consequences of these reactions for wine composition, quality or style.
- An increased capacity to preserve and enhance wine quality and ultimately to meet wine

specification in order to match consumer expectations and increase profitability.
- Tools that measure grape phenolic attributes of relevance to consumer wine preferences, and improved grape harvesting and delivery processes to preserve those attributes.
- Knowledge of grape and wine phenolics which enables: targeted optimisation of viticultural and winemaking practices; verification of grape potential pre-processing; and verification of risks associated with novel practices and new technology.
- Knowledge to establish and meet future phenolic specifications with importance to red wine quality and style.

4. Stream objectives:

The stream objectives were to:
- improve understanding of the types of compounds important to wine colour, mouth-feel and taste in red and white wine, and of conditions modulating their formation and degradation;
- develop an understanding of the transformations of grape seed and skin tannins into wine tannins during winemaking, and the influence of grape maturity on seed to skin tannin ratio and sensory properties of wine;
- develop a better understanding of the chemical reactions involved with the evolution (formation and degradation) of phenolic compounds and their physicochemical properties relevant to winemaking, e.g. monomeric polyphenols, anthocyanin-derived pigments and wine tannins;
- identify key compounds (including precursors) that are relevant for specific colour, mouth-feel and taste properties related to wine quality, style or consumer preferences;
- provide the knowledge for identifying attribute requirements of products to reflect market and consumer preferences in relation to wine sensory characteristics;
- improve the ability to meet preset specifications for product attributes, which in turn might reflect market preferences in relation to price and wine sensory characteristics;
- allow the improvement of processes used to target viticultural and winemaking techniques for optimisation;
- develop commercially practical rapid analytical techniques which can be widely adopted by the wine industry for the measurement of tannins and other phenolic compounds; and
- investigate the impact of novel yeasts on phenolics to improve quality attributed to wine.

Additional research into white wine phenolics, as first described in the AWRI's 7-year R,D and E Plan, was undertaken in a separate GWRDC-funded project 'Identification of the major drivers of ' phenolic' taste in white wines' (http://www.gwrdc.com.au/completed_projects/identification-of-the-major-drivers-of-phenolic-taste-in-white-wines/). While the white wine phenolics project complements the research described here, its results and outcomes are summarised in a separate report (http://www.gwrdc.com.au/wp-content/uploads/2012/09/AWR-0901-FINAL-REPORT.pdf).

5. Methodology:

The aims of this stream required the development of several robust analytical methods as well as refinement of existing methods to achieve the challenges associated with quantifying tannins and elucidating tannin structures. The methods developed include those for measuring tannins in wine using the methyl cellulose precipitable (MCP) tannin assay, a reliable technique for determining the concentration of tannins in red wine (Mercurio et al. 2007). As part of this development, the existing Somers colour measures for wine were modified to make them simpler and more robust (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

(McRae et al. 2013a). 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. 2013b).

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 the acid-catalysed depolymerisation in the presence of phloroglucinol to determine the composition of the tannin subunits (Kennedy and Jones 2001). Other methods were developed through collaborations and involved the novel application of existing technologies for grape and wine tannin analysis. These included small-angle x-ray scattering (SAXS) through a collaboration with the Australian Synchrotron, to determine tannin molecular mass and shape in wine-like conditions (McRae et al. 2013b). Also, isothermal titration calorimetry (ITC) was used in collaboration with the University of Queensland to measure the protein-binding capacity, a likely proxy for astringency, of a range of young and aged wine tannins (McRae et al. 2010).

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. Experimental details are provided in the relevant publications listed below.

The research into 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, Bindon and Smith 2013a.

Best practice representative sampling, handling and processing of grape samples from viticultural trials was undertaken, for example as outlined in Holt 2008a. 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. Experimental details are provided in the relevant publications listed below.

6. Results and discussion:

Investigation of the types of compounds important to wine colour, mouth-feel and taste, in red and white wine

A body of research across red and white wine, investigated the sensory roles of phenolics, tannins, and polysaccharides, and the effects of pH and ethanol, and has contributed to the understanding of the molecular basis of textural perception of wine.

In white wine, it was demonstrated for the first time that in-mouth texture is influenced by phenolic composition and that phenolics were important in defining wine style (Smith and Waters 2012, Gawel et al. 2013a, 2013b, 2013d, 2013e). More detail on the influence of phenolics, pH, and alcohol on white wine sensory is provided in a separate report (http://www.gwrdc.com.au/wp-content/uploads/2012/09/AWR-0901-FINAL-REPORT.pdf).

In a scoping study undertaken as part of Stream 1.1 (reported here because of its relevance to texture), the interactions between alcohol and glycerol on wine flavour and mouth-feel were investigated. The research showed that these compounds had only small and inconsistent effects on wine aroma and flavour, but a more pronounced and consistent impact on textural characters such as hotness, drying and overall textural perception (Jones et al. 2008). Reconstitution experiments with ethanol and glycerol were undertaken as these compounds are major products of alcoholic fermentation and can be controlled through fermentation management. Also, as pure compounds, glycerol is sweet and viscous, and ethyl alcohol can be perceived as both bitter or sweet and produces palate hotness. The sensory studies of alcohol and glycerol at wine-realistic levels (Gawel et al. 2007, Gawel and Waters 2008) showed that:

- alcohol concentration had only a small (positive) effect on perceived viscosity, and on overall white wine body;
- glycerol did not contribute to perceived viscosity even at the highest level found in dry white table wine (after the inherent sweetness of glycerol had been blocked at the receptor level); and
- neither ethanol nor glycerol, when assessed at a concentration range typical for wine, consistently affected aroma or flavour intensity.

These results indicate that palate viscosity in dry white wine is unlikely to be enhanced by employing traditional winemaking approaches that elevate glycerol levels (Gawel et al. 2007, Gawel and Waters 2008). After ruling out a major impact of ethanol and glycerol, the focus was shifted towards polysaccharides.

Polysaccharides are one of the most abundant macromolecules in dry white table wine and work in this stream has contributed to understanding their roles and practical ways to manipulate them in white wine. While their presence is usually associated with longer juice settling times and reduced filtration efficiency, they are also thought to aid protein and tartrate stability and to possibly contribute to the perception of palate fullness. Polysaccharides are highly complex molecules and their isolation and analysis are difficult. The standard method for preparative-scale polysaccharide extraction was significantly improved to increase purity levels and provided polysaccharide preparations that were much lower in phenolics and tartrates (Gawel et al. 2013c). This enabled sufficiently pure quantities for sensory experiments to be isolated and characterised; these experiments showed that augmenting white wine with up to 70 mg/L native polysaccharides, i.e. adding approximately 50% of the polysaccharides found in a typical white wine, resulted in:

- small but inconsistent increases in perceived viscosity;
- no reduction in their phenolic tastes; and
- reduced perception of palate hotness (Gawel et al. 2013c).

In subsequent sensory trials, it was shown that adding 200 mg/L (a realistic amount for a real wine) of whole polysaccharides to white wine also reduced palate hotness (presumably from alcohol), confirming the previous result (Gawel et al. 2013c) . This new discovery means that manipulating polysaccharides in wine can influence the perceived palate hotness (Gawel et al. 2013c).

The phenolic composition of red wine impacts upon the color and mouth-feel and thus quality of wine. An investigation of the impact of wine tannin structure on sensory perception was undertaken to determine how red wine tannin structure influences the perception of wine mouth-feel (McRae et al. 2013a). This study showed that larger and more water-soluble wine tannins are more astringent, while smaller wine tannins that are less water-soluble, more coloured and have more oxidative structures, were perceived as hotter, more bitter and less astringent. These results prove that different types of tannins can influence mouth-feel and confirmed that modifying wine tannin structures during winemaking is a practical path to altering the wine mouth-feel.

For a given type of tannin, it has been recognised that other components of the wine (such as ethanol concentration, ionic strength and pH) affect the sensory perception of that tannin. Small angle x-ray scattering (SAXS) techniques were used to demonstrate that the wine matrix does not affect tannin molecular size and shape in solution (McRae et al. 2013b). This indicates that the different sensory characteristics conferred by different wine matrices reflect wine composition and interactions, and are less likely to relate to the impact of a wine's condition on the dissolved tannin size and shape.

Grape seed and skin tannins; influence of grape maturity and winemaking, and sensory impacts

Tannin concentration is 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. For the viticulturist and winemaker, a grape-based measure to predict wine tannin concentration would be a useful tool to manage this variability. However, wine tannin concentration is dependent not only on grape tannin concentration, but also its extractability. As such, fundamental studies into the factors that influence tannin extractability from grapes were undertaken in this stream with a particular focus on the cell wall (Bindon et al. 2010a, 2010b, Bindon and Kennedy, 2011, Bindon et al. 2012). In summary, multiple factors were discovered which influence extractability, including:

- The interaction of skin and seed tannin with cell wall material, which was identified as a limiting factor in tannin extraction during fermentation.
- Cell wall material was shown to have a greater affinity for seed tannin than skin tannin, most likely due to differences in the conformation of seed and skin tannins rather than their molecular mass.
- Suspended flesh (pulp) material was shown to bind and remove tannin as lees during settling after the tannin was extracted during fermentation.
- The composition and amount of tannin removed was found to be dependent upon the extractability of tannins from grape components.
- Seed tannin was shown to be poorly extractable during fermentation compared with skin tannin, but this was associated with great seasonal variability.
- The effect of pulp material on tannin removal is likely to be greater for grapes with a low overall tannin concentration and grapes with a higher seed-to-skin tannin ratio.

The work described above has highlighted the role of cell wall material in limiting wine tannin concentration during fermentation due to issues related to its solubility and loss. This is a significant step forward in explaining the commonly observed lack of correlation between grape and wine tannin concentration.

In addition, a body of research demonstrated how grape maturity links to wine composition and to sensory and consumer preference (Bindon et al. 2013b, d). In studies on Cabernet Sauvignon and Shiraz, skin tannin concentration was found to increase with grape ripening in seasons where grapes reached commercial ripeness (>21°Brix) (Bindon et al. 2013b). Seed tannin concentration was confirmed to generally decrease during ripening, and seed tannin was poorly extractable during fermentation of ripe grapes, such that wine tannin composition reflected a trend of increasing skin tannin and decreasing seed tannin. Skin tannin extractability was shown to decrease during ripening due to increases in skin cell wall porosity, which causes tannin to be bound in micropores (Bindon et al. 2013c). However, skin tannin concentration in wine was enhanced with advancing grape ripeness, primarily due to increases in its concentration in grapes and because of decreases in tannin affinity for binding to cell wall materials (Bindon and Kennedy, 2011), resulting in an overall increase in wine tannin concentration with ripening. The sensory and consumer preference impacts of maturity are discussed in a subsequent section.

Properties of, and chemical reactions involved with the formation and breakdown of polyphenols relevant to winemaking.

Chemical modifications of grape polyphenols begin immediately once grapes are crushed and continue through fermentation and then during ageing. At different stages of wine production different types of reactions happen, at different rates. The outcome of these reactions determines a wine's final colour and taste properties and this project has discovered several key changes that occur at different points in wine production. For example, tannins are one critical polyphenol class that undergo significant changes during winemaking, in particular the conversion of grape tannins into chemically complex wine tannins. Oxygen has long been known to modify the astringency of red wines, but the chemical basis of what and how this occurs was unclear. Work undertaken in this stream has elucidated the changes wine tannins undergo; winemaking experiments injecting air into red wine fermentations demonstrated the impact of oxygen on tannin concentration and chemical structure that led to sensory differences. In particular, air reduced the concentration and size of tannins, increased stable colour formation and reduced the astringency of the wines relative to winemaking treatments without air addition (Day et al. 2013).

During red wine ageing, changes to the colour profile of the wine occurs; the desirable outcome is formation of long-term stable, red colour in wine. Yet in some situations, colour is inadequate from the beginning or is unstable and fades fast. While some of the factors influencing stable colour formation are known, others remain a mystery. One of the main reasons for the colour change in wine as it ages has been shown to be due to the decrease in anthocyanins derived from grapes, as well as changes to pigmented tannins. Analysis of 50 and 30 year vertical series of Cabernet Sauvignon and Shiraz wines showed that wine colour density was more strongly associated with pigmented tannins over the 30 and 50 year series than with anthocyanins (after the first two years post-bottling), clearly indicating the importance of pigmented tannins to wine colour. In general, by four years no coloured anthocyanins were left in red wine (McRae et al. 2012).

In a separate experiment, wine colour density was tracked through three years of ageing in wines of both high phenolic potential (high anthocyanin, high tannin), and low phenolic potential (low anthocyanin, low tannin). Higher wine tannin and anthocyanin led to enhanced wine colour density and non-bleachable pigments, which was retained through ageing. Formation of non-bleachable pigments was found not to increase beyond two years of ageing. The incorporation of colour with ageing was proportional to tannin concentration. Non-bleachable pigments were formed via multiple routes, such that high-tannin, high-anthocyanin wines formed greater quantities of both polymeric and non-polymeric red pigments (Bindon et al. 2013e).

Analysis also clearly indicated that aged red wines can have similar tannin concentrations to young wines, dispelling the anecdote that changes in red wine astringency with ageing are due to the loss of wine tannins through precipitation (McRae et al. 2012). Such changes in astringency are due to compositional changes in tannins (McRae et al. 2013a), although the rate of these changes is unknown. In addition, one experiment demonstrated that tannin structure in Shiraz wines is not influenced by storage at different pH values (3.3, 3.5 and 3.7) or under different screw cap closures with specific oxygen transfer rates after two years of bottle ageing (McRae et al 2013c) , even though wines of lower pH are often reported as more astringent. This suggests that a lower pH has a direct effect on sensory perception of the wine examined, but doesn't necessarily influence wine tannin evolution. These results provide improved insight into the stability of tannins in wine and underscore the importance of wine matrix influences on perceived mouth-feel.

Tannins isolated from older wines were shown to only weakly associate with saliva-like proteins compared to tannins from younger wines; this resembles the sensory impact of changes in tannin structure with wine ageing (McRae 2010). In addition, analysis of the structure of aged wine tannins revealed a large proportion of tannin that cannot be broken apart (i.e. tannin which is non-hydrolysable), unlike grape tannin which is readily broken apart into the comprising monomers (McRae 2010). This non-hydrolysable proportion of wine tannin has been shown to have a very weak association with saliva-like proteins and accounts for a larger proportion of aged wine tannins than young wine tannins (McRae 2010). These results may, at least in part, explain why red wines 'soften' with age.

Improvement of processes used to target viticultural and winemaking techniques for optimisation of phenolic profiles

A comprehensive study into the influence of pruning treatments on berry composition, wine composition and wine quality was completed (Holt et al. 2008a, 2008b). The study revealed that the smallest berries do not always produce the highest quality wines. The smaller differences in berry size due to vintage variability caused larger differences in wine quality, while larger differences in berry size due to viticultural treatments caused only small differences in wine quality scores. This indicates that the relationship between berry size and berry composition and wine quality is neither simple nor direct. In addition, while the improvements to wine quality from changes to pruning treatments are likely to be small, they might still be very important in achieving a desired berry and wine style (Holt et al. 2008a p, 2008b).

Observations from white winemaking trials show that white juice extraction methods can be used to affect both the total amount of polysaccharides (up to two-fold) and polysaccharide profile. This result suggests that reduction in hotness can be achieved by using winemaking practices that favour polysaccharide extraction and retention.

The effects on red grape, juice and wine polysaccharides were also observed in the grape maturity trial described previously (Bindon et al. 2013b). Gains in yeast mannoproteins and losses in grape-derived polysaccharides were associated with increases in viscosity in wine made from later-harvested grapes. Together with the observations about seed and skin tannins described above, this research highlights that an understanding of the interactive effects of the multiple changes that occur during grape maturation is critical to helping winemakers manipulate textural drivers in red wine.

One of the tools commonly used to optimise wine clarity and manage tannin levels to acheive the desired textural attributes is ‘fining’. This process removes particles and macromolecules in the wine to clarify it and also, depending on the type of fining agent, removes tannin. However, the performance of some fining agents can be unpredictable and many have mandatory allergen labeling implications such as a requirement to disclose their source as either milk, egg or fish for example. The search for effective fining agents, that do not have labelling requirements and seeking to potentially re- use winery by-products, led to the discovery that fibre from grape marc (and also apples) shows promise as a fining agent (Guerrero et al. 2013, Bindon and Smith 2013a). In terms of performance in comparison with proteinaceous fining agents, a higher dose of fibres is required, but fibres were able to more reproducibly remove tannin. Furthermore, the benefit of using fibres is their insolubility, compared to some protein fining agents which remain in wine after the application (Guerrero et al. 2013, Bindon and Smith 2013a). In summary: tannin removal by fibre relies on a different mechanism to protein fining, occurring by adsorption not precipitation; a higher dose of fibres is required to remove a similar amount of tannin compared to proteins, but overfining does not occur ; all fining agents reduced anthocyanins, total phenolics and wine color density together with tannin; generally, protein and fibre fining agents reduced wine tannin average size; and proteins and apple fibres were more selective for larger tannins than grape fibres. Wine volume loss as lees could be a potential problem with fibre addition, and further work is required to optimise wine recovery after fining (Guerrero et al. 2013, Bindon and Smith 2013a).

Knowledge for identifying attribute requirements of wines to reflect consumer preferences in relation to sensory characteristics

Wines made for research projects in this stream were also used for market and consumer preference research, and the results are reported in detail under Stream 3.1. In brief, liking of top-selling white wines was more associated with higher alcohol content and sweetness than with phenolic level, and the acceptability of those characters was dependent on wine style. Phenolic content was shown to be one of the important factors which differentiated between the two recognised styles of Pinot Gris/Grigio. Cabernet Sauvignon wines produced from grapes at different maturity stages were found to have significant differences in their phenolic profile, with low-alcohol (12%) wines having lower tannin, a lower proportion of skin to seed tannin, and lower colour and polymeric pigment compared to higher-alcohol wines (15%). Astringency increases were correlated with higher tannin for later-harvested wines and a consumer study showed that wines of 13% alcohol or higher were more preferred compared to wine made from grapes harvested at lower Brix, and no further increases in consumer liking were observed between 13% and 15% alcohol (Bindon et al. 2013d). Specifically, early harvested grapes were shown to produce wines of lower tannin concentration, higher seed tannin, higher perceived acidity, lower perceived astringency ,and were poorly rated by consumers. Wines produced from later harvested grapes (≥13% v/v alcohol) had higher tannin concentration, higher skin tannin, higher perceived astringency, were less acidic, and were more preferred by consumers. Generally wines produced from later harvests (14-15% v/v alcohol) enhanced the trends in tannin and sensory perception described above, but did not increase consumer liking (Bindon et al. 2013d).

Knowledge to better enable the meeting of preset specifications for product attributes

There continues to be demand from industry for understanding as to how wine composition influences quality and/or wine grading and/or price, and this stream addressed the role of tannins in the quality/grading/price nexus (Smith et al. 2008). Quality (defined as winemaker-assessed allocation gradings which ultimately relate to market price for wine) in young red wine, was positively correlated with tannin concentration, size, higher proportion of skin-derived tannins and overall wine colour (Mercurio et al. 2010, Kassara and Kennedy 2011). This correlation suggests that maximising skin tannin concentrations and/or proportions in wines can contribute to an increase in projected wine bottle price, but this must be done in balance with the other wine components which contribute to the desired wine style.

In order to find new molecular drivers that influence wine style, there was a requirement to develop high-throughput tannin purification methods for grape extracts and wines (Jeffery et al. 2008 p).

Development of commercially practical rapid analytical techniques that can be widely adopted by the wine industry for the measurement of tannins and other phenolic compounds

Measurement and understanding of tannin is considered very important by many winemakers because tannin has a major influence on texture and colour properties of red wines. However, most wine producers do not routinely analyse tannin and have no objective specification for grape or wine tannin.

Recognising the need for faster, simpler tannin analysis, the AWRI optimised and validated an innovative and robust assay for the measurement of tannins. Known as the Methyl Cellulose Precipitable (MCP) tannin assay, it enables the measurement of tannins in red wines and grape extracts using cheap, non-toxic and easily sourced reagents and instruments (Mercurio et al. 2007). The project validated a high-throughput assay format and developed a database of tannin concentrations in wines from Australia and internationally; the database now represents in excess of 6,000 samples. The MCP tannin assay was also scientifically compared to the Adams-Harbertson tannin assay, providing the first joint validation of both practically accessible tannin assays to enable evidence-based decisions on which method to use (Mercurio and Smith 2008). The MCP tannin assay is now published in the widely used 'Chemical analysis of grapes and wine; techniques and concepts' handbook by Iland et al. (2004), along with data from the Australian wine tannin survey which communicates what can be considered low, medium and high tannin concentrations. Finally, this assay formed the basis for developing the 'AWRI Tannin portal' now known as the AWRI WineCloud (Smith et al. 2010); the first web-based wine and grape tannin 'calculator' to be successfully developed, validated and commercialised; the process of which is further reported under Streams 2.3 and 4.4. The web- based interface is enabled by a spectral calibration that uses UV-Vis spectroscopy (Dambergs et al. 2012) and allows simple access to measurement of phenolics such as tannin and removes geographical barriers to accessing such analyses.

Investigations of the impact of novel yeasts on phenolics to improve wine quality

The wide ranging impact of different yeast on wine aroma has been well established over many years. However, the effects of yeast on macromolecules and texture has been less explored and this stream has shown that choice of yeast can also have a major affect on these attributes in wine. A range of yeasts were investigated for their effects on colour and tannin and were shown to influence final tannin concentration by up to 50%, and to significantly affect colour properties of the wines (Blazquez Rojas et al. 2012). A further study which evaluated the impacts of fermenting Shiraz must with different yeast strains also showed that choice of yeast strain had a strong influence on a number of wine compositional parameters, including tannin (Holt et al. 2013). This work demonstrates that the phenolic style of red wines can be clearly modulated by choice of commercially available wine yeast.

7. Outcomes and Conclusion:

A number of key parameters and compounds important to wine colour, mouth-feel and taste, in red and white wine have been identified and their impacts assessed. These include pH, ethanol, glycerol, polysaccharides, total phenolics, grape reaction product (GRP), caftaric acid and tannins.

At the outset of work in the stream, polysaccharides were hypothesised to contribute to white wine viscosity and fullness. In order to test this, new methods were developed and are now available for measuring and characterising polysaccharides, and for quantifying the roles they play in white wines and the influence that winemaking practices have on their concentration and composition. It has now been proven that polysaccharides play a minimal role in white wine viscosity, but a new role in reducing hotness has been discovered. This result shows that increases in palate fullness and reduction in hotness can be achieved by using winemaking practices that favour polysaccharide extraction and retention. When considered together with other project findings this means that, in the absence of variations to matrix composition, both phenolics and polysaccharides allow winemakers to ‘dial down’ white wine hotness, at least in some circumstances.

The influence of grape maturity, winemaking and sensory impacts of red grape seed and skin tannins has shown that the processes which control the extraction of tannins from grapes and the retention of those tannins in wine are very complex. In particular, the research has identified that grape cell wall material can bind tannins and modify the quantity and composition of tannins retained in wine. This binding of tannin by cell walls can limit extraction during fermentation. It presents an important new factor for consideration in grape selection and processing during winemaking, which might allow winemakers to more rigorously control colour and mouth-feel in red wines. The understanding developed through the research described above forms the basis from which to develop new methods for winemakers to predict the extractability of tannin in a particular batch of grapes.

Monitoring of grape skin tannin concentration has been shown to be an objective measure of grape maturity and final wine tannin concentration. Contrary to previous studies, it was demonstrated that skin tannin concentration in grapes increases with advancing ripeness, while seed tannin is poorly extractable and generally declines during ripening. Monitoring of grape maturity in Cabernet Sauvignon showed that changes which occurred in grape tannin composition were also translated into the wine. This also confirmed that wines from later-harvested grapes had higher total tannin, due to an increased skin tannin contribution, and proportionally decreased seed tannin. This research highlights opportunities for assessment of the extractability of tannin as it changes during maturity, so that decisions about phenolic maturity can be supported with more objective measurements than are currently available. This would facilitate the development of industry-practicable methods to determine phenolic maturity and grape tannin extractability.

New properties and chemical reactions involved with the formation and breakdown of polyphenols relevant to winemaking have been discovered. For example, in terms of colour properties of red wine, tannin concentration and composition affect the formation of non-bleachable pigments which are important for colour stabilisation in older red wine. Tannin was shown to be important for capturing anthocyanins and formation of long-term stablecolour, but it appears that additional pathways to pigmented polymer formation are occurring to quite variable levels and further work is required to understand how these alternative mechanisms for stable pigment formation can be harnessed. In summary, low tannin concentration doesn't necessarily negate the formation of non-bleachable pigments, but high tannin concentration maximises the likelihood of long-term colour stability.

Astringency of red wine was demonstrated to depend on the type of tannin (i.e. composition) as well as the concentration, and the molecular basis for decreased salivary protein binding in older wines has also been established. In particular, research revealed that tannin fractions with larger size and greater water-solubility were more astringent, but fractions with more colour, lower water solubility and more oxidative structures were less astringent. These latter characteristics are also associated with aged wine tannins; hence it is likely that the differences in tannin structure that occur during wine ageing account for the reduced astringency of aged red wine. In addition, many older wines have similar tannin concentrations to younger wines. The absence of age-related decreases in tannin concentration indicates that the astringency changes characteristic of older red wines are not necessarily related to a decrease in the amount of tannin, but more likely a consequence of these structural changes to the tannin. The influence of pH on modification of tannin composition during ageing seems negligible when low oxygen transmission rate closures are used, indicating that other factors are key to tannin modification in bottled wine. Fibres from winery waste streams might become a viable alternative to proteins for tannin fining, as the addition to wine of fibres isolated from by-products (apple and grape pomace) effectively removes tannin in a similar manner to proteins, but with less variability and without being subject to mandatory labelling requirements. In the future, by targeting the creation and retention of the types of tannins identified in this stream, it should be possible to create wines that have the mouth-feel of an aged wine, but at a much younger age.

The wine industry is now more able to meet preset specifications for key product attributes because of this research, which identified a number of important factors that contribute to red wine quality. For example, the ability of novel and commercially available yeasts to modify phenolics, and thus wine style, has been demonstrated, thereby providing winemakers with a practical tool with which to modulate the phenolic profile of their wines. Also, the first objective evidence that wine tannin concentration is one of the important factors that can differentiate between wine styles targetting different price points has been generated. The continued development of an 'index' of multiple objective quality measures is essential and requires knowing what chemical compounds are key to individual wine styles. Consequently, tannin and total phenolics concentration, as well as colour and flavour parameters, should be considered in the development of an index of objective measures for grape or wine quality.

Development of commercially practical rapid analytical techniques that can be widely adopted by the wine industry for the measurement of both grape and wine tannins and other phenolic compounds has been achieved. The AWRI Tannin Portal is based on research methods developed by this stream and provides a web-based interface which allows simple access to measurement of phenolics such as tannin. The AWRI Tannin Portal also removes geographical barriers to accessing such analyses; it is now just as easy for winemakers in regional areas who are a long distance from specialised laboratories to measure tannins, as it is for those based close to metropolitan centres. In addition, the AWRI Tannin PortaI secures and stores data from the samples analysed, and contains data from over 6,000 samples. These data can be used by winemakers to benchmark their own products, in order to understand what is high, medium or low tannin for a variety, region or vintage. While it is only at early stages of adoption, the AWRI Tannin Portal, as a rapid tool to measure key wine compositional trends, such as tannins and pigments, has already led to cost savings in analytical testing and better supported winemaking decision-making.

8. Recommendations:

This research project has identified several areas with potential to return high value; knowledge gaps for future research; and opportunities for synergies and leveraging from existing research. Research is recommended into colour stability of red and rosé wines to discover how to improve colour properties of wines made from lower tannin, lower anthocyanin grapes, likely through non-tannin driven formation of non-bleachable and stable pigments. The research has also highlighted the importance of understanding how the macromolecules – tannins, proteins, polysaccharides –work together in wine. Research into the effects of macromolecular interactions and the influence of colloids on texture, filterability, colour and protein stability is recommended. The effects on mouth-feel and taste of polysaccharides in red and white wine are more diverse and relevant than originally thought and, together with the need for discovering the molecular drivers of bitterness in red and white wines, represent a research opportunity with significant potential to support the production of desired wine styles (to be addressed in Project 3.1.4 of the AWRI's 2013-2018 R,D&E plan).

Development and implementation of a grape-to-wine extractability assay could be pursued in order to provide industry with an objective and practical solution to working out the value that is extractable, and actually extracted, from their grapes. Such a tool might support a practice change in some instances towards harvesting at earlier maturity for red wines (to be addressed in Project 3.1.4 of the AWRI's 2013-2018 R,D&E plan).

Practical solutions to the application of grape or apple fibre fining agents are required, which would likely allow effective modulation of tannin in wines without labeling implications. In addition the use of yeast for modulating texture and tannin in red wines should be explored (to be addressed in Project 3.3.6 of the AWRI's 2013-2018 R,D&E plan).

The use of oxygen to modulate tannin structure and concentration could be explored more widely in commercial-scale fermentations, including the effects of different ways to introduce the oxygen (to be addressed in Project 3.3.2 of the AWRI's 2013-2018 R,D&E plan).

Ongoing extension and communication efforts are required to maximise the value from the research outlined in this project. One significant opportunity is continuing to communicate the informal feedback from producers about the value being generated in their businesses by applying tannin measurement or using the AWRI Tannin Portal (to be addressed in Projects 4.1.1, 4.1.3, 4.1.4 and 4.1.5 in the AWRI's 2013-2018 R,D&E plan). Another is conducting case studies with individual producers to effective modulation of texture and phenolics in a diverse range of products (to be addressed in Projects 4.2.1 and 4.3.1 in the AWRI's 2013-2018 R,D&E plan).

In addition as producers currently assess the value of the grapes in a wide range of ways which are mainly subjective, there is scope to improve utilisation of objective chemical measures that research has shown are directly related to wine attributes that confer value such as key compounds responsible for taste, aroma, texture and appearance. Assessing the relationship between existing grading systems and fruit composition for analytical measures linked to wine value should improve uptake of available objective measures (to be addressed in Project 3.1.2 of the AWRI's 2013-2018 R,D&E plan).

9. Budget reconciliation:

Financial Year	Receipts / Income ❶	Outgoings / Expenditure ❷
Year 1: 2006/2007	$754,752	$754,752
Year 2: 2007/2008	$784,482	$784,482
Year 3: 2008/2009	$808,981	$808,981
Year 4: 2009/2010	$821,088	$821,088
Year 5: 2010/2011	$687,857	$687,857
Year 6: 2011/2012	$935,116	$935,116
Year 7: 2012/2013	$842,715	$842,715
TOTAL	$5,634,991	$5,634,991

❶ Note that the GWRDC – AWRI Investment Agreement budget was established and approved at an aggregate level, with variances to budget (i.e. annual overspends and underspends) reported and considered at that same aggregate (i.e. whole of agreement) level. The receipts / income relating to a Stream for any year therefore equate to the outgoings / expenditure within that Stream for that year, as any variances between total Investment Agreement funding received and total funds expended were considered at the whole of Agreement rather than individual Stream level.

❷ Includes a pro-rated share of Theme 5 *Executive management and administration*.

I hereby certify that this statement is true and accurate.

Signature of duly authorised representative..

Chris Day — Group Manager – Corporate Services — 29/11/2013

Name: — *Title:* — *Date:*

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