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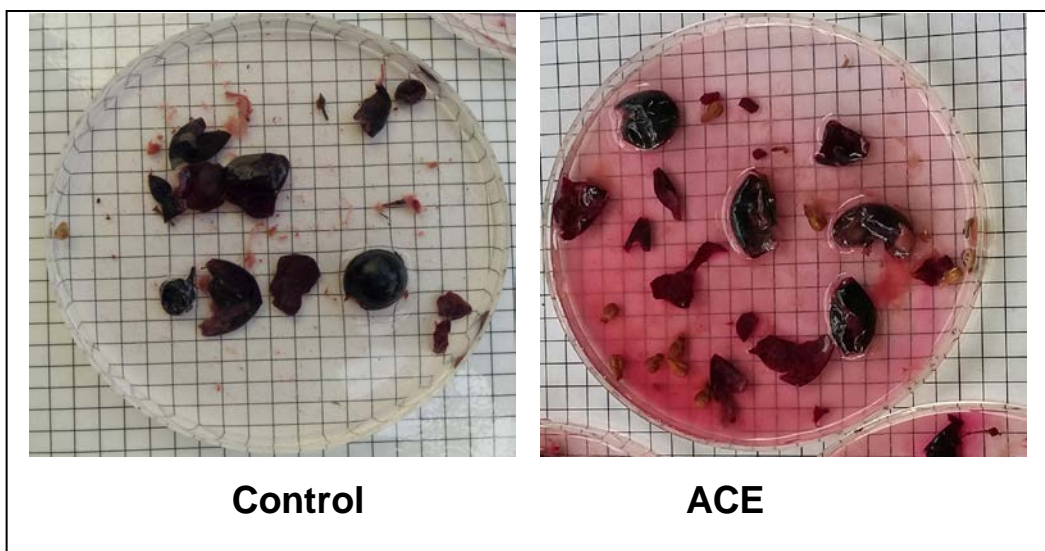
Australian Grape and  
Wine Authority



*Vinventive*

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## ACE WINERY TRIALS



Project Number: **VIN 1501**

Principal Investigator: **Dr Angela Sparrow**

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Research Organisation: **VINVENTIVE**

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Title: ACE WINERY TRIALS

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In submitting this report to *Australian Grape and Wine Authority* the author, Angela Sparrow attests that the project and its report is an original contribution, and no article that is substantially similar has been published or submitted in any language for publication elsewhere.

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## 1. Abstract

Accentuated Cut Edges (ACE) maceration reduces grape skin particle size thereby increasing the rate of extraction of components from skins. Subsequently, the wine can be pressed earlier (PE) than usual, and the fermentation completed in a smaller tank to make PE-ACE wine. The technique optimises fruit characteristics and saves time, space and labour in the winery. ACE maceration was tested on five red wine grape varieties at six commercial wineries located in four Australian states. PEACE treated wines had similar phenolic composition and sensory scores to the untreated wines. ACE maceration has the potential to significantly improve processing efficiency, profitability and competitiveness in the global market.

## 2. Executive summary

AGWA funded research project (UT1301) pursued the practical application of reducing the skin particle size of wine grapes during vintages 2014 and 2015, with very good results. Presentation of the research at the ASVO seminar (19 Nov 2015) and the CRUSH symposium (20 Nov 2015) brought enquiries from winemakers from a number of wine growing regions as to how they might access the technology. Wine Australia accepted a proposal by Dr Angela Sparrow to take the technology from state to state during vintage 2016. Winemaker networks were used to confirm participants at six wineries from four Australian states investigating the response to ACE maceration of red wine varieties: Grenache, Merlot, Shiraz, Cabernet Sauvignon and Tempranillo. In total, eight separate ACE trials were conducted during vintage 2016.

The trials included a control (the standard commercial treatment applied by the winery) and an ACE treatment (grape skins fragmented by a cutting device fitted into the must line between the crusher and the fermentation tank). Fermentation volumes ranged from 300 L to 13,000 L. The majority of winemakers elected to pursue the early press off option for ACE macerated wine, with the intention of reducing the time in the fermentation tank. These 'pressed early' (PE) ACE wines are hereafter referred to as PEACE wines.

A must sample was taken to assess the extent of skin fragmentation and seed damage occurring for both the control and ACE treatments. This work showed that skins of mechanically harvested grapes were already partially fragmented and seed damage was in the order of 7-12% whereas for hand-harvested fruit, seed damage was 2%. ACE maceration was found to increase skin fragmentation two-fold in machine harvested grapes and four-fold in hand harvested grapes; in neither case did ACE maceration cause additional seed damage. The results for seed damage in mechanically harvested fruit are cause for concern. Earlier studies (Sparrow et al. 2015) showed that high concentrations of seed tannin in the wine compromised the development of stable pigment e.g. an increase in tannin concentration of 1g/L reduces stable pigment by 30%, and this is the reason why the PEACE technique was developed.

At some of the larger wineries, the decision to press off the ACE macerated grapes was made at a designated time e.g. after three days on skins. At other wineries, the ferments were more closely monitored and grape solids pressed off after four or five days. Two wineries elected to leave their ACE macerated grapes on skins for the same length of time as for the control treatment. Fermentation kinetics showed that ACE maceration was inclined to increase the rate of fermentation, presumably as yeast cells could more readily access grape sugars and nutrients. Early press off from the grape solids had no significant effect on fermentation dynamics.

The most important of the phenolic parameters extracted from grapes to make red wine are the colour pigments (anthocyanins) and tannins. Anthocyanins can remain free in the wine solution or form molecular complexes with tannins or phenolic acids to form stable 'pigmented tannins'. Stable pigment reflects wine colour that is not susceptible to oxidation and is a good indicator of the intensity and longevity of colour pigment in the wine. Phenolic samples taken daily and assessed after vintage, showed that ACE maceration caused a dramatic increase in the extraction of colour and tannin in the first two days of fermentation and at the end of the fermentation period following early press off of the grape solids (i.e. PEACE wines), the stable pigment content ranged from 0 to 30% higher than the control. By contrast the tannin concentration of the PEACE wines was on average 20% lower than the control. Tannin concentration in the control treatments increased throughout the fermentation, the later extraction likely to be due to the release of tannin from the inner tissues of the grape seed.

A preliminary wine tasting held for the winemakers involved in the trial was held in Adelaide in July 2016, with the majority of wines then being evaluated by the Qualitative Assessment (QA) panel at the Australian Wine Research Institute (AWRI). In confirmation of the winemaker findings, this

independent panel found that there was no significant difference in the sensory scores of control and PEACE treated wines in any of the varieties tested. From the descriptors used by the QA panel, the PEACE wines were generally found to be more purple in hue and have more fruit flavours than the control wines, these being mostly described as dark berry fruit. They also described softer or finer tannins in the PEACE wines. Some reductive characters were noted by the sensory panel in both control and treatment wines, most likely as a function of aeration management. The only significant difference between control and treated wines determined by the QA panel occurred where the winemaker chose to leave the ACE macerated wine on skins throughout fermentation. The QA panel scored ACE wine slightly lower than the control wine as it was considered to be more astringent than the control.

In summary, the winery trials showed that ACE maceration in combination with early press off has considerable potential as a technique to help commercial winemakers manage compressed vintages. There were no significant differences in wine scores and both fruit and mouthfeel characteristics were somewhat improved. Reducing the time in the fermentation tank by 25 to 50% is likely to provide significant advantages for the Australian wine industry. Added to this are the savings in labour and energy costs as cap management requirements are also reduced in the production of PEACE wines. These savings have been outlined in project UT1301.

The author would like to acknowledge the financial support from Wine Australia for this project.

I also acknowledge the generous enthusiasm of winemakers, cellar hands and laboratory staff at participating wineries Yalumba and Barossa Valley Estate (South Australia), Best's Wines and De Bortoli Wines (Victoria), Lerida Estate (New South Wales) and Houghton's (Western Australia) and the supply of finished wine for sensory analyses and subsequent tasting workshops. I am indebted to the University of Tasmania, for use of laboratory time and equipment in the Viticulture and Oenology Laboratory at Prospect Tasmania for phenolic analyses, and to the Department of Primary Industries Water and Environment Tasmania for access to the Mt Pleasant Laboratories.

### **3. Background**

AGWA funded research project (UT1301) pursued the practical application of reducing the skin particle size of wine grapes during vintages 2014 and 2015, with very good results. Following articles in Daily Wine News and on ABC Radio at the time the trials were conducted, there were enquiries from around the world asking for details on the procedure, particularly as it does not damage the seeds.

The winemakers involved in vintage 2015 trials were quite intrigued by the ACE wines made at their own wineries. Presentations on the research at the ASVO seminar (19 Nov 2015) and CRUSH symposium (20 Nov 2015) were well received and brought enquiries from winemakers from a number of wine growing regions as to how they might access the technology. An article titled 'ACE maceration delivers quality and space' was subsequently submitted for inclusion in the ASVO proceedings. Enquiries were received from the research and commercial sectors asking for details on the procedure, however the project proponents felt that the Australian industry should be given the first opportunity to take advantage of the technology, through practical trials.

#### 4. Project Aims and Performance targets:

Year 1	Output	Target Date	Activities
a.	Plans for ACE winery trials finalised	5/02/2016	<ul style="list-style-type: none"> <li>Use Wine Australia networks to invite participation in ACE trials for 2016.</li> <li>Confirm trials at up to six wineries and for up to five varieties, including at least one winery in each of WA, SA and either VIC or NSW and at least two grape varieties at each winery. Confirm harvest schedule.</li> <li>Meet WHS induction requirements</li> </ul>
b.	Data documenting the impacts of ACE treatment on relevant winemaking parameters during fermentation	30/04/2016	<p>Conduct trials in WA, SA and either VIC or NSW at up to six wineries and for up to five varieties, including at least 1 winery in each of state and at least two grape varieties at each winery. Trials will include a control (standard commercial treatment applied by winery) and an ACE treatment. Post-ACE treatment may also be included, dependent on winemaker agreement.</p> <p>Document standard winemaking information for each wine, including fermentation kinetics and phenolic measurements throughout the trial.</p>

#### Outputs and Activities 2016–17

Year 2	Output	Target Date	Activities
a.	Set of bottled experimental wines	31/08/2016	Bottle sufficient quantities of treated and control wines for several tasting and analysis events.
b.	Data documenting the impacts of ACE treatment on wines 6 months after completion of fermentation	14/10/2016	<p>Sensory and wine phenolics analysis six months post fermentation</p> <ul style="list-style-type: none"> <li>Taste all wines with winemakers at a workshop in Adelaide and collect data on whether the treatment made a discernible difference and what winemaker preference was.</li> <li>Conduct phenolic and other analyses on wines and obtain an independent quality assessment of the wines.</li> </ul>
c.	Final report to AGWA	28/10/2016	Collate data from all trials to identify benefits of ACE per region and/or grape variety.

## 5. Methods

### *Winery recruitment*

Winery participants for the trials were recruited across four Australian states, with assistance and input from Wine Australia, regional Industry Development Officers, winemaker networks and other wine industry contacts. The harvest schedule for the batch of fruit dedicated to the trial was estimated by the winemaker approximately one week ahead and these were coordinated to fit eight separate ACE trials into the 2016 vintage.

### *Procedure*

A copy of the procedure for the trials was prepared and sent to each participating winemaker during February 2016 (Appendix 5.1).

### *Grape varieties*

Merlot, Grenache, Shiraz, Tempranillo and Cabernet Sauvignon grapes were harvested across southern Australia during the period 25 February to 22 March 2016. At five of the six wineries involved in the trial, the grapes were machine harvested; only the NSW Merlot was hand-harvested. Each batch of fruit was destemmed and crushed separately into two parcels, which were run in parallel: 1. Control and 2. ACE maceration.

### *Winemaking protocol*

To minimise disruption to commercial winery operations, the winemaking protocol at each winery was left to the discretion of the winemaker. The details were provided by the winemaker for reference but for reasons of confidentiality the procedure for individual wineries has not been included in this report. Rather, a summary from participating wineries has been prepared. Fermentation kinetics for each treatment are reported for six of the eight trials. The only difference between the control treatment and the ACE treatment was the temporary incorporation of the ACE maceration device into the winery infrastructure between the crusher-destemmer and the fermentation tank. The timing of press off from grape solids following ACE maceration was discussed prior to the start of the trial, and most winemakers elected to press the grape solids off part way through the fermentation. These Press Early ACE macerated wines are referred to by the acronym PEACE. Two wineries elected to ferment their ACE treated musts to dryness to make ACE wines.

### *Skin and Seed assessment*

A two-litre sample of grape must of each variety was collected as the must was pumped into the fermentation tank and used to assess skin fragmentation and seed damage. From this sample, two 200g samples and one 500g sample were taken and stored in sealed food grade plastic containers.

**Seed damage:** Using the 500g sample the must was suspended in a 1% solution of Decon 90® (Decon Laboratories Limited, Gillman, South Australia) and passed through a series of sieves (mesh size 0.7 to 0.1 mm<sup>2</sup>) to separate the grape solids from the liquid, and the seeds and seed fragments from the skins and grape pulp. The seeds and fragments were isolated using forceps and aired dried for 48 hours, then separated into whole and damaged fractions. The percentage of damaged seeds was calculated as a percentage of the total number of seeds and fragments.

**Skin fragmentation:** From one of the 200g must samples, three 5g samples of grape solids were taken and each placed in a 90mm petri dish, 15 mL of water was added to each dish and the fragments carefully separated from one another. The petri dish was placed on a piece of graph paper with 5 x 5mm grid and the entire contents of the petri dish photographed. Image analysis software (Image J 1.51a Wayne Rasband National Institutes of Health, USA) was used to calculate the extent of skin fragmentation for each sample.

### *Quantifying the effect of ACE maceration on grape and wine phenolics*

On the second 200g must sample the total phenolic composition of the grapes was assessed using the method described for the AWRI grape tannin portal (see AWRI "Sample prep grape portal" procedure (Appendix 5.2). Samples were taken for phenolic analysis prior to treatment application, daily during fermentation, then again at a preliminary tasting four to five months after treatment application. Frozen samples of fermenting or finished wine were thawed at room temperature and clarified by centrifugation at 5000 rpm for 5 mins. Spectral analysis was performed on the sample using a UV-VIS Spectrophotometer (Model Genesys™ 10S Thermo Fisher Scientific Inc., Madison, WI, USA) scanning at 2 nm intervals for wavelengths 200 - 600 nm. Total tannin concentration of the samples was determined using the rapid tannin analysis and wine colour using a modification of the

Somers assay described for the AWRI wine tannin portal (see AWRI “Sample prep wine portal” procedure (Appendix 5.3).

### *Sensory Evaluation*

Wines were submitted for quality assessment to the AWRI Technical Quality sensory panel, to provide an indication of sensory characteristics and quality scores. Ten or 11 judges assessed each wine in duplicate during four sessions over two days, with the wines presented in six pairs (control and treatment), identified by a three-digit code. Three pairs of wines were presented per session, with a minimum ten minute break between sessions. The judges were informed of the vintage and variety but had no other knowledge of the wines. An analysis of variance of the quality score data for the effect of treatment, judge, presentation replicate and their two-way interactions was conducted separately for each pair of wines. (Appendix 5.4).



## 6. Results and Discussion

The fruit composition at harvest, yield data and phenolic composition of the grapes used in the trial are shown in Table 1. There was a large variation in fruit ripeness in terms of sugar concentration and pH but less variation in titratable acidity (with the exception of Tempranillo). While the sugar concentration of the Merlot from South Australia and that from NSW were similar there is a notable difference in the phenolic components of the fruit, with much higher concentrations of tannin and anthocyanin in the NSW Merlot which was harvested three weeks later. Of particular interest is the diversity among the fruit composition characters and the fermentation size across the trials, yet the results for ACE maceration with early press off were consistent.

**Table 1. Fruit composition and yield data at harvest**

<b>Harvest parameter</b>	Merlot (SA)	Merlot (NSW)	Grenache 1 (SA)	Grenache 2 (SA)	Shiraz (SA)	Shiraz (VIC)	Cabernet Sauvignon (WA)	Tempranillo (VIC)
Region	Barossa Valley	Collector	Barossa Valley	Barossa Valley	Barossa Valley	Great Western	Colombero	King Valley
Yield (t/ha)	NA	NA	NA	7.0	15.8	NA	8.0	10.5
Ferment size (t)	8	0.3	0.25	13-16	20	0.8	8.4	10-14
Must flow rate (t/hr)	20	10	20	20	20	NA	18	20
Total Soluble Solids (Brix)	22.9	23.0	NA	26.5	25.6	30.2	24.5	25.2
Juice pH	NA	3.6	NA	3.52	3.58	4.07	3.52	3.50
Titratable Acidity (g/L)	NA	5.7	NA	4.63	6.09	6.45	5.26	9.1
Anthocyanin (mg/g)	1.1	1.78	0.4	NA	NA	1.78	1.41	1.09
Total tannin (mg/g)	8.5	11.3	6.6	NA	NA	11.3	7.06	6.0
Total phenolics (AU/g)	1.5	2.22	1.1	NA	NA	2.22	1.52	1.31

NA, Not available. Yield data and grape phenolics were not recorded in some cases.

### Seed damage assessment

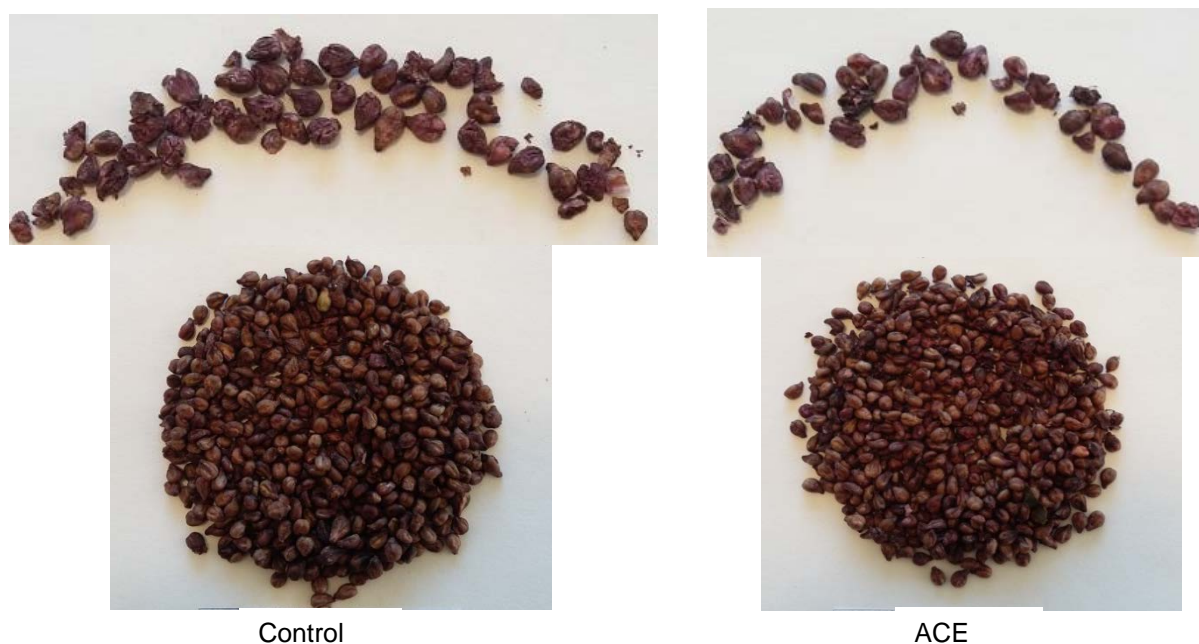
The percentage of seed damage caused by the ACE maceration treatment was determined for each grape variety. The results indicate that damage caused by the ACE macerator was minimal. The seed damage noted for the control treatments was greater for the mechanically harvested fruit than for fruit that was hand-harvested. Consequently, it appears that either the harvester is damaging the seed or the seeds of mechanically harvested fruit are more susceptible to damage during the crushing-destemming step.

**Table 2 Seed damage assessment: sample size 500g grapes**

Variety	Treatment	Whole seeds	Damaged seeds	Total Seeds	damage %
Merlot (SA)	Control	585	57	642	8.9
	ACE	570	36	606	5.9
Merlot (NSW) <sup>a</sup>	Control	271	5	276	1.8
	ACE	347	21	368	5.7
	<sup>b</sup> ACE 2	310	7	317	2.2
Grenache 1 (SA) <sup>a</sup>	Control	372	5	377	1.3
	ACE	315	3	318	0.9
Grenache 2 (SA)	Control	NA	NA	NA	NA
	ACE	NA	NA	NA	NA
Shiraz (SA)	Control	530	36	536	7.5
	ACE	620	50	670	6.7
Shiraz (VIC)	Control	864	67	536	7.2
	ACE	880	70	670	7.4
Cabernet Sauvignon	Control	150	13	163	7.9
	ACE	150	5	155	3.2
Tempranillo	Control	174	24	198	12.1
	ACE	200	24	224	10.7

<sup>a</sup>Hand-harvested fruit

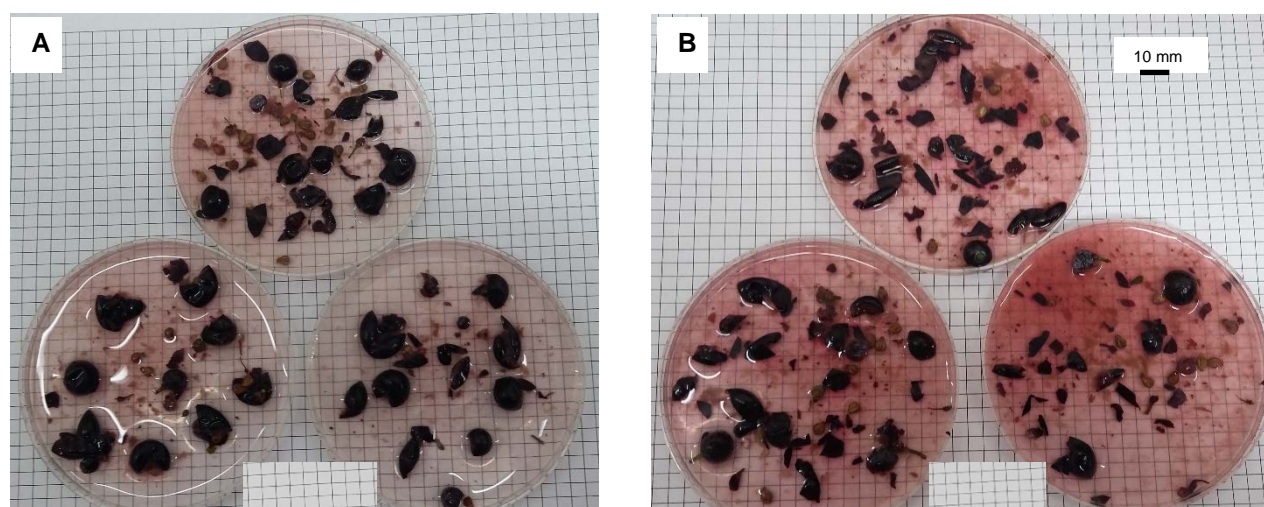
<sup>b</sup>Two Merlot ACE maceration treatments were included at this winery to compare different alignments of the ACE macerator within the must-line. NA, not available.



**Figure 1 Example of seeds and seed fragments isolated from grape must. (Damaged seeds magnified four-fold)**

### Skin fragmentation assessment

The extent to which the grape skins became fragmented by the ACE device was compared with the degree of grape skin fragmentation observed in the control treatment (Figure 2 and Table 3).



**Figure 2** Example of skin fragmentation for (A) control and (B) ACE treated musts.

A degree of fragmentation was observed for the control treatment in each case. This is likely to have occurred during machine harvesting or in the crushing-destemming step.

**Table 3** Skin fragmentation assessment

Variety	Treatment	Berry no.	Berry diameter (mm)	Fragment no.	SA/P	Fragmentation index <sup>b</sup>
Merlot (SA)	Control	6.3 ± 1.2	11.0 ± 1.0	7.3 ± 1.5	2.0 ± 0.2	1.2 ± 0.3
	ACE			15 ± 4.5	1.5 ± 0.3	2.4 ± 0.7
Merlot (NSW)	Control	3.0 ± 0	12.3 ± 2.5	4.0 ± 1.4	2.9 ± 0.5	1.1 ± 1.2
	ACE			4.5 ± 0.7	2.5 ± 0.2	1.6 ± 0.2
	<sup>a</sup> ACE 2			8.0 ± 2.8	2.5 ± 0.1	2.6 ± 0.7
Grenache 1 (SA)	Control	3.0 ± 0	13.7 ± 1.2	4.3 ± 1.2	2.5 ± 0.5	1.4 ± 0.4
	ACE			17 ± 0.6	1.7 ± 0.1	5.6 ± 0.2
Grenache 2 (SA)	Control	5.5 ± 0.7	12 ± 1.2	6.0 ± 1.4	2.5 ± 0.3	1.3 ± 0.3
	ACE			12 ± 1.4	1.7 ± 0.1	2.3 ± 0.3
Shiraz (SA)	Control	6.5 ± 0.7	15.3 ± 3.5	10 ± 1.4	2.1 ± 0.0	1.5 ± 0.1
	ACE			14 ± 4.2	1.9 ± 0.1	1.9 ± 0.7
Shiraz (VIC)	Control	11	10 ± 1.0	13 ± 0.0	1.9 ± 0.3	1.5 ± 0.5
	ACE			41 ± 13	1.3 ± 0.3	3.1 ± 0.8
Cabernet Sauvignon	Control	5.5 ± 0.4	11 ± 1.6	6 ± 0.7	2.5 ± 0.2	1.1 ± 0.1
	ACE			10 ± 2.8	2.3 ± 0.1	2.2 ± 0.4
Tempranillo	Control	3.0 ± 0.7	15.7 ± 2.1	3.0 ± 2.1	2.8 ± 0.2	1.1 ± 0.4
	ACE			9 ± 0.0	2.6 ± 0.1	2.3 ± 0.0

Data are Mean and Standard Deviation for 3 x 5g sub-samples of grapes. The berry number is of a 5g sample. SA/P, Surface area to perimeter ratio. <sup>b</sup>Fragmentation index is the number of fragments per berry. <sup>a</sup>Two Merlot ACE maceration treatments were included at this winery to compare different alignments of the ACE macerator within the must-line. ACE 2 was selected for sensory evaluation.

The degree of skin fragmentation varied both between and within grape varieties from different locations and appeared to be independent of berry size. The average degree of fragmentation for machine and hand-harvested control treatments was similar at 1.3, while the average for ACE treated

musts from hand-harvested fruit was 3.3 and for machine harvested fruit was 2.4. It is interesting to note that machine-harvested fruit did not appear to fragment the grape skins, but it did reduce the degree of fragmentation caused by the cutting device, this may be a consequence of the berries being deflated following machine harvesting and therefore less turgid when intercepted by the cutting blades. Deflated berries may also be the cause for greater seed damage in the control treatments, suggesting that the seeds were damaged in the crushing-destemming step (either by the action of the auger against the wall of the crusher or by the gate valve at the end of the crusher).

The degree of skin fragmentation for the ACE treated musts was apparently influenced by the location of the ACE device within the must line, namely whether the outgoing must line was level, elevated or lower than the incoming must line. This factor was the only difference between the ACE treatments for Merlot (NSW); when the outgoing must line was elevated, the fragmentation index increased by 60%. The most plausible explanation for this observation is that back pressure exerted on the cutting assembly by elevating the outgoing must line meant that the must line was full allowing at least 50% of the must to be intercepted by the cutting blades.

#### *Cap management*

There was considerable variation in the cap management practices of the wineries involved in the trial (Table 4).

**Table 4 Cap management procedure**

Variety	Treatment	Process	Frequency (per day)	Duration (min)	Number of days
Merlot (SA)	Control	N <sub>2</sub> /Air Pulsing	4-8	2	8
	ACE	N <sub>2</sub> /Air Pulsing	4-8	2	3
Merlot (NSW)	Control	Punch down	3	3	
	ACE	Punch down	3	3	
	<sup>a</sup> ACE 2	Punch down	3	3	
Grenache 1 (SA)	Control	Punch down	3	3	6
	ACE	Punch down	3	3	3
Grenache 2 (SA)	Control	Pump-over	3	20	8
	ACE	Pump-over	3	20	5
Shiraz (SA)	Control	Pump-over	3	20	6
	ACE	Pump-over	3	20	4
Shiraz (VIC)	Control	Punch down	2	5	3
	ACE	Punch down	2	5	7
Cabernet Sauvignon (WA)	Control	Pump-over	1-2	5-8	6
	ACE	Pump-over	1-2	5-8	4
Tempranillo (VIC)	Control	Pump-over	2	20	6
	ACE	Pump-over	2	20	6

#### *Winemaking*

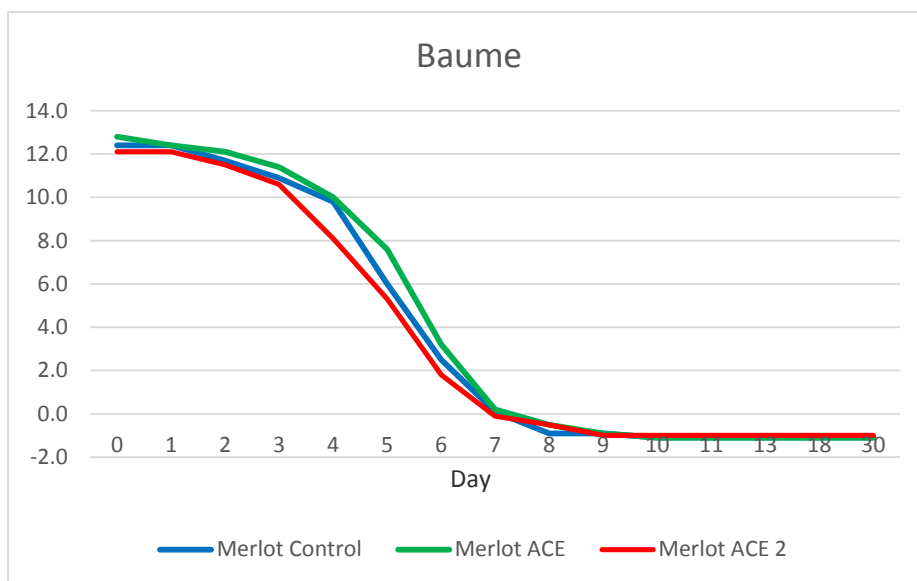
ACE maceration (on skins throughout fermentation) was selected by two wineries each using a different grape variety (Merlot NSW) and (Tempranillo VIC). The response to ACE treatment is discussed first. The four remaining wineries elected to trial PEACE maceration (Merlot (SA); Grenache SA 1 and 2; Shiraz SA and VIC and Cabernet Sauvignon WA) and will be considered second. PEACE maceration refers to ACE wines pressed from grape solids after three to five days on skins with fermentation completed in smaller stainless vessels with no further cap management procedures applied.

NOTE: Results that are not reported reflect data that were not provided by the winemaker.

### *Fermentation kinetics*

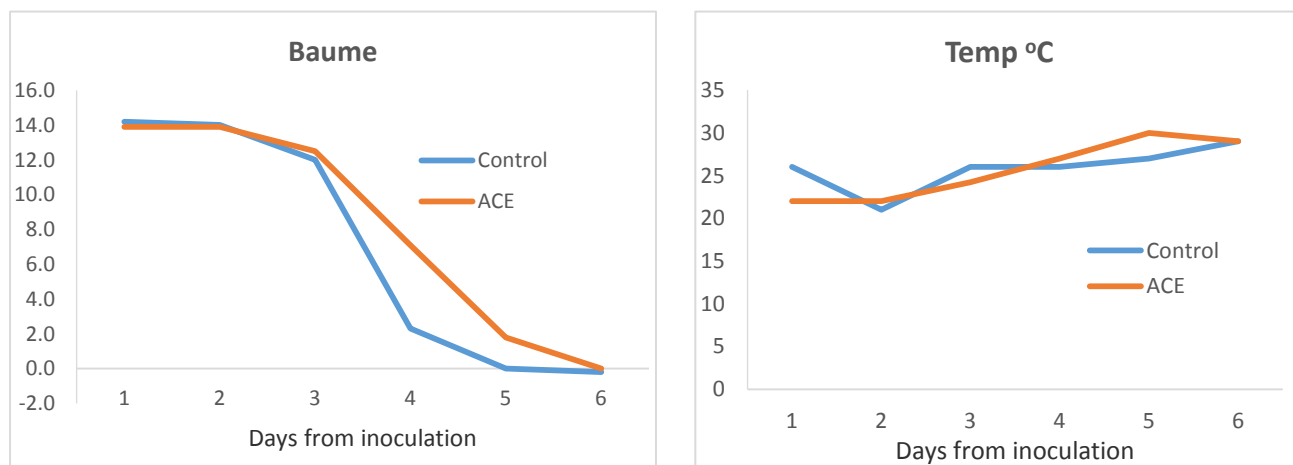
The temperature and Baumé of the fermenting musts were recorded daily at the majority of wineries involved in the trial (Figures 3-7).

Merlot (NSW): The fermentation kinetics show that the second ACE maceration treatment had the effect of slightly speeding up the rate of fermentation, but all ferments were dry after seven days (Figure 3).



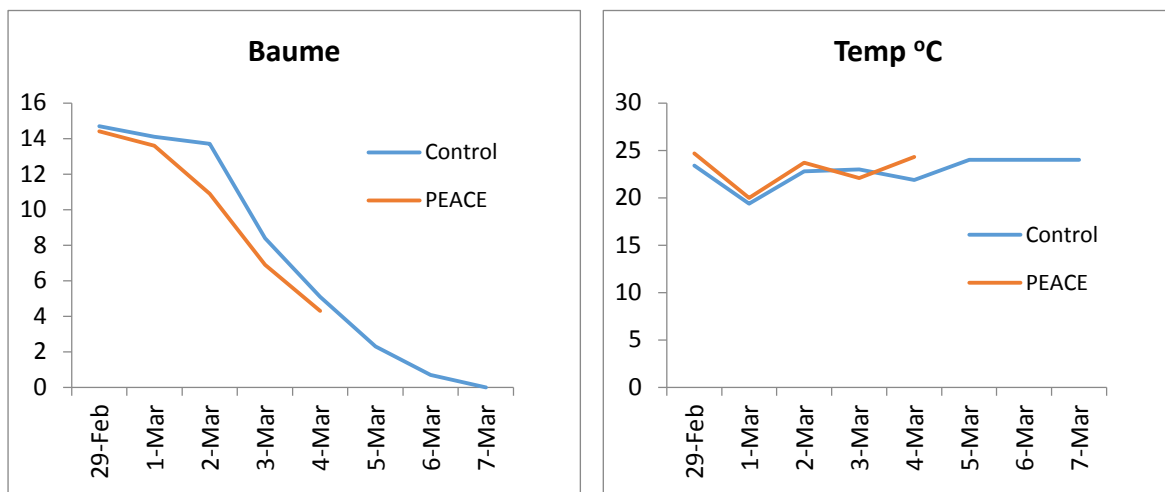
**Figure 3 Fermentation kinetics of control and ACE treated wines Merlot (NSW)**

Tempranillo (VIC): The fermentation kinetics show that ACE maceration had the effect of reducing the rate of fermentation (Figure 4). This may have been due to the smaller volume of fruit in the fermentation vessel for the ACE treatment (10 t) compared to the control wine (14 t) which lowered the fermentation temperature of the ACE treatment in the first three days.



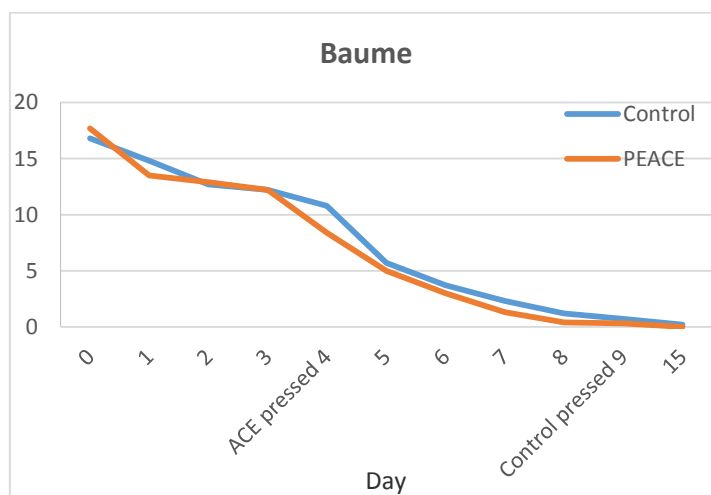
**Figure 4 Fermentation kinetics of control and ACE treated wines Tempranillo (VIC)**

Grenache 2 (SA): Fermentation kinetics show that ACE maceration had the effect of slightly speeding up the rate of fermentation. Early press off of grape solids to make PEACE wines did not compromise the fermentation dynamics (Figure 5).



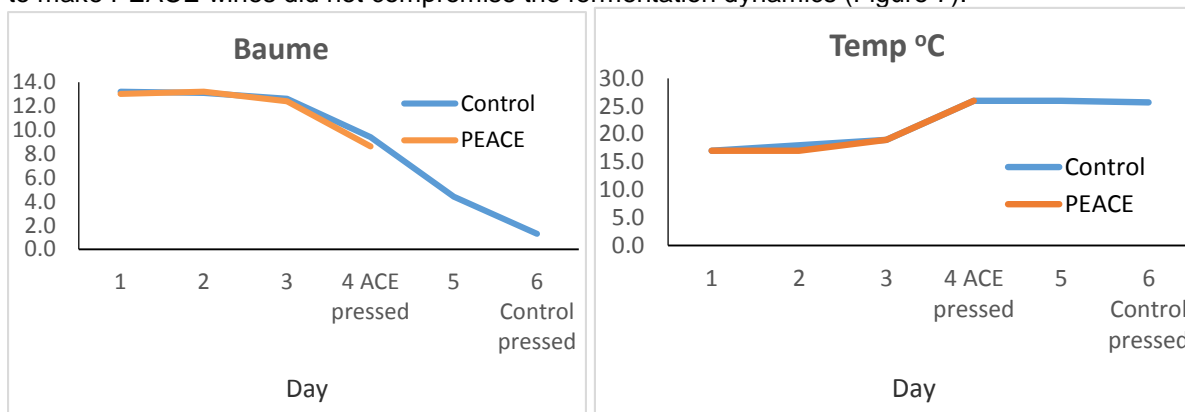
**Figure 5 Fermentation kinetics of control and PEACE treated wines Grenache 2 (SA)**

Shiraz (VIC): The fermentation kinetics show that ACE maceration had the effect of slightly speeding up the rate of fermentation. Early press off of grape solids to make PEACE wines did not compromise the fermentation dynamics (Figure 6).



**Figure 6 Fermentation kinetics of control and PEACE treated wines Shiraz (VIC)**

Cabernet Sauvignon (WA): The fermentation kinetics show that ACE maceration had the effect of slightly speeding up the rate of fermentation (Baumé lower at day 4) yet early press off of grape solids to make PEACE wines did not compromise the fermentation dynamics (Figure 7).



**Figure 7 Fermentation kinetics of control and PEACE treated wines Cabernet Sauvignon (WA)**

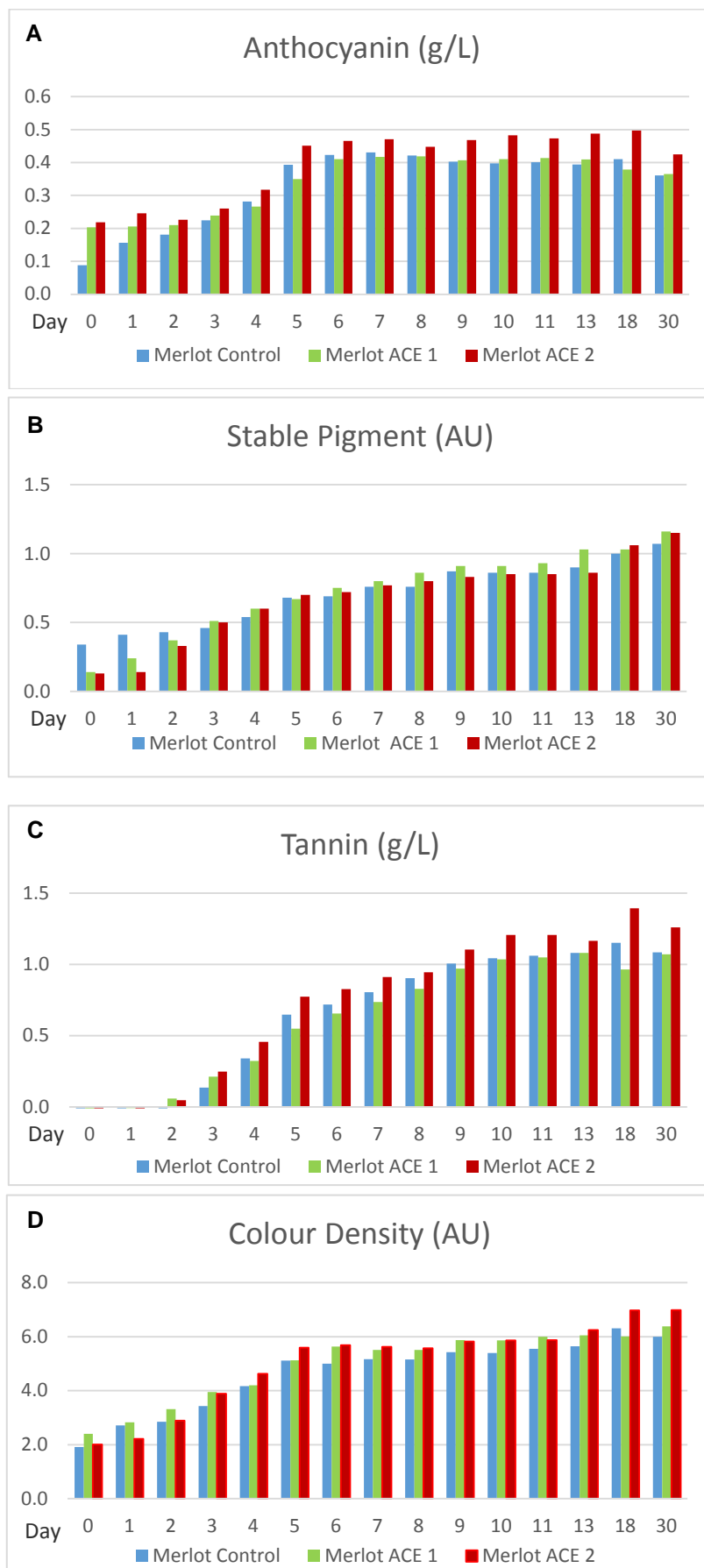
### *Extraction of wine phenolics*

The most important of the phenolic parameters extracted from grapes to make red wine are the colour pigments (anthocyanins) and tannins. Anthocyanins can remain free in the wine solution or form molecular complexes with tannins or phenolic acids to form stable 'pigmented tannins'. Stable pigment reflects wine colour that is not susceptible to oxidation and is a good indicator of the intensity and longevity of colour pigment in the wine. It is differentiated from total pigment that is seen with the naked eye that includes free anthocyanin, which becomes colourless as the pH increases with wine age and is oxidised as the wine ages causing browning. The tannin content of the wine is important for the mouthfeel of the wine; the correct balance of tannin and colour pigment is important, as excessively tannic wines not only increase the astringency of the wine but also jeopardise the production of more stable pigment as the wine ages. The colour density of the wine indicates the intensity of the colour and can range from pale to deep. The hue of the wine indicates the colour tone, which in red wine ranges from garnet to purple, the latter imparting greater resistance to oxidation as the wine ages.

Merlot (NSW): Extraction of anthocyanin was enhanced by ACE maceration for either placement of the ACE macerator, however only the ACE 2 position appreciably increased the anthocyanin, tannin and colour density of the wine. The wine for each treatment was pressed after nine days on skins and the final sample was taken three weeks later. At this stage the ACE 2 wine had anthocyanin and tannin concentrations that were 17% higher than the control, stable pigment was 8% higher and colour density 9% higher than the control. It is interesting to note that at day 4 the anthocyanin and tannin concentrations of the ACE 2 wines were 19% higher than that of the control wine (Figure 8 A and C) while the stable pigment and colour density were 11% higher than the control wine. One of the features of ACE maceration is the option for early press off from the grape solids to limit the concentration of seed tannin in the wine. This option was not chosen for the trial at this winery. The tannin extracted early in fermentation is usually skin tannin, with seed tannin being released from intact seed later during fermentation (Sparrow et al. 2015).

It would be interesting to observe the response to ACE maceration were the grape solids pressed off after four days. The higher concentrations of anthocyanin and tannin would continue to form stable pigments as the wine matures. The increase in tannin in all treatments after day 4 may in part be due to extraction of tannin from seeds, in the latter stages of fermentation.

The trial demonstrated that seed damage and skin fragmentation caused by the ACE device was dependent on the position of the macerator relative to the outlet from the crusher and the heat exchanger. Even in the second position (ACE 2) quite modest skin fragmentation was achieved relative to the control treatments. The phenolic responses were promising for both ACE 2 treatments relative to the control treatment with increases in the majority of phenolic parameters measured compared to the conventional treatment.

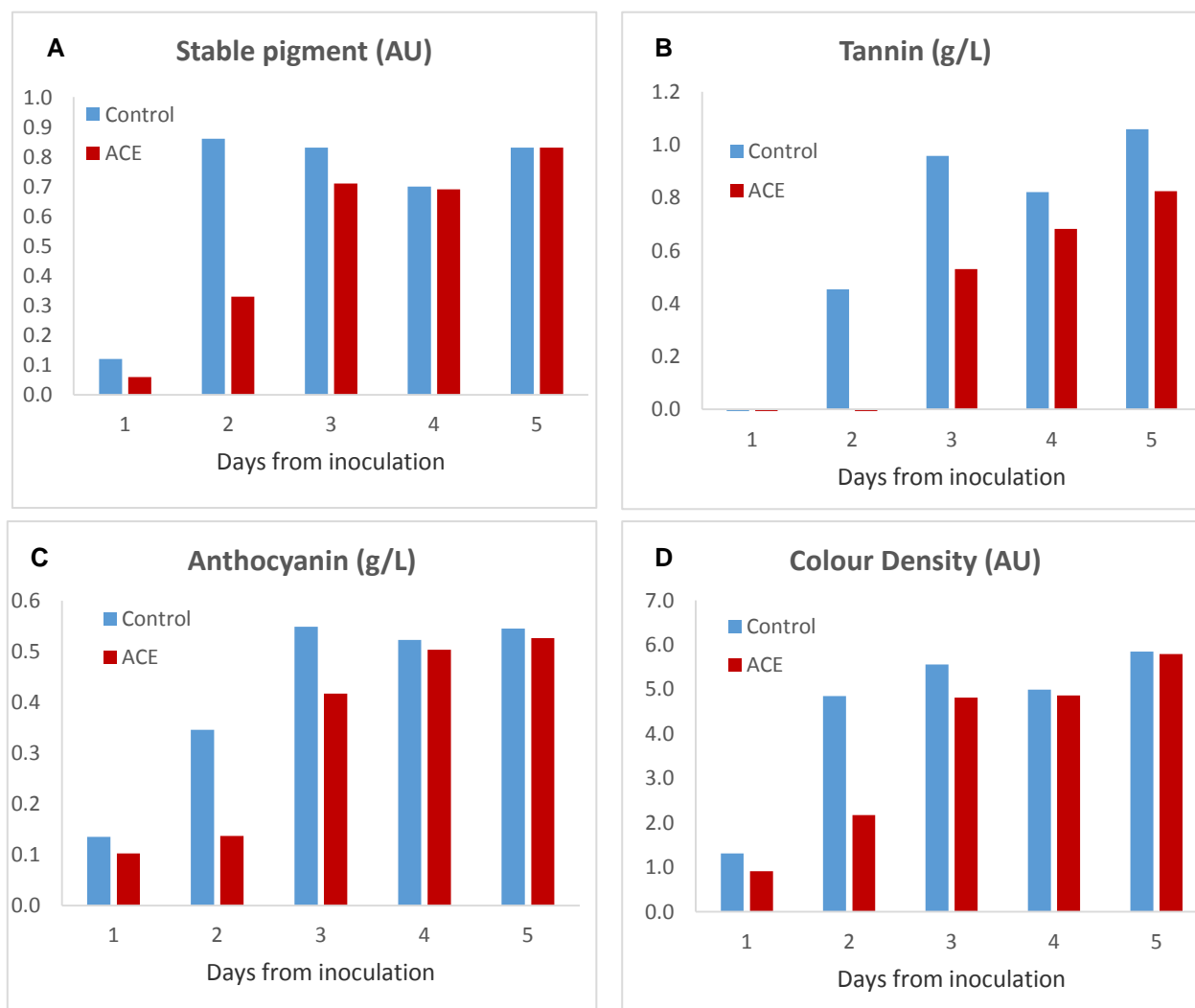


**Figure 8 Phenolic responses of control and ACE treated Merlot (NSW) wines.**



**Tempranillo:** There was no substantial difference in any of the colour related parameters between the control and ACE maceration treatments (Figure 9 A, C and D) for Tempranillo grapes. By contrast there was a 20% decrease in the tannin concentration of the ACE wine. This may be a function of the fermentation temperature, with the concentration of the control treatment being higher than the ACE treatment on day 1 (4°C) and day 3 (1.8°C) of fermentation (Figure 3). Alternatively, the slightly lower seed damage observed for the ACE treatment (13% less than control treatment) may have contributed to the difference in tannin concentration as damaged seeds release tannin more readily than whole seeds.

It would be interesting to observe the response to ACE maceration were the grape solids pressed off after four days. The anthocyanin and tannin would continue to form stable pigments as the wine matures. The increase in tannin in all treatments after day 4 includes tannin from seeds which is extracted in the latter stages of fermentation and can keep the colour pigment as free anthocyanin in the wine (Sparrow et al. 2015).



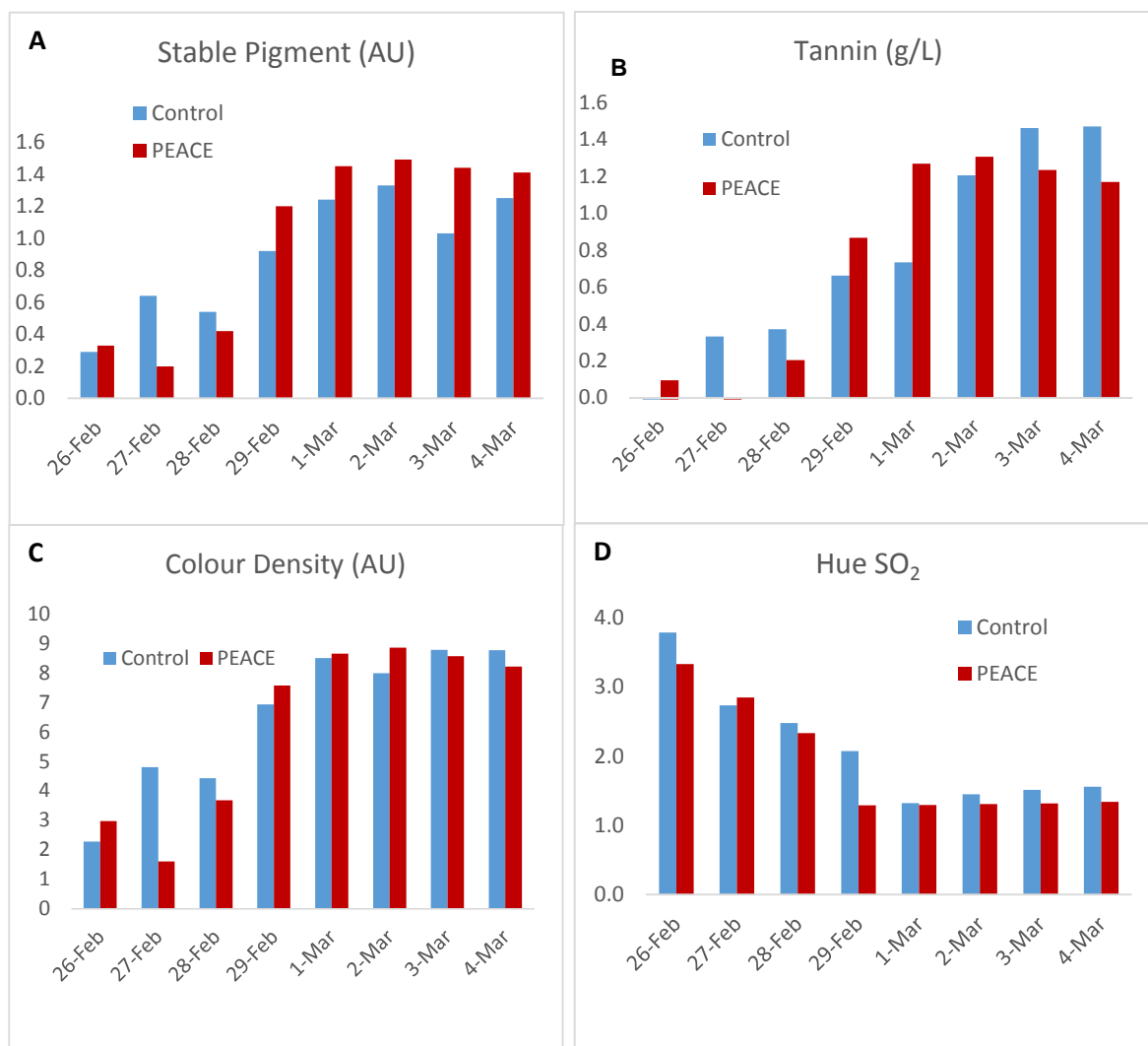
**Figure 9 Phenolic responses of control and ACE treated Tempranillo wines.**

A, Stable pigment; B, Tannin; C, Anthocyanin; D, Colour Density

**Merlot (SA):** A clear difference between the response of Merlot grapes to control and PEACE maceration occurred for both stable pigment and tannin beyond day 3 (28 Feb) which was the day the ACE treated wines were pressed from the grape solids (PEACE maceration) (Figure 10 A and B). The stable pigment content of the 'PEACE' wine was greater than for the control wine through to the end of fermentation when it was 13% higher than that of the control wine.

The tannin content of the ACE treated wine was lower than the control for the first few days, however by day 3 the tannin concentration of both control and ACE treatments increased significantly. The tannin concentration of the 'PEACE' wine then became relatively stable while the tannin concentration of the control wine gradually increased until it was 25% higher than that of the PEACE wine.

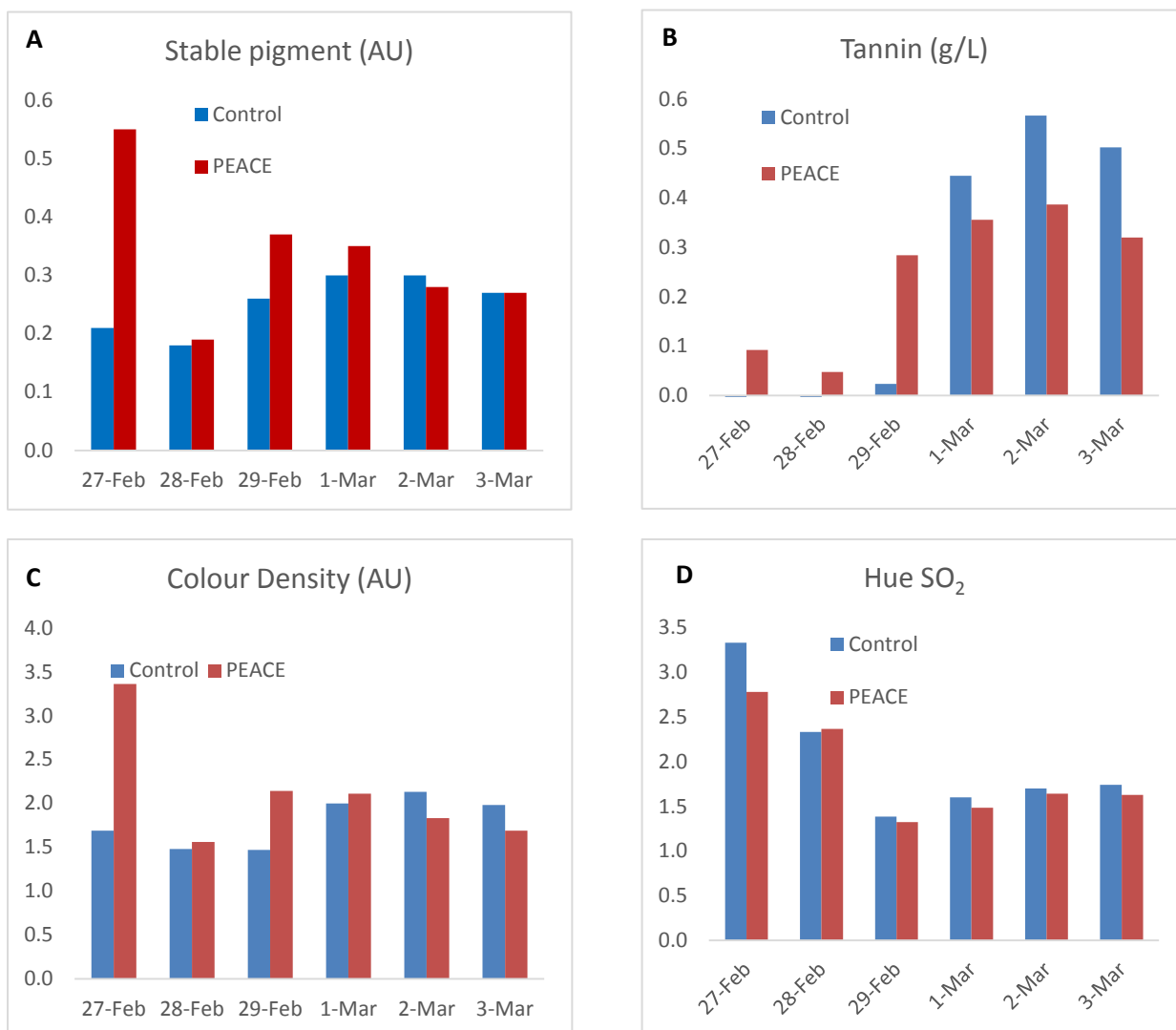
At the conclusion of fermentation, the colour density of the PEACE wine was 7% lower than that of the control wine (Figure 10 C) and the colour of the PEACE wine had an intensity of blue colouration (hue SO<sub>2</sub>) that was 16% greater than that of the control wine (Figure 10 D). Considering each of the phenolic characteristics described, the PEACE wines are more likely to retain their colour for longer than the control wines.



**Figure 10 Phenolic responses of control and PEACE treated Merlot wines (SA).**

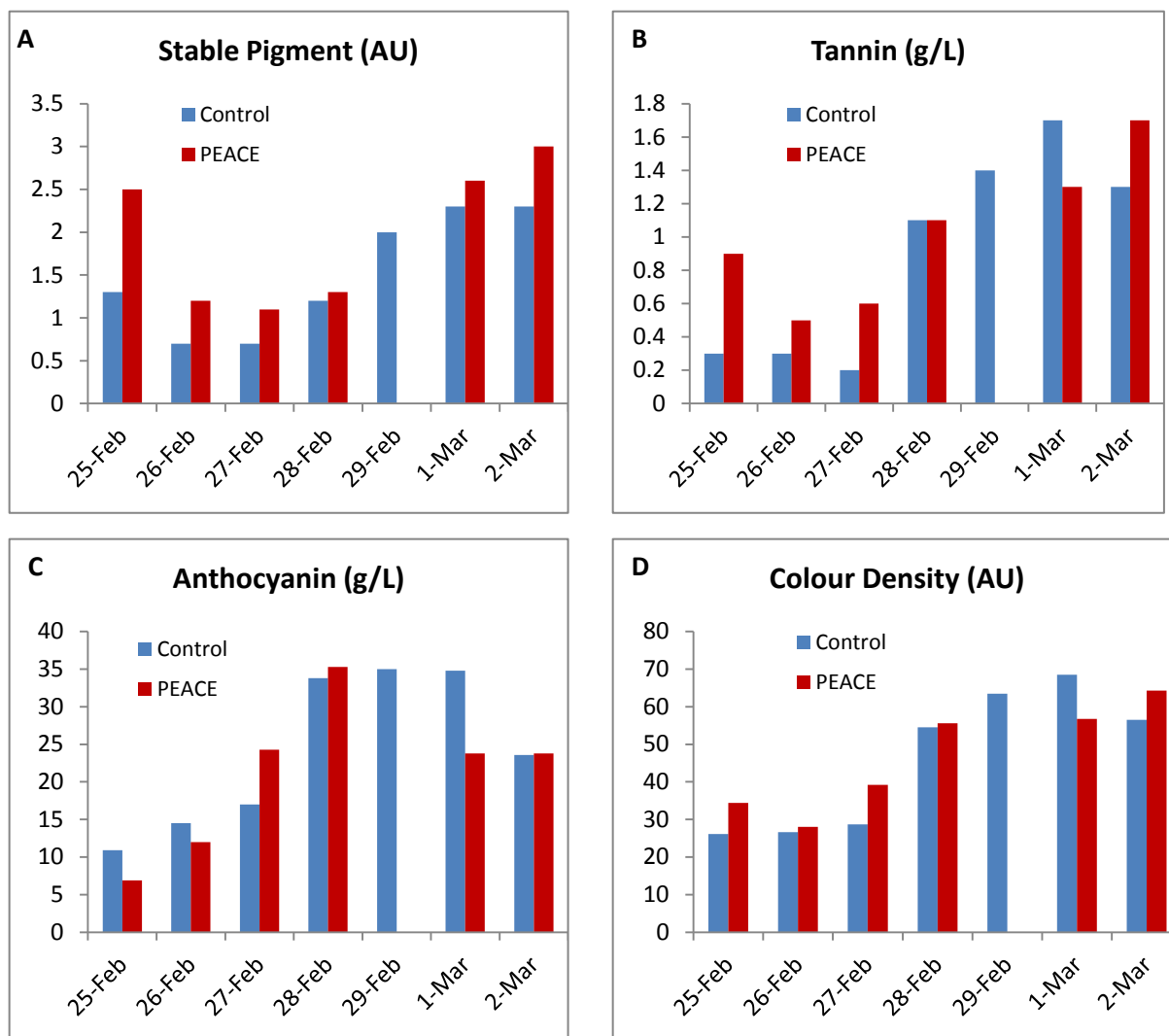
A, Stable Pigment; B, Tannin; C Colour Density; D, Hue SO<sub>2</sub>, (refers to the stable hue of the wine, a lower number corresponds with more blue colouration).

Grenache 1 (SA): The early extraction of phenolic components from the ACE treated must is shown clearly in Figure 11. Fermenting wine was pressed off the grape solids on 29 February after three days on skins. By the end of fermentation there was no difference in the response to ACE-PEACE maceration for stable pigment concentration (Figure 11 A), whereas the tannin concentration of PEACE wine was 40% lower than that of the control wine and colour density, 17% lower than the control (Figure 11 C). A reduction in the hue SO<sub>2</sub> value indicates that the hue of the PEACE wine was more blue (7%) than the control wine (Figure 11 D).



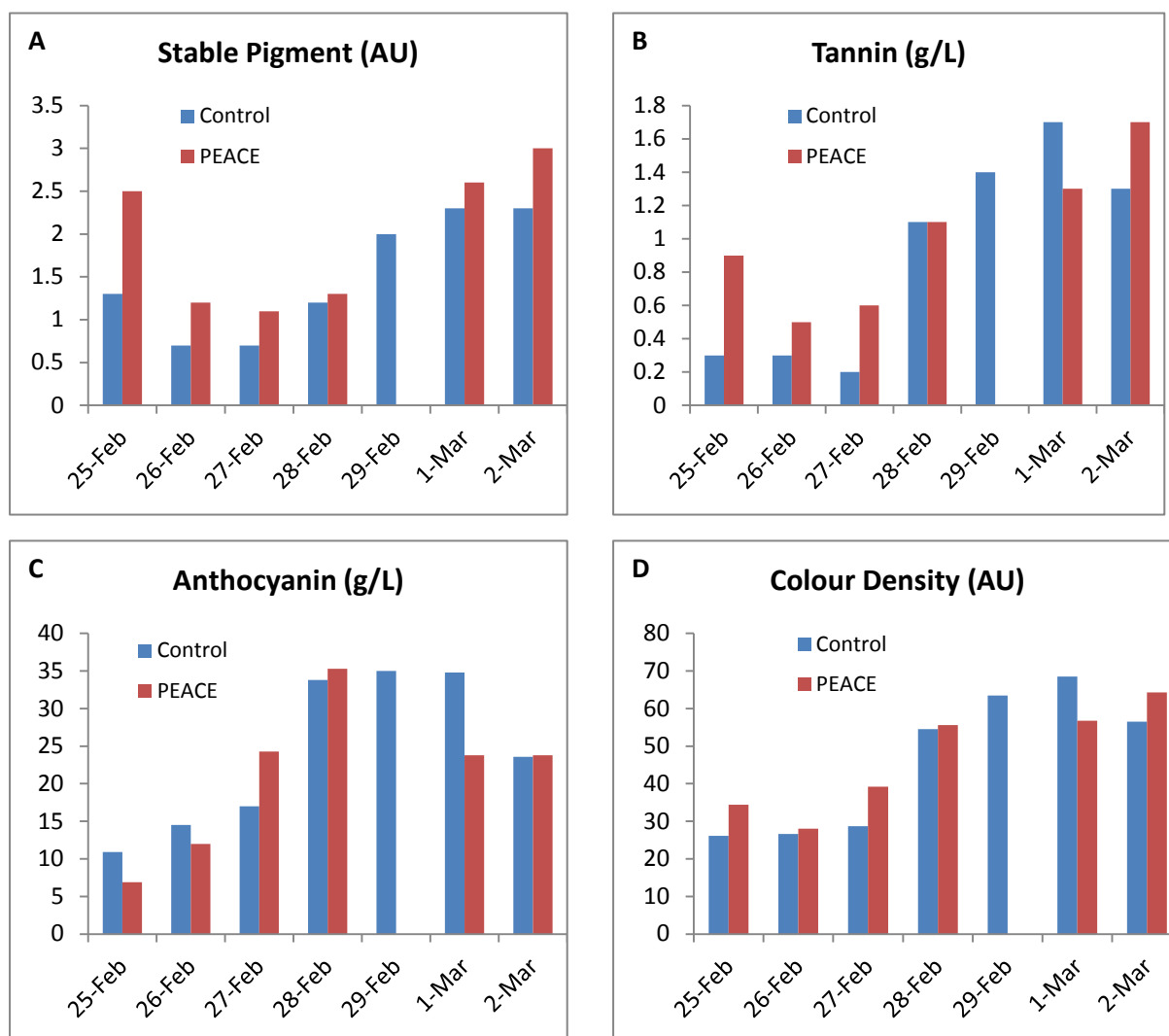
**Figure 11 Phenolic responses of control and ACE-PEACE treated Grenache 1 wines (SA).**  
A, Stable Pigment; B, Tannin; C Colour Density; D, Hue SO<sub>2</sub>.

Grenache 2 (SA): There was a three-fold increase in the concentration of tannin extracted by application of ACE maceration on the first day of sampling and stable pigment concentration was almost double that of the control (Figure 12 A and B). Fermenting wine was pressed off grape solids on 28 February after four days on skins at which time the concentration of both stable pigment and tannin were similar for both treatments. At the end of fermentation, the concentration of both stable pigment and tannin in the PEACE treated wine was a 30% greater than the control while colour density had increased by 12%.



**Figure 12 Phenolic responses of control and ACE-PEACE treated Grenache 2 wines (SA).**  
A, Stable Pigment; B, Tannin; C Colour Density; D, Hue SO<sub>2</sub>.

**Shiraz (SA):** Both tannin extraction and stable pigment formation were promoted by ACE maceration with the concentration of both these components being two to three-fold higher than for the conventionally treated control on day 1 (Figure 13 A and B). The control wine achieved the same concentration of tannin by day 4 of fermentation and stable pigment by day 5 of fermentation. ACE treated wines were pressed after four days on skins to make 'PEACE' wine. At the end of fermentation, there was a 30% increase in both tannin and stable pigment relative to the control wine (Figure 13 A and B), and a 13% increase in wine colour density (Figure 13 C). There was no difference in the anthocyanin concentration of the control and PEACE wines at the end of ferment. The presence of free anthocyanin at the end of fermentation indicates that more stable pigments can be expected to form as the wine matures.



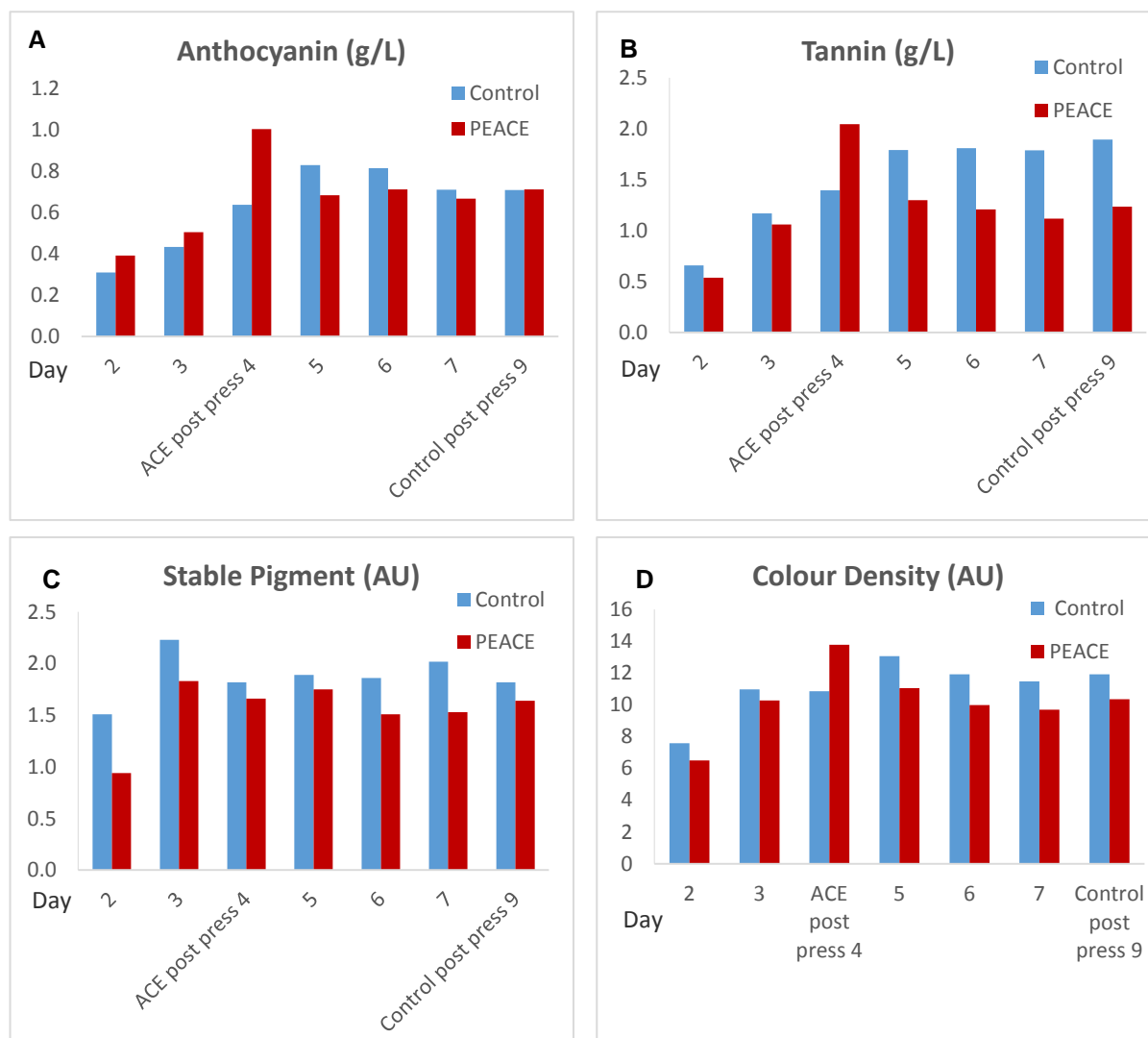
**Figure 13 Phenolic responses of control and PEACE treated Shiraz (SA) wines.**

A, Stable Pigment; B, Tannin; C Anthocyanin; D Colour Density.

Shiraz (VIC): Extraction of anthocyanin was enhanced by ACE maceration with the concentration post pressing being 40% higher than that of the control wine at the same time (Figure 14 A). It is interesting to note that at the time the control treatment was pressed five days later, the anthocyanin concentration of the control and PEACE treatments was identical (0.7 g/L). Tannin extraction was also enhanced by ACE maceration, however, early press off from grape solids (three days after yeast inoculation) effectively reduced the tannin content of the wine. By the time the control wine was pressed five days later it had 54% more tannin than the PEACE wine (Figure 14 B). Early press off of ACE treated wine caused some compromise to the concentration of stable pigment (10% reduction) and wine colour density (15% reduction) however as the presence of free anthocyanin was not affected, the formation of stable pigment is likely to continue as the wine matures.

It would be interesting to observe the response to ACE maceration were the must inoculated on the same day that the treatment was applied, or if it was pressed off one day later. The increase in tannin in the control treatment from day 5 to the end of ferment may in part be due to extraction of tannin from seeds, which takes place in the latter stages of fermentation (Sparrow et al. 2015).

As there was minimal damage to the seeds caused by the ACE treatment (Table 2) it is quite likely that the tannin present in the PEACE wine was derived mainly from the grape skins, thereby optimising the development of more stable pigment which should improve the wine colour density.

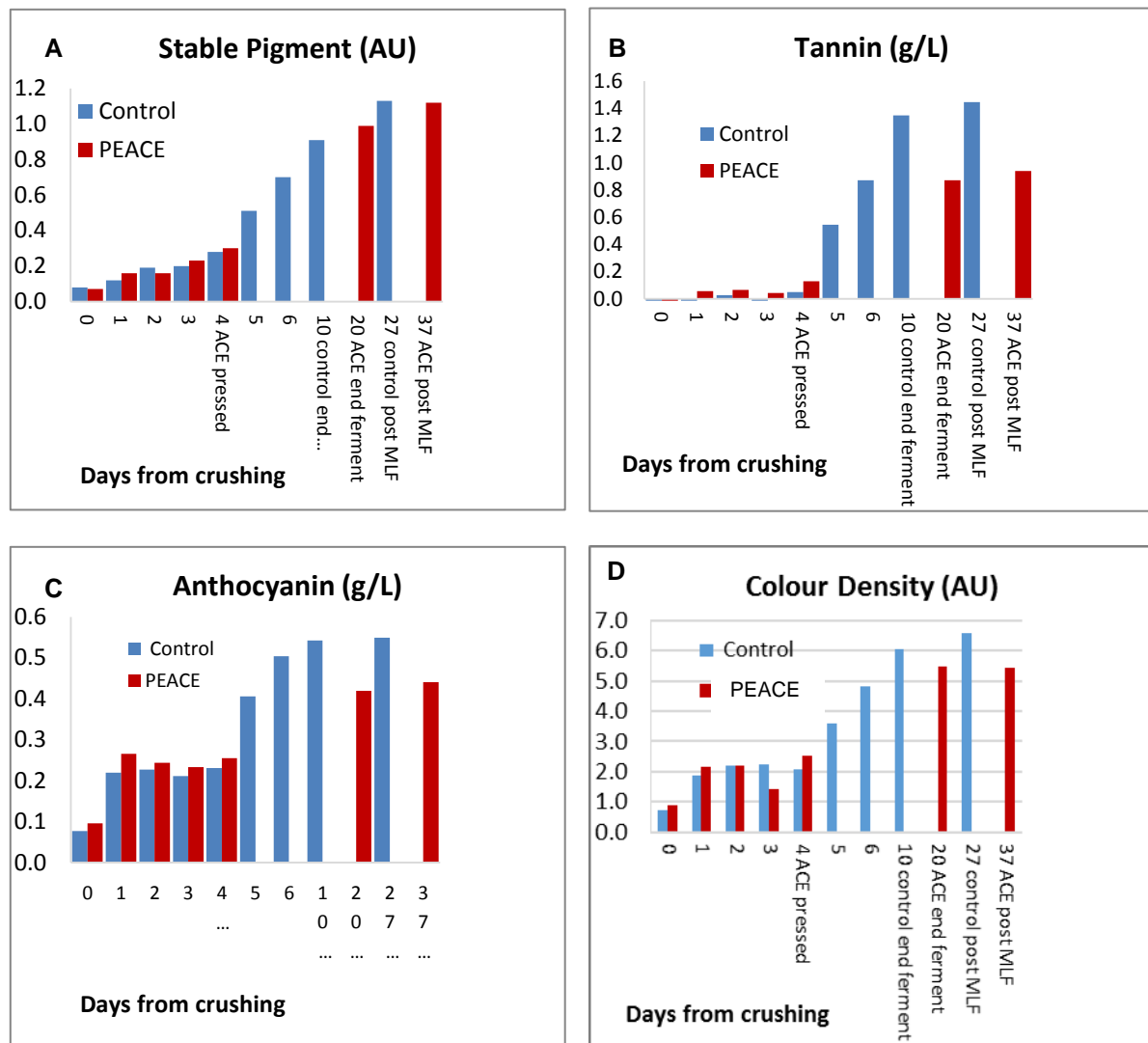


**Figure 14 Phenolic responses of control and PEACE treated Shiraz (VIC) wines.**  
A, Anthocyanin; B, Tannin; C, Stable pigment; D, Colour Density.

Cabernet Sauvignon: Extraction of both anthocyanin and tannin were enhanced by ACE maceration. The decline in anthocyanin for ACE treated ferments through to day 4, when the ACE treated ferment was pressed to make PEACE wine, corresponded with an increase in formation of stable pigment. The PEACE wine was slower to finish fermentation than the conventional ferment (10 days longer than conventional ferment). This may have been due to a reduction in yeast activity caused by a reduction in yeast population. The post MLF concentration of phenolic components demonstrates that there was little difference in the stable pigment content of the conventional and PEACE wines (<1%; Figure 15 A). By contrast, the tannin concentration of PEACE wines was reduced by ~50%, anthocyanin by ~25% and colour density by ~20% relative to the conventionally treated control (Figure 15 B, C and D). The increase in tannin from day 5 to the end of ferment may in part be due to extraction of tannin from seeds, which takes place in the latter stages of fermentation. The fact that the anthocyanin concentration of the conventionally treated wine was similar post MLF to that at the end of fermentation is also an indication of phenolic extraction from the seed which has been shown to keep anthocyanin free in the wine matrix thereby restricting the formation of stable pigment (Sparrow et al. 2015).

It is expected that the concentration of stable pigment in both the conventional and PEACE treated wines will continue to increase as the wine matures. As there was minimal damage to the seeds caused by the ACE treatment it is quite likely that the tannin extracted early in fermentation was derived mainly from the grape skins, thereby optimising the development of more stable pigment in the PEACE wine which will improve the colour density.

If a reduction in tannin concentration of Cabernet Sauvignon wine is seen as an advantage, without compromising the colour stability of the wine, then PEACE maceration may be considered beneficial.



**Figure 15 Phenolic responses of control and PEACE treated Cabernet Sauvignon wines.**  
A, Stable pigment; B, Tannin; C Anthocyanin; D Colour Density.

A tasting workshop for the ACE trial wines was conducted on 29 July 2016 in Adelaide, South Australia. The panel consisted of two of the winemakers involved in the trial, and four independent wine experts, the remaining winemakers were unable to attend. At this workshop the panel selected which trial wines should be included for Qualitative Assessment by the Australian Wine Research Institute. The results of the sensory assessment together with a phenolic summary of the wines at this stage are shown in Table 5.

Table 5. Mean phenolic attributes and wine scores for control and ACE treated wines.

Variety		Colour density	% stable pigment	Blue colouration	Tannin (g/L)	Score/20	P-value (score)
Merlot (NSW)	Control	5.6	7.5	0.89	1.1	13.61	NS
	ACE 2	6.4	7.2	0.94	1.2	13.92	
Merlot (SA)	Control	11.0	8.3	0.79	1.4	14.45	0.015
	PEACE	9.2	7.8	0.70	1.1	14.10	
Grenache 2 (SA)	Control	4.5	15.4	0.92	1.1	14.10	NS
	PEACE	5.0	19.6	1.01	1.0	14.40	
Shiraz (SA)	Control	12.2	8.1	0.89	1.6	13.15	NS
	PEACE	9.4	7.6	0.86	1.3	13.28	
Shiraz (VIC)	Control	12.5	8.2	0.79	1.8	14.53	NS
	PEACE	12.2	7.3	0.74	1.4	14.08	
Cab. Sauvignon (WA)	Control	8.6	6.0	0.99	1.6	14.00	NS
	PEACE	7.4	7.2	1.02	1.1	14.08	

Results for the phenolic analysis are unreplicated. Those for wine score are mean values (11 judges x two sessions).

The wines were made in a single batch at each winery so statistical analysis of the phenolic data was not possible. However, ACE treated Merlot (NSW) wines at five months showed an increase in colour density, blue colouration and tannin yet the sensory score was higher for the control wine. The phenolic response of PEACE treated wines from the other states was variable with an increase in colour density apparent for Grenache 2 (SA). An increase in the percentage of stable pigment and blue colouration was noted for Grenache 2 (SA), and Cabernet Sauvignon (WA) PEACE wines relative to the control while the tannin concentration was lower for all PEACE treated wines.

In general, the Qualitative Assessment Panel scored the sensory attributes of the PEACE wines higher than the control wines, but this result was not statistically significant at  $P < 0.05$  (Table 5).

The panel described more fruit flavours in the PEACE wines relative to the control, in particular dark berry fruits were noted for aroma and palate characteristics. Differences in acidity and astringency were not consistent between treatments although the PEACE wines generally had softer or finer tannins than the control, while the single Merlot ACE treatment had harsher tannins. The Qualitative Assessment Panel is particularly aware of taints and faults in wines and both control and PEACE wines evaluated were considered to display some faults including smoke, bitterness and reductive notes, although these were mostly detected as a slight occurrence. As these were young wines (six months old) that had not been subjected to normal pre-bottling quality control checks these findings are not surprising. Reductive characters were described more often for the PEACE treated wines and an explanation for this was found in the winemaker records which show that once the wines were pressed, no further aeration treatments were applied. In the case of the PEACE wines which by the very nature of the treatment were still fermenting, this was not ideal and might be easily rectified by occasionally aerating the must until fermentation is complete. The complete Qualitative Assessment report prepared by Australian Wine Research Institute appears in Appendix 5.4.



## 7. Outcome/Conclusions

Overall the project performed well against planned outputs and performance targets with only minor modifications required. These are summarised below:

- a. Plans for ACE winery trials were finalised by 5 February 2016. Trials were scheduled at six wineries encompassing four states and five red wine varieties and commenced three weeks later. However due to the constraints for tank space within the winery, only two wineries were able to test ACE on two different grape varieties, the remaining wineries chose one red grape variety.
- b. Data documenting the impacts of ACE treatment on relevant winemaking parameters during fermentation. The winemaker's notes were collated and compared by 11 May 2016. The extent of skin fragmentation and seed damage imposed by ACE maceration was compared with the conventionally produced control treatment. Fermentation kinetics and daily phenolic samples were analysed for each of the eight trials and individual reports were prepared and delivered to each winemaker.
- c. 31/08/2016. Bottled wines of control and ACE/PEACE treated wines were delivered to Adelaide for wine tasting by participant winemakers on 29 July 2016 to coincide with the Australian Wine Industry Technical Conference 2016. At this stage the wines were 4 - 5 months old, and not six months as originally planned.
- d. 14/10/2016. Data documented the impacts of ACE treatment on wines six months after completion of fermentation. At this time wine phenolics were again assessed and the wines tasted by participant winemakers. At the conclusion of this preliminary tasting, 12 of the 17 wines were submitted to the Australian Wine Research Institute for sensory evaluation by the Qualitative Assessment Panel. Sensory evaluation by Qualitative Assessment is shown in Appendix 5.4.

The five wines not included for this evaluation were:

1. Tempranillo, as the control and ACE treatments were blended by the winery at the conclusion of fermentation.
  2. The winery that elected to make ACE Merlot wine, experimented with two configurations of the ACE device, only one of these was forwarded for qualitative assessment.
  3. The winemaker of Grenache 1 elected to use a wild yeast ferment. Fermentation was very slow to commence and early press off of ACE was premature. The winemaker panel considered neither the control nor the PEACE wine from this trial suitable for further analysis.
- e. Final report to AGWA 28/10/2016 collating data for each region and variety.

Conducting commercial trials presents a challenge to wine scientists as winemaking procedures vary both between and within wineries. This includes fruit composition, temperature at harvest, winery equipment, processing rate, fermenter size, cap management procedures, yeast and nutrient additions. As the ferment sizes are large, replication is not possible so there is no opportunity to conduct statistical analyses. To conduct a nationwide trial in which all such variables are prescribed seems unlikely and probably unreasonable. Consequently, the outcomes of this and subsequent trials are likely to be relevant for their practical advantages even if they cannot scientifically proven.

This project demonstrated that the overall impact of the new technology was consistent, this in itself becomes a form of replication. In six of the eight ACE maceration trials across three states, the winemakers elected to use the Press Early ACE maceration option - a good indication that re-use of the fermentation tanks in a shorter time is a priority for commercial wineries. The responses of four different grape varieties were favourable in terms of phenolics and sensory properties of the wine. The practical implications of the research results are that ACE maceration and in particular PEACE maceration offers commercial wineries the opportunity to reduce the time taken for each ferment to occupy a fermentation tank by as much as 50% without compromising the quality of the wine. This effectively doubles the rate of turn-over of red wine fermentation tanks, the mostly costly type of fermenter in the winery. In economic terms, instead of having to manage compressed vintages by purchasing more fermentation tanks, PEACE maceration can be implemented, and after early press off, the fermenting wine can be transferred to smaller tanks such as those usually used for white wine, or directly to oak barrels.

In addition to increasing the rate of turn-over of the fermentation tanks, cap management procedures such as punch-down and pump-over are not required during the latter part of fermentation under PEACE maceration conditions, resulting in energy and labour savings.

- The financial savings of this technology have been calculated as part of a concurrent project (UT 1301) which is due for completion in February 2017. Part of that project included the investigation of ACE and PEACE maceration as a means of improving processing efficiency. According to these calculations, the cost of purchasing a suitable ACE maceration device coupled with early press off can be recovered in one year.
- Improving the processing efficiency of winemaking is likely to have flow-on benefits for the entire wine sector. Fruit wastage during compressed vintages due to lack of winery capacity can be avoided without large investments in infrastructure extensions. The estimated cost of an ACE device suited to commercial wineries is \$25,000 to \$60,000 depending on the size of the crusher-destemmer unit used and therefore the rate of fruit processing. The high rate of return on this investment means that the price of the finished product will not be inflated by production costs.

## 8. Recommendations

Future research directions arising from this research that will bring the greatest benefits are those that:

### 1. *Disseminate the outcomes of the research project at nationwide industry workshops.*

Considerations: At the commencement of the project participating wineries were asked to provide one dozen bottles of each treatment to be used in workshops around the country when the wine was twelve or more months old. These should be conducted in each of the states involved in the trial, in order to explain the simplicity of the technique and its economic benefits. Sufficient wine has been supplied from each winery to provide tastings for participants at four workshops.

### 2. *Explore the development of a commercially robust ACE device.*

Considerations: Sustained use of a cutting device capable of fragmenting skins while not damaging seeds;  
Optimum positioning of the ACE device within the must line; (location and orientation) within existing winery infrastructure.  
Sampling port to assess degree of skin fragmentation and seed damage in-line.

### 3. *Resolve the source of seed damage in mechanically harvested grapes.*

Considerations: An unexpected finding from this project was the extent of seed damage in mechanically harvested grapes. As extracts from seed have been shown to compromise the production and stability of pigmented tannins in red wine, this issue needs to be resolved. Whether the seed damage occurs during harvest or during the crushing and destemming step for mechanically harvested fruit is worthy of investigation.

### 4. *Encourage daily monitoring of wine phenolics.*

Considerations: The technology provided by the Australian Wine Research Institute through their grape and wine portal appears to be under-utilised with only one of the wineries involved in this project using the technology;  
Routine analysis of these parameters would assist in optimising the time of early press-off for ACE treated wines.

### 5. *Develop sensory descriptors typical of popular Australian wine varieties.*

Considerations: Sensory evaluation remains very subjective and this becomes especially problematic when analysing commercial trials. Different terms are used by different tasters to describe a similar attribute e.g. red fruit may be described as cherry, strawberry, raspberry or simply 'red'. In the case of commercial evaluation, a general term such as 'red fruit' to differentiate these from 'dark fruit' such as blackberry, mulberry, blackcurrant would make comparisons of treatments in commercial trials more meaningful. Similarly, terminology

surrounding wine hue (is there a difference between ruby, crimson and cherry?) and tannin descriptors would be beneficial.

The primary outcome of this research is the commercial evaluation of an innovative technique (ACE) that had previously proven advantageous for Pinot Noir winemaking. Pinot Noir wines are known for their delicate flavour and challenges with colour stability. As the majority of other Australian red wine varieties generally do not lack colour pigments the project was able to explore a further use of ACE maceration. Pressing the grape solids from fermenting red wine sooner than is the usual practice, caused no detriment to wine quality and has the potential to realise significant production efficiencies in the winery. The project demonstrated that these efficiencies include increased tank space plus savings in labour and energy requirements during the busiest part of the vintage thereby increasing profitability in the winery and has the potential to improve Australia's competitiveness in the global market.

## **9. Appendices**

### ***Appendix 1: Communication***

The project was very short, and was completed in less than 10 months.

- Each of the six mainland wineries that participated in the project have been provided with a 16-18 page report of the trial conducted at their winery including: skin fragmentation, seed damage, phenolic extraction during fermentation and sensory evaluation.
- As the project describes the results of commercial trials the data cannot be statistically analysed. The findings are more appropriate for a trade journal and publication in the Australian and New Zealand Wine and Viticulture Journal has been scheduled for early 2017.
- The project outcomes lend themselves to further communication such as industry workshops or seminars, to explain the simplicity of the technique and its economic benefits.

### ***Appendix 2: Intellectual Property***

No new intellectual property was generated by the project; rather it represented industry evaluation of existing IP. An innovative approach to incorporating ACE maceration into the production of popular red wine varieties (early press off of grape solids) was explored and was consistently shown to be advantageous in terms of maintaining wine quality by reducing production costs.

### ***Appendix 3: References***

Sparrow, A.M., R.G. Dambergs, K.A. Bindon, P.A. Smith and D.C. Close. 2015. Interaction of grape skin, seed and pulp tissues on tannin and anthocyanin extraction in Pinot noir wines Am. J. Enol. Vitic. 66 (4) 472- 481.

AGWA Project UT1301. Principal Investigator Dr Anna Carew, University of Tasmania, Australia

### ***Appendix 4: Staff***

Dr Angela Sparrow - Vinventive, Lanena, Tasmania

#### Industry participants:

Peter Gambetta, Natalie Cleghorn, Alana Seabrook - Yalumba, Barossa Valley, South Australia

Ryan Waples, Angus Seabrook - Barossa Valley Estate, South Australia

Stephen Webber, Sarah Fagan - De Bortoli Wines, King Valley, Victoria

Justin Purser, Leanne Thompson - Best's Wines, Great Western, Victoria

Jim Lumbers, Malcolm Burdett - Lerida Estate, Collector, NSW

Ryan Aggiss, Courtney Treacher - Houghton's wines, Nannup, WA

#### Wine sensory evaluation experts:

Francesca Blefari, Leigh Francis, Wies Cynkar - Australian Wine Research Institute, South Australia

## **Appendix 5: Other relevant material**

### **Appendix 5.1 Procedure Red wine ACE Trial 2016**

AIM: Compare control, ACE and PEACE maceration techniques

ACE= Accentuated Cut Edges

PEACE= Press Early, Accentuated Cut Edges

NOTE: not all wineries have elected to do both ACE and PEACE treatments

#### **Materials**

*Supplied by commercial winery*

Red wine grapes;

2-3 fermenters of equal size (0.5 to 30 t capacity);

Crusher/de-stemmer;

Variable speed must pump;

2 x 250 mL plastic jars with screw cap for must samples;

40 x 10 mL plastic centrifuge tubes for daily samples;

2 X 3" or 4" hose must lines with BSM fittings (one end of hose 1, male; one end of hose 2, female)

Power supply (single phase)

*Supplied by Angela*

ACE macerator (3" or 4" diameter) with male and female BSM fittings at either end;

2 x reducers (4" to 3") male and female BSM fittings at either end.

#### **Harvest**

Call Angela Sparrow three days prior to crushing the grapes to arrange delivery of ACE device and time of Angela's arrival.

Angela will bring the ACE macerator to the winery on the day the grapes are crushed and supervise operation of the device in conjunction with the winemaker who is operating the must pump.

### **WINEMAKING PROCEDURE**

#### **Winemaker to record**

- Grape variety, clone number
- Vineyard site location (irrigation method, yield, harvest details (machine/hand; harvester rate (t/hr))
- Date of harvest
- Baume, pH, TA of juice sample at harvest
- Wine extractable grape colour and tannin using AWRI commercial services

<http://www.thewinecloud.com.au>

(See AWRI "Sample prep guide grape portal" procedure attached)

**NOTE: If you don't yet subscribe to the AWRI Grape and Wine Portal, discuss with Angela optimum press off date. Freeze the samples and send to Angela within 3 months.**

#### **Ferments**

<b>Grape variety</b>	<b>Treatment</b>	<b>Must weight (kg)</b>	<b>Treatment Details</b>
<i>Red wine</i>	Control	0.5 to 30 t	De-stem & crush
<i>Red wine</i>	ACE	0.5 to 30 t	De-stem & crush; Apply ACE device in must line
<i>Red wine</i>	PEACE	0.5 to 30 t	De-stem & crush; Apply ACE device in must line Press early e.g. half-way through normal ferment

#### **DAY 0**

- De-stem & crush fruit.
- Control treatments: Pump must of known weight into fermentation vessel.
- ACE treatments: install the ACE macerator in the must line and pump the same weight of must into a second fermentation vessel via the ACE maceration device.
- Take 10 mL juice sample from each fermentation vessel. Freeze the samples.

- Record details of SO<sub>2</sub> and yeast additions for winemaking protocol determined by winemaker (e.g. time of addition, concentration, rate, form of SO<sub>2</sub>, yeast strain).
  - Record method of mixing the ferment as determined by winemaker.
- Send this information to Angela WITHIN 3 MONTHS of starting the trial:**  
[angela@vinventive.com](mailto:angela@vinventive.com)

## FRAGMENTATION ASSESSMENT

From both the control and ACE macerated must take one ~2L sample determine:

1. percentage of seed damage;
2. degree of skin fragmentation;
3. concentration of wine extractable tannin in the grapes.

### Procedure:

- Mix the must and take a sample from it using an open vessel (e.g. measuring jug) of ~ 2L capacity.
- From this take 2 x 200g samples and place each in a 250 mL plastic screw capped sample jar and one 500g sample for seed assessment.
- Using the 500g sample pass the must through a sieve to separate the grape solids from the liquid, then separate all seeds and seed fragments from the grape solids.
- Place the seeds and seed fragments in a 50 mL plastic screw capped sample jar. Weigh the seeds and record the weight and date on the sample jar. Count the seeds and record the percentage of damaged seeds in the sample. (The seed sample can be frozen and seeds counted at a later date).
- From one of the 200g must samples take three 5g samples of grape solids (if time is short this 200g sample can be frozen for later skin fragmentation analysis)
- To the fresh or thawed 5g sample of grape solids, add 15 mL of water. Mix by inverting to suspend all the solids in the water then pour the contents into a 90mm petri dish and carefully separate the fragments one from another. Place the petri dish on a piece of graph paper with 5 x 5mm grid. Photograph the entire contents of the petri dish.
- Calculate percentage skin fragmentation using Image J analysis software or equivalent.
- On the second fresh 200g sample conduct grape tannin (see AWRI "Sample prep guide wine portal" procedure attached).

**NOTE: If you don't yet subscribe to the AWRI Grape and Wine Portal, freeze the grape sample and send to Angela within 3 months.**

## DAYS to END of fermentation

- Record fermentation kinetics daily (alcohol concentration/Baumé, temperature).
- Continue regular sampling of each treatment after daily mixing; 10 mL samples can be frozen for later analysis through AWRI Wine Portal;
- Record the date the wine was pressed from the grape solids.
- Record the date the fermentation is completed.
- Test for dryness and record residual sugar.
- Record the type of fermentation vessel (stainless steel, PVC, wooden barrel) and the date on which the wine was moved from one to the other.
- Record sample analyses normally conducted by lab for this trial (e.g. sugar/alcohol, pH, TA).

**Send this information to Angela WITHIN 3 MONTHS of starting the trial:**  
[angela@vinventive.com](mailto:angela@vinventive.com)

## RACKING AND BOTTLING

1. Take 10 mL sample from each treatment immediately after racking (record the date) and another immediately prior to bottling (record the date). Freeze for phenolic analysis.
2. Keep 12 x 750 mL bottles of each treatment separate for phenolic analysis and tasting at >= 6 months bottle age.

## PHENOLIC EXTRACTION ASSESSMENT

This requires the use of a UV-Visible Spectrophotometer, and can provide results on the same day that the samples are prepared for analysis. If UV-Vis spectro is not available the samples can be sent off-site to an analytical laboratory for preparation.

If you have a UV-Visible Spectrophotometer, subscribe to AWRI for use of the Grape and Wine portal through: <http://www.thewinecloud.com.au>

**Procedure:**

Thaw the daily wine samples, prepare them for phenolic extraction using AWRI procedure (attached).

**Send the results to Angela WITHIN 3 MONTHS of starting the trial for interpretation relevant to**

**ACE maceration:** [angela@vinventive.com](mailto:angela@vinventive.com).

## Appendix 5.2 Sample preparation for AWRI grape portal

Sample preparation guide – analysing grape samples for tannin, colour and phenolics measures using the Grape Portal

### Equipment

- UV/Visible Spectrophotometer capable of measuring in the range 250 – 520 nm.
- 10 mm path length **Quartz** cuvette (**plastic cuvettes cannot be used for tannin analysis**).
- Sealed QC reference standard cuvette (supplied by AWRI).
- Calibrated pipettes capable of delivering 1 mL and 10 mL volumes. (You could also use a 10 mL dispenser to deliver the acidified ethanol.)
- Homogeniser appropriate to sample size:
  - For samples 50-100g: Ultra-Turrax T25 high speed homogeniser with an S25 N - 18 G dispersing element (Janke & Kunkel GmbH & Co, Germany).
  - For samples 200-250g: Retsch Grindomix GM200 Homogeniser (Retsch GmbH & Co KG, Germany), fitted with a floating lid.

(The homogeniser used must be capable of breaking up grape seeds so that the seed tannin can be extracted. Both the Retsch and Ultra-Turrax can do this, but the Retsch is easier to use. A general kitchen blender is not powerful enough.)

- Centrifuge capable of radial centrifugal force (RCF) of at least 1800 G.
- Mixing device e.g. Chiltern rotating wheel, shaker table or roller mixer (optional).
- Centrifuge tubes.
- Analytical balance with minimum scale reading 0.01g.

### Reagents

- 1.0M Hydrochloric Acid.
- **Acidified** 50% v/v ethanol in Milli-Q (or equivalent) water, prepared by adding 4.4 mL concentrated HCl (10N) to 1 L of 50% v/v ethanol.

**Sample preparation, storage and holding times** Grape samples can be analysed fresh or be frozen (-20°C) prior to analysis. Fresh samples must be stored cool (~4°C) and analysed within 24 hours of collection. Grape samples can be stored frozen, as whole berries, for a maximum three months before analysis.

### Sampling

Take a representative sample just prior to homogenisation, using the following procedure:

- If the sample contains bunches, remove all berries from the rachis by hand and place into tray or container. If the berries are loose, just place all berries into a tray or container.
- Gently mix the berries by hand being careful not to split the skins of any of them.
- Randomly take berries from different areas within the container until you have the required amount and place them into a clearly labelled container. If using the Retsch homogeniser, choose approximately 200 berries/200g and if using the Ultra-Turrax homogeniser, choose approximately 100 berries /100g. If berries are frozen, they should be thawed overnight in a refrigerator and processed cold (below 10°C) to minimise oxidation of colour components.

### Homogenisation

Homogenise the sample using the settings appropriate to the model of homogeniser:

- Ultra-Turrax – 30s at 24000 rpm, then scrape the homogenate from the shaft into the vessel, then a further 30s at 24000 rpm.
- Retsch – 20 seconds at 8000 rpm.

Ensure that all seeds are thoroughly macerated and, if using the Ultra-Turrax homogeniser, ensure all homogenate is scraped from the shaft and collected in the homogenising vessel. **Once homogenised, samples must be extracted within four hours.**

### Extraction

- Mix the homogenate well and then weigh approximately 1g into a 10 mL centrifuge tube, recording the homogenate weight.

- Add 10 mL of acidified 50% Ethanol/Milli-Q.



- Allow samples to extract for 1 hour with constant mixing. If a mixing device is not available, mix by inverting the tube approximately every 10 minutes over a period of one hour. Ensure that mixing is efficient and that the pellet does not become lodged in the bottom of the tube.
- Centrifuge homogenate/ethanol mixture at your centrifuge's maximum speed until fully clarified. Check after five minutes whether or not you have fully clarified your sample, and if not, keep centrifuging. Once clarified, the supernatant can be considered the homogenate extract. Homogenate extracts can be stored frozen (at -20 °C) for up to three months without significant loss of colour.

### **Incubation**

- Add 10 mL of 1.0M HCl to 1 mL of homogenate extract in a new tube and mix well (total volume will be 11 mL).
- Incubate for at least 1 hour and no longer than 24 hours, preferably in a dark place.
- During the incubation period, turn on your spectrophotometer to ensure adequate warm up, and perform instrument diagnostics if available.

### **QC standard check**

On any day that you wish to analyse grape or wine samples for tannin, phenolics and colour, you will need to measure your QC standard at seven wavelengths and upload the data to the WineCloud. This allows you to monitor the performance of your instrument. The QC standard check only needs to be done once per day, not with every set of samples analysed. To do this:

- Set your spectrophotometer for measurements at 250, 270, 280, 290, 315, 320 and 520 nm.
- Zero the instrument with air (i.e. no cuvette present).
- Measure your QC standard cuvette at the seven wavelengths listed above and enter your data directly into the Samples page of the Grape Portal.

### **Reading your diluted extracts and uploading your data**

- Zero with 1.0M HCl in 10 mm quartz cuvette.
- Measure diluted grape extracts at 280, 320 and 520 nm (11 mL will allow two rinses of the cuvette between samples) and record the absorbance readings.
- Once a set of samples has been completed, add the data to the Grape Portal either via the sample upload spreadsheet or by direct data entry onto the Samples page. Your results will be calculated immediately.

### **Help?**

If you need help, you can email the AWRI at [thewinecloud@awri.com.au](mailto:thewinecloud@awri.com.au) or phone the AWRI on +61 8 8313 6600.

### **Appendix 5.3 Sample preparation for AWRI wine portal**

Sample preparation guide - analysing wine and ferment samples for tannin, colour and phenolics measures using the Wine Portal

#### **Part (a) – Calculation of tannin, total pigment and total phenolics**

##### **Materials**

- 10 mm path length **quartz** cuvettes (plastic cuvettes cannot be used for tannin analysis)
- 1 M HCl
- 10mL test tubes
- Pipettes, tips, wipes
- UV/VIS Spectrophotometer
- Sealed QC Reference standard cuvette – provided by the AWRI

##### **Method**

NOTE: This method is suitable for analysing wines that have completed alcoholic fermentation (classified as post-ferment) and ferment samples from Day 3 of fermentation onwards. It is not suitable for analysing juices or ferments earlier than Day 3. If ferments or wines are hazy, clarify first by centrifugation.

##### **Dilution and Incubation**

- Add 10 mL of 1M HCl to a 10 mL test tube.

- Add 200 µL ferment or wine sample to the tube and mix. Ensure at least one tube is set aside containing only 1M HCl to use as a blank.

- Incubate blank and diluted samples at room temperature (18 -21°C) for at least one hour. Note that a longer incubation time than one hour is fine, but absorbance readings should be taken on the same day as samples are diluted.

- During incubation period, turn on the spectrophotometer to ensure adequate warm up and perform instrument diagnostics if this facility is available.

##### **QC standard check**

On any day that you wish to analyse grape or wine samples for tannin, phenolics and colour, you will need to measure your QC standard at seven wavelengths and upload the data to the WineCloud. This allows you to monitor the performance of your instrument. The QC standard check only needs to be done once per day, not with every set of samples analysed. To do this:

- Set your spectrophotometer for measurements at 250, 270, 280, 290, 315, 320 and 520 nm.

- Zero the instrument with air (i.e. no cuvette present)

- Measure your QC standard cuvette at the seven wavelengths listed above and enter your data directly into the Samples page of the Wine Portal.

##### **Reading your samples**

- Set your instrument for measurement at 250, 270, 280, 290, 315 and 520 nm.

- Zero with 1M HCl (Blank) in 10 mm pathlength quartz cuvette.

- Measure diluted samples at 250, 270, 280, 290, 315 and 520 nm using a 10 mm pathlength quartz cuvette.

NOTE: if the spectrophotometer is double beam, zero the instrument with no cuvettes in either path, then place a 1M HCl blank in the reference beam and take readings with samples in the sample beam.

- Data can then be uploaded via spreadsheet or entered directly into the Samples page of the Wine Portal for immediate calculation of results.

#### **Part (b) – Optional additional step to calculate pigmented tannin and free anthocyanins**

If you wish to calculate free anthocyanins and pigmented tannins for your samples, you will also need to carry out the following additional step. This part of the method cannot be performed in isolation; it should always be done at the same time as part (a) because readings from part (a) are used in the calculations for part (b).

**Additional materials**

- 5M NaOH
- Tartaric Acid
- AR Ethanol
- Sodium metabisulfite
- pH meter

**Buffer preparation (100 mL, scale accordingly for larger amounts)**

- Dissolve 0.5 g of tartaric acid in approximately 50 mL of water.
- Add 12 mL of ethanol and then make up total volume to 100 mL.
- Using a pH meter adjust the overall solution pH to 3.4 by dropwise addition of 5M NaOH.
- Add 0.38 g of sodium metabisulfite and dissolve.
- Place in a tightly stoppered container and store in cool dark place.

Please note that this buffer solution has a shelf life of **one week** after which it must be freshly prepared.

**Sample preparation and measurement**

- Place 1 mL of wine or ferment sample in a 10 mL test tube and then add 9 mL of the buffer solution.
- Mix sample.
- Set aside one 10 mL test tube containing just the buffer solution. This will be your blank.
- Incubate blank and diluted samples at room temperature for at least 1 hour. As in part (a), a longer incubation time is fine, but readings should be taken on the same day that samples are diluted.
- After you have completed reading the absorbance of the samples diluted in acid as described in part (a), re-zero your spectrophotometer using the blank buffer solution and then measure the absorbance at 520 nm of your samples diluted in buffer. This reading should be recorded as A<sub>520</sub> buffered, and should be entered into the Samples page of the Wine Portal along with the readings generated in part (a).

**Safety**

- Ensure that laboratory staff members wear adequate personal protection equipment at all times, including lab coat, safety glasses, closed-in footwear and disposable gloves.
- Diluted samples from part (a) are strongly acidic. Neutralise or dilute with copious amounts of water when disposing. Do not pipette any reagents by mouth.

**Help?**

If you need help, you can email the AWRI at [thewinecloud@awri.com.au](mailto:thewinecloud@awri.com.au) or phone the AWRI on +61 8 8313 6600.

#### ***Appendix 5.4 Sensory Evaluation Technical Quality Report***

(Note this data was provided by AWRI in pdf format and is attached to electronic copy)

#### ***Appendix 6: Budget reconciliation***

Submitted on-line 27 October 2016