

Cooperative Research Centre for Viticulture



Australian Government

Grape and Wine Research and Development Corporation

Wine grape tannin and colour specifications

FINAL REPORT to

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1 Abstract

The project has successfully evaluated the role of phenolic compounds present in red grape and wine for colour, astringency, mouth-feel and taste. In response to the wine industry's need for objective indicators of grape and wine quality, the project has delivered a practical standardised methodology for quantifying tannins in grape and wine samples. A wide range of other techniques were also investigated and methods for measurement, structural characterisation and sensory evaluation of phenolic compounds, including anthocyanins, grape tannins and pigmented polymers have been established. Viticultural and winemaking experiments have enabled us to better understand how some grape phenolics are formed and extracted, and how they are modified or degraded in wine.

2 Executive summary

Many key phenolic compounds of wine remain poorly explained or yet to be identified, and the factors affecting red wine colour, mouth-feel, astringency and taste are poorly defined on a molecular level. Therefore, the project efforts concentrated on improving our understanding of the basis of red wine colour, identifying target compounds that are relevant for specific mouth-feel properties, and developing a detailed insight into transformations of grape compounds during berry development and winemaking. As phenolic compounds in wine constitute a hugely heterogenic mixture and reference substances are largely not available from commercial sources, separation, isolation and characterisation of pure compounds are a significant challenge.

The development of reliable analytical methods for identification and quantification of important grape-derived non-volatile phenolic compounds was a high priority and we have successfully established generic and widely used HPLC methods for the quantitative determination of anthocyanins, tannins and pigmented polymers in grape seed, skin and wine samples. Subsequently, the HPLC methods provided the reference data for the development of robust and fast techniques such as the tannin assay, or successful application of near infrared spectroscopy. To characterize grape tannins, we have adopted a complex protocol for the hydrolysis of these polymers in the presence of phloroglucinol followed by HPLC analysis of the sub-units. This enables to describe the overall composition and average molecular mass of tannin samples and allows us to monitor tannin development during berry ripening. An even more detailed picture on the composition of complex grape tannin samples can be gained from novel mass spectrometric experiments for 'tannin finger-printing' which allow the detection and identification of individual grape tannins on a molecular level.

Our studies on red wine mouth-feel initially concentrated on the sensory evaluation of purified grape tannins and have been extended to evaluate the influence of polysaccharides, anthocyanins, and ethanol concentration on tannin perception. Instrumental for the success of all our sensory studies is the 'Mouth-feel Wheel', the formal terminology developed to describe and communicate the mouth-feel characteristics of red wine. Sensory evaluation of purified grape tannins confirmed that tannin composition and concentration influenced astringency most strongly. The findings demonstrated that larger grape tannins are more astringent at a given mass concentration than smaller tannins, and that seed tannins are perceived as more coarse and astringent than skin tannins of comparable size. Highly purified grape pigments, anthocyanin glycosides, were not perceived as bitter or astringent. From the "red wine reconstitution" investigations that have been carried out, there are some clear outcomes that add to our previously scarce knowledge regarding what components present in wine contribute particular mouth-feel characteristics when a wine is tasted. Although the experiments, by necessity, were carried out in a model system and further work is required to confirm these effects in actual wines, the data point to some important advances. For example, a fraction of anthocyanin dimers and trimers isolated from both wine and marc were not perceived as astringent. This interesting result could suggest that anthocyanins might carry their lack of mouth-feel properties with them into oligomeric and polymeric structures.

Research into factors that potentially could affect red wine colour has demonstrated that copigmentation reactions are strongly influenced by pH, while ethanol decreases the copigmentation interaction when studied in model experiments. Self-association of the major grape pigment malvidin-3-glucoside appears to be the major cause of colour enhancement by copigmentation. It could be established that intermolecular copigmentation depends on the concentration of anthocyanins and is likely reflecting substantial anthocyanin concentrations typically found in young wines. However, copigmentation interactions of anthocyanins with other red wine components are a minor phenomenon in red wine compared to anthocyanin self-association.

Considering the reactive nature of the grape-derived anthocyanins, substantial effort in the research program on red wine colour has been on the formation of new and more stable wine pigments from anthocyanins, and the influence of tannins and other phenolic compounds on stabilising wine colour. We successfully demonstrated the presence of numerous potential red pigments, identified novel polyphenol derivatives by electrospray ionisation - tandem mass spectrometry and confirmed the structural assignment by extensive NMR and MS experiments. We also demonstrated that many red wine pigments, which are formed during winemaking and

ageing, including some pigmented polymers, are almost inert to SO_2-mediated bleaching. While grape anthocyanins are instantly decolourised, anthocyanin-derived wine pigments usually show superior colour properties under wine-like conditions when compared to grape anthocyanins. Higher pigmented polymer concentrations in red wine samples were in most instances positively related to higher wine colour densities. In addition, the chemical reactivity of anthocyanins with grape-derived oenotannins was studied in model reactions in the presence of different SO_2 and acetaldehyde concentrations. In summary, it could be demonstrated that formation of pigmented polymers from anthocyanins and tannins was significantly enhanced by fermentation or fermentation metabolites such as acetaldehyde, and that pigmented polymers were the most prominent red wine pigments in older wines.

The viticultural studies investigated the effects of different levels of sunlight intensity at the bunch zone on seed development, phenolic composition of seeds and skins of grapes and phenolic composition and sensory properties of wines. Also, effects of pruning and harvest timing on phenolic composition of grapes and red wine, and wine sensory properties were studied in a separate experiment.

The first viticultural study involving Shiraz grown in the Barossa Valley provided further evidence that improved wine quality ratings may be related to viticultural conditions which result in higher concentrations of anthocyanins and skin tannins in berries, a lower ratio of seed tannins to anthocyanins and a higher ratio of skin tannins to anthocyanins. As one might expect, excessive canopy shade (<100 PPFD) was detrimental to berry composition, wine composition and wine sensory properties. As part of this research project, a seed colour chart was developed to describe the changes in colour of the seed coat. These seed colour changes were highly correlated with changes in seed monomers and tannins as well as the accumulation of total soluble solids, total anthocyanins and skin total phenolics and indicated that the external appearance and colour of the seed coat may be used as an additional indicator of seed maturity and overall berry ripeness.

The main result from the viticultural trial using Clare Valley Cabernet Sauvignon to study the effects of berry size on grape and wine phenolics and wine sensory properties is that vines from all pruning treatments provided grapes of high colour (more than 1.5 mg/g). Compared to spur, and rod-and-spur pruned grapevines, machine pruning consistently resulted in grapes with the highest colour in all vintages and all maturity levels. However, wines produced from the machine pruned vines were consistently identified as the less preferred wines in sensory analysis by winemakers. This supports the hypothesis that at high grape colour values, grape colour, when looked at in isolation, is not necessarily a suitable marker to differentiate between grape or wine quality of premium grapes. In addition, this trial has also highlighted effects of grape maturity and irrigation and seasonal effects on grape and wine composition, and on wine style.

3 Background

While tannins are found in many plants, condensed tannins (proanthocyanidins) are particularly important for mouth-feel and astringency attributes in wine and are believed to be responsible for colour development in red wines. Red wine tannins play a large and important role for the sensory characteristics of red wines. The fascinating and subtle changes that occur in red wine during aging are believed to be due in part to the dynamic and ever changing nature of the pigments and tannins. It is thought that tannins react with anthocyanins, catechins and other wine components and thus the wine 'softens' and becomes more pleasant to drink.

According to their structure, tannins can be broadly divided into two classes of macromolecules, termed hydrolysable tannins and condensed tannins. Hydrolysable tannins, comprise numerous polygalloyl esters of glucose, the so-called gallotannins, and esters of ellagic acid in ellagitannins and complex tannins. Hydrolysable tannins present in wine – typically at concentrations which are five to ten times smaller compared to condensed grape tannins - are extracted from oak, or were added as oenotannins during winemaking, traditionally to remove undesirable proteins. Oenotannins from oak and other plant sources such as chestnut, tara or quebracho can react like grape tannins with salivary proteins in the mouth; they also contribute to the mouth-feel and astringency of wine. The term 'tannin', therefore, defines a very heterogenous group of polyphenolic compounds which are all identified by their ability to complex with, and precipitate proteins. It is the characteristic interaction of tannins with proteins that differentiates tannins from other polyphenolic compounds, which are ubiquitous in red wine. 'Non-tannic' polyphenols in red wine include anthocyanins such as malvidin-3-glucoside, various cinnamic acid derivatives and the flavonol quercetin, which is formed by grapes in response to light exposure as a glycoside to various sugars. As opposed to hydrolysable tannins, all grape-derived tannins of enological importance are condensed tannins. Condensed tannins are large macromolecules formed by polymerisation of flavan-3-ol subunits, such as catechin, epicatechin, epicatechin gallate and/or epigallocatechin. The subunit composition varies among tannins from grape skins, seeds and stems. These monomers form chains of variable length and it has been shown that as grape tannins become larger, they become increasingly astringent at a given concentration. Occasionally, condensed tannins are referred to as proanthocyanidins, which characterises their ability to form red pigments in concentrated acids upon oxidative heating. It is important to note, however, that pure grape tannins are colourless molecules, and only very small concentrations of pigmented polymers can be detected in grapes at maturity. Once grapes have been crushed in the winery or homogenised in the laboratory, grape tannins will start reacting with anthocyanins to form pigmented polymers.

In Australia, a major research effort on grape and wine polyphenolic compounds was performed by Dr Chris Somers from 1966 to 1990 at The Australian Wine Research Institute. Subsequently, a collaborative project between the Australian Wine Research Institute and the Department of Horticulture, Viticulture and Oenology, The University of Adelaide was initiated at the request of industry and this 'Tannin Project' formed the nucleus of the CRCV project 1.2 'Wine grape tannin and colour specifications'.

The objectives of the 'Tannin Project' were:

- to gain an understanding of the types of compounds important to red wine colour and mouth-feel and of conditions favouring their presence in wine;
- to determine whether the interaction of proanthocyanidins (tannins) with other grape and wine compounds modifies their sensory properties; and
- to establish viticultural and oenological techniques that enhance wine colour and desirable mouth-feel.

4 Project Aims and Performance targets

4.1 Performance targets from original project application

The foreshadowed performance targets for the project as submitted in the original application are shown below.

This project was established with a number of key objectives for years 1 to 4:

- To gain an understanding of the types of compounds important to red wine colour and of conditions favouring their formation.
- To gain an understanding of the role of grape seed and skin proanthocyanidins in the development of red wine colour and mouthfeel.
- To determine whether the astringent sensation results from modification of the lubricating properties of saliva following binding of polyphenolic compounds with salivary proteins.
- To determine whether the interaction of proanthocyanidins with grape and wine polysaccharides modifies the sensory properties of proanthocyanidins.
- To establish viticultural and oenological techniques that enhance wine colour and mouthfeel.

The outcomes foreshadowed through satisfaction of the objectives were:

- Recommendations for viticultural and oenological techniques to deliver improved red wine colour and mouthfeel.
- Identification of grape and fermentation products to act as targets for future programs to genetically modify vines and microorganisms for industry relevant outcomes.

The foreshadowed performance targets for the four years were as follws:

Outputs	**Performance Target**
1. Refereed paper and better methods to enhance our evaluation of red wine.	1. Publication on sensory methods to describe mouthfeel properties of red wines by February 2000.
2. Refereed paper and better methods to further evaluate the role and management of seeds during red winemaking.	2. Publication on methods to quantify tannins in grape seeds in refereed scientific journal by June 2000.
3. Refereed paper and essential information on the nature of stable pigments in red wine. A basis for influencing the production of stable pigments during vinification.	3. Submitted publication on structures and isolation of oligomeric wine pigments in refereed scientific journal by June 2000.

Outputs	Performance Target
1. The Australian Wine Research Institute (AWRI) and the Department of Horticulture, Viticulture and Oenology, The University of Adelaide (HVO, UA)	1. Publication in an Australian refereed scientific journal describing a proposed comprehensive mouthfeel terminology – a 'mouthfeel wheel', by August 2000.
2. PhD thesis and information on the role of wine pigments derived from grape anthocyanins in the total colour expression of wine and potential ways to increase the formation of such pigments in wines.	2. PhD thesis by Robert Asenstorfer on the formation of vitisin A during fermentation and maturation, and its properties, submitted by September 2000.
3. Refereed paper and an understanding of the formation of pigmented tannins and their sensory properties. A basis for influencing the colour, tannin levels and mouthfeel properties of wines.	3. Publication in a refereed scientific journal of experimental data investigating the conditions required to produce high molecular weight pigmented phenolics in a model system and data relating to their chemical properties, by December 2000.
4. Refereed paper and an understanding of the reactivities of the main anthocyanins derived from grapes and of their contribution to wine colour. A basis for influencing the colour of wines.	4. Publication in a refereed scientific journal of experimental data about the structures and equilibria between different forms of malvidin-3-glucoside and of it acetylated derivatives, under wine-like conditions, by December 2000.
5. Refereed paper, methods to quantify wine tannins and information on the role and management of seeds during red winemaking.	5. Publication on the impact on red wine composition and sensory properties of fermenting with additional seeds in refereed scientific journal by June 2001.

Outputs	Performance Target
1. PhD thesis and information on the role of polymeric wine pigments derived from grape anthocyanins in the mouthfeel properties of wine and potential ways to modify the formation of such compounds in wines.	1. PhD thesis by Anita Oberholster on the sensory and chemical properties of polymeric wine pigments, submitted by December 2001.
2. Refereed paper and an understanding of the formation of pigmented tannins and their sensory properties. A basis for influencing the colour, tannin levels and mouthfeel properties of wines.	2. Publication in a refereed scientific journal of experimental data investigating the conditions required to produce high molecular weight pigmented phenolics in a model system and data relating to their chemical properties, by June 2002.
3. Referred paper and improved methods to investigate tannin structure. A basis for linking the chemical properties of tannins to their sensory characteristics.	3. Publication in a refereed scientific journal of methods using phloroglucinol to characterise the structure of grape and wine tannins, by June 2002.
4. Improved ability for winemakers and researchers to communicate attributes of red wines when tasted.	4. Have carried out a workshop and oral paper on red wine mouthfeel description as part of 11th Australian Wine Industry Technical Conference by October 2001
5. Improved ability for winemakers and researchers to understand the impact of simple winemaking parameters on red wine taste properties and composition.	4. Have carried out a workshop illustrating the impact of temperature and oxygen addition on red wine composition and sensory attributes as part of 11th Australian Wine Industry Technical Conference by October 2001

Outputs *(for 2002/2003)*	**Performance Target** *(for 2002/2003)*
1. An understanding of the formation of pigmented tannins and their sensory properties. A basis for influencing the colour, tannin levels and mouthfeel properties of wines.	Publication in a refereed scientific journal of experimental data investigating the formation of high molecular weight pigmented phenolics (pigmented polymers) in commercial scale fermentations, submitted by December 2002.
2. An understanding of the reactivities of the main anthocyanins derived from grapes and of their contribution to wine colour. A basis for influencing the colour of wines.	Publication in a refereed scientific journal of experimental data about the structures and equilibria between different forms of malvidin-3-glucoside and of its acetylated derivatives, under wine-like conditions, by December 2002.
3. Information on the role of polymeric wine pigments derived from grape anthocyanins in the mouthfeel properties of wine and potential ways to modify the formation of such compounds in wines.	PhD thesis by Anita Oberholster on the sensory and chemical properties of polymeric wine pigments, submitted by December 2002.
4. Improved methods to investigate tannin structure. A basis for linking the chemical properties of tannins to their sensory characteristics.	Publication in a refereed scientific journal of mass spectrometric methods to characterise the structure of grape and wine tannins, by December 2002. Publication in a refereed scientific journal of studies describing sensory properties of purified tannin preparations, by December 2002.
5. Information on changes of phenolic compounds during commercial scale red wine fermentations. A basis for influencing the colour, tannin levels and mouthfeel properties of wines.	To carry out replicated fermentation trials at the WSL with focus on yeast effects on colour and mouthfeel properties. Chemical and data analysis to be completed by December 2002. Availability of analytical methods to study oligomeric proanthocyanidins in wine by December 2002. Analysis of phenolic compounds in fermenting wine: adoption of research methods for fast routine analysis and development of methods for high-throughput analysis by December 2002.
6. Establish the effects of bunch exposure on grape phenolics levels, wine composition and sensory properties.	Chemical and preliminary sensory analysis of wines made from grapes derived from viticultural trials by December 2002. PhD thesis by Renata Ristic completed by June 2003.

4.2 Performance targets from follow-on 3 year project (years 5-7)

Subsequently, a follow-on 3 year project (years 5-7) was approved which had the following objectives:

- To improve the understanding of the types of compounds important to red wine colour and mouthfeel and of conditions favouring their formation.
- To develop an understanding of the transformations of grape seed and skin tannins into wine tannins during winemaking.
- To provide the knowledge basis to assist with the design of future trials that will aim to achieve specific wine colour and mouthfeel properties based upon the targeted optimisation of viticultural and winemaking techniques.
- To determine if volatile constituents influence sensory perception of mouthfeel properties of non-volatile constituents such as tannins and polysaccharides.
- To find which, if any, volatile and non-volatile compounds interact to affect overall 'in mouth' retronasal flavour impression and aftertaste.

Associated with these objectives, the following performance targets were identified:

Output	Performance Targets	Date
The formation of stable red pigments from anthocyanins		
1. Information on oxidative processes involved in wine pigment formation: The basis to improve the understanding and control of factors affecting stable colour.	1.1. Adoption of innovative physical methods for the separation and analysis of stable red wine pigments: Successful application of Field Flow Fractionation methods to study pigments in young and aged Shiraz wines. 1.2. Understanding of reaction pathways for wine pigment formation involving various flavan-3-ols. Completion of studies to identify products and reaction pathways from experiments using acetaldehyde as the linking agent. 1.3. Completion of studies on the nature and relevance of oxidative reactions that can be enhanced by presence of condensed tannins or ellagitannins.	Manuscript submitted by July 2004. PhD thesis of David Lee accepted by 2004. December 2005.
Specifying the contribution of pigments to overall wine colour		
2. An estimation of the contribution of individual red pigments to red wine colour based on HPLC analysis, CIELAB measurements and colour dilution assays: The basis for developing colour specifications	2.1. Completion of feasibility studies applying CIELAB methods for the specification of red wine colour and the characterisation of colour properties of purified pigments. 2.2. Availability of CIELAB data to describe colour properties of individual pigments and a minimum of a hundred randomly selected Australian red wines. 2.3. Development of an experimental approach to predict red wine colour on the basis of compositional data.	December 2003. Outcomes drafted by December 2004. PhD thesis of Maria de Sa accepted by 2006.
The structural and sensory characterisation of wine tannins		
3. An understanding of the formation of pigmented and non-pigmented tannins in wines and their sensory properties: The basis for linking the chemical properties of tannins to their sensory characteristics.	3.1. Completion of experiments describing sensory properties of pigmented and non-pigmented polymers produced under model conditions. 3.2. Completion of case studies describing structural characteristics and sensory properties of pigmented and non-pigmented polymers from wine.	July 2005. Drafted for publication by June 2006.
The impact of viticultural practices on tannin development in grapes		

4. Identification of viticultural practices that lead to defined ratios of anthocyanins and tannins in skins and seeds, and the sensory impacts of these levels on wines.	4.1. Information on the effects of viticultural practices, in particular canopy architecture, pruning levels and water status on bunch exposure, berry size, grape and wine composition. 4.2. Completion of sensory studies describing the colour and mouthfeel properties of wines made under 4.1.	Manuscript submitted by December 2005. June 2006
Winemaking to link the above studies from 'vine to bottle'		
5. Information on changes of phenolic compounds during red wine fermentations and an understanding of the interaction between skin and seed composition during fermentation: A basis for influencing the colour, tannin levels and mouthfeel properties of wines.	5.1. Availability of analytical methods to monitor the changes of phenolic compounds during red wine fermentations. 5.2. Application of the methods developed under 5.1. to describe the kinetics of anthocyanin extraction and degradation, and formation of stable red pigments and 'pigmented polymers' during experimental winemaking. 5.3. Determination of the degree of extraction of skin and seed tannins from berries with organic solvents and wine-like model medium, during fermentation and periods of extended skin contact. Winemaking trials, data analysis, sensory studies, publication in a refereed scientific journal and PhD thesis.	Manuscript submitted by July 2004. Manuscript submitted by December 2005. Trials completed by 2005, manuscript submitted by July 2006; PhD thesis submitted by 2007.

Output	Performance Targets	Date
1. Information disseminated on the likely role of wine tannin-like compounds, simple wine pigments predominantly present in young red wines, and glycerol. A step towards identifying wine compounds important to wine mouthfeel properties.	Publication on counter current chromatographic methods to fractionate grape and wine pigments and tannins Publication on the mouth-feel properties and chemical characterization of acetaldehyde linked catechin polymers Data on the mouthfeel properties of purified anthocyanin glycosides at typical red wine ethanol content and acid levels. Publication describing this data Data on the sensory impact of varying concentrations of glycerol in at least two wine styles of importance to Australia. Publication describing this data	December 2003 December 2003 December 2004 June 2005 December 2005 June 2006
2. Information on the likely most important volatile and non volatile components responsible for the typical style of full bodied barrel fermented white wines and wood aged red wines. The basis for developing specifications and management practices.	Preparation of crude fractions of volatiles from at least two full bodied wine styles Data from time intensity assessment of mouth feel perception of mixtures of volatile and tannin fractions in model wine Preparation of crude fractions of tannins, pigments, polysaccharides and other non volatiles from at least two full bodied wine styles Characterization of crude fractions of volatiles from at least two full bodied wine styles	December 2004 December 2005 December 2005 June 2006

All outputs have been achieved and as advised in annual progress reports the following changes were made.

Output 1.1 was changed, given that FFF was assessed of not being an option for studying the structures of wine pigments, to 'Application of novel methods to study pigments; (1) use of radiolabelled anthocyanin, (2) use of cyanidin rutinoside to monitor reactions involved in pigment transformation (Manuscript submitted by July 2005)'.
Output 1.3 was abandoned due to resignation of Ewald Swinny and delays in recruiting a replacement. Priority was given to finalize research described under Outputs 1.1 and 1.2.
Output 2.2 was delayed due to the maternity leave of Maria Birse.
Output 3 was limited to chemical characterisation with no sensory studies being conducted due to lack of sufficient material of known identity.
Output 5.3 was substantially reduced in scope due to the difficulty of appointing an appropriate PhD student. Some of the work was achieved with the help of Mario Mazza, a Masters student with Chris Ford at the University of Adelaide.

5 Method

The research methodologies used in the project are outlined below under the various headings of the broad objectives. Further detail of experiments, methods and techniques are given in the journal articles listed in Appendix 3.

5.1 Development of analytical methods for important phenolic compounds in grape and wine samples

Reliable analytical methods for identification and quantification of important grape-derived non-volatile phenolic compounds have been developed using HPLC methods for the quantitative determination of anthocyanins, tannins and pigmented polymers in grape seed, skin and wine samples. Where reference compounds were not commercially available, compounds had to be isolated by preparative column chromatography, gel permeation chromatography or liquid-liquid countercurrent chromatography (MLCCC). In some instance, reference compounds and starting materials had to be prepared by organic synthesis, following established protocols. Subsequently, the HPLC and HPLC-MS methods were applied to provide reference data for the development of robust and fast techniques such as the tannin assay, or for the development of calibrations which were essential for the successful application of near infrared spectroscopy.

5.2 Formation of stable red pigments from anthocyanins

Formation of stable pigments was studied by HPLC analysis of pigment profiles in red wine fermentations, and model ferments and model reactions using purified precursor molecules on a small scale. Pigments were further characterised by HPLC-MS analysis, and some prominent pigments including several vitisins, pyranoanthocyanins and ethyl-linked anthocyanins were prepared in mg-amounts to robustly corroborate the assigned structures through NMR spectroscopy. To characterize the contribution of individual pigments to wine colour, red wine pigments were analysed by HPLC and the data were compared to spectral data and CIELab data of red wine. A modification of the routine HPLC method for pigment analysis allowed us to establish colour properties as CIELab data of individual pigments in complex mixtures under wine like conditions at a pH value of approx. 3.5 and in presence of SO_2.

5.3 The structural characterisation of tannins

To characterize grape tannins, we have adopted a complex protocol for the hydrolysis of these polymers in presence of phloroglucinol followed by HPLC analysis of the sub-units. This enables us to describe the overall composition and average molecular mass of tannin samples and allows to monitor tannin development during berry ripening. An even more detailed picture on the composition of complex grape tannin samples can be gained from novel mass spectrometric experiments for 'tannin finger-printing' which allows the detection and identification of individual grape tannins on a molecular level.

5.4 Sensory investigations

The "red wine reconstitution" investigations involved isolation and purification of Shiraz skin anthocyanins, grape seed and skin proanthocyanidins of varying size and wine polysaccharides, as well as investigating a set of apple proanthocyanidins as a comparison. The tannins were chemically characterized and also tasted in model wine separately and together with polysaccharides and anthocyanins.
The "white wine reconstitution" experiments involved separating wine component groups, such as proteins and polysaccharides, from wine and subjecting combinations of these purified component groups in aqueous solutions together with aroma compounds, glycerol, tartaric acid and ethanol.
The "white wine adulteration" experiments involved increasing the ethanol and glycerol content to realistic levels, together and separately, of three commercial Riesling wines by addition.
In all studies, a trained sensory panel rated the intensity of specific sensory attributes and the data were subjected to statistical analysis.

5.5 The impact of viticultural practices on tannin development in grapes, wine tannins and sensory properties

The influence of canopy shading and bunch exposure on the composition of grape seeds was studied with a trial involving Barossa Valley Shiraz. These experiments have focussed on determination of the morphological characteristics of grape seeds during development, understanding the changes that occur in phenolic compounds of grape seeds during berry development and determination of the sensory properties of wines made from fruit from different levels of canopy shading. In 1999/2000 a viticultural trial was established by wrapping vines with bird nets to obtain shaded canopies or training shoots up and down to obtain open canopies. In the 2000/2001 season an additional experiment was conducted by enclosing bunches in specially designed boxes to obtain different levels of bunch exposure. Samples of eight replicates from each treatment have been collected weekly, starting a week after fruit set until harvest maturity. Wine was made on a small scale from fruit from different levels of canopy shading and mouth-feel and aroma attributes were characterised through sensory assessment.

Distinct effects of seed components and/or varied anthocyanin/tannin ratios on wine composition and sensory properties may occur if there were differences in berry size between treatments and we have investigating this aspect in Cabernet Sauvignon in collaboration with Leasingham Wines during the 2003 to 2006 vintages. Grape samples (six per treatment) were collected from three different pruning treatments (spur, rod-and-spur and machine pruned vines) at two irrigation levels in three consecutive seasons at commercial harvest, and also at veraison, pre-harvest and post-harvest. All samples have been analysed for maturity (°Brix, pH and TA), and grapes have been randomised and sub-sampled for colour and phenolic analysis. Replicated mini lot ferments of all grapes collected at harvest and some samples from pre- and post harvest for all six treatments (three pruning and two irrigation levels) have been conducted. Chemical analysis of anthocyanins, pigmented polymers and tannin and informal sensory analysis has been completed for mini-lot wines. In addition, sensory analysis was conducted on replicated large scale wines from the three pruning treatments with a small group of winery personnel.
In addition to the winemaking trials required for the Clare Valley Cabernet Sauvignon study, experimental winemaking trials to determine the effects of pre-fermentation (cold soak) and post-fermentation extended maceration treatments on the tannin composition of red wines have been conducted at the Hickinbotham-Roseworthy Wine Science Laboratory.

6 Results/Discussion

The results from the 'Tannin Project' are well documented in the Annual Reports of the CRCV and by numerous peer-reviewed publications as summarized in Appendix 3.

6.1 Mechanisms of pigment formation in wine and cofactors involved in the formation of stable pigments

Research into factors that potentially could affect red wine colour demonstrated that copigmentation reactions are strongly influenced by pH, while ethanol decreases the copigmentation interaction when studied in model experiments. Self-association of the major grape pigment malvidin-3-glucoside appears to be the major cause of colour enhancement in red wine by copigmentation. It could be established that that intermolecular copigmentation depends on the concentration of anthocyanins and is likely reflecting substantial anthocyanin concentrations typically found in young wines. However, copigmentation interactions of anthocyanins with other red wine components are a minor phenomenon in red wine compared to anthocyanin self-association.

Copigmentation and self association of anthocyanins and anthocyanin pigments were further investigated as a result of current literature proposing the benefits of adding co-pigments in increased anthocyanin extraction from grapes during red winemaking. Circular dichroism (CD) studies of younger red wines and solutions of malvidin-3-glucoside in model wine-like media show that the intact wines and solutions of the individual anthocyanin have very similar CD spectra and do not show the antisymmetry which has been proposed to indicate chiral stacking. Lowering of pH to approximately pH 1 did not affect the CD spectra of either malvidin-3-glucoside or any of the red wines studied. Similarly, varying the ethanol concentrations of reconstituted wines showed that ethanol does not affect the CD spectra of these wines. These data illustrate the ineffectiveness of CD to identify self-association, or the lack of it. Given our previous data on the self-association of anthocyanins and the strength of copigmentation interactions with red wine components it is clear that copigmentation is a minor phenomenon in red winemaking compared to anthocyanin self-association.

An improved, more rapid and effective purification method for anthocyanin-epicatechin ethyl-linked pigments was developed using the parent anthocycanins, malvidin-3-glucoside and cyanidin-3-rutinoside. The temporal stability of these ethyl-linked pigments was studied in wine systems. When added to a white wine base, they decompose rapidly to the parent anthocyanins as evidenced by LC-MS. Small-scale red wine fermentations made with added ethyl-linked pigment of malvidin-3-glucoside and epicatchin showed a rapid decline in the levels of this pigments during fermentation releasing the parent anthocyanin to undergo further reactions. More temporally stable pigments (eg vitisins) were observed to be formed. When the epicatechin-ethyl-linked pigment of cyanidin-3-rutinoside was added to a white wine base, the pigment decomposed rapidly but in this case yielded the aglycone of cyanidin-3-rutinoside. Similarly, when cyanidin-3-rutinoside was added to a red wine ferment there was no evidence of any cyanidin-containing pigments being formed, a result attributed to the lesser stability of cyanidin-3-rutinoside in comparison with that of malvidin-3-glucoside. These data illustrate the ineffectiveness of using cyanidin-rutinoside or compounds made from it as tracers to follow complex pigment formation in red winemaking.

Experimental winemaking trials were performed to determine the effects of pre-fermentation (cold soak) and post-fermentation extended maceration treatments on the tannin composition and colour properties of Pinot Noir wine. Wine was produced in triplicate 50-kg ferments, and analysed by spectrophotometer, HPLC and sensory trials during the period of the present report. Wine colour was found to be significantly different across treatments with the cold soak and cold soak with plunging treatments obtaining the highest chroma (C*) values. Maceration treatments had no affect on the total amount of anthocyanins, but did affect pigmented polymer concentrations with the three week extended maceration treatment having the lowest content at 176 days post crush. Neither anthocyanin nor pigmented polymer concentration was correlated with the spectral measurements in this study. Total tannin content as well as catechin and epicatechin concentrations was affected by maceration treatment. Catechin and epicatechin concentrations increased dramatically as a function of time on skins after alcoholic fermentation. Despite the differences, a trained sensory panel did not rate the wines as significantly different with regards to bitterness or astringency when the wines were tasted 6 months following fermentation.

6.2 The structural characterisation of grape and wine tannins

According to their structure, tannins can be broadly divided into two classes of macromolecules, termed hydrolysable tannins and condensed tannins. Hydrolysable tannins comprise numerous polygalloyl esters of glucose (so-called gallotannins), and esters of ellagic acid (ellagitannins and complex tannins). Hydrolysable gallotannins present in wine have been extracted from oak, or were added as oenotannins during winemaking (traditionally to remove undesirable proteins). Oenotannins, which (like grape tannins) can react with salivary proteins in the mouth, also contribute to the mouth-feel and astringency of wine. As opposed to hydrolysable tannins, all grape-derived tannins having enological importance are condensed tannins. Condensed tannins are large macromolecules formed by polymerisation of flavan-3-ol subunits, and our research has confirmed that subunit composition and molecular mass varies among tannins from grape skins and seeds, and changes between veraison and harvest. Occasionally, condensed tannins are referred to as proanthocyanidins, which characterises their ability to form red pigments in concentrated acids upon oxidative heating. It is important to note, however, that pure grape tannins are colourless molecules. Only in ripe to over-ripe berries trace amounts of pigmented polymers could be found occasionally, which most likely resulted from chemical reaction of anthocyanins with tannins in partially damaged berries. However, once grapes have been crushed in the winery or homogenised in the laboratory, grape tannins will start reacting with anthocyanins to form pigmented polymers.

The research has confirmed that wine tannins are essential for astringency, and for stabilising colour in red wine through formation of anthocyanin-tannin conjugates. It was shown that a large portion of pigmented polymers are formed from anthocyanins and tannins during alcoholic fermentation, and that the pigmented polymer concentration in red wine remains largely unchanged after completion of malolactic fermentation. Further experimental evidence for the direct condensation between anthocyanins and tannins was found when a variety of analytical techniques including gel permeation chromatography (GPC), NMR spectroscopy, mass spectrometry and acid-catalysed depolymerisation were combined. Based on mass spectral data, this study has also highlighted the possible presence of non-coloured anthocyanin-tannin conjugates besides the pigmented polymers. The ability of condensed tannins to break apart in acidic solutions is worth considering in view of the differing views held on the fate of tannins during vinification and ageing. During ageing, it is generally considered that wine tannins 'mellow' and 'soften'. The structural basis for this, however, remains unknown. Many consider that tannins continually polymerise during ageing, becoming larger and larger until they precipitate from solution and, therefore, no longer elicit astringency. This concept is supported by research from other groups which have shown that monomeric and oligomeric flavanols polymerise in the presence of acid to higher molecular weight species. Also, other wine compounds such as acetaldehyde or furfural have been demonstrated in model studies to react with and polymerise flavanols and tannins, thus contributing to increases in tannin size. An alternate viewpoint is that tannins might actually become smaller with ageing and – based on sensory data from grape tannins – smaller wine tannins could be perceived as less astringent. Recent evidence from model studies has demonstrated the decrease in mean degree of polymerisation for seed and skin tannins in the presence of flavanol monomers and under mildly acidic conditions as found in wine. Without doubt, acid catalysed depolymerisation and repolymerisation reactions of grape tannins are occurring in wine all the time. The identification of conditions that favour increasing or decreasing tannin size, and their impact on sensory properties and stability of red wine are questions future research will have to answer. It is also important to note that this depolymerisation phenomenon is characteristic of grape tannins, but might not be distinctive of wine tannins. Attempts to depolymerise wine tannins are often only successful on a small percentage of the total wine tannins in the sample and in our hands as much as 80-90% of some wine tannin fractions from experimental wine (which did not contain oak and oenotannins) were incapable of depolymerisation.

Once monomeric polyphenols have been removed and the tannins have been enriched, the characterisation of condensed tannins by depolymerisation was employed. Treatment of a condensed tannin with acid, in the presence of a nucleophile such as phloroglucinol allows the subunit profile to be analysed by HPLC and the average molecular mass (expressed as 'mean degree of polymerisation'; mDP) to be calculated. Most of our current knowledge about general composition and structure of grape tannins has been obtained by the depolymerisation method. For example, it has been shown that grape tannins derived from the skin, seeds and stems differ in their length, overall subunit composition and sensory properties. In general, seed tannins are shorter and have a higher percentage of subunits bearing gallic acid esters, a so-called higher 'degree of galloylation' due to more epicatechin gallate subunits being present. Skin tannins are generally larger and comprise a significant proportion of trihydroxylated epigallocatechin subunits. One

notable limitation of all of the depolymerisation methods is their inability to identify specific subunit sequences.

While the depolymerisation method allows the study of the majority of grape tannins, it is only of limited used for the characterisation of wine tannins. In our experience, the majority of wine tannins resist the acid mediated depolymerisation, thus allowing only a minor portion of wine tannins to be characterised. In addition, it is worthwhile to note that we still remain challenged to isolate individual pure tannins from grape and wine, which limits the value of the depolymerisation method in the context of sensory studies. Samples described as purified tannins are commonly free of other polyphenols, but can still resemble mixtures of individual tannins. When studied by the depolymerisation method, such composite samples containing, for example, a mix of 50% of small tetrameric tannins and 50% larger octameric tannins, would yield an mDP of six, the same as a sample consisting of 100% hexameric tannins. As sensory data have indicated that the size of tannins (expressed as mDP) is an important factor for their astringency properties we recommended to augment mDP values from the depolymerisation method with other chromatographic or spectrometric data, when characterising tannin samples for structure-activity relationship studies. In addition, we and others suggest to always state the degree of conversion when reporting compositional data based on the depolymersiation methods.

The chromatographic methods separate smaller tannins from larger tannins and monomeric 'non-tannic' polyphenols quite well, but do not usually resolve individual tannins present in complex mixtures from natural sources such as grape and wine samples. At the same time, we encounter a lack of chemically defined tannins, which are essential as reference standards. This limits us, as we can only assume that recovery and stability of different tannins during the extraction process, and the UV absorbance and mass spectrometric signal used for detection after the chromatographic separation are consistent across a wide range of tannins. Clearly, further progress will require improved techniques for the isolation, purification and characterisation of tannins and particularly wine tannins; availability of chemically defined reference tannins, which can be obtained only by chemical synthesis; and, most importantly, robust methods for the efficient quantification of wine tannins.

6.3 Sensory investigations

In the various "red wine reconstitution" sensory investigations, we have found that tannin composition influenced astringency strongly: larger tannins were more astringent at a given mass concentration than smaller tannins; and grape seed tannins were perceived as more coarse and astringent than grape skin tannins of similar size. For all the tannin fractions, bitterness was rated similarly to the model wine.

When assessing a tannin fraction of one size, it was found that tannin concentration had the biggest effect on the intensity of astringency; ethanol concentration slightly increased the intensity of bitterness and decreased the intensity of astringency; the polysaccharide, rhamnogalacturonan II, reduced astringency, particularly the coarse, chalky and pucker attributes; and the crude anthocyanin fraction slightly increased fullness, which was most likely due to other species in the fraction than pigment.

The phenolic polymers (catechin subunits linked through ethyl bridges) with mDP 5 were rated as intermediate in astringency between that of apple tannins (catechin oligomers linked through direct inter-flavan bonds) with a mDP 3 and 9. The grape monomeric pigments, anthocyanin glucosides and their corresponding coumarates, and wine or marc derived pigment oligomers were not perceived as different in astringency to the model wine. In addition, only the catechin oligomers linked through ethyl bridges were perceived as more bitter than the model wine.

In summary, in these wine-like solutions
- tannin size and concentration appeared to be the major factor affecting the level of perceived astringency;
- the polysaccharide, rhamnogalacturonan-II decreased tannin astringency;
- linkage type between subunits of tannins influenced the perception of bitterness of these compounds; and
- anthocyanins did not contribute to wine astringency, bitterness or fullness.

The "white wine reconstitution" experiments indicated that ethanol has a direct enhancing effect on many 'unpleasant' palate attributes such as hotness, bitterness, metallic and drying. The overall

perceived flavour was also higher at high ethanol levels (13% v/v) in the presence of glycerol compared to samples without glycerol. The effect of polysaccharides, glycerol and ethanol on viscosity were not simple and it was not clear from this first experiment how perceived viscosity relates to other commonly used sensory terms/descriptors such as fullness, body and density.

The "white wine adulteration" experiments suggested that ethanol and glycerol level in wine realistic ranges had a small but inconsistent positive effect on the body and viscosity of Riesling wines. The perceived hotness of the wines was strongly influenced by alcohol level, while sweetness, flavour and aroma intensity were relatively unaffected by either glycerol or alcohol. Assessors, given a broad definition of wine fullness, were idiosyncratic with regard to what features of the wine contributed to its fullness. However, flavour and perceived viscosity were most frequently and most strongly correlated with the fullness of these wines.

6.4 The impact of viticultural practices on tannin development in grapes, wine tannins and sensory properties

The influence of canopy shading and bunch exposure on the composition of grape seeds was studied with a trial involving Barossa Valley Shiraz. These experiments have focussed on determination of the morphological characteristics of grape seeds during development, understanding the changes that occur in phenolic compounds of grape seeds during berry development and determination of the sensory properties of wines made from fruit from different levels of canopy shading. In summary, this research on Barossa Shiraz provided further evidence that excessive canopy shade is detrimental to berry composition, wine composition and wine sensory properties. Excessive canopy shading caused lower wine colour density than could be expected from the measure of berry colour and anthocyanins. Thus a determination of berry colour values needs to be assessed in conjunction with an assessment of canopy microclimate, and the development of a simple method for determination of skin tannins would help to advance the research. The study also highlighted the potentially detrimental effects of excessive bunch exposure in warm to hot climates on anthocyanin concentration in berries, and for warm to hot viticultural climates adoption of practices which lead to a moderate degree of bunch exposure are recommended. Finally, in these studies modification of the degree of light interception at the bunch zone had little effect on concentration of seed tannins on a per gram berry weight basis. Generally, berry weights were not affected by the applied treatments and hence contribution of seed components to wine made from those berries was likely to be similar. The experiments demonstrated that the moderately and highly exposed bunches had a higher ratio of skin tannins to anthocyanins than extensively shaded bunches. This might explain why wines made from berries that developed and ripened under moderate to high sunlight intensity had higher levels of pigmented polymers. Development of a simple method for determination of skin tannins would certainly help to advance research on factors, which contribute to the formation of pigmented polymers in wines, and thus can enhance wine colour density at a constant concentration of anthocyanins.

Distinct effects of seed components and/or varied anthocyanin/tannin ratios on wine composition and sensory properties may occur if there were differences in berry size between treatments and we have been investigating this aspect in Cabernet Sauvignon in collaboration with Leasingham Wines during the 2003 to 2006 vintages. The main result from the pruning trial with Clare Valley Cabernet Sauvignon is that vines from all pruning treatments provided grapes of high colour (approx. above 1.5 mg/g). Compared to rod, and rod-and-spur pruned grapevines, machine pruning consistently resulted in the smallest grapes with the highest colour in all vintages and all maturity levels. However, wines produced from the machine pruned vines were consistently identified as the less preferred wines in sensory analysis by winemakers. This supports the hypothesis that at high grape colour values, grape colour, when looked at in isolation, is not necessarily a suitable marker to differentiate between grape or wine quality of premium grapes. As one might expect this trial has also highlighted the effects of grape maturity, and seasonal effects on grape and wine composition, and on wine quality.

7 Outcome/Conclusion

Significant progress has been made towards understanding the relationships between grape and wine phenolic compounds, and wine composition and sensory attributes. The project team has achieved most Outputs and Performance targets and in many cases exceeded them.

7.1 Analytical methods

The project has successfully evaluated the feasibility of many methods for the analysis of key phenolic compounds found in grapes and red wine. In response to the wine industry's need for rapid analytical methods for the determination of objective indicators of grape quality, the project has concentrated its efforts on development of a robust method to quantify total tannins in grape and wine samples. Aided by the initially developed HPLC reference method the tannin assay has been developed, validated, and extensively trialled with industry partners. To foster up-take, exploration and adoption by industry a user-friendly SOP has been made available to interested parties, and the tannin assay is currently available as a commercial service from AWRI Analytical Services. The research has also identified areas in need of further development, such as characterisation and quantification of wine tannins.

7.2 Mechanisms of pigment formation in wine and cofactors involved in the formation of stable pigments

Research into factors that potentially could affect red wine colour has demonstrated that self-association of the major grape pigment malvidin-3-glucoside appears to be the major cause of colour enhancement by copigmentation and is likely reflecting substantial anthocyanin concentrations typically found in young red wine. Based on our data on the self-association of anthocyanins and the strength of copigmentation interactions with other red wine components such as tannins it is reasonable to conclude that copigmentation is a minor phenomenon in red winemaking.
Considering the reactive nature of the grape-derived anthocyanins, formation of new and more stable wine pigments from anthocyanins was studied. The presence of numerous novel red pigments was established. Furthermore it was demonstrated that red wine pigments other than grape anthocyanins are formed during winemaking and ageing and that many red wine pigments, as opposed to grape anthocyanins, are almost inert to SO_2-mediated bleaching. Formation of pigmented polymers from anthocyanins and tannins was significantly enhanced by fermentation or fermentation metabolites such as acetaldehyde, and pigmented polymers were the most prominent group of red wine pigments in older wines, thus warranting further research into formation of pigmented polymers from grape phenolics.

7.3 Sensory studies

In summary, the "red wine reconstitution" investigations that have been carried out in collaboration with Dr Cheynier's group at the Institut National de la Recherche Agronomique in Montpellier are very valuable additions to our previously scarce knowledge regarding what components can contribute particular mouth-feel characteristics when a wine is tasted. Although the experiments were carried out in model systems, and further work is required to confirm these effects in actual red wines, the results point to some important advances.

As might be expected, tannin composition and concentration influenced astringency most strongly, with the findings demonstrating that larger tannins were more astringent at a given mass (rather than molar) concentration than smaller tannins and that seed tannins were perceived as more coarse and astringent than skin tannins of comparable size. The grape pigments, anthocyanin glucosides and their corresponding coumarates, were not perceived as bitter or astringent. In addition, they did not influence the fullness or viscosity of the solutions they were tasted in. It remains to be confirmed whether anthocyanins carry their lack of mouth-feel properties with them when they are incorporated into oligomeric and polymeric structures, and whether condensation of tannins with anthocyanins could reduce tannin-related astringency. With regard to the effect of polysaccharides, the two types studied conferred the sensation of fullness in the absence of tannins, but not in the model system with tannins present.

The "white wine reconstitution" and follow up "adulteration" experiments clearly showed that wine components interact with one another to influence the sensory outcome. Wine body or fullness is an

important attribute of both red and white wines and whilst our studies have shown that the volatile wine components, tannins, polysaccharides, ethanol and glycerol contribute to this attribute, the role of the volatile flavour compounds is most probably highly relevant and its relative importance could well vary depending on wine composition and style.

7.4 The impact of viticultural practices on tannin development in grapes, wine tannins and sensory properties

The viticultural study involving Shiraz grown in the Barossa Valley provided further evidence that improved wine quality ratings may be related to viticultural conditions which result in higher concentrations of anthocyanins and skin tannins in berries, a lower ratio of seed tannins to anthocyanins and a higher ratio of skin tannins to anthocyanins. As one might expect, excessive canopy shade was detrimental to berry composition, wine composition and wine sensory properties. Seed colour changes were highly correlated with changes in seed monomers and tannins as well as the accumulation of total soluble solids, total anthocyanins and skin total phenolics and indicated that the external appearance and colour of the seed coat may be used as an additional indicator of seed maturity and overall berry ripeness. The data indicate the importance of skin tannins for red wine colour and mouth-feel, and highlight the need for specific measures of skin and seed tannins, to augment data on total tannins from grape and wine samples.

The main result from the trial with Clare Valley Cabernet Sauvignon is that at higher grape colour values, grape colour, when looked at in isolation, is not necessarily a suitable marker to differentiate between grape or wine quality of premium grapes. Obviously, smaller berries are not necessarily resulting in red wine of improved phenolic composition and sensory properties. As one might expect this trial has also highlighted the effects of grape maturity, irrigation and seasonal effects on grape and wine composition, and on wine style. The information from this study will enable us to design experiments for assessing the impact of harvest timing on grape composition and wine style, and while good progress has been made in this area we expect further advances with the characterisation of additional quality markers in the next years.

8 Recommendation

Many analytical methods for key phenolic compounds from grape and wine samples have been established with this research. These methods allow the specific detection of anthocyanins and other red wine pigments such as vitisins and pyranoanthocyanins, and condensed tannins however, so far, not the quantification of individual grape and wine tannins. Clearly, further progress will require improved techniques for the isolation, purification and characterisation of tannins and particularly wine tannins; availability of chemically defined reference tannins, which can be obtained only by chemical synthesis; and, methods for the efficient quantification of individual wine tannins. One might infer that sensory properties of wine tannins are similar to grape tannins, but wine tannin structures are not all the same as grape tannin structures. How does the degree of oxidation of wine tannin influence its perception? How do we characterise the degree of oxidation of wine tannins? Many questions remain unanswered and to achieve an improved understanding of wine tannins it is recommended to foster development of enhanced tools for characterisation of wine tannins, including techniques to assess hydrolysable oak tannins and oenotannins.

The unresolved biosynthesis of tannins and the 'maturation reactions' of tannins between véraison and harvest are amongst the last frontiers in plant secondary metabolism. It is recommended to advance the knowledge of how key enzymes of grape tannin biosynthesis operate and can be controlled, and further research investment is warranted to establish, as example, the production of red grapes (or selection of grape varieties) with lower astringency, but retained colour stabilisation properties. Also, one could be hopeful to reprogram tannin biosynthesis and to reduce the concentration or extractability of galloylated seed tannins.

This project has demonstrated the value of mini lot and small scale winemaking to ensure sufficient numbers of replicates allow statistical analysis to link grape composition to wine composition and wine sensory properties. Given the limited resources and panel time available for complex sensory studies it is recommended to consider development of analytical techniques to reduce sample numbers ('de-replication'), ie. to adopt and validate techniques that allow identification of key samples (or groups of samples) with distinct properties for priority sensory assessment.

Sensory studies in which pure components are assessed are important to identify key compounds important to astringency, mouth-feel and taste, and to establish sensory properties of key compounds. In isolation, however, such experiments may give an incomplete picture of the overall importance of particular wine components on wine sensory properties. It is recommended that wine reconstitution and adulteration experiments be part of any sensory experimental of specific wine components.

9 Appendix 1: Communication

The results of the project have been widely communicated through numerous scientific and technical papers, oral and poster presentations at conferences and symposia, and oral presentations to grape growers and winemakers throughout Australia via the AWRI's 'road show' seminar program and at the Australian Wine Industry Technical Conferences, as summarised in the Annual Reports of AWRI, CRCV and GWRDC, Appendix 3 and CRCV Attachment 2.

10 Appendix 2: Intellectual Property

The project has generated significant IP building from a base of background IP contributed by participants. IP includes assays, extraction techniques, methods for isolation, measurement and identification of phenolics, techniques for characterisation of tannins, SOPs, knowledge regarding design of viticultural, winemaking and sensory trials, and knowledge regarding viticultural and winemaking factors of importance to phenolic composition and wine specifications. The vast majority of this has or will be published.

11 Appendix 3: References

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12 Appendix 4: Staff

1999–2000

AWRI	Dr Liz Waters, Dr Leigh Francis, Dr Zhong Kui Peng, Dr George Skouroumounis, Mr Yoji Hayasaka, Ms Gayle Baldock, Ms Mariola Kwiatkowski, Mr Kevin Pardon, Ms Anita Oberholster, Ms Stella Kassara
Adelaide University	Dr Graham Jones, Mr Patrick Iland, Dr Stephanie Lambert, Mr David Lee, Ms Renata Ristic, Dr James Kennedy, Mr Robert Asenstorfer, Dr Richard Gawel

2000–2001

AWRI	Dr Liz Waters, Dr Leigh Francis, Dr Zhong Kui Peng, Dr George Skouroumounis, Dr Stéphane Vidal, Mr Yoji Hayasaka, Ms Gayle Baldock, Ms Mariola Kwiatkowski, Mr Kevin Pardon, Ms Anita Oberholster,
Adelaide University	Dr Graham Jones, Mr Patrick Iland, Dr Stephanie Lambert, Mr David Lee, Ms Renata Ristic, Dr James Kennedy, Dr Robert Asenstorfer

2001–2002

AWRI	Dr Markus Herderich, Dr Leigh Francis, Dr Zhong Kui Peng, Dr George Skouroumounis, Dr Stéphane Vidal, Dr Liz Waters, Mr Yoji Hayasaka, Ms Gayle Baldock, Mr Holger Gockowiak, Ms Mariola Kwiatkowski, Mr Kevin Pardon, Ms Mango Parker,
Adelaide University	Dr Graham Jones, Dr Patrick Iland, Dr Stephanie Lambert, Mr David Lee, Ms Renata Ristic, Dr Ewald Swinny, Dr Robert Asenstorfer

2002–2003

AWRI:	Dr Markus Herderich, Dr Leigh Francis, Dr Patrik Jones, Dr George Skouroumounis, Dr Paul Smith, Dr Stéphane Vidal, Dr Liz Waters, Mr Yoji Hayasaka, Ms Gayle Baldock, Ms Maria de Sa, Ms Heather Donnell, Mr Holger Gockowiak, Ms Mariola Kwiatkowski, Ms Kate Lattey, Mr Kevin Pardon, Ms Mango Parker,
Adelaide University	Dr Graham Jones, Dr Patrick Iland, Dr Stephanie Lambert Mr David Lee, Ms Renata Ristic, Dr Ewald Swinny

2003–2004

AWRI	Dr Markus Herderich, Dr Leigh Francis, Dr Helen Holt, Dr Patrik Jones, Dr George Skouroumounis, Dr Paul Smith, Dr Liz Waters, Mr Yoji Hayasaka, Ms Gayle Baldock, Ms Maria de Sa, Ms Heather Donnell, Mr Holger Gockowiak, Ms Mariola Kwiatkowski, Ms Kate Lattey, Mr Kevin Pardon, Ms Mango Parker
Adelaide University	Dr Graham Jones, Dr Chris Ford, Mr Mario Mazza, Mr David Lee, Ms Renata Ristic, Dr Ewald Swinny

2004–2005

AWRI	Dr Markus Herderich, Dr Leigh Francis, Dr Helen Holt, Dr Patrik Jones, Dr George Skouroumounis, Dr Paul Smith, Dr Liz Waters, Mr Yoji Hayasaka, Ms Gayle Baldock, Ms Maria Birse, Ms Heather Donnell, Mr Holger Gockowiak, Ms Mariola Kwiatkowski, Ms Kate Lattey, Ms Mango Parker
Adelaide University	Dr Graham Jones, Dr Chris Ford, Mr Mario Mazza, Mr David Lee, Dr Renata Ristic, Dr Ewald Swinny, Dr Natalie Williamson

2005–2006

AWRI	Dr Markus Herderich, Dr Leigh Francis, Dr Helen Holt, Dr Paul Smith, Dr Liz Waters, Ms Maria Birse, Ms Heather Donnell, Ms Kate Lattey
Adelaide University	Dr Graham Jones, Dr Natalie Williamson

13 Appendix 5: Data

All raw data has been archived at the AWRI according to the policies and practices in place at the time. Retrieval can be arranged on request.

14 Appendix 6: Budget reconciliation

(Gaye Jones from CRCV will include information)

CRCV Attachment 1: Project Summary & Impact Form





Project Leaders are required to submit reports to Program Managers for compilation for the information of the Executive Management Group, the CRCV Board and the Australian Government's CRC Program

The format and reporting dates have been developed to satisfy the requirements of the CRC Program and the CRCV. This form seeks to collect information from Project Leaders about how the project has performed against the CRCV Commonwealth Agreement.

Project Title	Wine grape tannin and colour specifications
Period Report Covers	1st November 2005 – 30th June 2006
CRCV Project Code Number	Project 1.2
Organisation	Cooperative Research Centre for Viticulture

Objectives

To improve the understanding of the types of compounds important to red wine colour and mouthfeel and of conditions favouring their formation.
To develop an understanding of the transformations of grape seed and skin tannins into wine tannins during winemaking.
To provide the knowledge basis to assist with the design of future trials that will aim to achieve specific wine colour and mouthfeel properties based upon the targeted optimisation of viticultural and winemaking techniques.
To determine if volatile constituents influence sensory perception of mouthfeel properties of non-volatile constituents such as tannins and polysaccharides.
To find which, if any, volatile and non-volatile compounds interact to affect overall 'in mouth' retronasal flavour impression and aftertaste.

Progress over the life of the project

Many key phenolic compounds of wine remain poorly explained or yet to be identified, and the factors affecting red wine colour, mouth-feel, astringency and taste are poorly defined on a molecular level. Therefore, **the project efforts concentrated on improving our understanding of the basis of red wine colour, identifying target compounds that are relevant for specific mouth-feel properties, and developing a detailed insight into transformations of grape compounds during berry development and winemaking.** As phenolic compounds in wine constitute a hugely heterogenic mixture and reference substances are largely not available from commercial sources, separation, isolation and characterisation of pure compounds are a significant challenge.

The **development of reliable analytical methods** for identification and quantification of important grape-derived non-volatile phenolic compounds was a high priority and we have successfully established generic and widely used HPLC methods for the quantitative determination of anthocyanins, tannins and pigmented polymers in grape seed, skin and wine samples. Subsequently, the HPLC methods provided the reference data for the development of robust and fast techniques such as the tannin assay, or successful application of near infrared spectroscopy. To characterize grape tannins, we have adopted a complex protocol for the hydrolysis of these polymers in presence of phloroglucinol followed by HPLC analysis of the sub-units. This enables to describe the overall composition and average molecular mass of tannin samples and allows to monitor tannin development during berry ripening. An even more detailed picture on the composition of complex grape tannin samples can be gained from novel mass spectrometric experiments for 'tannin finger-printing' which allow the detection and identification of individual grape tannins on a molecular level.

Our studies on **red wine mouth-feel** initially concentrated on the sensory evaluation of purified grape tannins and have been extended to evaluate the influence of polysaccharides, anthocyanins, and ethanol concentration on tannin perception. Instrumental for the success of all our sensory studies is the 'Mouth-feel Wheel', the formal terminology developed to describe and communicate the mouth-feel characteristics of red wine. Sensory evaluation of purified grape tannins confirmed that tannin composition and concentration influenced astringency most strongly. The findings demonstrated that larger grape tannins are more astringent at a given mass concentration than smaller tannins, and that seed tannins are perceived as more coarse and astringent than skin tannins of comparable size. Highly purified grape pigments, anthocyanin glycosides, were not perceived as bitter or astringent. From the **"red wine reconstitution" investigations** that have been carried out, there are some clear outcomes that add to our previously scarce knowledge regarding what components present in wine contribute particular mouth-feel characteristics when a wine is tasted. Although the experiments, by necessity, were carried out in a model system and further work is required to confirm these effects in actual wines, the data point to some important advances. For example, a fraction of anthocyanin dimers and trimers isolated from both wine and marc were not perceived as astringent. This interesting result could suggest that anthocyanins might carry their lack of mouth-feel properties with them into oligomeric and polymeric structures.

Research into **factors that potentially could affect red wine colour** has demonstrated that copigmentation reactions are strongly influenced by pH, while ethanol decreases the copigmentation interaction when studied in model experiments. Self-association of the major grape pigment malvidin-3-glucoside appears to be the major cause of colour enhancement by copigmentation. It could be established that that intermolecular copigmentation depends on the concentration of anthocyanins and is likely reflecting substantial anthocyanin concentrations typically found in young wines. However, copigmentation interactions of anthocyanins with other red wine components are a minor phenomenon in red wine compared to anthocyanin self-association.

Considering the reactive nature of the grape-derived anthocyanins, substantial effort in the research program on red wine colour has been on the **formation of new and more stable wine pigments** from anthocyanins, and the influence of tannins and other phenolic compounds on stabilising wine colour. We successfully demonstrated the presence of numerous potential red pigments, managed to identify novel polyphenol derivatives by electrospray ionisation - tandem mass spectrometry and confirmed the structural assignment by extensive NMR and MS experiments. We also demonstrated that many red wine pigments, which are formed during winemaking and ageing, including some pigmented polymers, are almost inert to SO_2-mediated bleaching. While grape anthocyanins are instantly decolourised, anthocyanin-derived wine pigments usually show superior colour properties under wine-like conditions when compared to grape anthocyanins. Higher pigmented polymer concentrations in red wine samples were in most instances positively related to higher wine colour densities. In addition, the chemical reactivity of anthocyanins with grape-derived oenotannins was studied in model reactions in the presence of different SO_2 and acetaldehyde concentrations. In summary, it could be demonstrated that formation of pigmented polymers from anthocyanins and tannins was significantly enhanced by fermentation or fermentation metabolites such as acetaldehyde, and that pigmented polymers were the most prominent red wine pigments in older wines.

The **viticultural studies** investigated the effects of different levels of sunlight intensity at the bunch zone on seed development, phenolic composition of seeds and skins of grapes and phenolic composition and sensory properties of wines. Also, effects of pruning and harvest timing on phenolic composition of grapes and red wine, and wine sensory properties were studied in a separate experiment.
The first viticultural study involving Shiraz grown in the Barossa Valley provided further evidence that improved wine quality ratings may be related to viticultural conditions which result in higher concentrations of anthocyanins and skin tannins in berries, a lower ratio of seed tannins to anthocyanins and a higher ratio of skin tannins to anthocyanins. As one might expect, excessive canopy shade was detrimental to berry composition, wine composition and wine sensory properties. As part of this research project, a seed colour chart was developed to describe the changes in colour of the seed coat. These seed colour changes were highly correlated with changes in seed monomers and tannins as well as the accumulation of total soluble solids, total anthocyanins and skin total phenolics and indicated that the external appearance and colour of the seed coat may be used as an additional indicator of seed maturity and overall berry ripeness.
The main result from the viticultural trial using Clare Valley Cabernet Sauvignon to study the effects of berry size on grape and wine phenolics and wine sensory properties is that vines from all pruning treatments provided grapes of high colour (more than 1.5 mg/g). Compared to spur, and rod-and-spur pruned grapevines, machine pruning consistently resulted in grapes with the highest colour in all vintages and all maturity levels. However, wines produced from the machine pruned vines were consistently identified as the less preferred wines in sensory analysis by winemakers. This supports the hypothesis that at high grape colour values, grape colour, when looked at in isolation, is not necessarily a suitable marker to differentiate between grape or wine quality of premium grapes. In addition, this trial has also highlighted effects of grape maturity and irrigation and seasonal effects on grape and wine composition, and on wine style.

Progress Against Milestones

Milestone	Year							Progress	Revised Date for Achievement of Milestone
	1	2	3	4	5	6	7		
Elucidate the mechanisms of pigment formation in wine.	X	X	X	X	X	X		Achieved	30 June 2006
Establish the cofactors involved in the formation of stable pigments.	X	X	X	X	X	X		Achieved	30 June 2006
Examine the variables influencing the formation of tannins in wines and characterise the tannins.	X			X	X	X	X	Achieved	
Understand the role of copigmentation in wine colour and the species involved.	X	X	X					Achieved	
Rationalise the impact of the interaction of flavour compounds, polysaccharides and phenolic compounds on the perception of wine palate sensory attributes.	X	X	X	X	X	X	X	Achieved	
Establish the effect of viticultural		X	X	X	X	X	X	Achieved	

	Year								
treatments on grape components involved in colour and tannin levels									

Amendments to Milestones

No amendments

Progress since the beginning of the project
The Report length is 500 word limit. Information provided in this Report will be used to compile CRCV Reports as public documents. The report must not contain information of a confidential nature concerning the Centre or its intellectual property.

The project has successfully evaluated the role of phenolic compounds present in red grape and wine for colour, astringency and mouth-feel, and taste. In response to the wine industry's need for objective indicators of grape and wine quality, the project has delivered a practical standardised methodology for quantifying tannins in grape and wine samples. A wide range of other techniques were also investigated and methods for measurement, structural characterisation and sensory evaluation of phenolic compounds, including anthocyanins, grape tannins and pigmented polymers have been established. Viticultural and winemaking experiments have enabled us to better understand how some grape phenolics are formed and extracted, and how they are modified or degraded in wine.

Likely use of research outcomes by Industry and Others

How successful was the project in meeting its scientific objectives (please underline)

SOMEWHAT MOSTLY COMPLETELY

Who is currently using the outcomes of the project? (please underline)

NO-ONE RESEARCHERS AUSTRALIAN GRAPEGROWERS

AUSTRALIAN WINERIES OTHER(please specify)

Who will be using the outcomes of the project within 5 years? (please underline)

NO-ONE RESEARCHERS AUSTRALIAN GRAPEGROWERS

AUSTRALIAN WINERIES OTHER(please specify)

Have there been any unexpected benefits of the research?
Yes or No

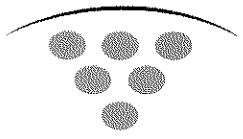
Please detail:
Aided by the initially developed HPLC reference method the tannin assay has been developed, validated, and extensively trialled with industry partners. To foster up-take, exploration and adoption by industry a user-friendly SOP has been made available to interested parties, and the tannin assay is commercially available.
Data generated with the help of HPLC reference methods were used to explore the potential of alternative methods for rapid determination of key phenolic compounds.
The research has also identified areas in need of further development, such as characterisation and quantification of wine tannins.

What is your best estimate of how long it will be before the Australian Industry is using the outcomes of this research project to best effect (maximum benefit)?

NOW 1-2 YEARS 3-5 YEARS 6-10 YEARS OTHER (please specify

CRCV Attachment 2: CRCV Annual Report Requirements





COOPERATIVE
RESEARCH CENTRE
for
VITICULTURE

Project Leaders are required to submit reports to Program Managers for compilation for the information of the Executive Management Group, the CRCV Board and the Australian Government's CRC Program

The format and reporting dates have been developed to satisfy the requirements of the CRC Program and the CRCV. This form seeks to collect information from Project Leaders for the CRCV's 05/06 Annual Report.

Project Title	Wine grape tannin and colour specifications
Period Report Covers	1st November 2005 – 30TH June 2006
CRCV Project Code Number	Project 1.2
Organisation	Cooperative Research Centre for Viticulture

Intellectual Property

Identify any items of new knowledge or know how arising from the work that could be of value to the CRCV as intellectual property. This
should be in enough detail for the CRCV to assess whether further action to register and protect the knowledge would be appropriate.)

No new intellectual property was identified.

Publications and Technical Communications (2005/2006 only)
(List within the following sections publications and technical communications arising from the Project To assist presentation it is requested that you adopt format for citation as shown)

Scientific Publications (refereed) 05/06 only

1. Asenstorfer, R.E., Lee, D.F. and Jones, G.P. (2006) Influence of structure on the ionization constants of anthocyanins and anthocyanin-like wine pigments. Analytica Chemica Acta, 563, 10-14.
2. Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich. M.J.; Smith, P.A. (2006) Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. Australian J. Grape and Wine Res. 12: 39-49.
3. Herderich, M.J., Smith, P.A., Analysis of grape and wine tannins: methods, applications and challenges, Aust. J. Grape Wine Res. 11, 205–214, 2005.

Research Conference, Seminars, Workshop Presentations and Technical Reports (non-refereed) (2005/2006 only)

Papers and/or Addresses

1. Paul Smith - An AWRI Research Overview, Flinders NMI collaboration workshop, 20 September 2005.
2. Paul Smith - Development and application of a simple and robust assay for quantification of tannins in wine and grape samples, 2005 King Valley Vigneron annual meeting, 3 October, 2005.
3. Markus Herderich - Grape and wine tannins: an overview on current research, emerging applications and future challenges, 2005 ASVO Tannin Symposium, 6 October, 2005.
4. Paul Smith - Development and application of a simple and robust assay for quantification of tannins in wine and grape samples, 2005 ASVO Tannin Symposium, 6 October, 2005.
5. Paul Smith - Analysis of Grape and Wine tannins, Lecture to Adelaide University HVO students, 19 October, 2005.
6. Paul Smith - Collaborative tannin assay research, Hardy Wine Company Technical Meeting, 26 October, 2005.
7. Paul Smith – Delivering the Goods: What factors generate perceptions of quality, Winedown, 24-25 October, 2005.
8. Meagan Mercurio, Development and application of a simple and robust assay for quantification of tannins in wine and grape samples, Stonehaven Winery, Padthaway, 28 March, 2006.
9. Paul Smith - Grape and Wine Tannins, Red Wine Colour and Mouthfeel – An Overview on Current Research, Emerging Applications and Future Challenges, AWRI roadshow, 5th May, 2006.
10. Paul Smith - An industry survey of grape colour, tannin, TSS (Brix and crop yield) – with some implications for winemaking, AWRI Roadshow, 5th May, 2006.
11. Lambert, S.G., Asenstorfer, R.E., Lee, D.F. and Jones, G.P. (2006) Copigmentation Interactions and the Impact of Anthocyanin Equilibria. 4th International Workshop on Anthocyanins, Rotorua, NZ, 14-17th February 2006.
12. Swinny, E.E., Lee, D.F., Hayasaka, Y., Williamson, N.M. and Jones, G.P. (2006) NMR Identification of Ethyl-Linked Anthocyanin-Flavanol Pigments Formed in Model Reaction Solutions. International Society of Magnetic Resonance, Murramarang National Park (NSW, Australia) 12-16 February 2006.

Posters

1. Daniel Cozzolino, Mango Parker, Robert G. Dambergs, Mark Gishen and Markus Herderich Qualitative monitoring red wine fermentation: combining chemometric and near infrared spectroscopy. In Vino Analytica Scientia, 7–9 July 2005, Montpellier France.

Technical Reports and Industry Journal articles (non-refereed)

Herderich, M.J., Birse, M., Dambergs, R.G., Holt, H., Iland, P., Lattey, K.A. and Smith, P.A. (2006) Grape and wine tannins – an overview on current research, emerging applications, and future challenges. Australian

Society of Viticulture and Oenology, Adelaide, South Australia; 4-10.
Mazza, MV and Ford, CM (2005) Unravelling the outcomes of extended maceration in red winemaking. The Australian and New Zealand Grape Grower and Winemaker Annual Technical Issue 2005, 56-61.
Smith, P.A. (2005) Development of an assay to quantify tannins in grape and wine samples. Australian Wine Research Institute Technical Review 154, 37–38.
Smith, P.A., Sarneckis, C.J., Jones, P., Dambergs, R.G., and Herderich, M.J. (2006) Development and application of a simple and robust assay for quantification of tannins in wine and grape samples. Australian Society of Viticulture and Oenology, Adelaide, South Australia; 30-31.

Leaflets/Brochures/Technical Guides

Other Communications

Public Presentations – 2005/2006 only

Presentations
(Provide details of any conferences, seminars or the like which the Project team convened or hosted for others)

Event	Date	Number of Attendees
AWRI 50th Anniversary Seminars: Renmark, SA Wodonga, VIC Bendigo, VIC Melbourne, VIC Coonawarra, SA Mildura, NSW Margaret River, WA Mount Barker, WA Swan Valley, WA Toowomba, QLD	 15 June 2005 28 June 2005 29 June 2005 30 June 2005 11 October 2005 3 November 2005 22 November 2005 23 November 2005 25 November 2005 29 November 2005	

Public Relations and Communication 2005/2006 only

Media Reports (Radio, Newspapers, Television
(Provide details)

Industry Contribution to the Project 13. 2005/2006 only	
14. Vineyard area (ha) made available by collaborating growers	
Vineyard staff (no. by category) made available by collaborating growers: (Insert grower name)	
Manager (s)	
Technical staff	
Field staff	
Materials (by item):	
15. Vineyard chemicals	
Vineyard products	
Laboratory apparatus	
Consumables	
Cash support ($).	

Personal in-kind participation associated with review or advisory activities (persons x hr p.a.)	

Contributions of Project Staff to Viticultural Education and Training 2005/2006 only
(Show time (hours) spent by project staff in an average working week
in education and training programs.

Name	Undergraduate Lectures	Postgraduate Supervision	Industry Training*
Markus Herderich		2h	
Paul Smith		2h	

*include seminars, workshops, demonstrations; show annual figure if more applicable.

Grants and Awards – 2005/2006 only
(List awards, honours or other special recognition to the project and/or team members)

Award	Recipient(s)

Project Team Meetings – 2005/2006 only
(Complete details under headings shown)

Date	Location	Purpose	Participants
Fortnightly	The Australian Wine Research Institute	Review of Progress	Group Members