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Consequences of extended maceration for red wine colour and phenolics

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UA 05/03**

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Project Summary – UA 05/03

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Executive summary

During red winemaking operations, strategies whereby skins are held in contact with juice, must or wine for extended periods have been used widely to provide enhanced colour, flavour and tannin structure to the finished wine. The main period of maceration, during the alcoholic fermentation, may be extended to the pre-ferment or post-ferment periods to achieve these ends. Pre-ferment extended maceration (EM), often termed cold soak, is commonly reported to increase the colour intensity of wines and to improve the levels of fruit flavours. Post-ferment EM is held to improve colour stability and tannin structure of wines. **While winemakers continue to use these approaches in their red winemaking strategies, there has been little data to support the claims for significant compositional differences arising from either treatment.** The principle benefits claimed for EM treatments may be considered extended functions of the differential extraction of phenolic compounds that takes place during conventional maceration. Analytical determination of these parameters and identification of the particular compounds that show modified levels in EM wines will permit more accurate targeting of winemaking activities to desired outcomes. By their nature, EM wines spend longer periods in fermentation vessels that are likely to be in high demand during vintage. Strategies that result in the ability to make wines with equivalent sensory properties in a reduced time period will present winemakers with improvements in process efficiency. **The research conducted during this project will examine the consequences of EM strategies for the composition of wines produced from the commercially vital varieties Shiraz and Grenache, in addition to further extending our existing research program with Pinot Noir.** Preliminary work in our laboratory resulted in successful demonstrations of the necessary small-scale winemaking and analytical procedures, and has provided preliminary data for the assessment of pre- and post-ferment EM treatments in Pinot Noir wines.

This project outcomes comprise a comprehensive investigation of the effects on red wine phenolics of extended maceration methods during winemaking, determined using a variety of chemical and sensory test methods.

Grenache and Shiraz experimental wines were produced in vintage 2006 and 2007, respectively. For both varietals, four replicates of each of three maceration protocols were produced. A three-day cold soak treatment at 10°C with plunging (CS), a control treatment (C), and a three-week post-fermentation extended maceration treatment (Post) were compared. Pinot Noir wines produced in a previous study

(triplicate wines for each of five maceration protocols) undergoing bottle aging were also analysed. For Pinot Noir two pre-fermentation (cold-soak) treatments and two post-fermentation treatments were compared against each other and a control treatment (C).

Results show the varietals used were good models to illustrate the consequences of extended maceration for red wine. For most measures, there was no significant difference between control and cold-soak treatments, and for Pinot Noir, there was no significant difference between cold-soak for three days with plunging and the same treatment without plunging, suggesting that if winemakers want to carry out cold-soak time and work can be saved by not plunging. In many instances the Pinot Noir three-week post-fermentation extended maceration treatment was significantly different (higher phenolic content, for example) than the one-week post-fermentation extended maceration treatment, suggesting the extra two weeks on skins significantly altered the phenolic profile of the final wine. Post-fermentation extended maceration generally resulted in lower colour intensity, higher wine colour hue, and higher total phenolics than the other treatments.

Introduction

In order to extend the functions of the differential extraction processes that occur during conventional maceration, and to achieve organoleptic properties beyond those offered by conventional maceration during fermentation, extended contact with skins may occur before (cold-soak) or after fermentation (post-fermentation extended maceration). Extended maceration (EM) regimes are playing an increasing role in winemaking, especially for premium red wines, in Australia and overseas. The nature of these techniques mean these wines spend longer in fermentation vessels during vintage, when vessels are in high demand. While many Australian winemakers would ideally use EM during vintage, many cannot afford to due to logistic pressures. This study started out with the aim of investigating how the effects of various extended maceration technologies, which are desirable for specified red wine outcomes and carried out by Australian red winemakers to emulate French wines, may be achieved without the requirement for an ‘extended’ maceration, and therefore, if there is a way of achieving a wine organoleptic outcome in a shorter time frame, and in a way that is more cost effective and process efficient for the industry.

The importance of maceration regimes for the phenolic composition of red wines has been long known – with the advent of large-scale mechanised winemaking, much effort continues to be devoted to effective extraction of grape components during fermentation. Excellent wines are often produced with maceration only during the alcoholic fermentation; EM techniques were developed in response to winemaking situations in which 'conventional' maceration proved inadequate to realise the fruit's full potential. Heatherbell et al. (1997) investigated the outcomes of pre-ferment EM on the composition of Pinot Noir wines, citing reports of pre-fermentation maceration in cool-climate Burgundian wineries. Similarly, Scudamore-Smith et al. (1990) reported attempts to elaborate on the use of post-fermentation EM, used

in production of Bordelais wines. In the former case, pre-ferment EM of Pinot Noir grapes gave wines with enhanced red colour intensity compared to control wines. The colour increase was due to an overall greater extraction of phenolic compounds from the skins rather than to an elevated level of anthocyanins in EM-treated wines. Sulfur dioxide used to protect juice during the EM period retarded formation of pigmented polymers in the early stages of maturation, but by 2 years no detectable differences existed in phenolic profiles of the wines produced by pre-ferment EM compared to control wines. Analysis of tannin monomer and dimer concentrations in Pinot Noir wines with 4 and 10-day pre-fermentation cold soaks suggested longer periods of pre-ferment EM did not change wine phenolics (Peyrot des Gaschons and Kennedy, 2003). This trial was unreplicated, and examination of the data shows a ca 20% increase in extraction of skin tannin components by day 8 of the fermentation using 10-day pre-ferment macerated grapes. Scudamore-Smith et al. (1990) suggested that post-ferment EM treatments of Cabernet Sauvignon wines gave little or no difference to the tannin structure of wines as assessed by a trained panel, but achieved earlier colour stability. This trial was compromised by the use of oak barrel maturation of the wines – the variability imparted is likely to have severely impacted across treatments. Reports exist that post-fermentation EM results in higher levels of total phenolics in the final wine (Auw et al. 1996; Watson et al. 1997). Nemanič et al. (2002), using Pinot Noir wines, reported that a 3 day post-ferment EM gave wines higher levels of total phenolics than controls. In each of the above references, and in others not listed, many factors contributed towards a lack of rigour in experimental design (e.g. replication of ferments) or analytical procedures. Recent work in our laboratory using Pinot Noir grapes with triplicated 50-kg ferments examined the effects of pre- and post-ferment EM on the phenolic composition of wines during vinification and subsequent maturation. We showed increased red colour intensity of wines produced by pre-ferment EM due to increased formation of pigmented polymers, not higher concentrations of anthocyanins (Mazza and Ford, 2005). Post-ferment EM similarly increased levels of phenolics, specifically tannins and their monomers.

Outcomes of the present research

1. Australian winemaker survey: The outcomes of extended maceration in red winemaking – winemakers' perspectives

A mail survey was sent to 700 Australian red winemakers in order to gauge the EM processes used and the extent of use in the Australian industry and, in particular, to further understand what winemakers believe are the consequences for red wine made using EM regimes. Winemakers targeted in the survey were sourced from the Wine Producers Database (available from Winetitles Pty Ltd). Selection of recipients aimed to encompass those winemakers who produce a range of red wine varieties. Thanks to the industry's drive and enthusiasm, evident in the feedback and comments received, the response rate was 15%.

Summary of survey results:

- Around 60% of the winemakers who responded to the survey reported the use of some form of EM
- Varieties used and the percentage of each variety undergoing EM were surveyed, and based on the myriad of responses, it seems someone, somewhere is using EM on a particular red wine grape variety
- Some wineries use EM only on premium parcels; others put all their red fruit through some form of EM
- The time on skins for cold-soak and post-fermentation extended maceration is as varied as the varieties on which it is used
- Results show that 90% of the winemakers who responded to the survey believe cold-soak affects red wine organoleptic properties and that 94% of winemakers believe post-fermentation extended maceration affects red wine organoleptic properties. Only a respective 4% and 3% of respondents believe cold-soak or post-fermentation extended maceration regimes do not affect red wine organoleptic properties; the others remain unsure
- Although 92% of winemakers who responded to the survey believe cold-soak affects red wine organoleptic properties there was no consistency in exactly what they believe are the particular consequences of cold-soak for organoleptic properties such as colour stability, structure, depth of flavour, and improved fruit characters
- Winemakers believe structure, mouth-feel and palate length are the organoleptic properties of red wine most affected by post-fermentation extended maceration
- 64% and 93% of respondents believe post-fermentation extended maceration affects bitterness and astringency, respectively. 73% of respondents to this question don't believe cold-soak affects bitterness but results are divided on whether it affects astringency (48% believe it does, 44% disagree, 8% undecided)
- If over 90% of winemakers who responded to the survey believe EM makes a difference to wine organoleptic properties, why do only around 60% of winemakers use it? Comments received in response to this question reflected that while more winemakers would ideally use EM strategies to achieve their desired wine outcome, many are unable to afford it "due to logistic pressures during vintage." Wineries strategically employing EM are likely to have higher running costs and investments compared to those using only conventional maceration treatments in their red winemaking
- Another reason is the insufficient information available on the consequences of EM regimes for red wine organoleptic properties. Of those winemakers not using one form of or neither form of the EM strategies surveyed, over 62% stated as at least part of the reason for this is the insufficient sound and practical information on EM. This suggests that if such information were available, an additional 62% of winemakers would consider using extended skin contact treatments to complement conventional maceration during red winemaking
- 84% of winemakers who responded to the survey believe the industry does not know enough about the consequences of EM for red wine organoleptic properties and the majority (88%) feel

there is a need for a more scientific understanding of the consequences of EM strategies for red wine, rather than the traditional “gut feel” approach based on “empirical and anecdotal evidence”, with data being translated to the industry with practical applicability

2. Experimental winemaking and standard wine analyses

The PhD research project conducted as the principal activity of this project represents is a continuation of research conducted by Mario Mazza, a University of Adelaide Masters (Oenology) student under the support of the CRC Viticulture Tannin Project. Mario made a Pinot Noir during vintage 2005, following a small-winemaking protocol developed by the AWRI. Mario produced triplicate wines for each of five maceration protocols. Two pre-fermentation (cold-soak) treatments and two post-fermentation treatments were compared against each other and a control treatment (C). Wines were analysed over the first 300 days post-crush.

Pre-fermentation treatments included a three-day cold-soak at 10°C with twice daily plunging (CSP) and a three-day cold soak at 10°C without plunging (CS). Post-fermentation extended maceration treatments included a one-week post-fermentation extended maceration (PS1) and a three-week post-fermentation extended maceration (PS3). Wines produced were analysed for colour, anthocyanins, pigmented polymers, tannin, (+)-catechin, (-)-epicatechin, proanthocyanidin subunit composition, tannin characterization by gel permeation chromatography, and for bitterness and astringency by a trained panel. For the present PhD research, Grenache and Shiraz experimental wines were produced in vintages 2006 and 2007, respectively, enabling further refinement of the small-scale winemaking protocol. For both varietals, four replicates of each of three maceration protocols were produced. A three-day cold soak treatment at 10°C with plunging (CS), a control treatment (C), and a three-week post-fermentation extended maceration treatment (Post) were compared.

Summary of results for wine chemical analyses of treatment replicates:

- Results from standard analyses of 2006 Grenache and 2007 Shiraz fruit following fruit randomisation into replicate treatments show fruit was randomised across all treatments with pH, Titratable Acidity (TA) and TSS values being similar for all treatments
- For both varietals, fermentation curves monitored twice daily show fermentation was maintained at a steady rate
- For both Grenache and Shiraz there was good uniformity among the fermentations after the final racking and prior to bottling. However, an error during Grenache SO_2 analysis resulted in a PMS addition error and significantly higher level of free and bound SO_2 in the control treatment. Adjustments were made to Grenache control wines in subsequent analyses to counteract the difference between treatments. Despite care taken the following vintage, Shiraz CS treatment had a lower final free SO_2 compared to the control and Post treatments

- MLF results show Grenache did not go through MLF, whereas the Shiraz did (both intended). Despite slow MLF progression in the Shiraz treatments the final malic acid levels were consistent across replicates and treatments
- In 2005, pre-bottling analysis of the Pinot Noir treatments revealed there was a significant difference between treatments for pH (Mazza 2005). The pH results showed the PS3 treatment was approximately 0.2 pH units higher compared to the other treatments. At the time, the decision was made to not adjust the pH of the PS3 treatments because the pH increase was accepted as a consequence of the extended maceration treatment and no adjustment was made in order to better simulate commercial practices. In contrast, during the 2006 and 2007 vintages, the pH of all treatments was monitored and adjusted with tartaric acid in order to achieve the uniform pH across all treatments
- Mazza (2005) suspected the higher PS3 treatment pH was due to the formation of tartrates as potassium and calcium; however pre-bottling analysis showed there was no difference in K or Ca between Pinot Noir treatments. In 2007, Pinot Noir K concentration was analysed again and Grenache K concentration was analysed for the first time. The results show no significant differences in K concentration between treatments for varietals. Potassium analysis was also carried out on the Shiraz while it was going through MLF. Results show there was a significant difference in K content between all treatments, and that in fact the CS treatment had the highest K content, followed by the control treatment
- Acetaldehyde concentration was measured in all varietals to see if the Post treatments had resulted in an increase in wine oxidation due to this treatment's greater potential exposure to oxygen. More specifically, to determine if the Pinot Noir Post treatments (PS1 and PS3) had elevated levels of acetaldehyde and therefore if oxidation (browning) may be a contributing factor to these wines being significantly browner and less red in colour than the other treatments (results discussed later). Results show there were no significant differences between treatments for either Pinot Noir or Grenache. This suggests oxidation is not a contributing factor to the Pinot Noir colour differences (although acetaldehyde is difficult to accurately analyse). In fact, for Shiraz the control treatment had a significantly higher concentration of acetaldehyde than the Post treatment, as did the CS treatment. Despite these differences, values are within the normal range for red wines (4-212 mg/L acetaldehyde)
- Due to a long period of MLF for Shiraz, acetic acid concentration was measured to determine if volatile acidity (VA) levels were becoming elevated. Although the enzyme test measures only acetic acid it still provides a good indication of VA production. Results show there was no significant difference between CS and control wines or between Post and control wines, but Post wines had a significantly higher acetic acid concentration (g/L) than CS wines. However, the values were still below 0.5g/L acetic acid (an acceptable level of VA in red wine)

3. Colour measurements of experimental wines produced using EM strategies

The consequences of EM regimes for red wine colour and total phenolics, determined by spectrophotometric methods are summarised. The following methods were used to analyse the experimental wines:

1. Somers measurements – Wine Colour Density, Wine Colour Hue, bisulfite resistant pigments
2. Folin-Ciocalteu assay for total phenolics and correlation between results for this assay and Somers measurements for total phenolics
3. CIE *L*a*b** measurements including:
   Wine Colour Density (WCD) and Wine Colour Hue (WCH)
   Degree of lightness (*L**)
   Redness to greenness (*a**)
   Yellowness to blueness (*b**)
   Hue Angle° (*h*°) expressed as degrees from 0° (red) to 90° (yellow) to 270° (blue)
   Chroma (*C**), the measurement of hue intensity or colourfulness, and correlation to WCD
   Log 10 of hue angle (*h*º), and correlation to Log10 of WCH
   Total colour difference (*ΔE*)

Summary of results from the summary of wine colour and total phenolic differences between treatment replicates:

- The PS3 Pinot Noir treatment consistently showed lower WCD and chroma compared to the CSP treatment, and in some instances the PS3 treatments showed lower WCD than the PS1 treatment, indicating the extra two weeks on skins significantly affected the wine phenolic profile
- The Shiraz Post treatment also consistently showed lower WCD compared to its CS treatment, and as for Pinot Noir, there is no difference between the CS treatment and control treatment for WCD
- Interestingly, the Grenache Post treatment has higher WCD compared to the CS treatment
- The Pinot Noir post-fermentation extended maceration treatments consistently show lower hue angles and lower per cent total red pigment compared to the control, CS and CSP treatments (Shiraz behaves similarly), but there is no significant difference between treatments (for any varietal) for chemical age index
- Grenache treatments showed no significant differences between CS and Post treatments for WCH
- For all varietals, the post-fermentation extended maceration treatments had highest total phenolics compared to the control and cold-soak treatments. For Pinot Noir, the PS3 treatment had significantly higher total phenolics than its one-week counterpart. Again, there was no difference between control and cold-soak treatments for this parameter

- The measurement of bisulphite resistant pigments (which provides an *estimation* of the polymeric pigments present in the wine - it is estimation because there are non-polymeric pigments that are also resistant to bisulphite bleaching) could only differentiate between Shiraz treatments where the Post treatment had a lower concentration of bisulphite resistant pigments compared to the control and CS treatments. As will be discussed, RP-HPLC analysis of pigmented polymers was more vague in determining differences between treatments, despite the belief that polymeric pigments are one of the primary phenolic components affected by EM.
- Pinot Noir and Shiraz post-fermentation extended maceration treatments were lighter in colour than the cold-soak treatments, whereas for Grenache, the opposite was true.
- The Pinot Noir and Shiraz CS treatments were significantly more red (higher *a**) than their post-fermentation extended maceration treatments, whereas for Grenache, the opposite was true (Grenache is a variety low in phenolic content and reasons for the deviation in results compared to Shiraz and Pinot Noir will be discussed in the PhD thesis).
- Delta E values indicated that all the Pinot Noir wines, with perhaps the exception of control and CS wines (ΔE=1) could be differentiated based on colour because all comparisons had a ΔE > 1. The largest difference at pH 3.60 and under SO_2 adjustment was between the CSP and PS3 treatments, followed closely by the CSP and PS1 treatments. These results back up the Sensory Descriptive Analysis (DA) results in which the PS3 treatment was significantly different in colour to the CSP treatment such that the PS3 treatment was browner and less red than the CSP treatment. Shiraz results followed the same trend, including a significant difference in colour intensity between treatments.
- Delta E values comparing the CS and Post Grenache wines at natural pH and without SO_2 adjustment suggested that there was only a slight difference in colour between the wines. ΔE values indicated that all the Grenache wines that were pH and SO_2 adjusted could be differentiated based on colour because all comparisons had a ΔE > 1. The largest difference at pH 3.60 and under SO_2 adjustment was between the control and Post treatments

4. Sensory analysis of experimental wines produced using EM strategies

Sensory analysis of all wines provided a compliment to the chemical analyses conducted. Simple difference tests for colour were carried out for Grenache and Pinot Noir, first between replicates of each treatment, then between treatments in order to determine if there was a significant visible difference in colour between treatments. Over the course of several months, a DA was carried out on the Grenache and Pinot Noir wines using a trained panel.

A DA was conducted on the Shiraz wines in 2007 by a GWRDC-supported University of Adelaide Bachelor of Oenology Honours student, Kelly Wellington, who performed a mouthfeel DA on these wines as part of her project. Instead, ranking tests for colour intensity and saltiness (as the grapes from which

the wines were made were salty, thereby making the wine taste salty). Ranking is more powerful than triangle tests because treatments can be assigned a rank as in addition to determination of significant differences between treatments.

Summary of results from sensory analyses:

- There was a significant difference in colour between all Pinot Noir treatments (triangle test) and DA showed that the Post treatment was browner and less red than the CSP treatment (only CSP, control and PS3 treatments underwent the DA).
- For Grenache there was no significant difference in colour between CS and Post treatments (triangle test and DA). Although elevated SO_2 levels in the control wine were ameliorated results for the control wines cannot be confidently included here.
- Shiraz control and CS treatments had greater colour intensity than the Post treatment (comments suggest the Post wines were browner and less red than the other treatments, similar to the Pinot Noir DA results). There was no significant difference between the colour intensity of control and CS treatments.
- Sensory data supports the chemical data in that there is no significant difference between control and CS treatments but both control and CS treatments have a greater colour intensity/chroma/WCD than the post-fermentation extended maceration treatments. There was no significant difference in saltiness between Shiraz treatments.

5 Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) and Methyl Cellulose Precipitable Tannin (MCPT) assay of samples taken from crush to bottle maturation

Reverse Phase-High Performance Liquid Chromatography was used to monitor and quantify concentrations of the monoglucosides delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside using pure standards, and pigmented polymers with results expressed in malvidin-3-glucoside equivalents (mg/L) at 520nm. Reverse phase-HPLC was used to monitor and quantify (+)-catechin and (-)-epicatechin using pure standards, and tannin with results expressed in (-)-epicatechin equivalents (mg/L) at 280nm. Wines of all varietals was analysed in order to explain the treatment differences in various phenolic parameters outlined in previous chapters. In addition, fermentation samples taken during the 2006 Grenache vintage and 2007 Shiraz vintage were analysed in order to examine the changes in phenolic profile from crush through to bottling. For interest, anthocyanin and tannin content of Grenache grape skins and catechin, epicatechin and tannin content of Grenache grape skins and seeds from samples collected during ripening were also quantified.

Tannin concentration was also analysed by the Methyl Cellulose Precipitable Tannin (MCPT) assay developed by the AWRI with results expressed in (-)-epicatechin equivalents (mg/L) in order to provide a comparison to RP-HPLC derived tannin results. While techniques to measure other phenolic compounds have been refined, there are few robust and efficient analytical methods to quantify wine tannins, making it difficult to compare tannin data obtained by different methods. Reverse-phase HPLC works by pumping mobile phases at varying gradients together with a small amount of wine through a column. Wine components interact with the surface of the column to different degrees based on their hydrophobic character, causing them to separate and elute at different times. The elution of a single compound is visualised as a peak and the compound quantified according to the area under the peak. Reverse-phase HPLC is effective at quantifying monomeric polyphenols such as (+)-catechin, but is less effective at quantifying condensed tannins due to their heterogeneous nature. While most condensed tannins elute in one large peak, a proportion of tannins elute prior to the peak. Only the tannins eluted in the large peak, and therefore only a sub-portion of the total tannin, are analysed and quantified by RP-HPLC. In contrast, the MCPT assay, based on a polymer-tannin interaction that enables the removal of all tannin from the matrix, analyses and quantifies all tannin, and is therefore believed to be more accurate than tannin quantification by RP-HPLC.

The following analyses were conducted:

1. RP-HPLC anthocyanins, pigmented polymers in Pinot Noir, Grenache and Shiraz wines.
2. RP-HPLC anthocyanins, pigmented polymers, (+)-catechin, (-)-epicatechin, tannin in Grenache and Shiraz samples collected during fermentation.
3. RP-HPLC (+)-catechin, (-)-epicatechin, tannin in Pinot Noir, Grenache and Shiraz wines, MCPT assay results, and correlation between RP-HPLC tannin results and MCPT assay results.
4. Anthocyanins and tannins in Grenache grape skins, (+)-catechin, (-)-epicatechin and tannin in Grenache seeds and skins during ripening.
5. Shiraz marc extraction experiment. This experiment involved the extraction and quantification in absorbance units and malvidin-3-glucoside equivalents of colour from Shiraz marc samples taken during fermentation. If under standard conditions, more colour can be extracted from the Post treatment's marc samples, this may suggest colour compounds might have bound back to grape cell walls during the extended time on skins and may be the reason for the lower Shiraz Post WCD (and possibly the decrease in the Pinot Noir PS3 WCD), and total anthocyanins (determined by RP-HPLC).

Summary of results from the summary of RP-HPLC anthocyanin, pigmented polymer, (+)-catechin, (-)-epicatechin and tannin, and MCPT assay differences between treatment replicates:

Anthocyanins and pigmented polymers

- At 31 months post-crush, the Pinot Noir PS3 treatment had a significantly higher per cent of malvidin-3-glucoside and a lower per cent of other monoglucosides compared to the other treatments, suggesting this treatment caused an accelerated loss of these monoglucosides, however there was no significant difference between Pinot Noir treatments for total anthocyanins.
- At the end of fermentation the Shiraz CS treatment had a higher total anthocyanin concentration compared to the Post treatment (due to a greater concentration of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside and peonidin-3-glucoside, rather than malvidin-3-glucoside), but was not significantly different to the control treatment. Further investigation shows that for these monoglucosides the CS treatment had a significantly higher concentration than either the control or Post treatments. It should be noted that the relative contribution to the total anthocyanin levels of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside and peonidin-3-glucoside was a minor proportion of that derived from malvidin-3-glucoside.
- While results were inconsistent throughout fermentation, by the end of fermentation, the Post Grenache treatment had a higher concentration of petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside compared to the CS treatment. After some time in the bottle, total anthocyanin results for Grenache wine showed no significant difference between CS and Post treatments.
- There was no significant difference in pigmented polymer concentration between treatments for any varietal at any time, except for Shiraz wine sampled at seven months post-crush when the CS and control treatments had a higher pigmented polymer concentration than the Post treatment, and for Grenache at the end of fermentation, when the CS treatments had a higher pigmented polymer concentration than the Post treatment.

(+)-catechin, (-)-epicatechin, tannin

- By the end of fermentation, the Grenache Post treatment had a higher concentration of tannin compared to the control treatment, whereas for Shiraz at the end of fermentation the control treatment had the highest tannin concentration and there was no difference between CS and Post treatments. After some time in the bottle, the Grenache Post wines were significantly higher in tannin than both the CS and control treatments and the Shiraz control treatment had remained higher in tannin than the CS treatment (but was not significantly different to the Post treatment).
- While at 25 months post-crush there was no difference in tannin concentration between Pinot Noir treatments, later analysis at 28 months post-crush showed CS and CSP treatments had significantly higher tannin concentration compared to the PS3 treatment.
- By the end of fermentation, the Grenache Post treatment had a higher concentration of catechin and epicatechin compared to the control and CS treatments, whereas for Shiraz

at the end of fermentation (and consistently throughout fermentation) the Post treatment had the highest catechin and epicatechin concentration compared to the CS and control treatments. After some time in the bottle, the Grenache Post wines were still significantly higher in catechin and epicatechin than the CS and control treatments and the Shiraz Post treatment had remained higher in catechin and epicatechin than the CS and control treatments (in addition, the CS treatment was higher in catechin than the control treatment).

- The Pinot Noir post-fermentation extended maceration treatments consistently (25 months post-crush and 28 months post-crush) had a higher catechin and epicatechin concentration compared to the control and cold-soak treatments. Again, there was no difference between control and cold-soak treatments for these flavanols.

MCPT assay

- While results show no significant differences in tannin content between Shiraz treatments, for Grenache and Pinot Noir the Post treatments were generally higher in tannin than both the control and CS treatments. For this data there was a poor correlation between the MCPT assay and RP-HPLC tannin results.

6 Phenolic content comparison of New and Old World Pinot Noir

Vitis vinifera cv Pinot Noir is native to the Burgundy region of France and is now planted in many other cool climate viticultural regions in the world. Pinot Noir, best-suited to cooler, marginal climates, is a variety notorious for difficulty associated with obtaining adequate colour extraction in the winery. The Pinot Noir grape is different to all other important dark-skinned *Vitis vinifera* varieties because it has no acylated anthocyanins, and therefore has low skin anthocyanin concentration. Pinot Noir also has lower tannin concentration than other varieties and is prone to oxidation during vinification.

Burgundy produces the finest Pinot Noir wines in the world. Burgundian Pinot Noir is known for its high phenolic content; astringency and complex tannins that provide the wine with body, structure and longevity. Cold-soak is traditionally used in Burgundy, where winemakers believe that colour is best extracted in the absence of alcohol and that tannins are best extracted during extended maceration post-fermentation. New World countries such as The United States (in particular Oregon), Australia (the Mornington Peninsula, Geelong and the Yarra Valley of Victoria, Tasmania and the South Australian Adelaide Hills) and New Zealand (particularly Central Otago) are beginning to challenge Burgundy in the production of premium Pinot Noir. While New World methods of making Pinot Noir wine vary, due regard is paid to the Burgundian style of vinification. While moving away from whole-bunch fermentations due to the astringency imparted to the wine by stalks, more and more New World Pinot Noir producers are using cold-soak and post-fermentation extended maceration to obtain the right balance of colour and tannin extraction.

This experiment compared a range of Pinot Noir wines from around the world, using analytical measurements to determine their precise phenolic compositions (analyses listed below), and these wines were presented to an expert panel of winemakers, oenologists and advanced students for their consideration, particularly seeking feedback on the structure and mouthfeel properties of the wines under examination. The aim was to determine if was possible to objectively discriminate such wines based upon sensory and analytical parameters associated with phenolic composition.

New and old world Pinot Noir wines of diverse provenance were sourced for this study including four wines from the Adelaide Hills (2005 vintage), three wines each from Tasmania (2005 vintage), Victoria (2005 vintage), New Zealand (2005 vintage), and Oregon (2004 vintage), plus seven wines from Burgundy (2004 vintage) including three Grand Cru and three Premier Cru wines.

The following parameters were analysed:

1. RP-HPLC for (+)-catechin, (-)-epicatechin, tannin
2. MCPT assay
3. Folin-Ciocalteu for total phenolics
4. Somers results for total phenolics and bisulphite resistant pigments
5. Measurement of pH, Titratable Acidity (TA), % (v/v) alcohol for each wine. Wines were not adjusted to a standard pH prior to analysis. As alcohol content, pH and TA also contribute to the perception of mouthfeel, these parameters were also measured
6. Discussion with tasting notes

Summary of Phenolic content comparison of New and Old World Pinot Noir:

- There was an excellent correlation (R^2 value of 0.885) between the Folin-Ciocalteu total phenolic data and the Somers total phenolic data, suggesting that these two measures of phenolic composition were each providing comparable data.
- For the two tannin measures (MCPT assay and RP-HPLC), even though the results are expressed in mg/L (-)-epicatechin equivalents for both, the values obtained were different, which is reflected in the weaker correlation between the MCPT assay data and the HP-HPLC tannin data (R^2 value 0.482).
- Relative to each other, wines performed similarly across analyses. The new world Pinot Noir wines generally had a lower (+)-catechin, (-)-epicatechin, tannin and total phenolic content than their French counterparts (although the distinction between countries was less for the bisulphite resistant pigment results). The one exception was the New Zealand wines, which on average,

produced similar results to the Burgundian wines. Two Grand Cru wines stood apart from the other Burgundian wines having higher levels of (+)-catechin, (-)-epicatechin and total phenolics.

- Despite the much lower average phenolic content of the Australian and Oregon Pinot Noir wines, some of these wines consistently had higher tannin and total phenolic contents than the other wines from their country of origin.
- On average, the Tasmanian Pinot Noir wines had the lowest pH (3.56, range: 3.51-3.61) and highest average TA (6.30 g/L, range: 5.84-6.70 g/L) whereas the Oregon Pinot Noir wines had the highest average pH (3.87, range 3.85-3.90) and lowest average TA (4.80 g/L, range: 4.57-5.02 g/L). The South Australian Pinot Noir wines had the highest average alcohol content of 14.44 % (range: 13.80-14.96 %) and the Burgundian wines had the lowest average alcohol content of 13.22 % (range: 12.81-13.67 %). The average pH, TA and alcohol content values for the other countries were similar.
- The analytical and tasting results supported each other. Intense, saliva-puckering and chewy/grippy tannins dominated the palate of the Burgundian wines. Panellists perceived these wines as being less fruit forward than the new world wines. The new world wines were perceived as having a greater presence of fruit on the palate, as being generally more delicate and as having a less aggressive and more approachable mouthfeel for their age. Perhaps during the fining of the new world wines, a greater amount of bitter polyphenols was removed. This may account for part of the reason why these wines generally had a lower level of (+)-catechin and (-)-epicatechin compared to the Burgundian wines. Tasting notes show the lower pH and higher TA of the Tasmanian wines did not negatively affect their acid balance. Tasters were also sensitive to the higher average alcohol content of the South Australian wines as they found these wines to have a prickly finish, which may have slightly masked their phenolic content.
- Of course, fine Pinot Noir wine is produced with ageing in mind and Burgundies are not supposed to be savoured until at least five to seven years after vintage. The Burgundian wines were only three years old when tasted, and the new world wines six months younger again. The greater extraction of phenolics into the Burgundian wines during vinification and subsequent higher phenolic content in the final wines compared to new world wines suggests these wines require longer ageing than our new world counterparts. Regardless of optimal drinking age of the wines selected for this study, the old world wines generally had the highest phenolic content and were matched only by two New Zealand wines.
- Wines used in this study were selected from regions with reputations for producing high quality Pinot Noir wines. Although the selection of wines from each region sourced for this study was necessarily small, results suggest that despite variability in the parameters measured within countries, overall, each country has its own style of phenolic profile.

Concluding remarks

- Pinot Noir and Shiraz provide good models for the consequences of EM for red wine
- For most measures, there was no significant difference between control and cold-soak treatments, and for Pinot Noir, there was no significant difference between cold-soak for three days with plunging and the same treatment without plunging, suggesting that if winemakers want to carry out cold-soak time and work can be saved by not plunging
- In many instances the Pinot Noir three-week post-fermentation extended maceration treatment was significantly different (higher phenolic content, for example) than the one-week post-fermentation extended maceration treatment, suggesting the extra two weeks on skins significantly altered the phenolic profile of the final wine
- Post-fermentation extended maceration generally resulted in lower colour intensity, higher wine colour hue, and higher total phenolics than the other treatments
- A more detailed discussion about the potential causes of treatment differences including an in depth discussion of industry relevant outcomes of this study will be presented in the final thesis

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