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TASMANIAN INSTITUTE OF AGRICULTURE

Improved red wine process efficiency and product quality through enhanced phenolic extraction by Controlled Phenolic Release (CPR) and berry pricking



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Improved red wine process efficiency and product quality through enhanced phenolic extraction by Controlled Phenolic Release (CPR) and berry pricking

Authors and Contributors

Tasmanian Institute of Agriculture (TIA)

Dr Anna L Carew

Dr Angela Sparrow

Dr Angela Merry

A/Prof Dugald C Close

Acknowledgements:

Dr Fiona L Kerslake (Tasmanian Institute of Agriculture)

Dr Robert Dambergs

Dr Paul Smith (Australian Wine Research Institute)

Dr Keren Bindon (Australian Wine Research Institute)

Dr Kai Knoerzer (CSIRO)

Mr Piotr Swiergon (CSIRO)

Mr Dylan McMahon (Seville Estate, VIC)

Mr Dan Buckle (Domain Chandon, VIC)

Mr Simon Black (Montalto, VIC)

Mr Franco D'Anna (Hoddles Creek Estate, VIC)

Mr Jeremy Dineen and Mr Ockie Myburgh (Josef Chromy, TAS)

Mr Brett McLean (Brown Brothers)

Ms Kellie Hoffmann (Treasury Wine Estates)

Tasmanian Institute of Agriculture, Private Bag 98, Hobart 7001.

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Abstract

Two novel approaches to Pinot Noir winemaking were investigated; Controlled Phenolic Release (CPR) by microwave maceration of must, and Accentuated Cut Edges (ACE) (initially referred to as ‘berry pricking’) to increase diffusion from grape skins. Industry engagement was achieved through Distributed Industry Trials conducted in working wineries, and wines were subjected to full chemical and sensory analysis. The novel wines were phenolically and sensorially acceptable. Each process demonstrated a relatively short payback period, and ACE moved to follow-on investigation (VIN 1501). CPR improved extraction efficiency, but human factors and the existence of competing technologies suggested CPR was unlikely to be developed further at this time.

Executive Summary

This project investigated two novel approaches to Pinot Noir winemaking; Controlled Phenolic Release (CPR) by microwave maceration of grape must and Accentuated Cut Edges (ACE) (initially referred to as 'berry pricking') processing to increase diffusion from grape skins. Early industry input and engagement was achieved by running trials in working wineries (Distributed Industry Trials) and wines were subjected to full chemical and sensory analysis. Of the approximately 36 trial wines made over the course of the project, only one was identified as faulty (due to a failure in SO_2 management), demonstrating robust winemaking processes across control and novel winemaking in this project.

Chemical and sensory analysis of the Pinot Noir wines produced in this project yielded significant differences associated with the novel maceration treatments applied. Wines made with microwave maceration (CPR) and fermented on skins showed differences in phenolics concentration out to two years post-bottling, with up to two-fold higher tannin concentration, and higher anthocyanin and total phenolics concentration.

In a comparison of seven different maceration treatments including CPR on skins, CPR with early pressing, ultrasound maceration and standard winemaking processes, phenolic, tannin and monosaccharide composition of Pinot Noir wines was shown to vary by maceration treatment. The most notable treatments in terms of impact on wine chemistry, were the two CPR treatments and the ultrasound treatment. CPR wines with early pressing were similar to control wines in terms of phenolics concentrations, although they had approximately half the time on skins. Formal sensory appraisal of CPR with early pressing wines showed they had unique sensory characteristics that were, on average, preferred to control wines.

While the relative impact of CPR treatments was the main focus of much of this project, the impact of ultrasound treatment on the composition of wines was investigated and may warrant further investigation. In particular, it would be interesting to examine sensory differences between control, CPR and ultrasound wines, and to ascertain if observed significant differences between control and ultrasound wines in terms of monosaccharide concentration had sensory significance. Another reason for further investigation of ultrasound is that the technology is established, relatively low cost, and offers few OHS risks in the industrial setting.

In contrast to the findings with Pinot Noir, the impact of CPR and ultrasound maceration treatments on Shiraz wine phenolics concentration was generally non-significant. The results suggest that existing wine making approaches for Shiraz are effective at extracting phenolics from this variety.

Accentuated Cut Edges (ACE) maceration involves the cutting of grape skins into smaller fragments than is usual, consequently increasing the rate of extraction of colour and flavour components from the broken edges of the grape skins. The procedure was demonstrated to increase the rate of phenolic extraction to such an extent that pressing the grape solids off prior to the end of fermentation with fermentation completed in a smaller tank was investigated. This is referred to as Press Early (PE) ACE maceration or 'PEACE maceration'.

Phenolics of samples taken daily in commercial trials showed that ACE maceration caused a dramatic increase in the extraction of colour and tannin in the first two days of fermentation. Six months after bottling, the average increase in tannin and stable pigment concentration, relative to conventionally made wine, was 45% for ACE wines, while for the PEACE wines the concentrations were 35% and 30% respectively. Sensory panelists judged PEACE wines to be superior in phenolic composition relative to their control and ACE counterparts and described PEACE wines as having more fruity and floral attributes than the control wines.

The ACE maceration technique in combination with early press off to make PEACE wines has considerable potential as a technique to help commercial winemakers manage compressed vintages. Reducing the time in the fermentation tank by 25 to 50% is likely to provide significant advantages for the Australian wine industry.

The business case for each of the novel processes examined in detail in this project with Pinot Noir wine making demonstrated acceptable payback periods (approximately four years for CPR, less than 12 months for ACE). The ACE process moved to wider trials with additional Wine Australia funding (VIN 1501). The CPR process was found to produce sensorially preferred wines in approximately half the time of standard winemaking processes, and have the potential to generate more strongly extracted wines from low colour and tannin fruit. However, human factors (e.g. OHS and operator familiarity, local capacity for design and maintenance of units) and established technologies that may offer similar extraction efficiencies (e.g. standard thermovinification, Flash Release) mean that microwave maceration is unlikely to be developed further at this time.

In addition to actively engaging winemakers in research ('embedded extension across the life of the project') and providing proof of concept for CPR and ACE winemaking, the project delivered fundamental wine science advances. Valuable new knowledge was generated on the analysis of red wines and formation of pigmented polymers, with a comparison of extended maceration and CPR wines showing differentiation of the size and conformation of polymers associated with different extraction pathways for the constituent colour and tannin compounds. Additionally, the control and CPR wines provided independently replicated wines of diverse character made from single batches of fruit. This allowed AWRI colleagues to test and prove the efficacy of a new red wine analysis (macromolecular complexes), and allowed the research team to undertake novel integration of chemical analyses data across phenolic, tannin composition, monosaccharide concentration, aroma and sensory analysis to build rich representations of wine character.

Background

TIA's research into rapid phenolic extraction was prompted by evidence that maceration was a constraint to winery operations. Winemakers reported skin contact regimes of two weeks or greater. Reducing the time red wine spends on skins will increase the efficiency of red wine production. TIA found microwave maceration to be highly effective for rapid extraction of phenolic compounds in red winemaking (Carew et al., 2013a) and to be superior to thermal maceration in impact on grape integrity (Carew et al, 2014). The final concentration of phenolics in Shiraz and Pinot Noir juice and wine was shown to vary with the duration of hold time after microwave maceration (e.g. Carew et al., 2013). Together, microwave maceration and managed hold time provided Controlled Phenolic Release (CPR). Proof of concept was established for Accentuated Cut Edges, ACE (initially referred to as 'berry pricking') during semi-commercial trials of skin fragmentation with Pinot Noir undertaken in 2013. Skin fragmentation produced wines with a significantly greater concentration of tannin and colour compounds, compared with control wines fermented on skins (Sparrow et al., 2016). Formal sensory analysis of these wines conducted by the AWRI at 18 months bottle age found that PEACE wines exhibited more fruit and floral characters and softer tannins. This project investigated CPR (15kW-200kg/hour) and ACE maceration processes with winemaker collaborators to establish how resulting musts and juices can become premium quality wines. Eight innovative winemakers volunteered and their active participation will aid future industry uptake. Engineers at CSIRO Animal, Food and Health Sciences, Victoria collaborated on CPR, opening pathways into CSIRO's extensive experience of novel food technologies that are not currently used in winemaking.

Wine phenolics concentration has been correlated with quality. Analysis of 1,643 red wines showed total phenolics and tannin to be positively correlated with wine grade (Mercurio et al., 2010) and retail price (Dambergs 2013). Pinot Noir grapes can be low in anthocyanins (Cliff et al., 2007) and those anthocyanins can be unstable (Heazlewood 2006). Analysis of 1,325 wines showed Pinot Noir and Shiraz were lowest in tannin out of five red varietals (Harbertson et al., 2008). For low phenolics varieties, more effective phenolics extraction could lead to better quality red wine. Cold soak and extended maceration are used to increase red wine phenolic concentration (Haeger, 2008, Ponte et al., 2004) but these constrain winery throughput. Thermal maceration is effective for fast phenolic extraction (Sacchi and Bisson et al., 2005) however current technologies like Flash Detente have not been adopted in Australia, perhaps due to the large capital cost and lack of familiarity. Flash Detente was shown to be ineffective for early press-off wine (Morel-Salmi et al., 2006), whereas CPR and ACE early press-off wines were equivalent to control wines for phenolics (Carew et al., 2014, Sparrow, 2016). Microwave has been demonstrated to be highly effective for extraction of compounds from plant material (Casazza, et al., 2010; Liu, et al., 2009; Routray and Orsat 2012) but has only recently been applied in winemaking for phenolics extraction (Carew et al., 2013). Microwave is a flexible food technology which is currently used in Australia and overseas for food processing (Schubert and Regier, 2005). While cutting whole fruit into small pieces is a common practice for many food processing industries, it has seldom been applied to winemaking.

The ACE maceration process does not homogenise the fruit but instead it fragments the grape skins without damaging grape seeds. (Sparrow et al., 2016 ab).

The business of winemaking is constrained by its intense seasonality with the vintage lasting around six weeks in most regions. In red wine fermentation, pomace occupies ~40% of tank space. The option to limit skin contact time or ferment off-skins offers wineries expanded processing capacity, without the need for further capital investment. Additionally, pressed-off CPR juice fermented about two days faster than controls fermented on skins while pressed-early ACE wines were found to halve the fermentation time without compromising wine quality, providing further scope for more efficient use of available winery capacity. The impact of CPR on laccase may relax constraints on the use of Botrytis-affected fruit, enabling greater fruit utilisation.

Project Aims

1. Design and optimise process control for CPR applied to red wine grape musts at pilot scale (15KW-200kg/hr)
2. Collaborate with winemakers to establish best practice CPR winemaking
3. Collaborate with winemakers to establish best practice ACE winemaking
4. Measure long-term wine phenolic and aroma development of CPR and ACE wines
5. Undertake formal sensory appraisal of CPR and ACE wines
6. Build the capacity to make premium wine from CPR musts and juices in commercial wineries (Victoria, Tasmania)
7. Familiarise winemakers with the advantages and limitations of ACE for red wine making
8. Evaluate the business case for ACE and CPR, compared with red wine making practices currently used
9. Disseminate the CPR and ACE processes across the Australian wine industry

Chapter 1. Using microwave heating to enhance phenolic extraction in on-skins fermentation

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Abstract

In this chapter we describe the first year of semi-commercial scale trials for controlled phenolic release (CPR) using microwave maceration of approximately 400 kg per replicate of fruit through a 15 MW pentagonal microwave unit. The first year of trials allowed us to establish microwave maceration processes to simulate an in-line operation that would be suited to wineries. We also engaged with three respected Victorian Pinot Noir winemakers to provide expertise, fruit, and make control and CPR wines. Control and CPR wines were fermented on skins for approximately 8 days, and plunged, pressed off and barrel-aged identically.

As expected with a Distributed Industry Trial (one pair of wines at each site for replication), no statistically significant differences were observed between control and CPR wines. Analysis of the phenolic fingerprint and wine aroma volatiles by Principal Component Analysis however, showed a similar pattern of separation between control and CPR wines, suggesting a relatively consistent effect between wineries. Informal sensory appraisal of wines in blind tastings with industry panels suggested that there were sensory differences between the wines, with CPR on-skins wines being perceived as more extracted in general than control wines.

The activity reported here provided proof of concept and technical process information which guided trial work and process analysis in subsequent years (see Chapters 2 to 6).

Method

Wineries, risk management and fruit

Four commercial wineries were recruited to participate in semi-commercial trials of microwave maceration of Pinot Noir fruit with the aim of enhancing phenolic extraction during a standardised eight-days skin contact winemaking process. Three wineries volunteered to participate during a tasting of small lot microwave maceration wines at a Yarra Valley Winegrowers Association meeting (May 2013). A fourth winery volunteered to participate following Dr Carew's presentation at the 15th Australian Wine Industry Technical Conference (July 2013). The rationale for conducting these trials in-house across industry sites, and with winemakers fully engaged was explained in a presentation on Distributed Industry Trials at Crush 2014 (Appendix 5.5).

The process of moving grape material from wineries in the Yarra Valley, across Melbourne to CSIRO Werribee, and back again required approval from the Victorian Department of Environment and Primary Industries due to phylloxera risk. Copies of the permit application and risk plan are provided in Appendix 5.2.

Fruit was harvested at commercial ripeness by each of the participating wineries during March 2014. Due to poor harvest volume, however, one of the wineries withdrew, leaving three participants: Domain Chandon, Hoddles Creek and Seville Estate. For each winery, two lots of approximately 400 kg of Pinot Noir fruit were crushed and destemmed into two picking bins supplied by the research project (Figure 1). For each winery, one bin of must was transported to CSIRO Werribee for microwave processing, and returned to the winery for initiation of alcoholic fermentation within 24 hours of harvest.



Figure 1: Winemaker Dylan McMahon tipping trial fruit to the crusher destemmer at Seville Estate, Victoria.

Simulated in-line process for microwave maceration of grape solids

During 2014 vintage work at the CSIRO Food Innovation Centre at Werribee (Victoria), the team trialed several processes for moving grape must through the CSIRO's semi-commercial scale 15MW pentagonal tunnel microwave unit. The unit was a custom-built array of 1MW magnetrons arrayed in three banks around a 100mm wide conveyor belt (see Figure 2). The path through the pentagonal microwave unit was 1800mm and microwave heating kinetics could be adjusted through switching individual magnetrons or banks of magnetrons on or off, or by adjusting the speed of passage (belt speed). The pentagonal microwave unit employed for this experimental work was approximately 20 years old, and demonstrated some of the limitations of the use of water cooling for magnetrons; magnetrons would trip out when overheated and this occurred often during the 2014 trial work.



Figure 2: Experimental semi-commercial scale 15MW pentagonal microwave unit at CSIRO Werribee, Victoria.

The initial methods trialed for moving must through the unit were unsuccessful. These included using a must pump to transmit must through a food grade line, which failed because the line needed to be full and the grape must reasonably homogenous to optimise microwave energy efficiency and attain even optimal heat (70°C). The nature of the grape must was that it separated and pumped in 'plugs' of solids, rather than as a homogenous liquid. In other words, it was a two-phase mixture for the purposes of pumping and microwaving. An internal auger developed by CSIRO's Clayton Workshops and designed to minimise physical damage to grape solids and move must through the unit in a steady flow to keep the line full (Figure 3), was also unsuccessful as solids became lodged in both the hopper and auger, which caused the juice to separate from the solids.



Figure 3: Pilot internal auger Grape Must Conveyor developed at CSIRO Clayton, Victoria to move grape material through conveyer passage of pentagonal microwave unit. Additional detail, including concept drawings is provided in Appendix 5.1.

In response to these early unsuccessful attempts at moving whole must through the experimental microwave unit at an even flow rate and maintaining a full line, the project team decided to separate grape solids from juice and apply microwave only to the solids. This offered the opportunity for greater energy efficiency by only heating the material from which we were attempting to increase the diffusion of phenolics, and also the opportunity to off-set energy use by chilling must after microwave treatment.

The project team used hand pressing against food grade stainless steel screens to simulate the separation of grape solids from juice that would be achieved using the initial conveyor section run of an incline drainer (Figure 4), and achieved grape solids that were approximately 62% the weight of whole must.

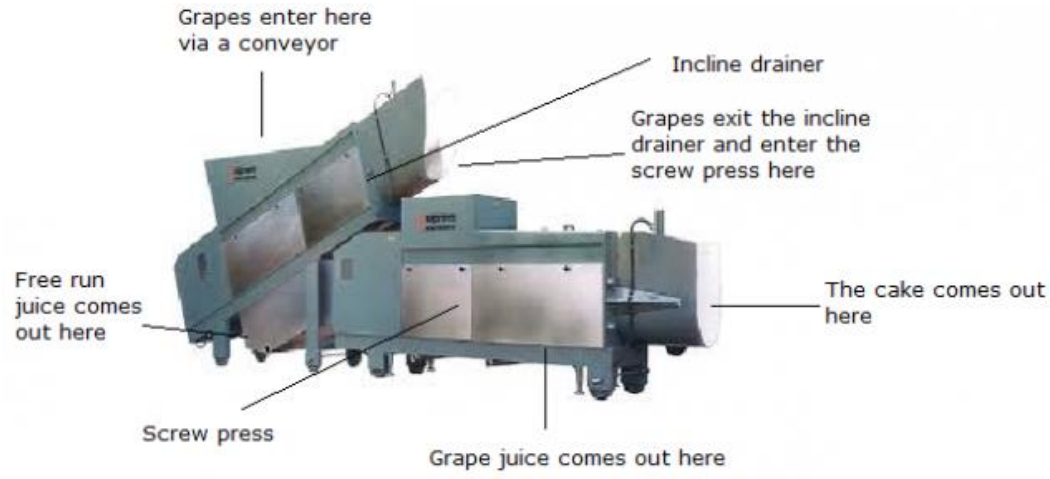


Figure 4: An incline drainer is a familiar piece of winemaking equipment offering in-line processing to separate grape solids from juice prior to in-line processing in a conveyor belt microwave unit (Image accessed 19/04/17 from http://simallwines.com.au/index.php/2017/02/24/typesofpresses/)

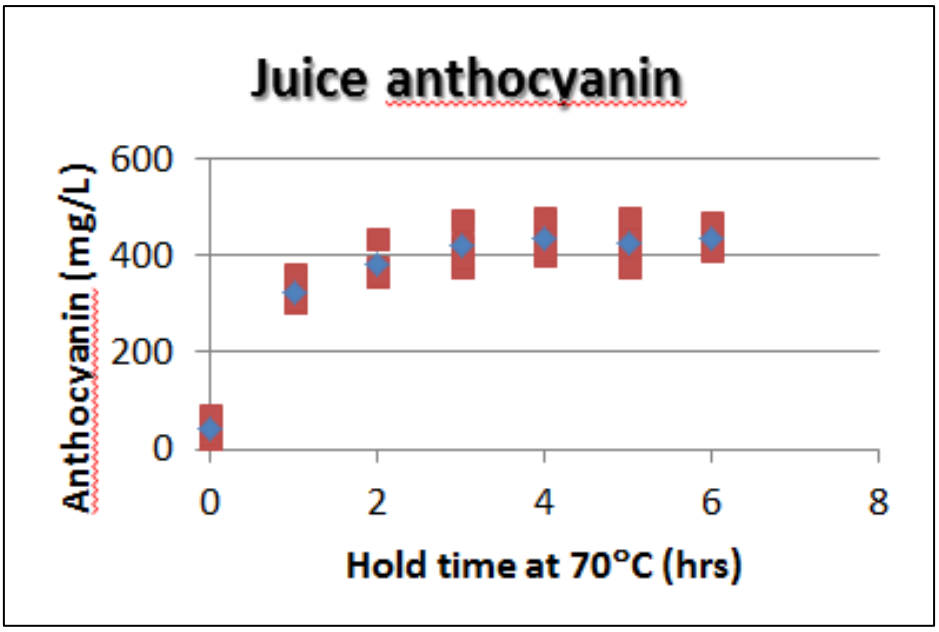
Grape solids were packed into Cryovac 124 x 94 x 64 mm food-grade polypropylene rectangular plastic tubs, and placed in succession on the microwave unit conveyor belt (Figure 5). Each approximately 20th tub had optic fibre thermal probes with four data-read points inserted in grape material, and the microwave belt speed was adjusted to ensure that grape material reached an optimum temperature of 70°C prior to exiting the microwave unit. During 2014 vintage work, transmission of grape material was slow because the microwave unit cooling system was malfunctioning, causing magnetrons to trip out from overheating. For grape solids processed in 2014, the transmission rate was 74 kg of drained grape solids per hour. This equated to a processing rate of approximately 150 kg of whole grape must per hour, or a potential processing rate for 1 tonne of whole must per 6.5 hours.



Figure 5: Drained grape solids packed into food grade tubs simulating in-line processing through a microwave unit conveyor belt.

Hold time and chilling of must after microwave maceration

Microwave macerated grape solids were aggregated into 20L food grade plastic buckets post-microwave, and held for 1 hour to optimise heat-mediated mass transfer of phenolics from grape solids into juice. The bulk density of the partially drained grape solids was approximately 650 kg/m^3 and while this was a multi-phase solution, the thermal resistance of grape solids sufficiently offset the juice component such that latent heat loss from buckets was minimal. The mean temperature of grape solids after a hold time of one hour was 63.4°C. Previous research demonstrated that grape solids microwave heated to 70°C and held for one hour yielded a six-fold increase in concentration of anthocyanins and an eight-fold increase in tannin concentration, compared with microwave macerated grape solids at 0 hours hold time (Figure 6). We have hypothesised that this extraction process has two mechanisms; microwave-mediated physical degradation of grape vacuolar integrity, and acceleration of mass transfer (diffusion) due to heat (Fick's Law).



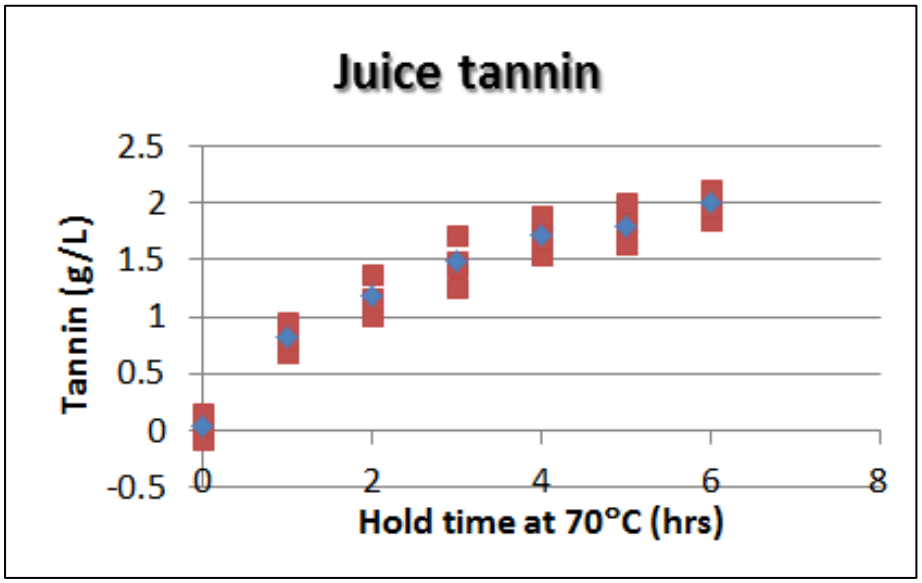


Figure 6: Pinot Noir grape must concentration of anthocyanin and tannin as a function of hold-time following microwave maceration to 70°C. Blue diamonds are mean concentration from three replicates, ochre bars show standard error (from Carew, 2014).

Following hold time, grape solids were combined with juice that had been held at -20°C for the approximate five hours between finish of hand-separating solids from juice, and finalisation of maceration and extraction time. Reconstitution was carried out in a Cleveland Kettle that was chilled with 14°C water circulated through an external sleeve (Figure 7). Must was decanted into a clean picking bin at 27°C, and transported back to the participating winery for alcoholic fermentation.



Figure 7: Chilling grape must in a kettle with slow sweep mixing, after reconstitution of microwaved solids and freezer-chilled juice.

Winemaking

The winemaking protocol was derived from that developed for small scale microwave winemaking in previous years (Carew et al., 2014). At each winery, microwave macerated must and control must were inoculated with cultured yeast supplied by Lallemand, which was rehydrated to the manufacturer's specification. The RC212 yeast strain was selected as it has been widely used for Pinot Noir winemaking in Australia (anecdotally) and North America (Haeger, 2008), and had proven reliable in previous experimental work (Carew, 2014). Control and treatment musts were fermented side-by-side in participating wineries (Figure 8), plunged identically, and pressed off after equivalent skin contact time. One of the wineries pressed both replicates off at day 7, the remaining two wineries pressed the wines off at day 8. The comparatively small scale of making wines in single bin or 400 kg lot size meant that the largest participating winery decided to use traditional means for pressing (Figure 9) which was labour-intensive and messy. In future, larger scale trials on a higher throughput and more reliable microwave unit would be more appropriate for participation of more substantial wineries.

Wines were cellared in matched four year old barrels for malolactic fermentation and bottled approximately six months after pressing. Wines for analysis were stored in TIA's temperature-controlled cellar at 14°C, and a new bottle was opened for analysis at each bottle age period examined.

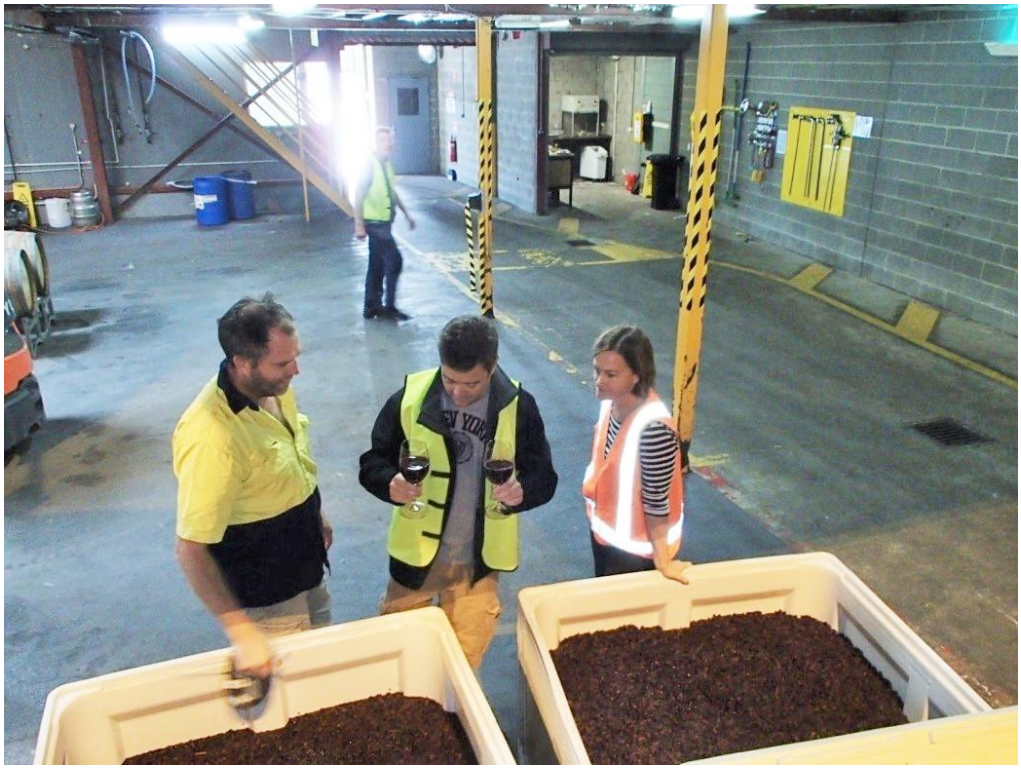


Figure 8: Winemaker Dan Buckle (Domain Chandon, Victoria) compares juices from microwave macerated and control musts, prior to inoculation of picking bins of fruit for primary fermentation.



Figure 9: Labour-intensive use of basket pressing in the largest participating winery due to 400 kg lots being too small for convenient use of standard large winery equipment.

Wine Chemical

Analysis of wine phenolics at six monthly intervals was by UV-Visible spectrophotometry as previously described (Dambergs, et al., 2012, Mercurio, et al., 2007; Carew, Close and Dambergs, 2015). Nine phenolic parameters were examined in wines: total phenolics (coloured and non-coloured tannin and anthocyanins, and low molecular weight, non-pigmented phenolics); total pigment (total red colour in the samples, including free anthocyanins and pigmented tannins); total anthocyanins (unbound and co-pigmented); colour density (pigment saturation); hue (higher values more ruby/garnet, lower values more purple); non-bleachable pigment (stable colour from pyranoanthocyanin formation, and anthocyanin and tannin complexing and has been correlated with pigmented tannin); and SO_2-resistant pigment (hue in the presence of high SO_2 concentrations, with low values indicating a greater concentration of purple-hued non-bleachable pigment); tannin by modified Somers method (pigmented and non-pigmented) and tannin by the MCPT method (measure of condensed tannins).

Volatiles

The analysis of wine volatiles was performed on an Agilent 7890 gas chromatograph equipped with Gerstel MPS2 multi-purpose autosampler and coupled to an Agilent 5975C XL mass selective detector. The gas chromatograph was fitted with a 30 m x 0.18 mm Restek Stabilwax – DA (cross-bond carbowax polyethyleneglycol) 0.18 µm film thickness column, and a 5 m x 0.18 mm retention gap. Helium was used as the carrier gas with a flow-rate of 0.8mL/min in constant flow mode. The oven temperature program started at 33 ºC for 4 mins, then heated to 60 ºC at 4 ºC/min, heated to 100 ºC at 16 ºC/min, then to 240 ºC at 25 ºC/min and held at this temperature for 2 mins. The volatile compounds were isolated using large volume headspace sampling and injected into a Gerstel PTV (CIS 4) inlet fitted with a Tenax TA liner. The injector was heated to 330 ºC at 12 ºC/min. Positive ion electron impact spectra at 70eV were recorded in scan mode. Wine samples (in triplicate) were diluted (2:5) in buffer solution (10% (w/v) potassium hydrogen tartrate, pH adjusted with tartaric acid to 3.4). A total of 16 authentic volatile compounds was analysed concurrently with the wine samples and each sample was spiked with deuterated internal standards.

Statistical Analysis

Means and standard deviation were calculated in Excel™ for phenolic concentration, and ANOVA in R (version 3.1.0) to assess for main effects and interactions. As this was a Distributed Industry Trial, we expected substantial vineyard-to-vineyard variation and so Principal Component Analysis (PCA) in R (version 3.1.0) was applied to data to establish whether treatments separated in a similar way regardless of vineyard, inferring treatment effects that might be reliable across a wide range of fruit and facilities.

Wine Sensory Appraisal

Due to the non-replicated nature of this trial, and as this was the first year of piloting the process, these wines were not subjected to formal sensory appraisal. Instead, they were used to gather feedback from participating winemakers and wider groups of winemakers through opportunistic tasting events in Tasmania, Victoria and South Australia. These included project presentations to the Yarra Valley Technical Committee meeting, an event run by the Pinot Massif (Mornington Peninsula), TIA Field Days and one-off meetings with Tasmanian winemakers.

Microwave maceration wines from an earlier trial were subjected to informal sensory appraisal by a panel of Yalumba staff late in 2014. The Yalumba panel included five experienced winemakers and three senior staff with approximately 10 years each experience in specialized aspects of wine production (e.g. laboratory analysis, vineyard management). Control, CPR on-skins and CPR early pressing wines (Carew et al., 2014) were tasted blind and ranked, and the tasting panel provided descriptive notes and blending/bottling recommendations as they were accustomed to doing during wine evaluation for routine decision-making as part of Yalumba winery operations. The descriptors of control and CPR wines were simplified and are presented as word clouds in Chapter 3.
Formal sensory analysis of microwave maceration wines is also presented in Chapter 2.

Results and Discussion

Phenolics

As expected from this form of Distributed Industry Trial, there were no statistically significant differences between vineyards/winery operations. This was expected and likely to be due to differences in climate, soil, clone and management practices being predictably dominant in effecting wine phenolic outcome. For this reason, trends using descriptive statistics within an individual vineyard are presented (at 0, 12 and 18 month bottle age; Tables 1, 2 and 3, respectively), interpreted and discussed given that we believe this will be of interest to industry.
At 0 month bottle age (Table 1) colour density, NBP and total phenolics were relatively (ca. 20-30%) higher in microwave wines at Hoddles Creek but not at other vineyards. In contrast, tannin was ca. 15-20 % higher at Domain Chandon and Seville Estate and ca. 150% higher at Hoddles Creek, in microwave wines relative to control wines.
In strong contrast to 0 months bottle age, at 12 months bottle age (Table 2), wines were generally similar across the various phenolic attributes although anthocyanins and tannins were increased and NBP decreased in microwave wines at Hoddles Creek, relative to control wines.
At 18 month bottle age, differences (Table 3) in phenolic attributes were not marked in Domain Chandon wines, in Hoddles Creek wines colour, hue, NBP and total phenolics were ca. 20-50 % increased and tannin ca. 90% increased in microwave wines and at Seville Estates colour, anthocyanins and total pigment were ca. 30-50% increased whilst hue and NBP were decreased in microwave wines relative to control wines.

Table 1: Phenolics concentration in commercially made control (ctl) and microwave (mwv) wines for each vineyard at 0 months bottle age.

	Domain Chandon		Hoddles Creek		Seville Estate	
	Ctl	Mwv	Ctl	Mwv	Ctl	Mwv
Colour density (AU)	6.58	6.58	5.36	6.35	4.88	4.49
Hue	0.731	0.759	0.673	0.699	0.784	0.79
Total anthocyanins (mg/L)	192	205	282	285	370	413
Non-bleachable pigment (AU)	1.16	1.197	0.77	1.02	0.753	0.707
Total Pigment (AU)	11.54	12.26	15.37	15.93	19.74	21.85
Non-bleachable pigment (%)	10.06	9.86	5.08	6.42	3.82	3.24
Total phenolics (AU)	33.2	35.9	20.7	23.8	30.7	32.2
Tannin (g/L)	0.66	0.759	0.052	0.133	0.28	0.337

Table 2: Phenolics concentration in commercially made control (ctl) and microwave (mwv) wines for each vineyard at 12 months bottle age.

	Domain Chandon		Hoddles Creak		Seville Estate	
	Ctl	Mwv	Ctl	Mwv	Ctl	Mwv
Colour density (AU)	6.56	7.295	5.245	5.03	5.045	5.005
Hue	1.13	1.15	0.80	0.81	0.97	0.95
Total anthocyanins (mg/L)	6	3.8	144.9	204.9	180.8	156.1
Non-bleachable pigment (AU)	2.61	2.36	1.39	1.165	1.14	1.1
Total Pigment (AU)	4.2	4.5	9.6	12.2	10.9	9.6
Non-bleachable pigment (%)	55.8	57.5	14.5	9.6	10.4	11.4
Total phenolics (AU)	48.1	48.1	31.1	38.6	42.1	40.9
Tannin (g/L)	1.59	1.58	0.55	0.83	0.98	1

Table 3: Phenolics concentration in commercially made control (ctl) and microwave (mwv) wines for each vineyard at 18 months bottle age.

	Domain Chandon		Hoddles Creak		Seville Estate	
	Ctl	Mwv	Ctl	Mwv	Ctl	Mwv
Colour density (AU)	3.22	3.38	2.97	4.65	2.82	3.63
Hue	1.39	1.40	0.89	1.07	1.33	1.01
Total anthocyanins (mg/L)	23	24	71	57	47	71
Non-bleachable pigment (AU)	1.11	1.1	1.1	1.48	1.12	1.15
Total Pigment (AU)	3.009	3.009	5.406	5.304	4.233	5.457
Non-bleachable pigment (%)	36.8893	36.557	20.3477	27.9034	26.4587	21.0738
Total phenolics (AU)	41.922	41.208	26.622	34.629	37.995	36.72
Tannin (g/L)	1.22749	1.2258	0.39400	0.76632	0.87152	0.90983

Phenolics analysis at 0 months bottle age was also examined by Principal Component Analysis (PCA), and a scores plot of these data demonstrated a consistent trend between wineries that microwave wines were more extracted at this time period, with an indication of overall higher phenolics concentration apparent (Figure 10).

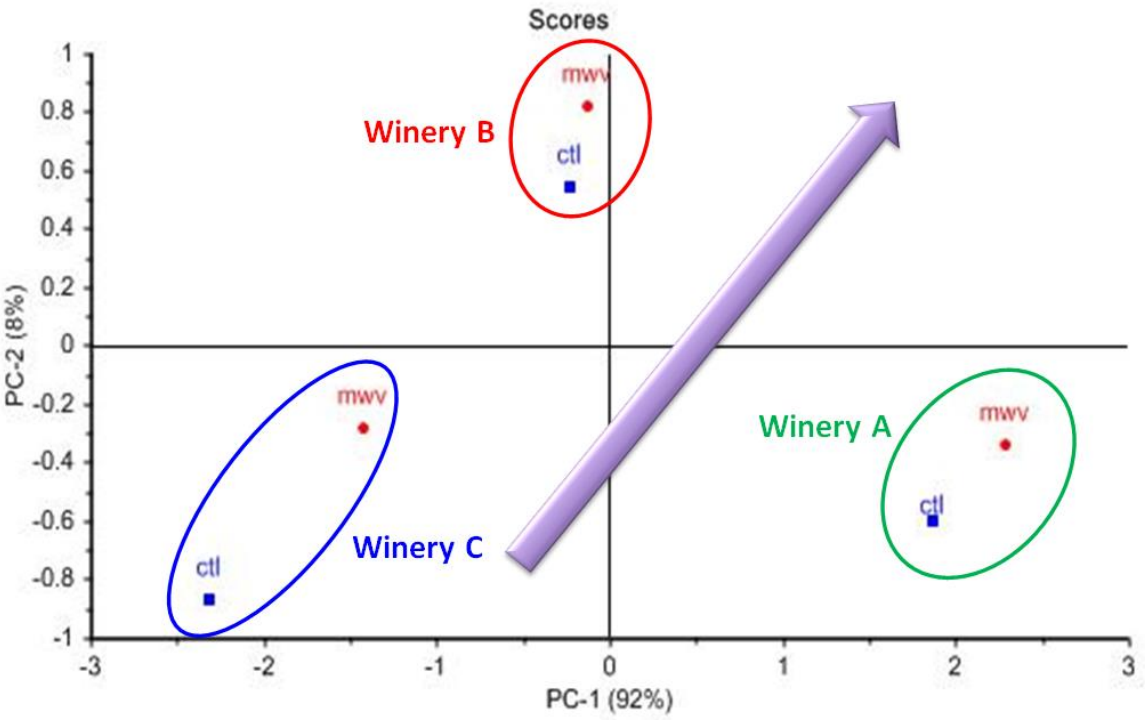


Figure 10: Principal Component Analysis scores plot showing consistent separation between control and microwave wines at 0 months bottle age.

Volatiles

Similar to phenolics in wines at 0 months bottle age, targeted analysis of wines for volatile compounds at 12 months bottle age showed a consistent direction of separation between treatments for control and microwave wines. In Figure 11 plate A, control and microwave treatment wines separate primarily on PC1 which explained 56% of the separation on the scores plot. The orientation of separation on PC2 (20% of separation) was not consistent between sites.

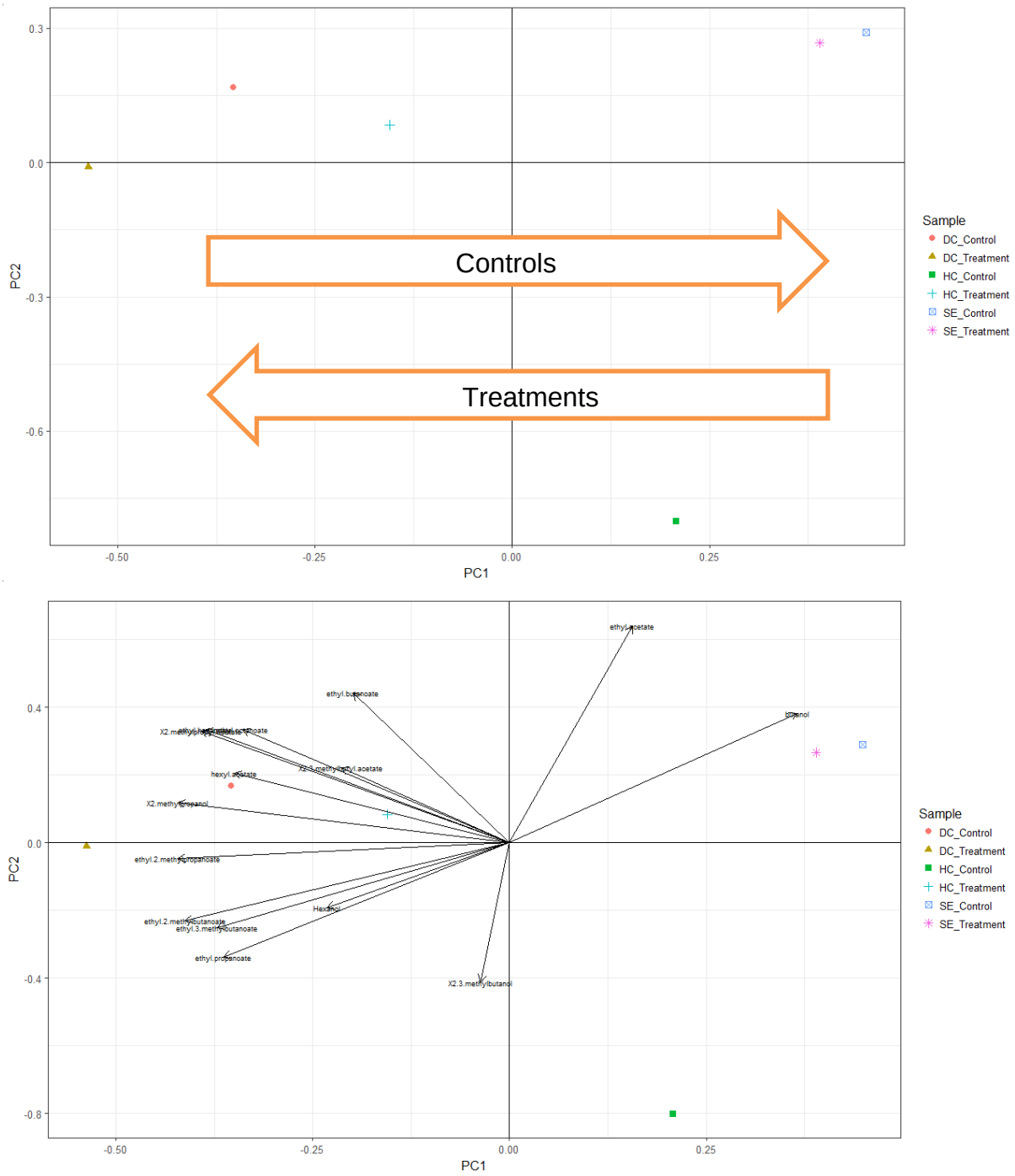


Figure 11: PCA scores plot for separation of control and microwave maceration wines for concentration of 16 volatile aroma compounds examined by GCMS. Plate A shows each replicate. Plate B shows eigenvectors for each of the aroma compounds examined. DC – Domain Chandon, HC – Hoddles Creek, SE – Seville Estates.

In addition to the results and learning generated by these Distributed Industry Trials, several small scale experimental winemaking trials were undertaken to compare control winemaking with winemaking using microwave maceration. Several of these trials are described in detail in Chapters 3 and 4, and one of these trials supported development of a novel analytical process to quantify and describe macromolecular complexes in red wine. The microwave macerated wine was selected for this analytical development on the basis that heat maceration was likely to have created a wine with unique chemical composition due to heat stress and physical degradation of grape solids. Figure 12, is the abstract from that published work, and the full paper is included as Appendix 5.3.

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Characterization of macromolecular complexes in red wine: Composition, molecular mass distribution and particle size

Keren A. Bindon [a,*], Anna L. Carew [b], Agnieszka Mierczynska-Vasilev [a], Stella Kassara [a], Fiona Kerslake [b], Paul A. Smith [a,c]

[a] The Australian Wine Research Institute, Hartley Grove, Urrbrae, 5064 Adelaide, SA, Australia
[b] Perennial Horticulture Centre, Tasmanian Institute of Agriculture, University of Tasmania, 165 Westbury Road, Prospect, Tasmania 7250, Australia
[c] Centre for Marine Bioproducts Development, Flinders University, Level 4, Health Sciences Building, Flinders University Registry Road, Bedford Park, SA 5042, Australia

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ABSTRACT

Precipitates were prepared from two compositionally different Pinot noir wines with addition of excess ethanol, and contained primarily polysaccharide, tannin and protein. The ethanol-soluble material was further fractionated into polymeric (tannin) and monomeric phenolics. Tannin associated with precipitates was of a higher molecular mass than that remaining in ethanolic solution. Wine fractions were reconstituted at the ratios of the original wine and analyzed using nanoparticle tracking analysis. The average particle size of the tannin fraction was 75–89 nm, and increased when combined with the precipitate (≅200 nm). Addition of the monomeric fraction to the tannin–precipitate complex increased both the incidence and concentration of smaller particles, reducing the average particle size. The formation of aggregates occurred in all fractions and only minor differences in particle size distribution were found between wines. Differences in particle concentration between wines appear to be due to differences in the total concentration of macromolecules rather than compositional differences.

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Figure 12: Abstract from Bindon et al. (2016) detailing differences in macromolecular concentration between control wines and wines made by microwave maceration.

Chapter 2. Using microwave heating to hasten production through early pressing

Acknowledgements

Mr Dan Buckle, Mr Dylan McMahon, Mr Simon Black, Mr Phillip Moraghan, Dr Angela Merry, Dr Kai Knoerzer, Mr Piotr Swiergon, Dr Paul Smith, Dr Keren Bindon

Abstract

This Chapter describes a highly efficient process for Pinot Noir wine production using Controlled Phenolic Release (CPR) by microwave maceration, and pressing off of wines after approximately 3 or 4 days skin contact time. Control and early pressing CPR wines were made at semi-commercial scale in four wineries in Victoria, Australia during the 2015 vintage. Batches of approximately 400 kg of fruit were treated by CPR and then fermented in winery side-by-side with control wines. Control wines were pressed off either 8 or 9 days after inoculation for alcoholic fermentation, whereas early pressing wines completed fermentation off skins.

Wine sensory and phenolics analyses demonstrated that CPR with early pressing is an effective option for production of Pinot Noir wine without faults, is richer in phenolic compounds, and has a distinct sensory character compared to control wines.

The main advantage offered by CPR with early pressing is relatively short skin contact time and the capacity to generate a more full-bodied wine from fruit that is light in colour and tannin. For those reasons, microwave with early pressing offers an opportunity for wineries to better diversify wines from blocks and available fruit, particularly in years with low colour. Wineries might also benefit from short skin contact time during high tonnage vintages, or years when vintage compression places severe pressure on tank space.

Method

Wineries, risk management, fruit and maceration

The major experiments examining early pressing were conducted during the 2015 vintage, with industry trials involving four Victorian wineries. Five wineries were recruited to participate including the three participants from 2014 vintage trials (Domain Chandon, Seville Estate and Hoddles Creek) and two wineries contacted via a tasting of wines made during 2014 trials which had been included in a tasting event at Moorooduc Estate (Mornington Peninsula, Victoria) in October 2014. The new wineries that volunteered were Montalto (Mornington Peninsula) and Curly Flat (Macedon Ranges).

Fruit was harvested at commercial ripeness by each of the participating wineries during March 2015. Due to vintage compression and Easter work stoppages at CSIRO, fruit from Hoddles Creek was unable to be scheduled for processing leaving four participating wineries. For each winery, two lots of approximately 400 kg of Pinot Noir fruit was crushed and destemmed into two picking bins supplied by the research project. For each winery, one bin of must was transported to CSIRO Werribee for microwave processing, and returned to the winery for alcoholic fermentation within 24 hours of harvest. Transportation risks associated with phylloxera were managed as in 2014 (Appendix 5.2). Microwave maceration was also undertaken in the same way as 2014 (see Chapter 1, Methods), with grape solids hand separated from juice, packed into tubs, microwaved to 70°C (Figure 1) and held at that temperature for one hour prior to reconstitution with juice that had been chilled. Musts were then transported back to the winery of origin for fermentation (Figure 2).



Figure 1: Grape solids packed into Cryovac 500 mL margarine tubs and heated to 70°C in the pentagonal microwave unit.



Figure 2: Winemaker Ben Kimmorley (Curly Flat, Victoria) comparing juice from microwave macerated must (left glass) with untreated control must (right glass), immediately prior to inoculation of musts in picking bins for primary fermentation.

Winemaking

At each winery, microwave macerated must and control must were inoculated with cultured yeast supplied by the winery. Yeast was rehydrated to the manufacturer's specification and applied in equal quantity to each picking bin of must. Control and treatment musts were fermented side-by-side in participating wineries, plunged identically until press off. For this trial, treatment wines were pressed off early; at day 3 in one winery and at day 4 in the remaining participating wineries. Early press off treatment wines completed alcoholic fermentation in barrel, under airlock. Control wines were pressed off at day 8. Wines were cellared side-by-side in pair-matched four year old barrels (Figure 3), and bottled off at approximately six months barrel age for cellaring at TIA's 14°C temperature-controlled cellar. For each six-monthly chemical analysis and for sensory analyses, a new bottle of each wine was opened.



Figure 3: Dr Carew with 'hot' and 'not' barrels of trial wine in the Domain Chandon cellar, Victoria.

Wine Chemical and Statistical Analysis

UV-Visible Spectrophotometry

Wines were examined for phenolics concentration at 12 months bottle age using a modified Somers method (Dambergs, Mercurio, Kassara, Cozzolino and Smith, 2012; Mercurio, Dambergs, Herderich and Smith, 2007). The phenolics quantified were: total tannin (pigmented and non-pigmented tannins); total phenolics (coloured and non-coloured tannins and anthocyanins, and low molecular weight, non-pigmented phenolic compounds); free anthocyanin (unbound or co-pigmented anthocyanins); non-bleachable pigment (stable colour from complexing of anthocyanins and tannins); colour density (the degree of pigment saturation of the wine); and hue (the nature of red wine colour - higher hue values being ruby-garnet, and lower values appearing more blue-purple). UV-Visible spectrophotometric analysis was conducted using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Samples were scanned in 10 mm cuvettes, at 2 nm intervals for the wavelength range 200-600 nm. Resulting absorbance data for selected wavelengths for each of the samples was exported to an Excel™-based phenolics calculator that had been developed by the Australian Wine Research Institute.

Sensory Analysis

Sensory evaluation of the wines at 24 months bottle age was carried out using the Australian Wine Research Institute's internal expert sensory panel. A 'compare and contrast' method was used to evaluate each pair of wine samples (treatment vs control). The tasters provided detailed tasting notes and rated each wine using the 20-point quality scoring system as used in Australian wine show judging. All tasters' comments and scores were provided for analysis. The panel was also instructed

to identify and rate the intensity of any perceived off-flavours or faults in the wines, and these notes are reported in the following section.

Statistical Analysis

Means and standard deviation were calculated in Excel™ for phenolic concentration. As this was a Distributed Industry Trial, we expected substantial vineyard-to-vineyard variation and so Principal Component Analysis (PCA) in R (version 3.1.0) was applied to data to establish if treatments separated in a similar way regardless of vineyard, inferring treatment effects that might be reliable across a wide range of fruit and facilities.

Results and Discussion

Phenolics

By 12 months bottle age, wines were examined for phenolic concentration (Table 1) and showed substantial variation across the two treatments and the four vineyard sites. For example, the Curly Flat microwave wine showed three-fold higher colour density and non-bleachable pigment, and eight-fold higher tannin concentration than the Domain Chandon control wine. As a non-replicated trial, the data presented in Table 1 were not analysed for within-vineyard treatment effects, but suggest a trend toward microwave macerated wines with early pressing showing higher phenolic extraction despite shorter skin contact time.

Principal Component Analysis (PCA) of the wines at 12 months bottle age demonstrated that 96% of the variance observed could be explained by the first two principal components (PC1 – 52%; PC2 – 44%). Figure 4 shows all microwave macerated wines separated out from control wines to the left hand side of the scores plot, suggesting this separation was in most part associated with PC1. Apart from Seville Estate where wines separated also on PC2, PC2 appeared to explain vineyard-to – vineyard separation. The four factors most associated with PC1 were tannin concentration, colour density, wine hue, total phenolics and non-bleachable pigment. Table 1 indicates that microwave wines tended to be higher in tannin, colour density, non-bleachable pigment and total phenolics than control wines, and that control wines tended to have higher hue value (more garnet than purple).

While not all wineries are seeking to make strongly coloured or tannic Pinot Noir wines, the use of microwave maceration in this trial demonstrated the capacity to make a more phenolically extracted wine but with shorter skin contact time (3 or 4 days, compared with 8 days), reinforcing the capacity to better manage extraction and winery constraints.

Table 1: Concentration of phenolics in 12 month old wines from four wineries having applied two winemaking treatments (control and microwave with early pressing).

	Chandon control	Chandon microwave	Curly control	Curly microwave	Montalto control	Montalto microwave	Seville control	Seville microwave
Colour density (AU)	2.11	3.73	3.76	6.36	3.33	5.41	4.06	4.89
Hue	0.93	0.90	0.87	0.78	0.77	0.71	0.74	0.74
Total anthocyanins (mg/L)	93.31	139.46	127.95	184.45	149.76	213.32	270.73	220.65
Non-bleachable pigment (AU)	0.48	0.85	0.94	1.54	0.76	1.24	0.65	1.00
Total Pigment (AU)	5.46	8.39	7.96	11.78	8.75	12.72	14.61	12.70
Non-bleachable pigment (%)	8.70	10.16	11.77	13.04	8.65	9.71	4.42	7.89
Total phenolics (AU)	24.28	37.41	37.43	51.38	28.82	40.80	35.37	41.41
Tannin (g/L)	0.22	0.84	0.91	1.67	0.38	0.89	0.50	0.86

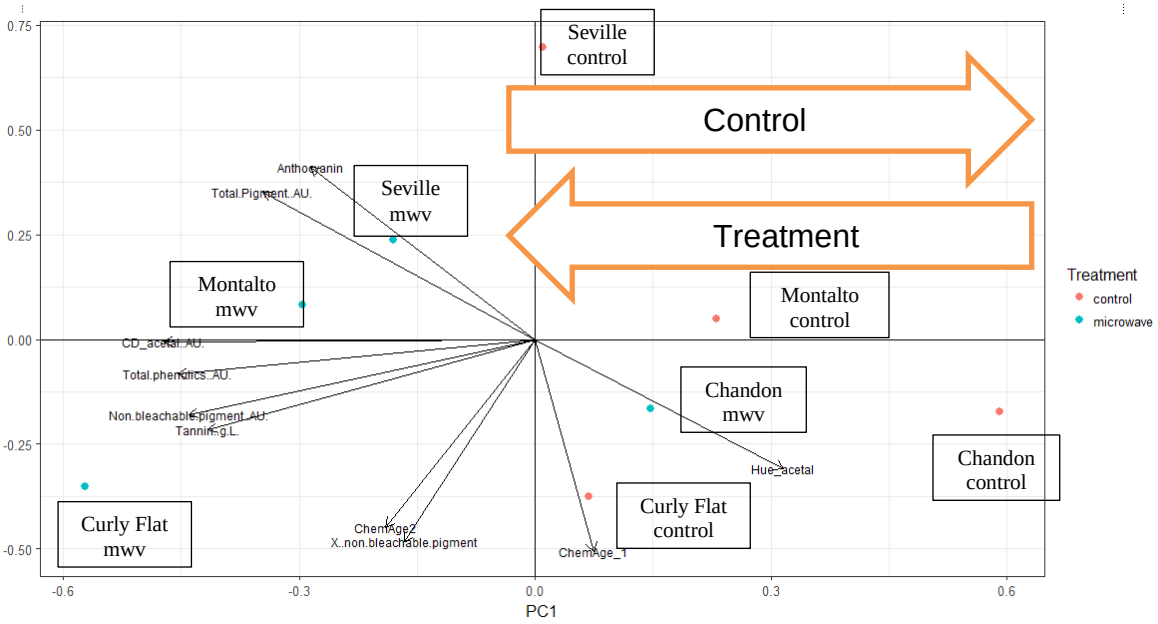


Figure 4: Principal Component Analysis scores plot showing separation of wines made by microwave maceration with early pressing, compared with control wines at 12 months bottle age.

At 24 months bottle age, the trend toward greater phenolic concentration in treatment wines was maintained. Table 2 shows treatment wines were numerically higher in phenolics concentration for all sites and all measures, apart from Seville Estate control wines being higher in residual anthocyanin.

Table 2: Wine matrix and phenolics analysis of control and early treatment wines at 24 months bottle age. (AWRI Commercial Services).

Attribute	Chandon		Curly Flat		Montalto		Seville	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Alcohol	11.9	12.2	13.9	14.1	12.5	12.5	12.7	13.2
Glucose + Fructose	0.35	0.40	0.40	0.60	0.40	0.40	0.65	0.80
Malic acid	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40
pH	3.61	3.69	3.65	3.63	3.44	3.44	3.73	3.42
Titratable acid pH 7.0	4.9	5.1	5.0	5.2	5.7	5.6	4.8	5.8
Titratable acid pH 8.2	5.4	5.7	5.6	5.7	6.2	6.2	5.3	6.3
Specific gravity	0.993	0.994	0.991	0.992	0.993	0.993	0.993	0.993
Volatile acidity as acetic acid	0.53	0.6	0.73	0.55	0.47	0.47	0.53	0.46
Sulfur Dioxide (free)	21.5	19.5	18.0	14.0	15.0	20.5	44.0	18.0
Sulfur Dioxide (total)	121	122	78.5	76	72	77	111	67.5
Colour Density	2.6	4.4	4.4	6.9	4.2	6.4	5.0	5.6
Hue	0.88	0.84	0.82	0.79	0.78	0.72	0.72	0.80
Free Anthocyanins	65.5	95.5	93	131	104	148	216	153
Total phenolics	23.0	35.0	36.5	48.5	27.0	38.0	33.0	38.0
Total pigment	4.2	6.3	6.4	9.2	6.55	9.6	11.9	9.3
Total tannin	0.18	0.73	0.86	1.55	0.31	0.81	0.40	0.75
Pigmented Tannin	0.52	0.91	1.06	1.62	0.82	1.32	0.70	1.00
% Pigmented tannin	12.4	14.4	16.5	17.6	12.6	13.8	5.8	10.7
Chemical Age 1	0.38	0.39	0.44	0.42	0.35	0.36	0.24	0.32
Chemical Age 2	0.13	0.15	0.17	0.18	0.13	0.14	0.06	0.11

Sensory Analysis

The Australian Wine Research Institute's sensory panel assessed wines at 24 months age using compare and contrast, a 20 point rating scale (Table 3), providing tasting notes and assessing for any off-flavours or faults. Wine sensory results are presented here by pair of wines from each participating winery.

Table 3: Sensory panel ratings of wine quality (20 point scale, nine trained tasters on the panel).

	Control		Treatment	
Wine	Average	St Err	Average	St Err
Chandon	14.1	0.4	15.1	0.4
Curly Flat	14.6	0.4	15.3	0.3
Montalto	15.3	0.4	15.6	0.5
Seville	14.7	0.3	14.3	0.4

Domain Chandon

These wines were relatively light, having been produced from fruit out of one of the less favoured blocks at this vineyard. Both treatment and control wines were identified as different from each other, and neither wine showed any discernible faults. Treatment wine was preferred on average by members of the tasting panel, scoring 15.1 points, compared with 14.1 points scored by the control wine. Control wine was perceived as light and salmon-coloured, with low intensity and red berry aroma and generally light or thin in the mouth by members of the tasting panel, whereas microwave wines made with early pressing were perceived by the panel as mid red colour and medium bodied with aroma featuring red and blue fruits, and some floral notes.

Seville Estate

Fruit used for these two wines delivered wines with relatively high anthocyanin and pigment content at 12 and 24 months bottle age, compared with other sites (Tables 1 and 2). This appeared to have translated into sensory perception of both control and treatment wines as medium bodied and mid-coloured, although the control wine was judged more tawny and the treatment wine ruby or garnet. Three members of the panel perceived a reductive fault in the treatment wine, which may have been associated with a strong discrepancy in total sulfur dioxide between the two wines (Table 2). In this pair of wines, the control and treatment rankings were not significantly different from each other (Table 3). Control wines were noticeably red fruit, savoury and spicy in aroma, and the treatment more berry fruit, unclean and rubbery.

Montalto

The Montalto wines were of the most highly ranked by the tasting panel, receiving rankings of 15.3 (control) and 15.6 (treatment), although the rankings did not differ significantly (Table 3). Control wines were judged light to mid-red with a ruby hue, and the aroma was dominated by red berry, blackcurrant and lolly notes. Treatment wines showed darker fruit intensity, with currant, morello and chocolate as aroma descriptors. Treatment wines appeared to be perceived as having length on the palate although both the control and treatment wines were judged as somewhat acid-driven.

Curly Flat

Treatment wines were more liked by the panel than control wines, with rankings of 14.6 and 15.3, respectively. This is an interesting result as an informal tasting with the winemaking team that produced these wines concluded that the treatment wine was overly extracted and outside the more subtle style of Pinot Noir for which this winery is renowned. The Curly Flat control wine was judged light to mid-red with some brick hues, and the aroma of control wine elicited widely different descriptors such that no dominant aroma description was apparent. Treatment wines were seen as mid to deep-red coloured with more dominant dark fruit (cherry and plum) aroma notes, and a persistent berry fruit palate but with some hotness or bitterness. The Control was perceived as somewhat bitter on the finish, with high acid and some thinness.

Conclusion

The sensory analysis and phenolics data presented here demonstrate that microwave maceration with early pressing is an effective option for production of Pinot Noir wine that is without faults, is richer in phenolic compounds, and of a distinct sensory character from wines made using traditional fermentation on skins for 8 to 9 days. While not all winemakers will favour the strong phenolic extraction and distinctive sensory character of microwave with early pressing wines, the advantage it offers is relatively short skin contact time (3 to 4 days) and the capacity to generate a more full-bodied wine from fruit that is light in colour and tannin (e.g. Domain Chandon fruit used in this trial). For those reasons, microwave with early pressing offers an opportunity for wineries to better diversify wines from the blocks and fruit available, particularly in years with low colour. Wineries might also benefit from short skin contact time during high tonnage vintages, or years when vintage compression places severe pressure on tank space. As is demonstrated in Chapter 6, the cost saving in pump-over labour and energy use renders investment in heat maceration technology feasible, with CPR in a greenfield site having a 3.5 year payback period.

Chapter 3. Microwave heating compared with other maceration processes*

***This is a draft manuscript. Authors Dr Fiona Kerslake, Dr Angela Merry, Dr Keren Bindon, Dr Paul Smith**

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Brown Brothers, Treasury Wine Estates, Hill-Smith Family Vineyards (formerly Yalumba), Dr Angela Merry, Mrs Hanna Westmore, Dr Jenn Lavers, Mr Piotr Swiergon, Dr Paul Smith, Dr Keren Bindon

Abstract

Maceration in Pinot Noir winemaking is designed to enhance the extraction of phenolics, but can often take up to two weeks or longer to achieve. Seven maceration techniques to reduce skin contact time, thus improving winery efficiencies, were compared including controlled phenolic release (CPR) on skins, CPR with early pressing, ultrasound maceration and standard winemaking processes. Phenolics, tannin and monosaccharide composition of Pinot Noir wines was shown to vary by maceration treatment. The most notable treatments in terms of impact on wine chemistry, were the two CPR treatments and the ultrasound treatment. CPR wines with early pressing were similar to control wines in terms of phenolic concentrations, although they had approximately half the time on skins. Formal sensory appraisal of CPR with early pressing wines showed they had unique sensory characteristics that were, on average, preferred to control wines.

Materials and Methods

Maceration and Microvinification

Two hundred and fifty kilograms of Pinot Noir grapes were harvested from a vineyard in Northern Tasmania, Australia at 12.5 °Baume and pH 3.25. Grapes were sampled for fruit characterisation and then bunches were randomly allocated into 21 replicates, each approximately 10 kg. Three replicates were allocated to each of seven maceration treatments (Table 1). Prior to application of the maceration treatments, grapes from each replicate were crushed and de-stemmed and the resulting must was decanted into a 20 L polypropylene food grade plastic bucket for fermentation. All replicates were treated with 50mg/L sulfur dioxide (SO_2) in the form of potassium metabisulfite solution. Control and extended maceration treatment replicates were moved to a 27°C (±3°C) constant temperature room in preparation for inoculation. Cold soak treatment replicates were lidded and stored at 4°C for four days prior to removal from the fridge, four hours of warming at ambient (20°C) and two hours warming at 27°C (±3°C) in preparation for inoculation on day 4. Must in the microwave on skins treatment was microwave macerated, following an adaptation of a published method (Carew, Sparrow, Curtin, Close and Dambergs, 2013). The adapted method involved separating grape solids from juice by gentle hand pressing, heating solids to 70°C by microwave, holding at that temperature for one hour, then reconstituting grape must with juice that had been cooled in a walk-in -20°C room for approximately three hours. Microwave with early pressing musts were prepared in the same way as on skins treatment musts.

In-line CO_2 replicates were attempting to simulate the Boreal® process (Air Liquide, Paris) which pumps dry ice into the must pump line to rapidly cool grape must prior to cold soaking. Our interest in this approach was the possibility of enhanced phenolic release through physical disruption; hypothesised spot-freezing of grape solids by powdered or particulate CO_2. In-line CO_2 replicates were stored at 4°C for four days and warmed for inoculation in the same way as cold soaking treatment replicates. Ultrasound maceration replicates were treated for 10 minutes each using a hand-held Branson Ultrasonic Sonifier 450A (Branson-Emerson, St. Louis) with a 13mm tapped horn at amplitude 90% with a pulse cycle of 0.7 seconds.

Table 1. Maceration treatments applied to Pinot must - skin contact time, day of inoculation and maceration process applied for each of six treatments.

TMT Number	Treatment	Skin contact time (days)	Day inoculated	Maceration process applied
1	**control**	8	1	Nil.
4	**microwave (on skins)**	8	1	Grape solids heated to 70°C, cooled after one hour.
7	**ultrasound**	8	1	Must treated by ultrasound for 10 minutes, pre-inoculation.
5	**microwave (early pressing)**	4	1	Must microwave macerated as above. Pressed off at day 4 after inoculation.
2	**cold soak**	12	4	Must stored at 4°C prior to warming and inoculation on day 4 of skin contact time.
6	**in-line CO_2**	12	4	Must stored and inoculated as above.
3	**extended maceration**	12	1	Wine held on skins for four days post-fermentation.

Approximately three hours after control, ultrasound, microwave and extended maceration treatments were applied, those replicates were inoculated with the active dried yeast strain *Saccharomyces cerevisiae* RC212 (Lallemand, Australia) that had been rehydrated according to the manufacturer's instructions. Cold soak and in-line CO_2 treatment replicates were inoculated in the same way at day 4 of the experiment. Microwave with early pressing treatment replicates were pressed off four days after inoculation for alcoholic fermentation, and fermentation was completed in 5 L glass carboys under airlock. This meant that control, ultrasound, and microwave treatments entailed eight days of skin contact time prior to completion of alcoholic fermentation, whereas cold soak, freeze and extended maceration treatments entailed 12 days of skin contact time, and microwave with early pressing had only four days of skin contact time.

All replicates were incubated at 27°C (±3°C) after inoculation and weighed regularly over the course of the 8 or 12 day ferment to track CO_2 loss (an indicator of the progress of fermentation). At day 3 after yeast inoculation, 60 mg/L of yeast assimilable nitrogen was added to each bucket in the form of diammonium phosphate solution. Microwave with early pressing replicates were pressed off on day 4 using a custom-made basket press. Control, ultrasound and microwave on skins treatment replicates were pressed off at day 8, and cold soak, in-line CO_2 and extended maceration treatment replicates were pressed off at day 12 using the same basket press. After pressing, wines were poured into screw cap 2 L glass flagons and incubated at 27°C (±3°C) for 12 hours to ensure completion of fermentation. Wines were cold settled for two weeks at 4°C and then racked to 500 mL Schott™ bottles under CO_2 cover and stabilised by the addition of 80 mg/L sulfur dioxide (SO_2) in the form of potassium metabisulfite solution. After two weeks, wines were bottled to green glass 375 mL bottles under CO_2 cover and crown sealed. Wines were stored for bottle aging in a constant temperature facility at 14°C and a fresh bottle of wine was opened for sampling at each bottle age analysis time period.

UV-Visible spectrophotometry

Wines were examined for phenolics concentration at 0, 12 and 19 months bottle age using a modified Somers method (Dambergs, Mercurio, Kassara, Cozzolino and Smith, 2012; Mercurio, Dambergs, Herderich and Smith, 2007). The phenolics quantified were: total tannin (pigmented and non-pigmented tannins); total phenolics (coloured and non-coloured tannins and anthocyanins, and low molecular weight, non-pigmented phenolic compounds); free anthocyanin (unbound or co-pigmented anthocyanins); non-bleachable pigment (stable colour from complexing of anthocyanins and tannins); colour density (the degree of pigment saturation of the wine); and hue (the nature of red wine colour - higher hue values being ruby-garnet, and lower values appearing more blue-purple). UV-Visible spectrophotometric analysis was conducted using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Samples were scanned in 10 mm cuvettes, at 2 nm intervals for the wavelength range 200-600 nm. Resulting absorbance data for selected wavelengths for each of the samples was exported to an Excel™-based phenolics calculator that had been developed by the Australian Wine Research Institute.

Tannin and monosaccharide composition

Wines were examined at 12 months bottle age for tannin and monosaccharide composition. For tannin composition, a 4 mL wine sample was loaded onto a solid phase extraction (SPE) cartridge and methyl cellulose precipitable tannin (MCPT) isolated following the method of Kassara and Kennedy (2011). Isolated tannin was subjected to acid-catalysed depolymerisation in the presence of phloroglucinol. Released tannin subunits as flavan-3-ol components and phloroglucinol adducts were analysed by a published HPLC protocol (Kennedy and Jones, 2003), and quantified as epicatechin equivalents using (-)-epicatechin as the quantitative standard (Sigma Aldrich, St Louis, USA). Tannin isolates were also analysed by gel permeation chromatography following a published protocol (Kennedy and Jones, 2003) with the modifications reported in Bindon and Kennedy (2011). The percentage of tannin subunit yield from phloroglucinolysis as a proportion of total MCPT provided the measure 'percent conversion yield', which has been suggested as an indicator of the proportion of total tannin that was depolymerisable. Four tannin compositional measures were applied to tannin isolated from wine samples: % tri-hydroxylated (epigallocatechin, prodelphinidin) subunits (%triOH); % galloylated subunits (%gall); mean degree of polymerisation (mDp); and molecular size at 50% elution by gel permeation chromatography (50%GPC). GPC was used as an indicator of the median size of tannin polymers in each wine sample, mDp provided a measure of the mean number of polyphenol subunits in wine tannin polymers, % trihydroxylation has been proposed as an indicator of tannin of skin origin, and % galloylation has been proposed as an indicator of seed-origin of tannin (Kassara and Kennedy 2011).

Wines were analysed for concentration of monosaccharides following the method described in Li et al., (2017). Monosaccharides in wine may be of grape origin or may result from yeast lysis, and have the potential to influence wine stability, mouthfeel and wine flavour. Ten measures were evaluated; mannose, rhamnose, glucuronic acid, galacturonic acid, glucose, galactose, xylose, arabinose, fucose, and total polysaccharide.

Sensory Appraisal

The research team was invited to present trial wines to an industry panel. Due to limited time and the need to also evaluate ACE wines at this event, only three wines from this trial were presented. We selected CPR wines as the main focus of this research funding block. Hence, control, CPR on-skins and CPR early pressing method (Carew et al., 2014; Carew 2014), were subjected to informal sensory appraisal by a panel of Yalumba staff late in 2014. The Yalumba panel included five experienced winemakers and three senior staff with approximately 10 years each experience in specialised aspects of wine production (e.g. laboratory analysis, vineyard management). The wines were tasted blind and ranked by preference, and the tasting panel provided descriptive notes and blending/bottling recommendations, as they were accustomed to doing during wine evaluation for routine decision-making as part of Yalumba's winery operations. Mean rankings and bottling/blending recommendations are presented. Descriptors of control and CPR wines were simplified and are presented as word clouds. These informal sensory results are presented in this Chapter to illustrate the perceived differences that industry experts perceived between three of the wines made in this extensive maceration trial.

Statistical analysis

Means and standard deviation were calculated in Excel™ for phenolic concentration, and tannin and monosaccharide measures. ANOVA in R (version 3.1.0) was used to identify treatment effects, and LSD test was used for post-hoc analysis. Where ANOVA did not support a conclusion of treatment effects, Principal Component Analysis (PCA) in R (version 3.1.0) was applied to data to establish if treatments clustered and separated, inferring treatment effects.

In the following section, phenolics results are reported separately for each bottle age period as the periods represent distinct points at which the phenolic quality of wines would be judged independently – at bottling (0 months) and 12 months bottle age as times that winemakers might be evaluating wines for cellaring, blending and bottle price point decisions, and 19 months bottle age representing a time period after which a consumer might open wine for consumption.

Results and Discussion

The maceration treatments applied in this study were associated with significant differences in phenolics in wines, and in monosaccharide concentration and the composition of tannin at 12 months bottle age. Of significance to industry, the pattern of difference in phenolic concentration changed between the bottle age periods that were examined, and by 19 months bottle age, only microwave maceration wines that had been fermented on skins showed significant differences in phenolic profile.

Phenolics

At bottling, several of the maceration treatment wines were showing differences to each other but no wine phenolic measure was significantly different to control wine at this point of their maturation (Table 2). By 12 months bottle age however, extended maceration wines (3) showed precocious colour stabilisation with a significantly higher percentage of stable pigment in the non-bleachable form, compared with control wine. Wines made by microwave maceration with fermentation on skins were significantly higher in colour density, total phenolics and tannin than control wine. The only other treatment to show significant difference in phenolics to control at that bottle age was the microwave maceration treatment with early pressing; early pressing wines had 25% less total tannin on average than control wine.

Table 2: ANOVA on phenolic concentration for Pinot Noir wines at bottling made with different maceration processes.

	control	cold soak	extended maceration	microwave (on skins)	microwave (early pressing)	in-line CO_2	ultrasound	*P value*
Colour density (AU)	3.61 a	3.69 a	2.38 a	3.14 a	3.84	2.19	2.70 a	*0.243*
Hue	0.62 b a	0.64 b	0.62 b	0.67 b	0.707 a a	0.59 b a	0.64 b a	*0.012*
Total anthocyanins (mg/L)	297 b	217 b	181 b	368 a	311 b	308 b	304 b	*0*
Non-bleachable pigment (AU)	0.45	0.48	0.36	0.48	0.54	0.29	0.37	*0.191*
Total Pigment (AU)	15.5 a 9 b	11.6 6 b	9.66 b	19.23 a	16.44 b a	15.8 a 9 b	15.81 b a	*0.006*
Non-bleachable pigment (%)	2.96	4.22	3.65	2.71	3.36	1.77	2.34	*0.169*
Total phenolics (AU)	34.8 a 0 b a	26.6 0 b	27.40 b	44.90 a	34 b a	36.5 a 0 b a	36.20 b a	*0.009*
Tannin (g/L)	0.75 b	0.42 b	0.46 b	1.26 a	0.624 b	0.85 b	0.80 b	*0.004*

Different lowercase letters indicate significant differences among means (LSD=0.05)

Table 3: ANOVA on phenolic concentration for Pinot Noir wines at 12 months bottle age made with different maceration processes.

	control	cold soak	extended maceration	microwave (on skins)	microwave (early pressing)	in-line CO_2	ultrasound	*P value*
Colour density (AU)	3.88 c b	4.11 bc ab	3.57 c a	5.54 a ab	3.99 c b	4.42 b b	3.80 bc ab	*0.000 0.004*
Hue	0.71 c a	0.72 c	0.74 b	0.72 c	0.749 a a	0.71 c	0.72 c	*0.004*
Total anthocyanins (mg/L)	223 b	204 b	163 b	276 a	216 b	273 a	228 ab	*0.001*
Non-bleachable pigment (AU)	0.68	0.76	0.77	0.88	0.7	0.80	0.66	*0.087*
Total Pigment (AU)	12.2 a 7 b	11.4 4 b	9.40 b	15.28 a	11.97 b a	14.9 9 a	12.51 ab	*0.000 1*
Non-bleachable pigment (%)	5.54 b	6.63 ab	8.14 a	5.76 b	5.85 b	5.40 b	5.38 b	*0.008*
Total phenolics (AU)	36.8 0 b	35.0 0 b	36.10 b	49.40 a	34.3 b	48.2 0 a a	38.70 ab	*0.000 1*
Tannin (g/L)	0.89 c	0.82 c	0.90 c	1.57 a	0.663 c	1.44 b	0.95 bc	*0.000 0*

Different lowercase letter indicate significant differences among means (LSD=0.05)

Table 4: ANOVA on phenolic concentration for Pinot Noir wines at 19 months bottle age made with different maceration processes.

	control	cold soak	extended maceration	microwave (on skins)	microwave (early pressing)	in-line CO_2	ultrasound	*P value*
Colour density (AU)	3.48 b	3.86 b	3.45 b	5.18 a	3.86 b	3.90 b	3.46 b	*0.001*
Hue	0.73	0.72	0.74	0.72	0.761	0.72	0.73	*0.194*
Total anthocyanins (mg/L)	124 b	164 b a	130 b	215 a	152 b a	169 b a	156 b a	*0.027*
Non-bleachable pigment (AU)	0.73	0.82	0.80	0.87	0.8	0.82	0.79	*0.125*
Total Pigment (AU)	7.43 b	9.59 b	7.84 b	12.21 a	8.94 b	9.84 b	9.11 b	*0.019*
Non-bleachable pigment (%)	11.85	8.60	10.24	7.18	8.99	8.41	8.63	*0.487*
Total phenolics (AU)	24.70 b	33.70 b a	35.00 b a	46.00 a	29.70 b a	35.80 b a	32.00 b a	*0.002*
Tannin (g/L)	0.34 a	0.76 b	0.85 bc	1.43 c	0.505 c b	0.87 c b	0.68 c b	*0.000*

Different lowercase letter indicate significant differences among means (LSD=0.05)

Perhaps the most important results in terms of wine phenolics, were the concentrations of various phenolics at 19 months bottle age. This longer-term aging period tested whether the time and effort expended on application of alternate maceration processes had useful, noticeable and lasting impact on wine phenolic chemistry. The most substantial suite of differences was associated with the microwave wines that had been fermented on skins, compared with control wines. This highly extractive process yielded wines at 19 months bottle age that were significantly higher in mean anthocyanin, tannin and total phenolics concentration, and in colour density. This wine was not however, higher in stable non-bleachable pigment than control wines, which might indicate a wine vulnerable to oxidation of excess co-pigmented anthocyanins. The remaining high anthocyanin concentration at 19 months bottle age, in concert with the four-fold higher concentration of tannin in these wines also indicated a need to investigate the binding characteristics and composition of these tannins.

It was apparent from 19 months bottle age analysis that all of the maceration treatments applied had significantly increased tannin concentration in trial wines between two- and four-fold, compared with the control wine. While cold soak, extended maceration and in-line CO_2 treatments had been exposed to 12 days' skin contact time which may have explained these higher tannin concentrations, the ultrasound and microwave with early pressing treatments resulted in wines two-fold higher in tannin concentration than control, having had only eight and four days' skin contact time respectively.

Tannin composition

At 12 months bottle age, the size of tannin polymers and the number of subunits were examined, as was the percentage of tannin that may have originated from grape skin or grape seed. The conversion ratio for each of the wines was in the range of 45% to 49% which suggests similar progression of tannins from 'grape-like' to 'wine-like', and justifies direct comparison of results between treatments. Two treatments were notable for significantly smaller molecular mass tannin polymers than control wine (Figure 1; microwave with early pressing and ultrasound treatment wines), and extended maceration wines showed significantly higher molecular mass tannin polymers than control wine. Notably, the number of subunits in extended maceration wine tannin polymers was significantly smaller than control, suggesting fewer but more substantial sized subunits. Conversely, microwave with early pressing wines had relatively small molecular mass polymers made up of a similar number of subunits to control wine polymers, suggesting smaller sized subunits. These findings show that while tannin concentrations might have been similar in wines at this age (Table 4 above), the structure of tannin polymers may have been quite different, and current research from McRae and others suggests the form of tannin polymers can impact on perceived red wine astringency (McCrae and Kennedy, 2011; McRae et al., 2014).

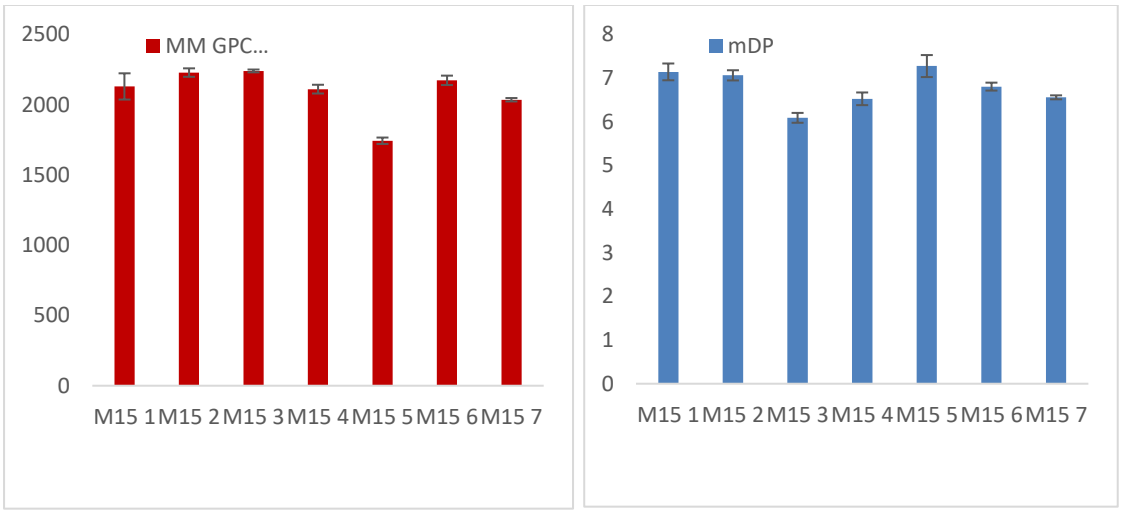


Figure 1: Molecular mass of tannin polymers (left graph) and number of tannin subunits in each polymer as mean Degrees of Polymerisation (right graph)

As is apparent from Figure 2 below, extended maceration and the two microwave maceration treatments had a significant impact on tannin composition of wines at 12 months bottle age. The proportion of tannin likely to be of skin origin was higher for the two microwave maceration treatment wines than in control wines, and lower for extended maceration wines. Microwave with early pressing wines were low in tannin likely to be of seed origin, which may be related to the relatively short skin contact period of four days, whereas extended maceration and microwave on-skins wines were significantly higher than control wines in tannin of seed origin.

The relative proportions of skin and seed-origin tannin may have been a factor in the differences in tannin polymer size and subunit structure discussed above (Figure 1). Further, research undertaken by Sparrow has indicated that in Pinot Noir, skin tannin may have unique colour stabilisation properties and may impact on perceived wine quality (Sparrow, 2014).

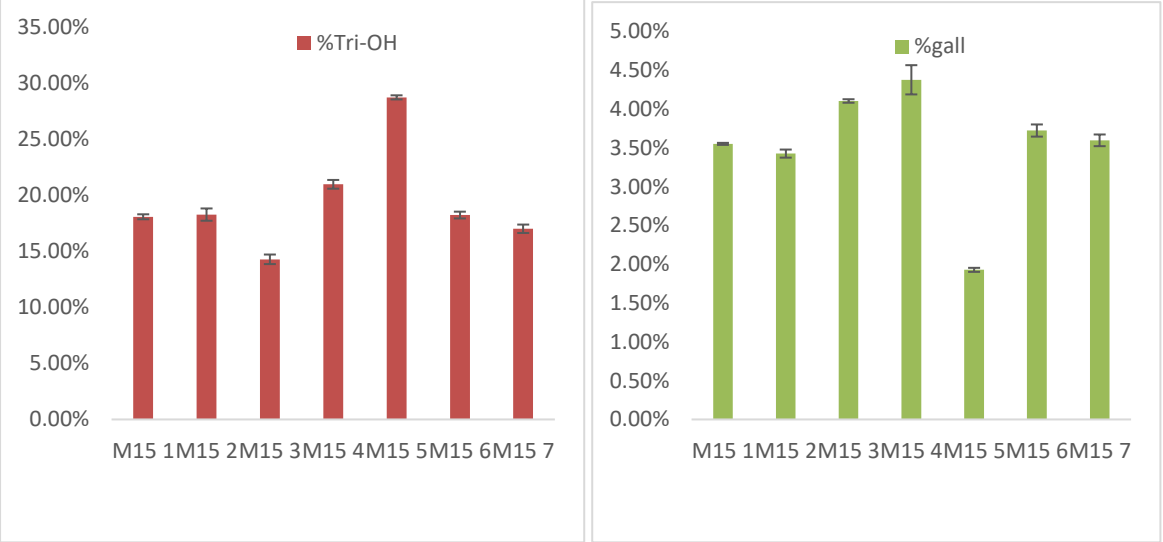


Figure 2: Percent of total tannin that was trihydroxylated indicating skin origin (left graph) and percent galloylated indicating seed tannin origin (right graph).

Monosaccharide composition

Characterisation of the monosaccharide profile of 12 month bottle age wines showed significant variation in profile associated with maceration treatment. The two microwave treatment wines were approximately 30% lower in rhamnose and arabinose, than control wines. Microwave wines fermented on skins were also lower in total residual polysaccharide and in galactose. These results suggest either that specific sugars were directly impacted by thermal or non-thermal microwave effects (e.g. heat labile, altered conformation by heating or microwave excitation of electrons), or that yeast communities that evolved in those ferments were more effective at metabolising those sugars (indirect effect). The ultrasound treatment was associated with significant differences to control for concentration of both glucuronic acid and xylose; xylose concentration was two-fold higher in ultrasound treated wines. The main source of xylose in grape material is hemicellulose, so this may indicate ultrasound had a direct effect on hemicellulose. Alternately, glucuronic acid is a dehydrogenated form of glucose, and xylose is a decarboxylated form of glucuronic acid (Figure 3), which may suggest that the ultrasound maceration process in some way impacted yeast and bacterial community to preference species that metabolised glucose to these two forms.

Table 5: Wine monosaccharide composition at 12 months bottle age.

	control	cold soak	extended maceration	microwave (on skins)	microwave (early pressing)	in-line CO_2	ultrasound	*P value*
Mannose	116.2 a	109.3 a	114.5 a	91.9 a	98.4 a	104.9 a	113.3 a	0.025
Rhamnose	29.83 a	28.86 a	28.41 a	19.5 b	17.29 b	28.17 a	29.71 a	0.000
Glucuronic.acid	11.83 b	12.77 ab	12.18 ab	10.33 b	9.45 b	12.33 ab	16.79 a	0.003
Galacturonic.acid	109.8	101.9	89.3	94.9	110	102.8	108.2	0.187
Glucose	35.4	33.6	32.9	36.2	38.2	31.9	37.4	0.238
Galactose	128.5 a	122.8 a	120.9 ab	81.2 b	79.6 ab	119.5 ab	125.5 a	0.006
Xylose	1.47 b	1.869 b	1.978 b	1.764 b	2.146 ab	2.041 ab	2.923 a	0.003
Arabinose	128.5 a	122.8 a	120.9 a	81.2 b	79.6 b	119.5 a	125.5 a	0.000
Fucose	2.548	2.968	3.007	2.703	2.49	2.474	2.377	0.338
Total.poly	539 a	513 ab	500 ab	415 b	443 ab	498 ab	537 a	0.008

Different lowercase letters indicate significant differences among means (LSD=0.05)

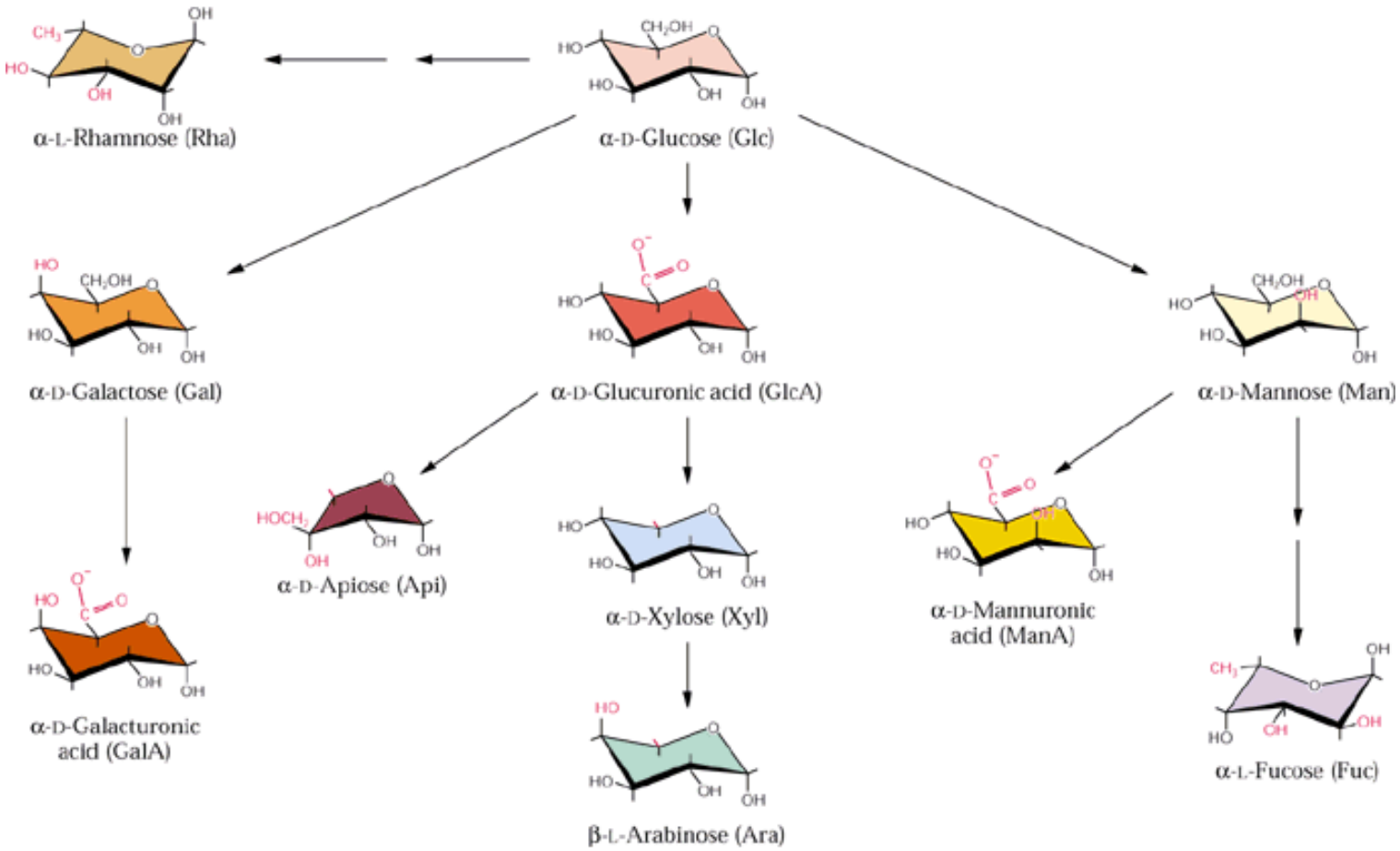


Figure 3: Pathways for metabolism of monosaccharides from glucose, indicating enzyme pathways needing to be active to achieve varied monosaccharide composition in wines (accessed May 2017 [http://www.uky.edu/~dhild/biochem/11B/lect11B.html)](http://www.uky.edu/~dhild/biochem/11B/lect11B.html).

Sensory appraisal of control, microwave on-skins and microwave with early pressing

The sensory appraisal undertaken was not formal sensory analysis and only focussed on three of the wines made in this trial. The following section however, does provide a view of the character and commercial potential of these wines, as judged by industry experts who are the main focus and end-users for applied research into novel winemaking approaches.

In terms of ranking preference for the three wines, the microwave with early pressing was preferred (mean ranking 1.1), on-skins fermentation wine was ranked second (mean ranking 2.3) and control third (mean ranking 2.6). The word clouds presented as Figures 4, 5 and 6 below, show that the control wine (Figure 4) was viewed as a bright, juicy wine with prominent red berry fruit and a confected flavour. The microwave wine with early pressing (Figure 5) was described as dark-coloured and purple with a complex extracted character and confected, fruit, plum and raspberry aroma prominent. The microwave wine that had been fermented on skins (Figure 6) was described as persistent, dark coloured complex and extracted, with blueberry, raspberry, stalky and caramel flavours. During discussion after the tasting, the wine-making team suggested that both the control and microwave on-skins wines would have a role as blenders, but that the early pressing wine would potentially warrant bottling as a complete wine.

Conclusion

The phenolic, tannin and monosaccharide composition of Pinot Noir wines was shown to vary by maceration treatment applied. The most notable treatments in terms of impact on wine chemistry, were the microwave treatments (on skins and early press) and the ultrasound treatment. While the relative impact of microwave treatments has been the main focus of much of this report, the impact of ultrasound treatment on the monosaccharide composition of wines may warrant further investigation. In particular, it would be interesting to ascertain if the source was direct degradation by ultrasound of specific grape material (e.g. hemicellulose) or was liberation of sugar precursors that were subsequently metabolised by yeast. The reason for expanded investigation of ultrasound is that the technology is established, relatively low cost, and offers few OHS risks in the industrial setting.



Figure 4: Word cloud for sensory appraisal of control wines by 8 wine industry experts

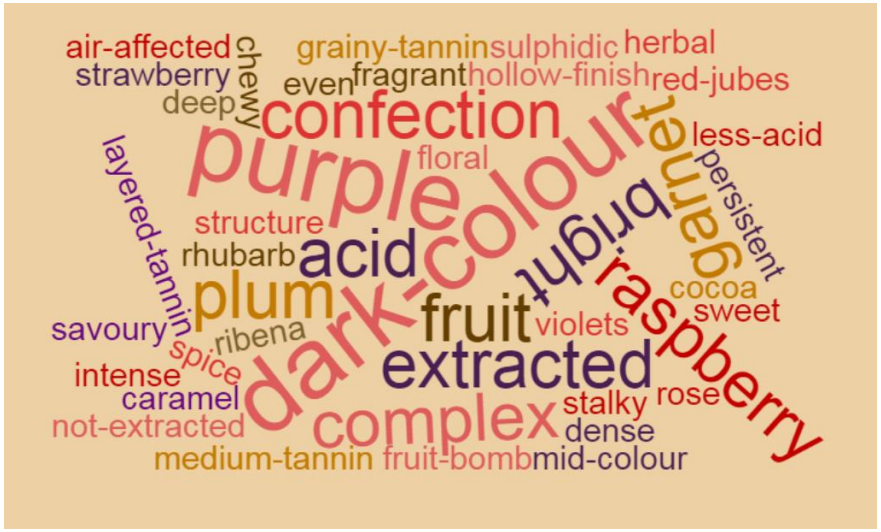


Figure 5: Word cloud for sensory appraisal of microwave with early pressing wines by eight wine industry experts

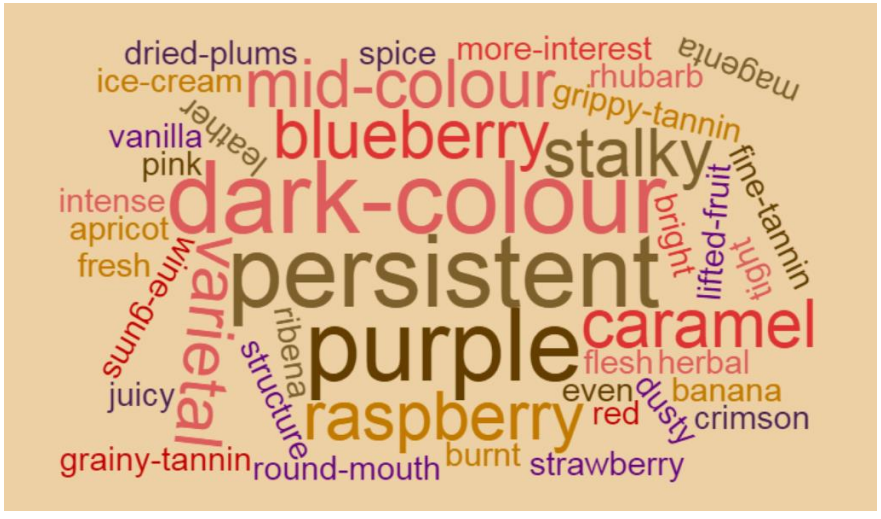


Figure 6: Word cloud for sensory appraisal of microwave with on-skins fermentation wines by eight wine industry experts

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Chapter 4. Assessing relative impact of microwave heating compared with viticultural intervention

* This is a draft manuscript currently in final preparation that will be submitted in 2017. It shows that microwave maceration of grape material results in wine with a higher concentration of phenolics, and that these effects are additive when applied in concert with leaf removal.

Title

Viticultural and winemaking treatments affect phenolics concentration and tannin composition in Pinot Noir wine

Authors

Dr Anna Carew – School of Land and Food, University of Tasmania, Private Bag 78, Hobart, Tasmania, Australia, 7001 email: anna.carew@utas.edu.au

Dr Fiona Kerslake - School of Land and Food, University of Tasmania, Private Bag 1375, Prospect, Tasmania, Australia, 7250

Dr Keren Bindon - The Australian Wine Research Institute, Post Office Box 197, Glen Osmond, South Australia, Australia, 5064

Dr Paul Smith (corresponding author) - The Australian Wine Research Institute, Post Office Box 197, Glen Osmond, South Australia, Australia, 5064

Dr Robert Dambergs – WineTQ, c/- Post Office, Monash, South Australia, Australia 5342

Associate Professor Dugald Close – School of Land and Food, University of Tasmania, Private Bag 78, Hobart, Tasmania, Australia, 7001

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Abstract

Pinot Noir fruit is often relatively low in the phenolics important for wine mouthfeel and colour stabilisation. Previous research has shown leaf removal can influence the concentration and composition of Pinot Noir fruit phenolics, but it is not clear to what extent these fruit effects translate to wine. A novel thermal treatment of must ('Controlled Phenolic Release') is effective for increasing wine phenolics concentration but there is limited information on the interaction between this process and viticultural interventions.

This study applied viticultural treatments to Pinot Noir (no leaf removal; leaf removal) in a commercial vineyard, and winemaking treatments in replicated microvinification (control; CPR with skin contact; CPR with early pressing). We examined fruit phenolics by the homogenate method, and wine phenolics using modified Somer's method and UV-visible spectrophotometry, and wine tannin composition and polymerisation by methyl cellulose precipitation and high performance liquid chromatography.

Viticultural and winemaking treatments were associated with significant effects on phenolics concentration in wines, and there was no interaction between viticultural and winemaking treatments (i.e. effects were additive). Of scientific and practical significance was the finding that fruit phenolics concentration measured by the homogenate method did not reliably predict Pinot Noir wine phenolics. Further, there was variation between treatments in the apparent source of tannins (composition), and the extent of tannin polymerisation and size of tannin polymers in wines.
The findings reported herein demonstrate that viticultural and winemaking interventions can affect Pinot Noir wine phenolics concentration, and are also likely to be important determinants of tannin composition, with potential mouthfeel and colour stability implications.

Keywords

Leaf removal; thermovinification; anthocyanin; maceration; canopy management

Introduction

The concentration of tannins and anthocyanins in red wine is associated with wine sensory effects, namely the perception of colour and astringency (Dambergs et al., 2012; Smith et al., 2007). In Pinot Noir grapes, the concentration and distribution of phenolics offers a challenge for winemakers in that the total anthocyanin concentration is low compared with many other red grape varieties (Cliff et al., 2007). There is also a limited number of anthocyanin types in Pinot Noir grapes (Cheynier et al., 2006), and all are non-acylated (Mazza et al., 1999). The acylation state influences anthocyanin colour stability in food and wine (Kirca et al., 2007; He et al., 2012), and non-acylated anthocyanins are vulnerable to decolouration under typical wine conditions such as: low pH, the presence of SO_2, and oxygen ingress (Cheynier et al., 2006). Red wine colour stabilisation occurs through formation of bisulfite-resistant pigments, of which a large component are pigmented polymers (McRae et al., 2012). Pigmented polymers result from complexing between anthocyanins, tannins and potentially other wine constituents (e.g. polysaccharides, proteins) (Bindon et al., 2016; Hayasaka and Kennedy 2003; Peng et al., 2002). Hence, anthocyanin and tannin concentration and composition are important for Pinot Noir colour stabilisation, and for the evolution of wine mouthfeel. For example, changes in perceived astringency and palate length.

Pinot Noir wine is typically low in tannin, compared with other well-studied red varieties. Analysis of 1,325 commercial red wines encompassing five varieties concluded that Pinot Noir wine had approximately half the tannin (as catechin equivalents) of Merlot and Cabernet Sauvignon (Harbertson et al., 2008). The physical distribution of tannin within Pinot Noir grapes may contribute to this low wine tannin concentration since approximately 90% of total tannin is located in the seed and the remaining10% in the grape skins (Kennedy 2008). Seed tannin tends to be slower to extract than skin tannin (Dambergs et al, 2012), and has been associated with bitterness in red wine (McRae, Falconer and Kennedy, 2010; Dambergs et al., 2012).

The challenge of managing winemaking for optimal phenolic composition in Pinot Noir is exacerbated by the challenge of cool climate growing conditions necessary for cultivation of ‘fine’ wine exemplars from this cultivar. Pinot Noir originates from the Burgundy region in France, situated at latitude 47°N, with a mean growing season temperature (GST) of 15.5 °C (Jones et al 2005). Under these climatic conditions, it can be difficult to reliably ripen fruit (e.g. accumulation of sugars, adequate anthocyanin concentration, maturation of tannins, maintenance of sufficiently low pH). Furthermore, similarly cool regions like Tasmania, Australia (GST 15.4 °C), often experience late-season rainfall and humidity which adds disease pressure to the challenge of Pinot Noir production (e.g. downy and powdery mildews, *Botrytis cinerea*).

Hence, winemakers need options to increase phenolic concentration in Pinot Noir wine. Two options are: intervention in the vineyard to affect concentration of phenolics in fruit, and application of wine making interventions to adjust phenolic extraction and stabilisation outcomes (Kennedy et al, 2006). Viticultural management techniques, such as leaf removal, are routinely applied to red grape varieties to influence berry colour and phenolic concentration (Diago et al., 2009; Smart 1991). Trials investigating shading and exposing Pinot Noir bunches have shown a reduction in flavonols in the fruit from shading, but little effect on anthocyanin concentration (Cortell and Kennedy, 2006; Price et al., 1995). A recent industry trend towards early season leaf removal, before flowering, has been examined in a range of varieties and was associated with an increase in anthocyanin concentration for Pinot Noir (Lemut et al., 2011; Lee and Skinkis, 2013; Sternad Lemut et al., 2015). However, Pinot Noir leaf removal trials for sparkling wine production demonstrated that the anthocyanin response can be site-sensitive, with an anthocyanin increase at one site from early leaf removal and an anthocyanin decrease from early leaf removal at another site (Kerslake et al., 2013). Changes in Pinot Noir fruit total tannin concentration (as epicatechin equivalents) in response to leaf removal has been reported, but was shown to vary according to the growing season (Kemp et al., 2011). In that study, there was no significant difference reported between two seasons of data for the mean degree of tannin polymerisation, which is an indicator of colour stabilisation and may moderate wine astringency (McRae et al., 2010; McRae and Kennedy, 2011).

Relatively few studies have examined whether Pinot Noir grape composition responses to leaf removal translate through to differences in wine phenolics. A study undertaken in a relatively warm Pinot Noir growing region (mean temperature in warmest month 23°C) reported effects on wine flavonols, anthocyanins and hydroxycinnamic acid profiles, but the observed response varied substantially over two successive growing seasons (2009, 2010) (Lemut et al., 2011). In line with those findings, Cortell and Kennedy (2006) reported reduced anthocyanin concentration from shaded cool-climate Pinot Noir fruit that translated into effects in a model wine. Neither of those studies reported associated effects on tannin composition from leaf removal, but Kemp and others (2011) showed a seasonal effect of leaf removal on wine total tannin and monomer flavan-3-ols in wines made from leaf removal fruit. Taken together, these studies suggest that leaf removal can influence final Pinot Noir wine phenolic concentration and composition, but the limited body of research to date means we lack clarity on the magnitude of effects, whether effects are reliable between seasons, vineyards and regions, and also what the optimal timing is for leaf removal to have a desired impact on wine phenolics.

Recent reviews of phenolic extraction during maceration have highlighted the importance of skin contact time (e.g. extended maceration) as an effective mechanism available to winemakers to influence final phenolic concentration in red wine (Casassa and Harbertson 2014; Smith et al., 2015). Extended skin contact time, however, represents an impost on winery facilities during the high pressure logistical challenge of vintage. An earlier review concluded that six winemaking interventions were effective for increasing phenolics in red wine (Sacchi et al. 2005), and a more recent review which focussed on tannin extraction identified practices such as: addition of exogenous enzyme, submerged cap, intensive pump-over, extended maceration and thermovinification to consistently increase tannin concentration in red wines (Smith et al., 2015). Those authors, however, offered the caution that complex interactions between grape material, phenolic and non-phenolic wine matrix components such as tannins, mannoproteins, polysaccharides, organic acids could result in tannin removal from wine in instances where the maceration process increased grape cell disruption (Smith et al., 2015). Two disruptive maceration processes commonly used by Pinot Noir winemakers are extended maceration and peak fermentation temperature in excess of 30°C (Haeger 2008).

Thermal maceration was identified as effective for phenolic extraction in red winemaking (Sacchi, et al., 2005) but is not commonly used in New World Pinot Noir winemaking (Haeger 2008; Joscelyne 2009). Controlled Phenolic Release (CPR) has proven effective for increasing the concentration of colour and tannin compounds in finished wine (Carew et al., 2013; Carew et al., 2014). CPR involves heating grape must to 70°C by microwave and managed diffusion of phenolics by maintenance of peak temperature for a defined period. Few studies have examined grape to wine impacts on phenolics, particularly whether viticultural impacts are additive with phenolic impacts attributable to maceration treatments.

Because of its challenging phenolic profile, Pinot Noir producers need a range of options to improve the phenolic profile of fruit in the vineyard, and to improve the efficiency and effectiveness of phenolic extraction in the winery. This study examined the impact on Pinot Noir phenolic profile of viticultural intervention (leaf removal) in concert with winemaking by CPR.

Materials and Methods

Grapevines and Viticulture

Two viticultural treatments were applied during the 2012/2013 season in a commercial vineyard in northern Tasmania, Australia (latitude 41°S). Own-rooted, *Vitis vinifera* L. cv. Pinot Noir clone MV6 grapevines trained to a divided canopy on a Scott Henry trellis were cane pruned to two arms per vine, 10 nodes per arm (total of 20 nodes per vine). Vines were eight years old with vine spacing of 2.75 m x 1.50 m (inter- and intra-row, respectively) and received 1.5 ML/ha of drip irrigation. Soil at the vineyard was a well-drained brown clay loam over basalt. The mean temperature of the warmest month of the growing season (February) was 17.3 °C, slightly below the 18.1 °C mean; and total rainfall during the growing season (Oct-Apr) was 290.6 mm, well below the 454.6 mm mean for the same period.

Two leaf removal treatments were applied; no leaves were removed (noLR), or leaves were mechanically removed both post-flowering and pre-veraison (LR). For LR, leaves were removed on 21 December 2012 and 21 January 2013 using a Pellenc Leaf Remover with barrel depth set to 380 mm to cover the fruiting zone. The experiment was a randomised complete block design, with two treatments and four replicates. Each experimental block was a separate row in the vineyard and treatments were applied along the entire vineyard row with two vines per row used as measurement vines. Fruit was harvested on 3 April 2013 ahead of commercial harvest. One hundred berries per replicate were sub-sampled for fresh fruit compositional analysis, and another 100 berries per replicate were sub-sampled and frozen at -20 °C for phenolic analysis.

Fruit and Winemaking

Bunches from each viticultural treatment (LR, noLR) were randomly allocated to 1 kg lots for vinification by three winemaking treatments; control (ctl), Controlled Phenolic Release by microwave followed by fermentation on skins (CPR-skins) and Controlled Phenolic Release by microwave with pressing before initiation of alcoholic fermentation (CPR-press). There were four 1 kg replicates for each combination of treatments (n=24). Control wines were made using the French Press method (Carew, et al., 2013; Dambergs and Sparrow 2011), with submerged cap fermentation at 28 °C for seven days in Bodum™ coffee plungers, followed by press-off and cold settling at 4 °C. CPR-skins wines were macerated following a previously published method (Carew et al., 2013), with grape must heated by microwave to 70 °C and held at that temperature for 1 h, rather than the 10 min previously described. CPR-skins must was then cooled to 28 °C, inoculated and fermented as described for the control treatment. CPR-press wines were macerated following a previously published method (Carew, Gill et al., 2014), with grape must heated to 70 °C by microwave, held at that temperature for 1 h, juice pressed off from grape solids and then cooled to 28 °C prior to inoculation and fermentation in 500 mL laboratory glass bottles (Schott Duran: Mainz, Germany) under airlock.

All wines were fermented to ≤ 2.5 g/L residual sugar, then cold settled at 4 °C for 14 days and were not subjected to malolactic fermentation. Wines were stabilised at first racking with 80 mg/L SO_2 and bottled under CO_2 cover to 50 mL amber glassware with wadded polypropylene capping. Wines were stored in the dark under ambient conditions at 22 °C for six months prior to analysis.

General Grape and Wine Analysis

Samples of 100 fresh berries were hand crushed and the juice was analysed for pH and titratable acidity (TA) (Crison, pH-Matic 23: Barcelona, Spain). Total soluble solids was measured using a digital hand-held pocket refractometer (Pocket PAL-87S: Atago, Japan). Grape total anthocyanins, total tannin and total phenolics were quantified by UV-Visible spectrophotometry (Genesys 10S UV-Vis: Daly City, USA) (Iland 2000; Australian Wine Research Institute, 2009), from frozen 100 berry samples prepared as previously described (Kerslake, Jones, Close and Dambergs, 2013).

Analysis of wine phenolics at six months bottle age was by UV-Visible spectrophotometry as previously described (Dambergs et al., 2012; Mercurio et al., 2007; Carew et al., 2015). Nine phenolic parameters were examined in wines: total phenolics (coloured and non-coloured tannin and anthocyanin, and low molecular weight, non-pigmented phenolics); total pigment (total red colour in the samples, including free anthocyanins and pigmented tannins); total anthocyanin (unbound and co-pigmented); colour density (pigment saturation); hue (higher values more ruby/garnet, lower values more purple); non-bleachable pigment (stable colour from pyranoanthocyanin formation, and anthocyanin and tannin complexing and has been correlated with pigmented tannin); and SO_2-resistant hue (hue in the presence of high SO_2 concentrations, with low values indicating a greater concentration of purple-hued non-bleachable pigment); and tannin by the spectral method (pigmented and non-pigmented).

Tannin Compositional Analysis

For each wine replicate at six months bottle age, a 4 mL sample was loaded onto a solid phase extraction (SPE) cartridge and methyl cellulose precipitable tannin (MCPT) isolated following the method of Kassara and Kennedy (2011). Isolated tannin was subjected to acid-catalysed depolymerisation in the presence of phloroglucinol. Released tannin subunits as flavan-3-ol components and phloroglucinol adducts were analysed by a published HPLC protocol (Kennedy and Jones, 2003), and quantified as epicatechin equivalents using (-)-epicatechin as the quantitative standard (Sigma Aldrich, St Louis, USA). Tannin isolates were also analysed by gel permeation chromatography following a published protocol (Kennedy and Jones, 2003) with the modifications reported in Bindon and Kennedy (2011). The percentage of tannin subunit yield from phloroglucinolysis as a proportion of total MCPT provided the measure 'percent conversion yield', which has been suggested as an indicator of the proportion of total tannin that was depolymerisable. Four tannin compositional measures were applied to tannin isolated from wine samples: % tri-hydroxylated (epigallocatechin, prodelphinidin) subunits (%triOH); % galloylated subunits (%gall); mean degree of polymerisation (mDp); and molecular size at 50% elution by gel permeation chromatography (50%GPC). GPC was used as an indicator of the median size of tannin polymers in each wine sample, mDp provided a measure of the mean number of polyphenol subunits in wine tannin polymers, % trihydroxylation has been proposed as an indicator of tannin of skin origin, and % galloylation has been proposed as an indicator of seed-origin of tannin (Kassara and Kennedy 2011).

Statistical Analysis

Phenolics and tannin composition data were analysed in the statistics package R (version number 3.1.0) using one-way and two-way ANOVA with post-hoc analysis by Tukey's test with significance at $p \leq 0.05$. Principal Component Analysis and linear discriminant analysis was undertaken in the package The Unscrambler X (version 10.3: CAMO, Norway).

Results and Discussion

The key finding of this study was that viticultural and winemaking interventions did not interact and so, in applied terms, could be viewed as potentially additive industry processes to better manage Pinot Noir wine phenolics. Of both scientific and practical significance was the finding that fruit phenolics analysis by the homogenate method (Iland 2000; Australian Wine Research Institute, 2009) did not reliably predict Pinot Noir wine phenolic concentration. This discrepancy has also recently been reported for Shiraz and Cabernet Sauvignon (Bindon et al., 2014). Further, methods used to quantify total tannin did not provide insight into variation between treatments in the apparent source of tannins (composition), and the impact that variation in tannin composition had on the extent of tannin polymerisation and size of tannin polymers in wines. These findings on tannin composition and polymerisation may demonstrate that viticultural and winemaking interventions could affect wine mouthfeel and colour stability, in different ways to simply impacting phenolic concentration.

Fruit composition and phenolics

Table 1 shows that basic fruit phenolics and maturity were not significantly impacted by leaf removal (LR), with the exception of total phenolics concentration, which was 12% higher in the LR treatment fruit. A seasonal- and site-specific response in total phenolics concentration was reported by Lee and Skinkis (2013), with an increased concentration associated with early- and mid-season leaf removal at one site, and no response to the same treatments at another site. However, in that study an increase in total anthocyanin concentration in fruit was observed at both sites associated with leaf removal, which was not observed in our study. The measure total phenolics may not offer useful insight into phenolic effects because this is a 'catch all' measure which does not distinguish between for example, anthocyanin, tannin and low molecular weight non-pigmented phenolic compounds (Dambergs et al. 2012). Hence, it is difficult to infer whether the significant increase in total phenolics from LR treatment that was observed in our study (Table 1) was associated with a possible trend toward higher values for anthocyanin and tannin with LR (Table 1), or if the significantly higher total phenolics result infers the LR treatment yielded fruit with a greater concentration of low molecular weight non-pigmented phenolics. By way of example, hydroxycinnamic acids were reported by Sternad Lemut and others (2015) to increase after early leaf removal.

Table 1. Phenolics concentration and composition measures for fruit with two viticultural treatments.

	Total soluble solids (°Be)	Titratable acidity (g/L)	pH	Total phenolics (AU/g)	Total anthocyanins (mg/g)	Total tannin (mg/g)
No leaves removed	13.0	8.89	3.30	1.15 ***a***	0.60	6.89
Leaves removed	13.4	8.39	3.29	1.31 ***b***	0.70	7.93

* different lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($P<0.05$).

Table 1 shows that LR was not associated with a significant difference in total tannin concentration in fruit, compared with noLR treatment. This lack of a treatment effect contrasts with previous research reporting increased total tannin as a result of bunch exposure in Pinot Noir (Cortell and Kennedy, 2006; Price et al., 1995). In a climate similar to that of our study, Kemp and others (2011) also reported no difference in total Pinot Noir fruit tannin concentration when leaves were removed as early as seven days after flowering, and Lee and Skinkis (2013) also reported no significant response in terms of tannin to leaf removal treatments applied to Pinot Noir vines from flowering onwards. Both studies, however, reported variation in mean total fruit tannin between vintages.

Several of these previous studies utilised different tannin analysis methods to the current study which could account for the discrepancies between studies in tannin effects. Based on these results from our study (Table 1), and previous studies, LR treatment could be concluded unreliable as a method for substantially increasing the concentration of colour and tannin compounds in Pinot Noir fruit.

Composition of wine phenolics and tannin

Analysis by two-way ANOVA showed no interaction between viticultural and winemaking treatments for phenolics (Table 2) or for tannin composition (Table 3) in wines at six months bottle age. The lack of interaction between viticultural and winemaking treatments suggests that the significant effects observed were additive, indicating potential intervention points for phenolic and tannin concentration adjustment in both the vineyard and winery. Phenolics concentration and tannin composition for wines from each treatment combination are reported in Tables 4 and 5.

Table 2. Main effects on phenolics concentration in Pinot Noir wine from two viticultural treatments and three winemaking treatments by modified Somers measure and total tannin by spectral method.

	Total Phenolics (AU)	Total Pigment (AU)	Total Anthocyanin (mg/L)	Colour density (AU)	Hue	NB Pigment (AU)	SO_2 – resistant pigment (AU)	Total tannin (g/L)
Viticultural treatment								
No leaves removed	24.3	4.25 ***a***	27.3	6.21 ***a***	0.94	1.73 ***a***	1.17	0.71
Leaves removed	25.2	5.16 ***b***	33.9	7.28 ***b***	0.92	2.08 ***b***	1.15	0.76
Winemaking treatment								
Control	23.2 ***a***	4.67 ***a***	43.4 ***a***	5.90 ***a***	0.96 ***a***	1.50 ***a***	1.24 ***a***	0.60 ***a***
CPR-skins	33.8 ***b***	5.57 ***a***	32.4 ***ab***	8.22 ***b***	0.96 ***a***	2.37 ***b***	1.17 ***a***	1.33 ***b***
CPR-early press	17.2 ***a***	3.87 ***b***	16.1 ***b***	6.10 ***a***	0.86 ***b***	1.84 ***a***	1.06 ***b***	0.29 ***a***
Two-way ANOVA (P values)~								
Leaf removal	0.67	**0.04**	0.41	**0.003**	0.33	**0.008**	0.33	0.67
Winemaking	**>0.001**	**0.01**	**0.03**	**>0.001**	**0.003**	**>0.001**	**>0.001**	**>0.001**
LR x Winemaking	0.73	0.60	0.79	0.35	0.41	0.65	0.29	0.77

* different lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD (P<0.05);

~ Two-way ANOVA results for main effects and interactions.

Table 3. Main effects on tannin composition in Pinot Noir wine from two viticultural treatments and three winemaking treatments.

	Mass conversion (%)	Trihydroxylated tannin (%)	Galloylated tannin (%)	Mean degree of polymerization (mDp)	Molecular mass at 50% GPC (g/mol)
Viticultural treatment					
No leaves removed	62 ***a***	22 ***a***	3.7 ***a***	6.2 ***a***	1822 ***a***
Leaves removed	58 ***b***	25 ***b***	3.2 ***b***	7.5 ***b***	1940 ***a***
Winemaking treatment					
Control	58	22 ***a***	3.4 ***a***	7.8 ***a***	1971 ***a***
CPR-skins	52	23 ***a***	4.4 ***b***	6.7 ***b***	2012 ***a***
CPR-early press	59	25 ***b***	2.7 ***c***	6.1 ***b***	1660 ***b***
Two-way ANOVA (P values)~					
Leaf removal	**0.02**	0.10	**>0.001**	**>0.001**	**0.02**
Winemaking	0.28	**>0.001**	**>0.001**	**>0.001**	**>0.001**
LR x Winemaking	0.99	0.10	0.07	0.94	0.90

* different lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($P<0.05$);

~ Two-way ANOVA results for main effects and interactions.

Table 4. Phenolics concentration for wines at six months bottle age made by three winemaking treatments (control, CPR with skin contact, CPR with early press off) from fruit with two leaf removal treatments (no leaves removed, leaves removed twice).

	Total Phenolics (AU)	Total Pigment (AU)	Total Anthocyanin (mg/L)	Colour Density (AU)	Hue	Non-bleachable Pigment (AU)	SO_2-resistant Pigment	Tannin by modified Somers (gm/L)
No leaves removed *								
Control	22.3 ***a***	4.0 ***a***	37	5.1 ***a***	0.99	1.29 ***a***	1.27 ***b***	0.54 ***a***
CPR-skins	34.6 ***b***	5.4 ***b***	32	8.0 ***b***	0.95	2.28 ***c***	1.16 ***ab***	1.36 ***b***
CPR-early press	16.0 ***a***	3.4 ***a***	13	5.5 ***a***	0.88	1.63 ***ab***	1.08 ***a***	0.23 ***a***
Leaves removed								
Control	24.2 ***ab***	5.4 ***b***	50	6.7 ***a***	0.94	1.71 ***ab***	1.21 ***b***	0.66 ***a***
CPR-skins	33.1 ***b***	5.7 ***b***	33	8.4 ***b***	0.97	2.47 ***c***	1.19 ***b***	1.29 ***b***
CPR-early press	18.4 ***a***	4.4 ***ab***	19	6.7 ***a***	0.85	2.05 ***bc***	1.04 ***a***	0.34 ***a***

* different lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($P<0.05$).

Table 5. Tannin composition for wines at six months bottle age made by three winemaking treatments (control, CPR with skin contact, CPR with early press off) from fruit with two leaf removal treatments (no leaves removed, leaves removed twice).

	Mass Conversion Ratio (%)	50% GPC	mdp	trihydroxylated tannin (%)	galloylated tannin (%)
No leaves removed*					
Control	64	1909 ***b***	6.62 ***a***	20.1 ***a***	3.6 ***b***
CPR-skins	65	1949 ***b***	6.17 ***a***	21.3 ***bc***	4.7 ***c***
CPR-early press	57	1608 ***a***	5.71 ***a***	23.9 ***bc***	2.9 ***ab***
Leaves removed					
Control	53	2034 ***b***	8.91 ***b***	23.3 ***abc***	3.1 ***ab***
CPR-skins	53	2077 ***b***	7.18 ***a***	24.8 ***bc***	4.1 ***bc***
CPR-early press	47	1711 ***ab***	6.46 ***a***	26.8 ***c***	2.5 ***a***

* different lowercase letters within columns represent significant difference in phenolic concentration by post-hoc analysis using Tukey's HSD ($P<0.05$).

Leaf Removal

Leaf removal treatment was associated with significant differences in total pigment, colour density and non-bleachable pigment concentration in wines. Total pigment and non-bleachable pigment were 17% higher in wines made from LR fruit, and this higher stable colour result was reflected in the 15% higher colour density observed in LR wines at six months bottle age (Table 2).

Total phenolics and colour

The lack of a significant difference for total phenolic concentration in leaf removal treatment wines (Table 2) contrasted with the observations for fruit analysis (Table 1), and supports research questioning the predictive value of commonly used methods for quantifying phenolics in red grapes (Bindon et al 2014). The work of Bindon and others (2014) emphasised the distinction between total available phenolics in grape material, and total phenolics extracted under winemaking conditions. Application of a wine-like extraction medium (Bindon et al., 2014) to the leaf removal fruit, instead of the homogenate method used in our study, might have delivered a different result in terms of the impact of viticultural treatment on grape total phenolics concentration; further research specific to Pinot Noir would be required to verify this assertion.

Phenolic compounds are generated in Pinot Noir fruit as a response to light exposure (Price et al., 1995) and the leaf removal regime applied in this study was estimated to increase fruit exposure by approximately 75%. Bunch exposure at key phenological stages is important (e.g. timing of leaf removal, as opposed to cumulative light exposure) as tannin precursors are synthesised during the first phase of berry growth, and anthocyanins accumulate during the ripening phase (Boss et al.,

1996). Based on the data in Table 2, leaf removal post-flowering and pre-veraison could be concluded effective for increasing stable colour in Pinot Noir wine at six months bottle age, however our results were not able to elucidate the mechanism which underpins these observations at the grape level. An alternate hypothesis would be that leaf removal had no impact on the synthesis and accumulation of phenolics, but rather changed the relative extractability of phenolics. This hypothesis would suggest physical cellular and sub-cellular effects with relevance to the retention or release of phenolics during fermentation.

The pattern of difference associated with leaf removal that we observed in these young trial wines may be promising for longer term colour stability as an analysis of a 30 year and a 50 year series of Shiraz wines showed that wine colour density was more closely correlated with concentration of pigmented polymers (non-bleachable pigment) than anthocyanin concentration, after the first two years of bottle aging, and that non-polymerised anthocyanins did not contribute significantly to visible colour in wines by four years bottle age (McRae et al., 2012). Given the tendency of some Pinot Noir wines to lose colour with bottle aging, the early stable colour results reported here may justify the cost of leaf removal during the growing season as a means to build colour and colour stability, particularly in vineyard blocks with a history of producing wine with poor colour or poor longer term colour stability.

Tannin concentration and composition

The observed lack of significant difference in fruit tannin concentration between leaf removal treatments (Table 1) was also apparent in wines, with no significant difference observed in wines from the two leaf removal treatments for the tannin measure applied in this study (total tannin by spectroscopy).

While differences in total wine tannin concentration between leaf removal treatments were non-significant (Table 2), Table 3 shows several differences in the tannin composition of wines measured at six months bottle age. Leaf removal treatment was a main effect for four out of the five tannin composition measures presented. The mean mass conversion ratio for LR wines was 4 % lower than for noLR wines, possibly associated with the incorporation of pigment within the tannin structure, as discussed previously, which may have indicated more of the tannin in LR wines had developed from grape-like (more readily converted) to wine-like (less readily converted) tannin. A more derived tannin structure might potentially impact wine sensory quality, given the established association between red wine aging and perceived astringency attributed to changes in tannin structure (McRae et al., 2012; McRae and Kennedy, 2011).

The difference in the size of wine tannin polymers as measured by gel permeation chromatography (50% GPC) was not significantly different between treatments, however tannin mDp was slightly lower in noLR wines compared with LR wines, possibly associated with an increased contribution of more polymerised skin tannin, reflected in higher tannin percent trihydroxylation with a corresponding drop in percent galloylation. This may have been due to a greater proportion of skin tannin associated with increased light exposure after leaf removal, but alternately reflects differences in skin tannin extraction resulting from the LR treatment. Retention of tannin in wine is a function of its relative extractability, but also net loss through selective re-adsorption to grape solids during alcoholic fermentation. Recent research showed wine tannin composition in Pinot Noir may have been impacted by preferential re-adsorption of seed tannins to grape pulp, compared with tannin of skin origin (Sparrow et al., 2015). LR treatment may have altered the physicochemical qualities of grape solids in a way that altered relative tannin extractability, or (re)adsorption properties of the grape for tannin, however this was not explored in our study.

The mDp values for wines in our study sit within the range of mDp reported in the literature for young Pinot Noir wines which is 5.0 – 8.6 subunits (Aron and Kennedy 2007; Kemp et al., 2011). Wine tannin polymers in aged wine are generally larger and less astringent than those of young red wine (McRae et al., 2012) however, polymers with a higher mDp have been associated with more astringent mouthfeel qualities in model wines (Vidal et al., 2003). Further investigation of the hydrophobicity of subunits from LR and noLR wines might have offered insight into the mechanism of mouthfeel effects associated with these apparently differing polymer constituents; McRae and others have established that a greater number of hydrophobic subunits may confer less mouthfeel astringency (McRae et al., 2013) but it is not clear if the changes observed here in tannin composition would be substantive enough to significantly impact sensory properties.

Given the important role of tannin in Pinot Noir mouthfeel and colour stabilisation, these viticultural effects on tannin composition may influence wine sensory quality and aging potential. The volumes of wine made in this trial precluded formal sensory analysis so future research should include sensory assessment of wines, given the tannin composition effects observed here. Further investigation may also be warranted to better understand if observed tannin composition effects were a contributing factor for the greater concentration of non-bleachable pigment observed in LR wines at six months bottle age. If so, this might suggest the viticultural treatment could be applied to generate a tannin composition associated with more effective colour stabilisation.

Winemaking Treatment

The two controlled phenolic release (CPR) winemaking treatments applied in this study were under investigation as alternate maceration approaches to manage Pinot Noir wine phenolics concentration and tannin composition, compared with standard vinification on skins (control). There were no interactions between viticultural and winemaking treatments so CPR winemaking treatment results are discussed separately from viticultural treatment results. Winemaking treatment was a main effect for all nine of the phenolic parameters examined in wines at six months bottle age (Table 2), and for all tannin composition measures, apart from percent conversion ratio which was not significantly different amongst winemaking treatments (Table 3).

Control versus CPR-skins

For one of the winemaking treatments, wines were made by CPR followed by fermentation on skins for seven days (CPR-skins). Table 2 shows that compared with control wines, the CPR-skins treatment wines had significantly higher total phenolics, non-bleachable pigment, colour density, and total tannin. The difference between control and CPR-skins wines for these parameters was substantial; CPR-skins wines had almost two-fold the non-bleachable pigment and total tannin of control wines. It is notable that the concentration of anthocyanin and total pigment in control and CPR-skins wines was not significantly different. Taken together with the observed effect of CPR on non-bleachable pigments this may indicate that the composition of compounds in the 'total pigment' fraction of wines differed between treatments, and this may have resulted in precocious development of stable pigmented products.

Tannin compositional analysis indicated that the higher tannin outcome in CPR-skins wines may, in part, have been attributable to greater proportional extraction of seed tannin; the percentage of galloylated tannin in the CPR-skins wines was approximately 25% higher than in control wines, and was associated with a drop in mDp. As previously noted, galloylated tannins have been associated with astringency in model red wines (Vidal et al., 2003) so this may have implications for the palatability of CPR-skins wines.

Similar to the results observed for noLR versus LR wines (Table 3), the molecular size of tannin in CPR-skins wines by 50%GPC was not significantly different to that of control wines, despite the lowered mDp. Should the observed differences in tannin composition have been associated with increased seed extraction, it would have been expected that tannin size might have been larger due to established differences in hydrodynamic volume associated with tannin origin in the grape (Kennedy and Taylor, 2003).

Control versus CPR-press

A set of treatment wines was made by CPR-press. In the current study, Control and CPR-press wines did not differ significantly in concentration of total phenolics, tannin, non-bleachable pigment, or colour density (Table 2), suggesting the CPR-press treatment was somewhat analogous to submerged cap alcoholic fermentation on skins for seven days in terms of colour development. This was also demonstrated in that CPR-press wines were significantly lower in total pigment (17%) and anthocyanin (63%) than control wines but had attained an equivalent level of non-bleachable pigment to control wines (Table 2). This suggests that as for CPR-skins, there may have been precocious formation of stable colour in CPR-press wines. The hue value for CPR-press wines was significantly lower than the control indicating a more purple hued wine, and had a corresponding lower SO_2-resistant hue value. This suggested that the purple hue of the CPR-press treatment coincided with a significantly higher concentration of purple pigmented polymers. Analysis of these wines over a longer bottle age period would establish whether CPR-press treatment wines, having attained this comparatively high early concentration of stable purple colour, might maintain this apparent 'young' purple colouration over time. The flavylium form of anthocyanin has been identified as a contributor to blueish wine hue (Cheynier et al., 2006; Sanchez-Ilarduya et al., 2012), as have ethyl bridged compounds (e.g. malvidin-3-glucoside dimer) (Pissarra et al., 2003; Sanchez-Ilarduya et al., 2012) and co-pigments formed through direct condensation (e.g. flavanyl-vinyl pyranoanthocyanins) (Cheynier et al., 2006; Mateus et al., 2003). Future analysis of CPR-press wines by liquid chromatography mass spectrometry or electrospray mass spectrometry is recommended to examine for differences in concentration of these compounds (Hayasaka et al., 2007; Pissarra et al., 2003). Such future research might establish which compounds and formation mechanisms are important in contributing to the purpleness of CPR-press wines.

Tannin composition varied between control and CPR-press wines (Table 3). CPR-press wine tannins had a higher relative proportion of trihydroxylation, which as discussed previously may point to a greater contribution of skin-derived material, possibly due to the limited contact time with seeds which tend to extract later during maceration. There were also notable differences in the size of polymers in CPR-press wines, compared with control wines, indicating that tannin polymers were smaller. This was associated with a lower mDp in contrast with the observed results for tannin size and mDp for the CPR-skins treatments. This again highlights that tannin origin within the grape (skin versus seed) has implications for tannin hydrodynamic volume which is correlated with, but not necessarily dependent upon, the absolute molecular mass. Longer term bottle aging and sensory assessment of wines would provide insight into the impact of these polymer differences on wine mouthfeel, tannin condensation and colour stability.

The industry potential of the CPR-press results reported here needs to be interpreted conservatively as a previously published study in which we compared control and CPR-press wines from two parcels of fruit vinified in the same way as this study demonstrated that the relative impact of control and CPR-press on wine phenolics could vary by parcel of fruit (Carew et al., 2014). CPR-press treatment may offer production efficiencies by allowing wines to be pressed off early and liberating winery tank space. Further development of this approach however, would depend on additional research on the sensory quality and long-term development of wine character made in this way.

Conclusions

Managing the extraction of phenolic compounds from grape solids into juice during alcoholic fermentation is key to producing the best possible quality Pinot Noir wine under the tight time constraints of vintage. The vagaries of cool climate growing conditions for Pinot Noir, and the capital cost of winery equipment and capacity mean that vineyard managers and winemakers need options to more accurately and efficiently achieve the desired phenolic character in wines. We report herein on two intervention options for adjusting phenolic concentration and composition in Pinot Noir wines, and demonstrate that those two interventions might be applied to achieve additive effects on wine phenolic concentration.

Leaf removal at post-flowering and pre-veraison (LR) was shown to impact significantly on wine colour measures and tannin composition. The finding of tannin compositional effects is interesting as an emerging consensus from the extensive work of McRae and others is that the role of tannin in mouthfeel and wine astringency is strongly influenced by the binding properties of tannin species, not simply a function of gross tannin concentration. This finding may warrant further investigation to scope the size of compositional difference at which sensory impacts are perceptible.
The data presented herein show that CPR offers two potential winemaking options: wine that is similar to control wine in phenolic profile, but fermented without pomace (hence approximately 40% saving in tank space, no cap management required), or wines with relatively high total tannin and non-bleachable pigment concentration after fermentation for a relatively short period of seven days (hence achieving highly extracted, phenolic wine from a short occupation of tank space).

An important conclusion from this research is the limitation of currently accepted methods for quantifying grape phenolics by homogenisation (Table 1), and the limited relevance of those results for describing wine phenolic concentration. This highlighted need for more effective, realistic measures with predictive power for wine outcomes. The Australian Wine Research Institute's wine-like extraction protocol, which aims to measure extractable- rather than total- tannin and colour (Bindon et al., 2015), may address the grape analysis shortcoming highlighted in our research findings.

Priority future research to build from the findings reported here would include further analysis of the long-term development of wine colour (stability) from the viticultural and winemaking treatments applied, and consideration of the relative mouthfeel effects of the range of tannin compositions observed in wines from this study. For example, further investigation of the properties of wine tannins associated with the maceration treatments could establish their potential impact on wine mouthfeel, the extent of folding in these polymers, impact on availability of hydrophobic sites for binding with salivary proteins, and resulting potential to mediate astringency perception (McRae and Kennedy, 2011). Recent research also highlights differences in macromolecular compounds in wines from alternate winemaking (Bindon et al., 2016), and the relative impact of leaf removal in combination with CPR might further elucidate the practical potential of novel treatments for managing wine macromolecular composition.

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Chapter 5. Ultrasound and microwave maceration in Shiraz winemaking

Abstract

Results of previous chapters demonstrated effects of increased extraction and higher phenolics concentration in Pinot Noir wines made by microwave maceration. The objective of this chapter was to investigate the effects of microwave and ultrasonic maceration on Shiraz. The impact of both maceration treatments on Shiraz wine phenolics concentration were generally non-significant at the two time periods examined (0 and 7 months wine age), with the exception of the control for ultrasound which had a higher hue value than the microwave maceration treatment wines at seven months bottle age. The results suggest that the maceration treatments tested do not increase phenolic release, relative to control maceration.

Materials and Methods

Fruit Preparation and Maceration

Three hundred kilograms of Shiraz fruit was hand-harvested at Shadowfax Winery on 10 March, 2016 at 13.2°Baume and pH 3.5. Hand harvesting was necessary due to a rain event preventing machine harvesting as planned. Bunches of fruit were randomly allocated to one of 21 food grade polypropylene 20L buckets, and crushed and destemmed using a Vaslin Bucher Delta E4 (Figure 1). Final must weight in bucket ranged from 12.4 to 15.3 kg. All replicates were treated with 50mg/L sulfur dioxide (SO_2) in the form of potassium metabisulfite solution and stored overnight in a 4°C cool room at the CSIRO's Werribee facility. Buckets of must were warmed at ambient temperature for approximately two hours at ambient temperature prior to application of maceration treatments the following day (Figure 2).

Buckets of crushed and destemmed fruit were randomly allocated to one of seven treatments (see Table 1). These included a control and two handling controls, a microwave maceration treatment and three ultrasound treatments. For the microwave handling control, grape solids were hand separated from juice and recombined after approximately three hours. This simulated both the handling and oxygen exposure factors that might contribute to the extraction and evolution of phenolics from grape solids in microwave maceration winemaking. The microwave treatment wine involved hand separation of grape solids, and juice being chilled for approximately three hours in a walk-in -20°C cool room. Solids were microwave macerated to 70°C in a domestic microwave unit (Sanyo EM5810WH; Sanyo, Tokyo), solids were held for one hour at that temperature, and then solids and juice were recombined in preparation for inoculation.

Ultrasound treatment was applied according to the parameters in Table 1. Ultrasound was applied using two different ultrasound units; the tunnel unit was used to simulate in-line processing with a low frequency application of ultrasound (28 kHz) (Figure 3), and both low frequency (40 kHz) and high frequency (1000 kHz) were trialled in a unit operations/batch processing simulation (Figure 4). For the ultrasound handling control, replicates were stirred in bath, or passed through the tunnel ultrasound, in the same way as replicates which were being subjected to ultrasound treatment.

Table 1: Winemaking treatments applied to Shiraz fruit in a replicated trial.

Treatment Number	Treatment Name	Treatment Details
1	Control	On skins fermentation for eight days, cap plunging every second day.
2	Microwave handling control	Grape solids separated and recombined with juice, fermentation as per control.
3	Microwave	Grape solids microwave macerated and recombined, fermentation as per control.
4	Ultrasound handling control	Grape must passed through ultrasound treatment system handling, fermentation as per control.
5	Low frequency ultrasound	Bath ultrasound of grape must at 40 kHz at 485 W for 600 seconds, fermentation as per control.
6	High frequency ultrasound	Bath ultrasound of grape must at 1000 kHz at 340 W for 856 seconds, fermentation as per control.
7	Low frequency ultrasound	Tunnel ultrasound of grape must at 28 kHz at 800 W for 420 seconds, fermentation as per control.



Figure 1: Crushing and destemming Shiraz fruit hand-harvested at Shadowfax Winery, Victoria (March 2016).



Figure 2: Twelve to 14 kilogram grape must replicates in food grade buckets arrayed at CSIRO Werribee Food Processing Module 5, prior to application of control, microwave and ultrasound maceration treatments.



Figure 3: CSIRO Werribee technicians emptying Shiraz grape must from the tunnel ultrasound (simulating in-line ultrasound treatment).



Figure 4: Mr Piotr Swiergon (CSIRO Werribee) monitoring Shiraz grape must maceration by bath ultrasound at right (simulating batch processing).

Winemaking

All 21 replicates were moved to the CSIRO 28°C constant temperature room and inoculated with the active dried yeast strain *Saccharomyces cerevisiae* RC212 (Lallemand, Australia) that had been rehydrated according to the manufacturer's instructions (Figure 5).



Figure 5: Dr Carew inoculating replicates in CSIRO Food Module 5, prior to fermentation for eight days at 28°C.

Ferments were plunged by hand every second day, and each bucket was dosed with 60 mg/L of yeast assimilable nitrogen in the form of diammonium phosphate solution at day 3. After eight days skin contact time, wines were pressed off using a custom-made basket press, and cold settled for two days in a 4°C cool store, prior to transportation to Tasmania for bottling and cellaring. Food grade plastic flagons were used for cold settling and transportation, in part due to CSIRO need to keep glass out of the Food Processing Modules and also to eliminate the possibility of breakage and loss of replicates during transportation (Figure 6).

Wines were bottled under CO_2 cover to 375 mL green glass bottles, and cellared for aging in the TIA temperature controlled cool store (14°C). At each bottle aging period, a fresh bottle of wine was opened for analysis.



Figure 6: Wines pressed off to plastic flagons at day 8, and cold settled for two days at 4°C prior to transportation to Tasmania for bottling, aging and analysis.

UV-Visible spectrophotometry

Wines were examined for phenolics concentration at 0 and 6 months bottle age using a modified Somers method (Dambergs, Mercurio, Kassara, Cozzolino and Smith, 2012; Mercurio, Dambergs, Herderich and Smith, 2007). The phenolics quantified were: total tannin (pigmented and non-pigmented tannins); total phenolics (coloured and non-coloured tannins and anthocyanins, and low molecular weight, non-pigmented phenolic compounds); free anthocyanin (unbound or co-pigmented anthocyanins); non-bleachable pigment (stable colour from complexing of anthocyanins and tannins); colour density (the degree of pigment saturation of the wine); and hue (the nature of red wine colour - higher hue values being ruby-garnet, and lower values appearing more blue-purple). UV-Visible spectrophotometric analysis was conducted using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Samples were scanned in 10 mm cuvettes, at 2 nm intervals for the wavelength range 200-600 nm. Resulting absorbance data for selected wavelengths for each of the samples was exported to an Excel™-based phenolics calculator that had been developed by the Australian Wine Research Institute.

Statistical analysis

Means and standard deviation were calculated in Excel™ for phenolic concentration, and tannin and monosaccharide measures. ANOVA in R (version 3.1.0) was used to identify treatment effects, and LSD test was used for post-hoc analysis. Principal Component Analysis (PCA) in R (version 3.1.0) was also applied to discern any clustering and separation between treatments.

Results and Discussion

The impact of maceration treatments applied in this study on Shiraz wine phenolics concentration were non-significant at the two time periods examined (0 and 7 months) (Tables 2 and 3), with the exception of the handling control for ultrasound which had a higher hue value than the microwave maceration treatment wines at seven months bottle age (Table 3). This result is mildly interesting but not really the focus of the research. Numerically, at seven months bottle age the ultrasound treated wines appeared to trend toward lower mean wine colour density, total phenolics and non-bleachable pigment, compared with the ultrasound handling control (treatment 4).

Table 2: ANOVA for wine phenolics in 0 month old Shiraz wines made by microwave (treatment 3) and ultrasound (treatments 5, 6 and 7). Control and handling treatments are in red.

	1	2	3	4	5	6	7	*P Value*
Colour density (AU)	11.38	11.08	12.02	11.98	9.63	9.11	8.39	*0.362*
Hue	0.583	0.58	0.598	0.616	0.575	0.582	0.595	*0.228*
Total anthocyanins (mg/L)	683	651	653	630	524	566	532	*0.417*
Non-bleachable pigment (AU)	2.115	2.013	2.242	2.45	1.8	1.653	1.638	*0.248*
Total Pigment (AU)	37.7	35.9	36.4	35.6	29.2	31.1	29.3	*0.413*
Non-bleachable pigment (%)	5.59	5.6	6.13	6.86	6.15	5.35	5.57	*0.065*
Total phenolics (AU)	57.4	54.7	55.9	53.6	45.3	47.4	44.7	*0.393*
Tannin (g/L)	1.24	1.21	1.25	1.09	0.90	0.90	0.84	*0.553*

Table 3: ANOVA for wine phenolics in seven month old Shiraz wines made by microwave (treatment 3) and ultrasound (treatments 5, 6 and 7). Control and handling treatments are in red.

	1	2	3	4	5	6	7	*P Value*
Colour density (AU)	13.85	13.08	13.39	14.14	10.47	10.43	10.71	*0.420*
Hue	0.60 ab	0.57 ab	0.53 b	0.63 a	0.567 ab	0.57 ab	0.58 ab	*0.031*
Total anthocyanins (mg/L)	415	436	507	411	396	435	381	*0.502*
Non-bleachable pigment (AU)	3.40	3.17	2.68	3.62	2.49	2.32	2.21	*0.197*
Total Pigment (AU)	26.42	27.08	29.84	26.57	23.97	25.60	22.73	*0.598*
Non-bleachable pigment (%)	12.76	11.72	9.01	13.60	10.34	9.25	9.69	*0.055*
Total phenolics (AU)	51.60	50.50	52.80	50.30	44.5	45.60	41.00	*0.483*
Tannin (g/L)	1.33	1.29	1.32	1.19	0.978	0.92	0.82	*0.369*

Principal Component Analysis appeared to support the contention that there may have been differences between control and treatment wines, which saw treatment wines less phenolically saturated than control wines, with some possible separation and clustering of the three sets of control wines to the top right quadrant of the PCA scores plot (Figure 7), and treatment wines possibly separating out to the bottom left of the plot. PC1 explained 56% of separation on the scores plot, and PC2 explained 37% of separation and hence, the diagonal separation reinforces a tentative conclusion of treatment effects that undermined phenolics concentration across the range of compounds examined.

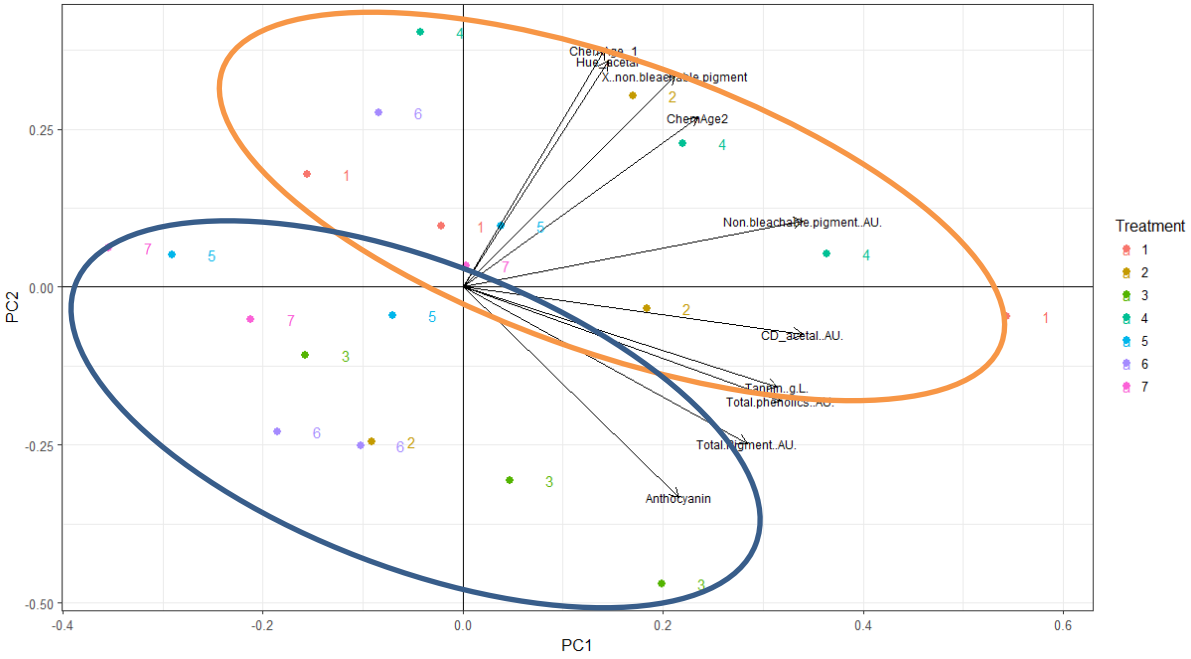


Figure 7: Principal Component Analysis of control, microwave and ultrasound maceration for Shiraz winemaking. Orange circle contains 8/9 control replicates. Blue circle contains 10/12 treatment replicates.

Conclusions

Based on the results from previous chapters that tended to demonstrate an effect of increased extraction and higher phenolics concentration in Pinot Noir wines made by microwave maceration, this set of results tends to suggest Shiraz may offer a less challenging matrix for phenolics extraction than Pinot Noir. Additionally, the three ultrasound processes applied in this study were different to the pilot-scale ultrasound treatment used in our earlier Pinot Noir maceration trial work (Chapter 3) which may explain the lack of significant impact here. Alternately, Shiraz may be a more generous variety in terms of its proclivity for releasing phenolics.

Chapter 6. Using ACE maceration

Authors

Dr Angela M Sparrow - 353 Rosevears Drive, Lanena, Tasmania, 7275 Australia. Email: angela@vinventive.com

Abstract

Accentuated Cut Edges (ACE) maceration reduces skin particle size thereby increasing the rate of extraction of components from grape skins. Commercial uptake of the method has the potential to improve the processing efficiency in Australian wineries. ACE maceration was tested on *Vitis vinifera* cv. Pinot Noir at five commercial wineries located in the south-eastern wine producing region of Australia. A small-scale trial was also conducted to assess the option of pressing the ACE treated Pinot Noir wine earlier than usual with the fermentation then completed in a smaller tank to make (Press Early) PE-ACE wine. PEACE treated wines had similar phenolic composition and sensory scores to the untreated wines. The technique optimises fruit characteristics and saves time, space and labour in the winery.

Executive summary

Accentuated Cut Edges (ACE) maceration is a new winemaking technique whereby grape skins are cut into smaller fragments than is usual, consequently increasing the rate of extraction of colour and flavour components from the broken edges of the grape skins. The procedure has been demonstrated to increase the rate of phenolic extraction to such an extent that pressing the grape solids off prior to the end of fermentation becomes an option, with fermentation then be completed in a smaller tank. Press Early (PE) ACE maceration or 'PEACE maceration' is a new technique with potential to assist in the management of compressed vintages where grape maturation times are shortened and pressure on winery infrastructure ultimately limits production capacity.

Following testing of the ACE technique in three semi-commercial trials during 2013, the current project investigated two further steps. In order to select the best time for pressing the fermenting wine from the grape solids, small scale trials were conducted during vintage 2014. A crusher-destemmer, modified to include cutting blades on the roller assembly was used to effect skin fragmentation, and grape solids were pressed off either 1, 2, 4 or 8 days post-inoculation. Each treatment consisted of 12 kg of fruit and was replicated three times. These were compared with an unfragmented control treatment that was fermented to dryness on skins in eight days.

In 2015, either ACE or PEACE techniques were applied in commercial trials in south eastern Australia. The five commercial trials included a control (standard commercial treatment applied by winery) and either an ACE treatment, a PEACE treatment or both. The grape skins were fragmented by a commercial stick blender as the must was pumped from the crusher to the fermentation tank. Fermentation volumes ranged from 300 L to 1,000 L. A must sample was taken to assess the extent of skin fragmentation for both the control and ACE treatments. ACE maceration was found to increase skin fragmentation three-fold in machine harvested grapes and up to nine-fold in hand harvested grapes.

Three of the winemakers elected to trial ACE maceration, with the intention of improving the stable pigment content of the wine, a fourth compared conventional and PEACE technology while the fifth compared both ACE and PEACE maceration with conventional methods. Wine composition parameters were assessed as the wines matured and ACE wines were found to have significantly greater colour density and stable pigment than wines made by conventional methods; PEACE wines made at the fourth winery was compromised by choosing a wild yeast ferment, whereas PEACE wine from the fifth winery showed improvements in colour density, stable pigment and blue colouration, while maintaining similar sensory attributes to the conventionally made wine.
Phenolic samples taken daily in commercial trials, showed that ACE maceration caused a dramatic increase in the extraction of colour and tannin in the first two days of fermentation. Six months after bottling, the average increase in tannin and stable pigment concentration relative to conventionally made wine was 45% for ACE wines, while for the PEACE wines the concentrations were 35% and 30% respectively.

The winemakers involved in the commercial trials were quite intrigued by the ACE wines made at their own wineries. The impact of reducing the particle size of the floating pomace cap coupled with early removal of the grape solids in Pinot Noir wines was shown to be considerable with respect to both the phenolic composition and perceived sensory properties of the wine. Not only were PEACE wines judged to be superior in phenolic composition relative to their control and ACE counterparts, the sensory panellists also described PEACE wines as having more fruity and floral attributes than the control wines.

As a result of articles in Daily Wine News and on ABC Radio at the time the trials were conducted in 2015, there have been enquiries from international wine growing regions asking for details of the procedure particularly as it does not damage the seeds.
Presentation of the research findings at ASVO seminar (19 Nov 2015) and CRUSH symposium (20 Nov 2015) were well received and brought enquiries as to how the technology could be accessed by winemakers in Australia. In 2016, Wine Australia funded a further project (VIN 1501) in which ACE and PEACE maceration were tested on five red grape varieties at six commercial wineries in four states of mainland Australia (Sparrow 2016).

In summary, the ACE maceration technique in combination with early press off to make PEACE wines has considerable potential as a technique to help commercial winemakers manage compressed vintages. Consumer evaluation of the commercial wines reported a 6:1 result in favour of PEACE/ACE wine. 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 (Chapter 7. The business case for adoption of microwave and ACE maceration in commercial red winemaking, P Smart).

The ACE maceration trials were conducted over two consecutive years, 2014 and 2015 and the results are presented in two sub-sections A) PEACE maceration 2014; and B) Commercial application 2015.

PEACE maceration 2014

Background:

The AGWA funded research project (GWR Ph1107) 'Comparative analysis of wine tannins from Pinot Noir grapes' investigated the effect of seed and skin tannins on the quality of Pinot Noir wine (Sparrow 2015). Increasing the extraction of skin tannin relative to seed tannin was found to be beneficial. Rather than removing the seeds, reducing the particle size of grape skins was shown to be a relatively easy and energy efficient means of enhancing phenolic extraction from the skins to promote both the development of stable pigment complexes (pigmented tannins and pyranoanthocyanins) prior to the extraction of seed components as these have been shown to limit the formation of stable pigments in the wine (Sparrow et al., 2015).

While ACE maceration has been associated with greater production of fruit flavour and aromas, in particular dark fruit (Sparrow et al., 2016b), a higher level of astringent and bitter characters in the wine, were noted by the qualitative assessment panel at AWRI. These characteristics have been associated with grape seeds (McRae and Kennedy, 2011). To offset these undesirable palate characteristics the current project coupled ACE maceration with early press off from grape solids to minimise exposure of the wine matrix to seed components. This report includes the phenolic and sensory attributes of wine made using a conventional maceration treatment with ACE technique and also with ACE in combination with early press off from the grape solids during fermentation. The acronym 'PEACE' is used to describe the latter treatment by which 'Pressed Early, Accentuated Cut Edge' wines were made.

Methods:

Grape sampling and analysis

Grapes of *Vitis vinifera* cv. Pinot Noir were hand-harvested at 22.5 Brix in April 2014 from 20 vines of a seven-year-old drip irrigated commercial vineyard in northern Tasmania, Australia (41.5^{o}S; 147.2^{o}E). Crop yield was 8 tonne/ha and bunches were randomly allocated to three 60 kg replicates.

Preparation of treatments for vinification

Grapes from each replicate were divided into five 12 kg batches which were independently de-stemmed and crushed using a Marchisio Grape Crusher/Destemmer (1000 to 1500 kg/h) to produce a total of fifteen, 10 kg samples of must, each of which was placed in a 20 L food grade plastic bucket. A control treatment that used crushed berries was compared to four different treatments in which the must was passed through a second Marchisio Grape Crusher/Destemmer (1000 to 1500 kg/h) that had the crusher rollers modified to form a series of rotating discs with serrated edges Figure 1. The discs interleaved as the rollers rotated thereby cutting the grape skins into small fragments (ACE). This device reduced the skin fragments to approximately 50% of their original size yet did not interfere with the integrity of the seeds. The four ACE treatments were each pressed from the grape solids on a different day during the fermentation (day 1, 2, 4 or 8) in order to determine the optimum time of exposure of the wine matrix to the fragmented must. All treatments were subjected to twice daily plunging using a broad wooden paddle until the day they were pressed off from the grape solids.

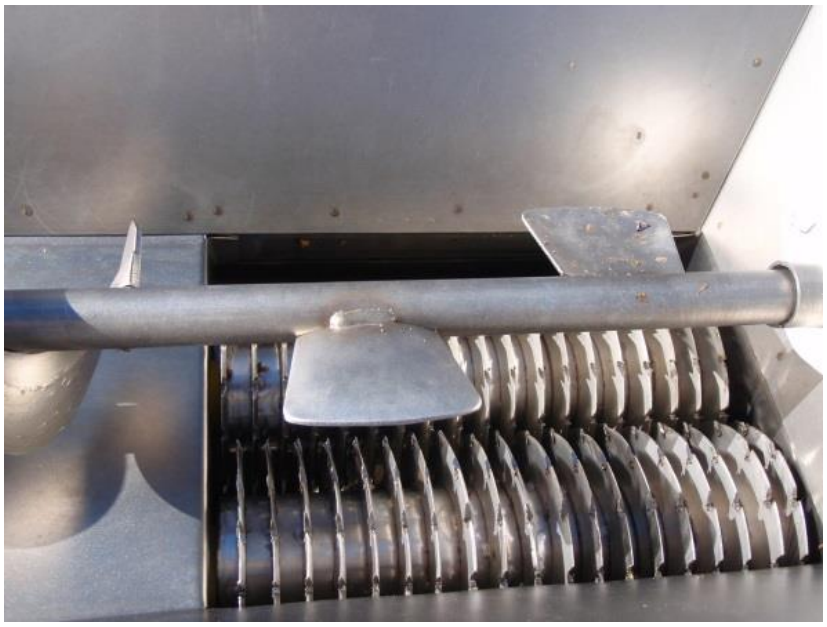
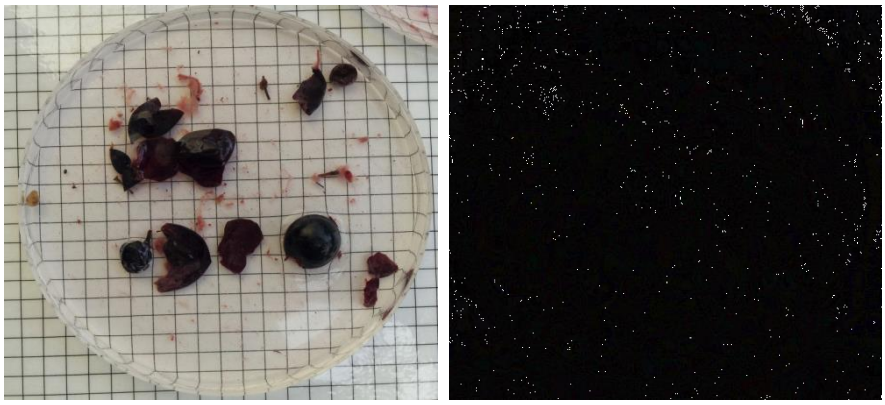


Figure 1 Cutting blades on rollers

The five treatments for this trial were: (1) Crushed berry control; (2) PEACE day 1; (3) PEACE day 2; (4) PEACE day 4; (5) ACE (fragmented skins remain throughout fermentation).

Skin fragmentation

For each experiment, a 200 g sample of grape must from each treatment was collected prior to yeast inoculation to assess skin fragmentation. From this 200 g must sample, 5 g sub-samples of grape solids were taken and each placed in a 90 mm 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 (ImageJ 1.51a Wayne Rasband National Institutes of Health, USA) was used to calculate the particle size and the extent of skin fragmentation for each sample (Figure 2).



Control **ACE**

Figure 2 Samples for skin fragmentation assessment

Vinification protocol

To the must in each 20 L fermentation vessel, 50 mg/L SO_2 was added in the form of potassium metabisulfite. Six hours later, the must preparations had equilibrated to ambient temperature and were inoculated with 300 mg/L RC212 yeast solution (Lallemand Australia Pty Ltd. Adelaide, Australia) and fermented under controlled temperature conditions at 27°C (±1°C). All fermentation vessels were covered with a loose fitting lid to allow adequate gas exchange. The treatments that were pressed early from the grape solids were pressed with a bench-top press into two 5 L glass demi-johns to recover a combined total of 6.0 L of fermenting juice. The grape solids were discarded and the fermenters were sealed with a water trap to prevent contamination. On day three of the fermentation, 300 mg/L of diammonium phosphate was added to each fermentation vessel. At the conclusion of fermentation residual sugar was confirmed at less than 2 g/L using Clinitest reagent tablets (Bayer Australia Ltd. Sydney, Australia). The control and final ACE treatment (ACE pressed day 8) were the last treatments to be pressed and 6.0 L of wine recovered which represented 60% (v/w) of the must weight. After fermentation, all wine was stored in 2 L glass bottles with screw caps (Schott Australia Pty. Ltd. Sydney, NSW, Australia) in the dark at 4°C for 14 days to settle lees, and then racked under CO_2 cover and 80 mg/L SO_2 added. After dark storage at 12°C for a further 30 days in fresh, screw-capped 2 L glass Schott bottles, the wine was racked a second time under CO_2 cover and transferred to 375 mL green glass wine bottles (Plasdene Glass-Pak Pty Ltd, Melbourne, VIC, Australia) and sealed with crown seals with neutral polyethylene under-cap (DrinkART Pty Ltd, Parkwood, QLD, Australia). Wines were stored upright at 12°C, with samples taken for phenolic analysis at bottling (50 days after inoculation) and at six, twelve and eighteen months bottle age (230, 400 and 580 days respectively, post-inoculation).

Phenolic analysis by spectroscopy

All samples were clarified by centrifugation at 4500 g for five minutes using an Eppendorf 5417C centrifuge with an Eppendorf F45-30-11 rotor (Crown Scientific, Melbourne, Australia). The phenolic composition of all samples was determined using the modification of the Somers assay described by Mercurio et al. (2007) and rapid tannin analysis calibrated against the methyl cellulose precipitable (MCP) tannin assay (Dambergs, et al., 2012). Wine phenolic parameters calculated from these assays were free anthocyanin, total tannin, non-bleachable pigment, wine colour density (WCD), hue and hue SO_2. A detailed definition of each of these phenolic parameters has been described elsewhere (Sparrow and Smart, 2015). An additional measure was taken using the model wine solution containing 0.375% sodium metabisulfite described by Mercurio et al. (2007). Absorbance of the wine sample in this solution at 580 nm was used as an indication of the proportion of pyranoanthocyanin in the wines.

Sensory evaluation

All treatment replicates were subject to sensory evaluation at 400 days post-inoculation by a panel of three experienced wine tasters. Subsequently three of the treatments: Crushed berry (control); PEACE day 2 and PEACE day 4, were selected for sensory evaluation when the wine had reached 580 days post-inoculation. Ten assessors from the Technical Quality sensory evaluation panel at the Australian Wine Research Institute (AWRI) were convened for this purpose. The samples were presented under blind tasting conditions using standardised sensory evaluation procedures. The tasters were instructed to comment in detail regarding the appearance, aroma and palate attributes of the wine, and to score the wine using the 20-point method of quality scoring as used in major Australian wine show judging.

Statistical analysis

Statistical analyses for phenolic components were conducted using GenStat 64-bit Release 16.1 (Copyright 2013, VSN International Ltd., United Kingdom). Mean, standard deviation and co-efficient of variation were calculated for fruit composition parameters. Data for individual phenolic components in wine samples were analysed using Repeated Measures Analysis of Variance (ANOVA) with treatment as the main plot and days from inoculation as the repeated measure. ANOVA was followed by post-hoc analysis using Fisher's protected least significant difference (LSD) test.
Wine scores were analysed using two-way ANOVA with treatment as the main plot and panellist as the sub- plot. ANOVA was followed by post-hoc analysis using Fisher's protected least significant difference (LSD) test.

Results and Discussion

This investigation examined the phenolic responses of wine made using both 'ACE' and 'PEACE' maceration techniques with qualitative sensory attributes assessed by expert panellists. Not surprisingly, reducing the particle size of the skins caused such disruption to cellular integrity, that the rate of anthocyanin leakage from the grape solids into the wine matrix increased considerably (Figure 1); moreover non-visible phenolic components and cellular compounds involved in aroma and flavour development were similarly affected.

Fruit composition

A juice sample taken from grapes used in this experiment had total soluble solids of 21 Brix, pH of 3.2 and titratable acidity of 7.7 g/L.

Skin fragmentation assessment

The modified roller resulted in skin fragments than were ~50% smaller than those of the control treatment which was sufficient to have a significant impact on the phenolic and sensory properties of the wines (Tables 1, 2 and 3).

Phenolic composition of wine

The trial showed that when the grape solids were pressed from the fermenting wine as early as two days after yeast inoculation, the stable pigment component of the wine at 50 days post-inoculation was twice that of the control wine, a feature that persisted in the wine through to 580 days post-inoculation. This increase in stable pigment contributed to a 24% increase in colour density of the PEACE day 2 wines and possibly contributed to the dark fruit character of the wine. When compared with the control and remaining PEACE and ACE treatments, PEACE day 2 wines showed a concomitant decrease in free anthocyanins. It would appear that the majority of the water-soluble anthocyanin pigments released by ACE maceration became directly incorporated into stable non-bleachable pigments and the remainder continued to develop as the wine aged (Table 1).

Table 1. Phenolic parameters of Pinot Noir wine from bottling (50 days post-inoculation) to 18 months bottle age (580 days post-inoculation) for control, skin fragmented (ACE) and press early skin fragmented (PEACE) maceration treatments.

Days post inoculation	**50**	**230**	**400**	**580**	LSD t x trt	*P-value* t x trt
			Tannin (g/L)			
Control	0.35	0.40	0.27	0.37		
PEACE day 1	0.44	0.51	0.51	0.57		
PEACE day 2	0.23	0.31	0.30	0.36		*NS*
PEACE day 4	0.56	0.63	0.68	0.69		
ACE	0.67	0.71	0.78	0.78		
			Anthocyanin (g/L)			
Control	0.25	0.18	0.12	0.11		
PEACE day 1	0.14	0.10	0.07	0.06		
PEACE day 2	0.18	0.13	0.10	0.08	*0.018*	*0.002*
PEACE day 4	0.24	0.17	0.13	0.09		
ACE	0.22	0.15	0.12	0.09		
			Non-bleachable pigment (AU)			
Control	0.28	0.48	0.50	0.56		
PEACE day 1	0.22	0.36	0.38	0.41		
PEACE day 2	0.49	0.86	0.86	1.01	*0.118*	*0.004*
PEACE day 4	0.38	0.85	0.79	0.87		
ACE	0.35	0.57	0.58	0.63		
			[a]Wine Colour Density			
Control	3.09	3.28	2.87	2.71		
PEACE day 1	1.91	2.11	2.25	1.93		
PEACE day 2	3.31	3.79	3.63	3.39		*NS*
PEACE day 4	3.58	4.05	3.49	3.38		
ACE	3.33	3.48	2.88	2.78		
			[b]Hue			
Control	0.57	0.72	0.80	0.83		
PEACE day 1	0.65	0.80	0.85	0.93		
PEACE day 2	0.65	0.75	0.83	0.85	*0.039*	*0.033*
PEACE day 4	0.59	0.69	0.79	0.82		
ACE	0.59	0.75	0.83	0.88		
			[c]Hue SO_2			
Control	1.65	1.45	1.44	1.43		
PEACE day 1	1.69	1.65	1.57	1.50		
PEACE day 2	1.41	1.24	1.25	1.23	*0.090*	*0.009*
PEACE day 4	1.55	1.16	1.22	1.20		
ACE	1.54	1.36	1.37	1.37		

Data are mean values; n=3; Fisher's LSD (0.05); [a]Wine Colour Density (A420+A520 in model wine solution); [b]Hue (ratio A420:A520 in model wine solution); [c]Hue SO_2 (resistant to sulfur dioxide bleaching); LSD, Least Significant Difference, NS, Not Significant, t, time; trt, treatment.

The concentration of non-bleachable pigments in PEACE macerated wines (pressed on day 2 or 4) were on average 70% higher than that of the control wines, suggesting that changes in wine hue with bottle age would be modest for the PEACE wines, consequently improving the longevity of the wine. Absorbance of wine samples at 580 nm in model wine solution containing sodium metabisulfite showed that at 580 days post inoculation the PEACE day 2 and day 4 wines had 50% more blue colouration than did the control wines (Table 2).

Table 2. Absorbance at 580 nm for five maceration treatments at 580 days post-inoculation. Data are Mean and Standard Deviation (n= 3).

Wine Age (days)	580
Treatment	A580 (AU)
Crushed berries (control)	0.027 ± 0.003
PEACE day 1	0.016 ± 0.001
PEACE day 2	0.042 ± 0.002
PEACE day 4	0.039 ± 0.003
ACE day 8	0.032 ± 0.003

The 17% increase in blue colouration in PEACE day 2 wine, relative to control wine at 50 days post-inoculation was a profound observation. The fact that the blue colouration continued to develop as the wine aged, such that it was 25% higher in PEACE day 2 wine by day 580 post-inoculation suggested that a considerable proportion of the stable pigment present in the wine consisted of pyranoanthocyanins (Alcalde-Eon et al., 2006; Cheynier et al., 2006). The preservation of vibrant purple colour of stable pigments in young wines is widely accepted to be a positive trait in red wine. Consequently the 50% higher absorbance value observed at 580 nm in PEACE day 2 and day 4 at 580 days post-inoculation when assayed in the presence of sulfur dioxide, was also considered a positive indicator of wine quality.

Stable pigment formation has been associated with greater development of polyphenolic compounds derived from the skin (Cortell et al., 2005; Cortell, Siversten, Kennedy and Heymann, 2008; Arnaud et al., 2013) and in this experiment the stable pigment concentration was also found to be strongly correlated with dark fruit aroma and flavour as described previously (Sparrow et al., 2016b).
With the exception of wines pressed off one day after yeast inoculation, both the PEACE and ACE treated wines had more intense colour than the control wine (Figure 3). Whilst this effect on ACE wines has been reported previously (Sparrow et al., 2016a,b), the result for PEACE wines was unexpected. Only the control and ACE treatments had been in contact with the grape skins throughout the fermentation.



Figure 3 PEACE trial wines
L to R: Control, PEACE day 1, PEACE day 2; PEACE day 4, ACE

Sensory evaluation of wine

Sensory scores for wines for each treatment at two time periods: 400 and 580 days post-inoculation, are shown in Table 3. Tasters involved in the preliminary evaluation conducted at 400 days post inoculation, confirmed the phenolic results, noting more intense colour for PEACE day 2 and ACE wines than for the control and PEACE day 4 wines. Sensory perception suggested greater berry fruit characteristics in the PEACE wines, with PEACE day 2 considered to have more dark fruit characteristics and PEACE day 4 having more red fruit characters. When the wines had aged a further six months (580 days post-inoculation) a subset of the wines from the five maceration treatments investigated was evaluated by the Australian Wine Research Institute Quality Assessment Panel. These were the control, PEACE day 2 and PEACE day 4 treated wines (Table 3).
More intense floral aromas were found in PEACE day 2 wine compared to both PEACE day 4 and control wines. These may be attributed to the retention of delicate aroma compounds that would otherwise be lost during cap management procedures. The sensory attributes described here for PEACE wines suggest that there may be a broad range of wine style options available to the winemaker when ACE maceration is employed. More rigorous sensory analyses are required to confirm this perception.

At this time, the PEACE wines were again noted for their colour intensity and fruit characters. Floral aromas appeared to be more pronounced in the PEACE day 2 wines, while fruit characteristics more dominant in the PEACE day 4 wines. The lower overall score for PEACE day 2 was ascribed to a reductive character in the wine, which appeared as the wine matured, possibly reflecting the time on lees required to ferment the PEACE day 2 wine to dryness.

Table 3. Sensory scores of microvinified Pinot Noir wine for five maceration treatments at 400 post-inoculation days (n= 9) and three maceration treatments at 580 days post-inoculation (n=30). Data are Mean and Standard Deviation.

Wine Age (days)	[a]**400**	[b]**580**
Treatment	**Score/20**	**Score/20**
Crushed berries (control)	12 ± 0.1	15 ± 0.3
PEACE day 1	13 ± 0.5	*NA*
PEACE day 2	13 ± 1.3	14 ± 1.1
PEACE day 4	12± 1.6	15 ± 1.1
ACE day 8	14± 0.9	*NA*

[a]Tasted by three panellists; [b]tasted by 10 panellists

Conclusions

The impact of reducing the particle size of the floating pomace cap coupled with early removal of the grape solids in Pinot Noir wines was shown to be considerable with respect to both the phenolic composition and perceived sensory properties of the wine. Not only were PEACE wines judged to be superior in phenolic composition relative to their control and ACE counterparts, the sensory panellists also described PEACE wines as having more fruity and floral attributes than the control wines. As early as two days post-inoculation, PEACE wines were pressed from the grape solids and transferred to a fermentation vessel that was 40% smaller than the original fermentation tank, representing a valuable space saving opportunity. In addition, the fact that the PEACE wines required no further plunging meant that labour and energy costs were reduced. In conclusion, PEACE maceration has not only been demonstrated to produce good quality wines as judged by an experienced sensory panel, but also represents an opportunity to realise a shorter and less expensive winemaking process.

Commercial trials 2015

Background

Preparations for the commercial trials began before the mature wines from the PEACE 2014 trial had been assessed, consequently only two winemakers were prepared to trial early press off of ACE macerated wines. The modified crusher roller used in 2014 was inappropriate for use in a commercial winery, and the design had problems with grapes becoming lodged between the cutting blades during its operation, effectively diminishing their cutting effect as more grapes were added to the hopper. Consequently, a commercial stick mixer set into the must line was used to cut the grape skins for the 2015 trials. The stick mixer had proven to be very effective in laboratory and semi-commercial trials conducted the course of Angela Sparrow's PhD candidature.

Method

Preparation of treatments for vinification

A trial procedure was sent to each winemaker involved in the commercial trial during February 2015. Grapes of *Vitis vinifera* cv. Pinot Noir were selected at a stage of ripeness determined by the winemaker and were either machine or hand-harvested. For each experiment a sub-sample of 100 berries was selected at random, to determine total soluble solids (Brix), pH, and titratable acidity. A further 200 g sub-sample of berries was frozen at -20^{o}C for later analysis of grape colour and tannin as described in Section A.

Vinification protocol

At each of the commercial wineries, grapes from a single batch of fruit were divided into two or three ferments that were run in parallel to compare conventional maceration with either the ACE maceration treatment or the PEACE treatment.

Grapes for the ACE and PEACE ferments were cut with a commercial stick blender as the must was pumped from the crusher to the fermentation tank. Must volumes for the control and ACE/PEACE wines at the five wineries ranged from 300 to 30,000 L.

The winemaking regime for the commercial wineries differed somewhat in terms of sulfite, yeast and addition of fermentation nutrient. Two of the commercial wineries involved in the trial, elected to press the wine from the grape solids after five days of fermentation which was then completed in neutral oak barrels. For the three remaining wineries, the wine was pressed from the grape solids at the conclusion of fermentation (residual sugar <2.5%) and the wine aged in neutral oak barrels. Samples were taken for phenolic analysis daily during fermentation, at pressing and six months after ACE maceration.

Statistical analysis

Statistical evaluation of wine sensory properties of the commercial wines was not relevant due to regional differences and winemaking procedures. Rather sensory evaluation was conducted by a panel of seven winemakers at a blind tasting, the wines were presented in coded, XL5 (ISO standard) glasses at 22-24°C. Each pair of wines (control and ACE or PEACE) was presented at random. The tasters were instructed to evaluate the appearance, aroma and palate attributes of the wine, and to score each of 10 attributes on a scale of 1 to 4, this score was then adjusted in parallel to the 20-point method of quality scoring as used in major Australian wine show judging. The sensory panel were also asked to indicate which wine they preferred.

Data for sensory attributes were analysed using a repeated measures design with a compound symmetry correlation matrix in which panellists were the experimental units on which repeated measures were recorded. The significance of these was calculated using Tukey's-tests.

Results and Discussion

Fruit composition

The fruit used in the trial showed some variation in ripeness with Brix ranging from 23.2 to 25.9, pH from 3.11 to 3.93 and titratable acidity from 5.0 to 10 g/L (Table 4). The anthocyanin content of the grapes at harvest, also varied from 0.7 to 1.6 mg/g fresh weight of grapes but was independent of the location of the winery. The tannin concentration of grapes from vineyards located on the Australian mainland (wineries A and B) was noticeably higher than that of the Tasmanian vineyards (wineries C, D and E).

Table 4. Fermentation size and fruit composition parameters

Winery	A	B	C	D	E
Fermentation mass (t)	30.0	2.5	3.0	20.0	3.0
Total Soluble Solids (°Brix juice)	25.9	23.2	25.6	24.1	24.7
Juice pH	3.93	3.33	3.67	3.11	3.53
Titratable acidity (g/L juice)	5.0	6.4	7.9	10.0	7.8
Anthocyanin (mg/g berry FW[a])	0.7	0.6	1.1	0.7	1.1
Total tannin (mg/g berry FW[a])	15.8	10.1	6.8	6.4	8.7

[a]FW, fresh weight;

Skin fragmentation assessment

Fruit from wineries B, C and E was hand-harvested and the low fragmentation index for the control was most likely caused during the de-stem and crush process. Fruit from wineries A and D was machine harvested and had a higher skin fragmentation percentage for the control treatments. The in-line ACE maceration device used in commercial wineries in 2015 appeared to be more effective in fragmenting the skin particles than the modified crusher roller option used in 2014 (Table 5). Skin fragmentation index for ACE treated grapes ranged from 3.6 to 11.9. The skin fragmentation index was found to be higher for the mainland wineries (A, B) than for the Tasmanian wineries (C, D and E) despite the difference in harvest methods, although the two machine harvested batches of fruit (A and D) were very similar.

Table 5. Skin fragmentation assessment

Winery	**A**		**B**		**C**		**D**		**E**	
Treatment	Control	ACE	Control	PEACE	Control	ACE	Control	ACE	Control	ACE
Av. Berry diameter (mm)	13 ± 3	-	12 ± 3	-	11 ± 2	-	11 ± 2	-	8 ± 4	-
No. of Fragments	15	27	5	36	3	18	9	25	10	20
Av. SA (mm^2)	45 ± 65	106 ± 77	160 ± 59	38 ± 35	66	35 ± 50	18 ± 27	78 ± 68	96 ± 38	39 ± 61
Av. P (mm)	39 ± 20	49 ± 27	49 ± 34	27 ± 15	22 ± 18	22± 20	18 ± 12	36± 19	31 ± 20	22 ± 25
Av. SA/P	11 ± 12	2 ± 0.8	13 ± 21	1.2 ± 0.6	99 ± 154	2 ± 3.6	41± 63	17 ± 75	39 ± 94	4 ± 12
Fragmentation index[a]	1.9	5.4	1.3	11.9	1.0	4.5	1.8	5.3	1.4	3.6

Standard Deviation for 5 g sub-samples of grapes. SA, Surface Area; P, Perimeter. SA/P, Surface area to perimeter ratio. [a]Fragmentation index is the number of fragments per berry.

Phenolic composition of wine

The most significant effect of ACE maceration in the commercial trial was the change in the tannin concentration and stable pigment content of the wine (Table 6). Increases in the tannin concentration with ACE maceration were apparent at each winery from the early stages of fermentation and remained higher than for control wines through to 6, 8 months post-inoculation. The development of stable colour complexes in ACE treated wines during wine maturation was observed, with the exception of PEACE treated wine from Winery B which did not follow this trend, most likely as this wine was fermented using a wild yeast and the fermentation process had only just begun when the wine was pressed off (Table 6).

It is noteworthy that the greatest impact of ACE maceration was not necessarily related to the degree of fragmentation of the skins, as in the commercial trials this was shown to cover a particle size range that was from 2.5 to 55% less than the crushed berry control (Table 5). It would appear that any increase in skin fragmentation beyond that of conventional wine making methods effectively improves the phenolic composition of the wine.

Table 6. Skin fragmentation effect on wine phenolic parameters at end of ferment and 6,8 months post ACE application.

Winery	**A**		**B**		**C**		**D**		**E**			
Treatment	Control	ACE	Control	PEACE	Control	ACE	Control	ACE	Control	ACE	PEACE	*p*-value
					Wine at end of fermentation (8,9 days post ACE application)							
Phenolic parameter												
Anthocyanin (g/L)	0.06	0.05	0.17	0.19	0.45	0.51	0.28	0.27	0.41	0.41	0.44	<0.001
Tannin (g/L)	0.2	0.31	0.54	0.92	1.07	1.18	0.74	1.09	0.99	1.20	1.10	0.007
[a]NBP (AU)	0.62	0.53	0.55	0.5	0.61	0.79	0.34	0.57	0.59	0.69	0.58	<0.001
NBP (%)	14.9	17.7	5.9	4.9	2.6	2.9	2.4	4.0	2.8	3.2	2.5	0.014
[b]WCD (AU)	3.38	3.17	3.29	3.38	0.73	0.74	1.7	1.38	4.79	5.63	5.35	0.007
[c]Hue	0.96	0.97	1.02	0.89	3.18	4.43	3.18	4.43	0.78	0.73	0.65	NS
[d]Hue SO_2	1.66	1.72	1.9	1.77	5.31	6.17	0.66	0.65	1.91	1.79	1.81	NS
					Wine at 6,8 months post ACE application							
Anthocyanin (g/L)	0.09	0.09	0.11	0.12	0.35	0.33	0.19	0.11	0.22	0.22	0.24	<0.001
Tannin (g/L)	0.72	0.80	1.23	1.21	1.03	1.19	0.85	1.63	0.73	1.19	0.99	0.009
[a]NBP (AU)	0.62	0.65	0.91	0.52	1.13	1.48	0.84	1.77	1.02	1.35	1.33	0.019
NBP (%)	11.4	12.0	12.9	7.4	5.9	7.7	7.8	21.3	8.2	10.0	9.4	<0.001
[b]WCD (AU)	2.74	2.92	3.85	2.90	6.33	7.12	3.60	5.64	4.88	6.08	6.30	<0.001
[c]Hue	1.07	1.12	1.03	0.89	0.76	0.77	0.82	0.79	0.86	0.81	0.79	<0.001
[d]Hue SO_2	1.60	1.69	1.57	1.66	1.47	1.32	1.40	1.01	1.46	1.28	1.32	NS

[a]Non-bleachable pigments resistant to bleaching in the presence of sulfur dioxide; [b]WCD, Wine Colour Density (A420+A520 in model wine solution); [c]Hue (ratio A420:A520 in model wine solution); [d]Hue SO_2 (hue resistant to sulfur dioxide bleaching). Significance ($P<0.05$) determined using Tukey's test.

Phenolic composition of wine

The changes in colour density, hue and percentage non-bleachable pigment of the ACE wines are all indicators of quality improvements in both the appearance and longevity of wine colour. The results indicated that the ACE wines from Tasmania (wineries C, D and E) were imbued with more red hues relative to the control wines, whereas the mainland ACE treated wines (wineries A and B) had more red-brown hues. Together with the relatively low concentration of anthocyanin in the grapes from the mainland wineries (Table 4) this suggests that the high tannin content of the mainland grapes was more likely to be seed tannin than skin tannin.

A comparison of control wine made at Winery E and wine that was subjected to skin fragmentation (ACE) then pressed early from the grape solids (four days of fermentation) to make PEACE wine, showed that the treatment conferred greater colour density, percentage of non-bleachable pigments, and both red and blue-purple pigments than for the control wine. These phenolic attributes were similar to the full-term ACE wines that had been on skins for an additional four days (Figure 4). Phenolic analysis conducted on samples collected during fermentation suggested that sufficient anthocyanin and tannin were extracted from the grape skins four days after ACE was applied, to produce a significant increase in desirable stable pigments conferring greater colour density and blue hue on the wine than was apparent for the control treatment.

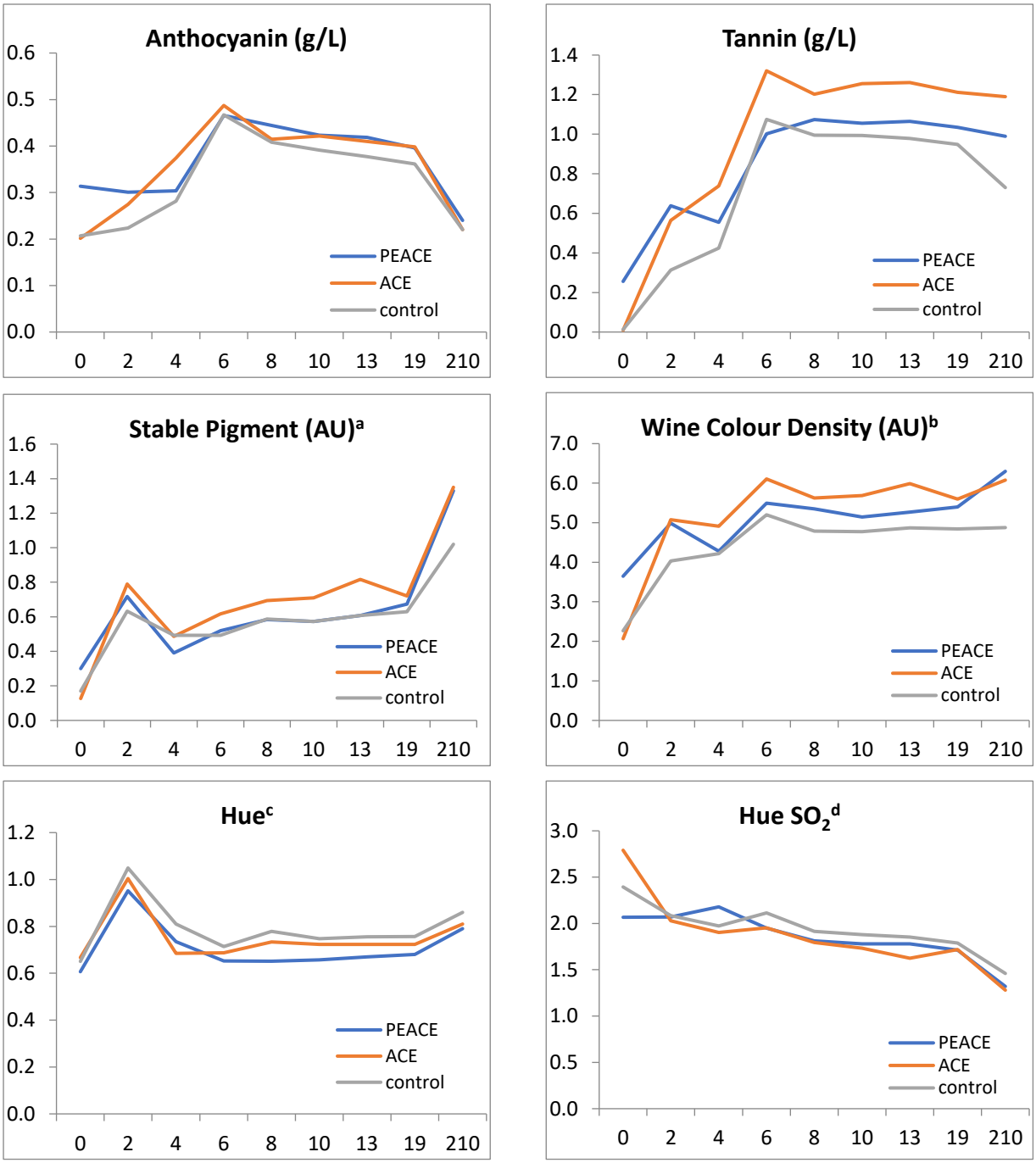


Figure 4. Phenolic progression for winery E from fermentation to six months bottle age. Days from crushing shown on horizontal axis; Control, pomace treated using conventional maceration; ACE, pomace cut prior to fermentation; PEACE, are ACE wines pressed off on day 5 of fermentation. [a]Non-bleachable pigments resistant to bleaching in the presence of sulfur dioxide; [b]WCD, Wine Colour Density (A420+A520 in model wine solution); [c]Hue (ratio A420:A520 in model wine solution); [d]Hue SO_2 (hue resistant to sulfur dioxide bleaching). Data are mean values of triplicate samples (n=24); For each parameter, numbers in the same column with different letters are significantly different at P ≤ 0.05 using Fisher's LSD test.

Sensory Evaluation

The sensory characteristics of the commercially made ACE and PEACE wines were assessed at a single event when the wines were approximately 6 to 8 months old (~ 210 days after ACE application) according to the commencement date of the trial at each winery (Table 7).

Table 7. Wine scores for ACE, PEACE and control wines (n=15) at 6, 8 months post fermentation.

	Winery				
Treatment	**A**	**B**	**C**	**D**	**E**
Control	10 ± 2.4	9 ± 1.2	15 ± 1.1	12 ± 2.1	14 ± 1.3
ACE	11 ± 1.9	-	16 ± 1.3	15 ± 1.5	16 ± 1.4
PEACE	-	9 ± 0.9	-	-	16 ± 0.8

Data Mean and Standard Deviation for seven panellists

When compared to the control wine, panellists agreed that ACE maceration imparted a more purple hue, greater colour density, confirming the results for phenolic analysis. They described more dark fruit attributes on the aroma and palate and considered the ACE macerated wines to have more structure than the control wine. Higher tannin was perceived on the palate, as was demonstrated for the phenolic analysis (Figure 4). The PEACE treated wines differed somewhat between wineries with the mainland winery (winery B) showing little difference in fruit aroma, dark fruit on the palate and tannin compared to the control wine (these wines were made used wild yeast), whereas PEACE treated wine from Tasmania (winery E) that used yeast inoculum RC212, showed improvement in all of the sensory characteristics relative to the control. Overall, the sensory panel preferred the ACE wines (90%) to the control wines (10%).

After 18 months in the bottle, wines from Winery E were presented at the International Cool Climate Wine Symposium 2016 at a blind tasting where a panel of 53 tasters nominated their preferred wines as PEACE wine (43%), ACE wine (36%), control wine (13%).

Conclusions

The opportunities presented by ACE to improve wine colour density and tannin in wine and PEACE maceration to economise on tank space, pump-over logistics and the labour required for daily punch down of the pomace cap, are clear. In view of the results described in this study, it is proposed that ACE maceration may have an effect similar to heat extraction but had much lower energy inputs. PEACE maceration has the added benefit of reducing the time taken for each batch of fruit to occupy the fermentation tank effectively reducing the tank space required for fermentation. Consequently, both ACE and PEACE maceration may represent a simple, fast and economic alternative to a range of conventionally practiced maceration techniques and could become a valuable asset to the wine industry in managing compressed vintages.

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ACE Project Staff

Project Manager:

Angela Sparrow, Tasmanian Institute of Agriculture, University of Tasmania

Technical assistance:

Mark Wilson, Tasmanian Institute of Agriculture, University of Tasmania.

Winemakers:

Anthony Murphy, Mark Holm, Trentham Estates, Gol, New South Wales

Frank Van de Loo, Mt Majura Vineyard, Majura, ACT.

Jeremy Dineen, Ockie Myburgh, Stewart Byrne, Josef Chromy Wines, Relbia, Tasmania

Alain Rousseau, John Bown, Frogmore Creek Winery, Cambridge, Tasmania

Julian Alcorso, Winemaking Tasmania, Cambridge, Tasmania

Wine sensory evaluation experts:

Francesca Blefari, Australian Wine Research Institute, South Australia

Leigh Francis, Australian Wine Research Institute, South Australia

Wies Cynkar, Australian Wine Research Institute, South Australia

Chapter 7. The business case for adoption of microwave and ACE maceration in commercial red winemaking

* This chapter was prepared by Mr Paul Smart, with input from Drs Anna Carew and Angela Sparrow*

Executive Summary

This report explores the business case for employing CPR (Controlled Phenolic Release) or ACE (Accentuated Cut Edges) in commercial wineries.
Some of the major flavour components of red wine occur in the skins of grapes. The extraction of these components is difficult and often requires both time and capital to extract.
The current method for extracting skin flavour compounds involves fermentation on skins. This is performed in conjunction with cap management techniques such as plunging (hand or mechanical, manual or automated), heading down boards or gas manipulation (Ganimede), rotary fermenters, pump-overs (manual or mechanical), or by the extreme thermovinification method.

All of these techniques require time on skins, labour hours or capital expenditure.
Two solutions have presented themselves from recent research. They are:

- CPR (Controlled Phenolic Release)
- ACE (Accentuated Cut Edges)

CPR (Controlled Phenolic Release)
CPR is the method of using heat to extract the flavour components from skin cells. The heat applied forces the skin cells to rupture and releasing the flavour components. This is a similar method to thermovinification, but differs in that only the solid part of the must is heated, not the entire must. This has proven to retain better delicate flavours in the juice, as well as releasing the skin-based flavour compounds. It also reduces the amount of time on skins that is required for extraction.

ACE (Accentuated Cutting Edges)
Crudely, ACE is red wine making with a giant mixer-blender. The blades of the machine cut the skins into segments, without damaging the seeds. This facilitates the extraction of the skin-based flavour compounds as the surface area is increased, promoting extraction rates. This also reduces the amount of time on skins

Both of these approaches reduce the time on skins required, which is an advantage, given the increasing trend for compressed vintages.

Cost-benefit Analysis

Assumptions for the business case are listed at the end of this chapter.

The reason for performing CPR or ACE on red must is to improve the extraction efficiency. This will reduce the time needed for skin contact and free up equipment, or in business terms, make better use of the fermentation capital expenditure. The extra capital cost that is needed to invest in buying the new equipment must be paid for by efficiencies in the system.

There are two methods we can use to determine whether buying a microwave unit or an ACE macerator is a good idea for a business. The first is to compare a hypothetical greenfield development of a 200 tonne crush of red grapes, with and without the new equipment. The second is to buy the piece of equipment for an existing winery.

All the assumptions that have been made are listed at the end of this chapter.

Greenfield Development

The major differences between the scenarios are the extra capital for the microwave unit or ACE macerator, the decreased capital requirement for fermenters, increased energy costs and the savings in labour due to less fermentation time.

Fermenters

Due to the decreased contact time, fewer fermenters would be required. The best time has been averaged to around three days, as opposed to the control of eight days. This time saving would result in 62.5% reduction in the number of fermenters required. The model proposed that originally the fermenters would be turned over (utilised) 2.5 times per vintage, this increased to 6.6 times per vintage.

CPR Equipment

A large microwave unit is expensive ($250k for a 100kW system), and uses a lot of energy, so it makes sense to only heat half the must, the solid half, where the skins, tannin and colour are located. A 50kW unit is considerably cheaper ($100k) and can facilitate heating half the must (total must flow of 2t/Hr) from 15°C to 70°C. This will also require the purchase of an inline drainer that can facilitate the separation into juice and wet solids.

A heat exchanger will also be required to cool the heated material down, but this requirement may be lessened as the juice is already at 15°C and the two streams will be mixed prior to loading the fermenter. There will also be some quality benefits in separating the juice from the heating element.

The 50 kW microwave unit modelled in this scenario can process a maximum of 2 tonnes of red grapes per hour.

ACE Equipment

The only piece of extra equipment required for ACE is the ACE macerator. This has been estimated to cost about $30,000 based on the prototype costing ~$7,000. The motor has been overestimated to be 10kW for a 2t/hr flow rate and no other equipment is needed as the unit sits inline, after the must pump. Also, no extra labour hours would be required to operate it as the controls could be connected to the must pump or control panel located for the crusher operator.

Pump-over Equipment

This has been estimated as $1,000 as more often than not, this equipment is usually already on site.

Depreciation

The cost of the capital required has been calculated using a straight-line depreciation over seven years, with a $0 residual value.

Staffing Costs

Pump-overs require a lot of labour to set up, execute and pack down. This is compounded due to the longer fermentation time for the control method. The average ferment needs two pump-overs a day, for two hours of labour each. This is where a large component of the savings with the new method could be achieved, as labour costs in Australia are very high.

The labour required for the microwave unit will only be needed on a per batch basis, as the treatment only happens during the crushing. It has been assumed that two hours per batch will be required for operating the microwave. The unit itself would be automated and in-line with the must pump, but extra labour would be required after the heating period to cool the hot wet solids, and to combine them with the juice prior to loading the fermenter.

The greatest labour savings would be for ACE, as the machine is operated at the same time as the crusher and must pump. There would be no other labour needs, as following the cutting, the must is pumped directly into the fermenter. A half hour per batch has been allocated to run the ACE macerator, to allow for clean-up and maintenance.

Energy

Heating and cooling is costly and the microwave method would require a large amount of power. To save on power, it was decided to separate the juice and wet solids stream (assuming a 50/50 separation) and to heat/cool only the stream that matters, thereby halving the energy requirements. Other energy savings that can be made are by increasing the separation so there is less juice in the solids stream, using a heat recovery exchanger to transfer heat from the post microwave-treated, heated wet solids to the incoming, pre-microwave-treated, cold wet solids.

The size of microwave unit investigated for CPR is 50kW, which can heat 1 t/hr of wet solids (2t /hr of must) from 15°C to 70°C. This is equivalent to 50 kWh for every two tonnes of grapes, or 5,000 kWh for the entire vintage.

The cooling requirement is less as the wet solids are cooled from 70°C to 30°C, fermentation temperature. This is equivalent to (assuming we are cooling water which has a higher specific heat) 4,666 kWh for the vintage.

The energy required to run the ACE macerator is significantly less. It is assumed, based on data from the prototype, that a motor of 10 kW would be more than sufficient to process 2t per hour. This equates to 1,000 kWh per vintage.

For pump-overs, it was assumed that the pump was rated at 2 kW, which equated to an energy use for the entire vintage of 2,560 kWh.

Summary - Greenfield Development

As shown in Table 1 below, there is a substantial saving in using either ACE or CPR. The CPR has a repayment period on the extra capital required of 3.2 years and an ROI of 31.6%. The ACE is the cheaper method as the method will pay for itself in the first year.

	Normal 200t	**With CPR**	**With ACE**
5t Fermenters	$80,000	$30,000	$30,000
Microwave	$0	$100,000	$0
Heat exchanger	$0	$7,000	$0
Drainer	$0	$12,000	$0
Pump-over equipment	$1,000	$0	$0
ACE macerator	$0	$0	$30,000
Total Capital	*$81,000*	*$149,000*	*$60,000*
Depreciation of Capital PA	$11,571	$21,286	$8,571
Cost to run P/Overs	$35,200.00	$0.00	$0.00
Cost to run MW	$0.00	$2,200.00	$0.00
Cost to run ACE	$0.00	$0.00	$550.00
Total Staffing	*$35,200.00*	*$2,200.00*	*$550.00*
Energy for heating	$0.00	$1,250.00	$0.00
Energy for cooling	$0.00	$1,166.50	$0.00
Energy for P/Over	$640.00	$0.00	$0.00
Energy for ACE	$0.00	$0.00	$250.00
Total Energy	$640.00	$2,416.50	$250.00
Total Costs	$47,411	$25,902	$9,371
Total Savings		$21,509	$38,040
Total Capital	$81,000	$149,000	$60,000
Extra Capital		$68,000	-$21,000
Repayment Period		3.2	-0.6
ROI		**31.63%**	**-181.14%**

Table 1 Greenfield Development

Retrofitting CPR or ACE to an existing winery

This scenario uses the same assumptions as before with the only adjustment being the addition of the equipment to an existing 300 tonne winery. The reason for changing the size used in the model is to force the repayment period to fall into the 4-5 year range, an acceptable range for buying equipment.

Depreciation

This has not been costed for the fermenters and pump-over equipment as the winery is either already paying for, or has fully depreciated these items, so it is not considered in this calculation.
Depreciation is calculated for CPR and ACE as these are new capital costs that need to be accounted for, and has been calculated using the straight-line method over seven years, with a $0 residual.

Energy and Staffing Costs

These costs have been calculated using the same method as above but have increased due to the increased tonnage used in the model.

Summary

From Table 2 below, it can be seen that adding a 50 kW microwave unit to a 300 tonne winery would repay itself in 4.0 years, due to significant labour cost savings associated with cap management of ferments.

Also, it can be seen that the cost of adding an ACE macerator to a 300 tonne winery would be repaid within one year.

It must also be noted that the winery can increase in capacity as there will be empty fermenters due to shorter ferment times. It is estimated that the increased capacity for both ACE and CPR, based on this 300 tonne winery model, and an increase in fermenter turnover from 2.5 per vintage to 6.7 per vintage, would mean that this winery could potentially process 800 tonnes. That could be achieved without buying any extra fermenters.

To put it another way, if the winery chose to stay at a 300 tonne processing capacity, the total length of vintage would decrease from 20 days to 7.5 days, reducing the duration of vintage by 266%.

	Without CPR	Extra CPR	Extra ACE
5t Fermenters	$120,000	$0	$0
Microwave	$0	$100,000	$0
Heat exchanger	$0	$7,000	$0
Drainer	$0	$12,000	$0
ACE macerator	$0	$0	$30,000
Pump-over equipment	$1,000	$0	$0
Total Capital	*$120,000*	*$119,000*	*$30,000*
Depreciation of Capital PA	$0	$17,000	$4,286
Cost to run P/Overs	$52,800	$0	$0
Cost to run MW	$0	$3,300	$0
Cost to run ACE	$0	$0	$825
Total Staffing	*$52,800*	*$3,300*	*$825*
Energy for heating	$0	$1,875	$0
Energy for cooling	$0	$1,750	$0
Energy for P/Over	$960	$0	$0
Energy for ACE	$0	$0	$375
Total Energy	$960	$3,625	$375
Total Costs	$53,760	$23,925	$5,486
Total Savings		$29,835	$48,274
Extra Capital		$119,000	$30,000
Repayment Period		4.0	0.6
ROI		**25.07%**	**160.91%**

Table 2 - Additional CPR or ACE equipment to an existing winery

Recommendations

Both CPR and ACE offer significant savings to the winery, both in time and costs.

CPR

Buying and operating a microwave unit is expensive, although this is offset by the labour savings. The quality of wines made by this method is equivalent to traditional methods. There also may be other uses for the equipment such as heat treating wine prior to bottling for preservative-free wine.

ACE

This is the cheaper alternative and is a very elegant solution to the red fermentation maceration problem. The quality of the wines made by this method is also equivalent to traditional models. It is simple to use and has minimal energy and labour costs.

Assumptions

This cost-benefit analysis only takes into consideration line items that would change between the different scenarios, such as fermenters, labour for pump-overs etc. It is not a full set of financial statements for a winery and only represents a hypothetical model. Professional financial advice should be sought before making any decisions.

Assumptions	Traditional	With CPR	With Ace	
Tonnes processed	200	200	200	
Number of fermenters	16	6	6	
Fermenter size	5	5	5	
Fermenter cost	$5,000	$5,000	$5,000	
Days fermenting	8	3	3	
Total days fermenting	20	20	20	
Microwave cost	$0	$100,000	$0	
Heat exchanger	$0	$7,000	$0	
Pump-over equipment	$1,000	$0	$0	
Drainer	$0	$12,000	$0	
ACE macerator	$0	$0	$30,000	
Staff hours per day p/over	64	0	0	(2 hr per tank, twice)
Staff hours to run MW unit	0	2	0	(2 hr per batch)
Staff hours to run ACE	0	0	0.5	
Staff hourly rate	$27.50	$27.50	$27.50	(including 25% on-costs)
Energy to heat solids 15°C to 70°C	0	50	0	kWh
Energy to cool solids 70°C to 30°C	0	0.04666	0	kWh/kg
Energy per P/over	4	0	0	kWh
Energy to run ACE macerator	0	0	5	kWh / tonne
Energy cost	$0.25	$0.25	$0.25	/kWh
Depreciation	7	7	7	years

Table 3: Cost-Benefit analysis assumptions

Outcomes and Conclusions

The project delivered strongly against the majority of objectives outlined in the original proposal to develop and trial CPR and ACE maceration processes in a commercial setting. It attempted to deliver both scientific value and industry impact. The objectives were broadly two-fold:

1. **Efficient Production** of sound wines by novel methods
2. **Intensive Engagement** with winemakers throughout the execution of trial work

Efficient Production

The chemical and sensory data, analysis and interpretation presented in this report demonstrate that the trial wines were produced and assessed for their chemical and commercial soundness. Consideration of the business case for each novel maceration approach ascertained that both offered acceptably short pay-back periods to justify investment, although human factors and technology design and maintenance were concerns for the CPR process.

In addition to thorough analysis of wines by established chemical and sensory methods, some of the trial wines were assessed using novel methods (e.g. macromolecular complexes, monosaccharide concentration) and hence provided value beyond the stated objectives in supporting expansion of fundamental wine knowledge and analytical capability. In terms of additional scientific benefit, analysis of the wines from novel processes against those made by more traditionally methods (e.g. cold soak, extended maceration) allowed integration and interpretation across related measures. For example, the relationship between total tannin concentration, tannin composition and the monosaccharide profile of wines provides new insight into the potential for differential targeting of components of grape cells with different maceration methods (e.g. hemicellulose may have been particularly susceptible to ultrasound maceration; tannin polymers from extended maceration wine were similar in the number of subunits but of different size to microwave wines, suggesting an alternate polymerisation pathway).

Where possible, wines were evaluated over a longer period of aging, to establish the enduring impact (or lack thereof) that would be required to justify industry investment in equipment, process adjustment and staff training/education to adopt novel maceration processes. This analysis emphasised a general principle for wine science that analysis of red wines for phenolics impact needs to consider both what size and scale of difference in phenolics concentration would be of significance or value to industry and consumers, and the time point at which that impact tends to be evaluated by winemakers. Winemakers would need to see significant impact at the time when blending and bottling decision are made (i.e. between six and 18 months barrel age). This emphasises the difference between an ‘industry significance’ standard for practical impact, and ‘statistical significance’ to demonstrate scientific impact.

Efficient Production

Key to the success of CPR experimental work was active engagement and participation by winemakers in their own facilities. Across the project timeline, five Victorian wineries directly participated in preparation of semi-commercial scale comparative ferments by harvesting, crushing and destemming fruit; negotiating timing for fruit to be taken off-site for microwave processing; commenting on the differences between the control and experimental must immediately after application of microwave maceration; running side-by-side ferments and monitoring the development of wine character during primary fermentation; handling pressing off; assessing control and microwave wines for their independent character; and deciding on the blending and bottling fate of the wines produced. ACE maceration trials were also undertaken in this intensely embedded way, with approximately seven wineries across Victoria and Tasmania participating. Additionally, project team members initiated approximately 14 informal industry tasting events whereby groups of winemakers who had gathered for other purposes were invited to taste, comment on and discuss wines produced using the novel methods. For example, during 2015, control and CPR wines were taken to a tasting event being run for 12 winemakers at Moorooduc Estate, Mornington Peninsula, Victoria. Wines were tasted blind amongst a run of trial wines from attending winemakers, and all tasters nominated the CPR as the preferred wine. This approach allowed for authentic and engaged conversations with winemakers about the practicality, acceptability and even the philosophy of using microwave maceration.

While this intense engagement was costly in terms of budget (e.g. staff time, travel and accommodation) it was geared to ‘repay’ the project in a range of important ways. Winemakers explained to project researchers that the opportunity to try novel processes in their own wineries allowed them to assess its immediate practicability, and the extent of production changes that would be required to implement novel processes on-site. They also identified times that frank conversations had been held between research team members and winery staff, which adjusted the project and research direction where operating conditions or desired wine outcomes differed from the research team’s assumptions. Head winemakers often brought inquisitive or creative members of their team together with researchers to engage in higher level discussions about wine science, seeing this as an ad-hoc professional development opportunity for staff, and smaller wineries tended to have a ‘drop in’ community of local winemakers who would often call around to taste novel wines or examine and comment on novel ferments as the wines developed. At field days and roadshow events, the opportunity to show pictures of respected winemakers participating in research activity, share trial wines made by those winemakers, and use winemaker quotations on process and wine qualities provided the research team with added credibility and authenticity, which can otherwise be difficult to garner with more laboratory-based research.

Recommendations

1. Research on thermal maceration technology could be continued with a view to further development of novel processes. However, while a good business case can be made for microwave processing from a production efficiency perspective, the investment in developing a unit/design that is robust, safe and well suited for winery operations may not be warranted given the availability of established thermovinification technologies that use conductive and convective heat transfer, particularly in Europe.
2. There would be great value in improving early press-off production efficiency by growing and refining the capacity of Australian winemakers to use established, and comparatively inexpensive, thermovinification units. This is particularly the case for varieties with a challenging phenolic extraction matrix, such as Pinot Noir.
3. Previous research and the sensory analysis data presented herein suggest that managing the 'juice' phase of alcoholic fermentation after early press-off requires additional research to mediate aroma qualities of finished wine (e.g. the relationship between oxygen ingress and CO_2 venting, and the confected and red fruit aroma which is often observed in thermo-vinified red wines).
4. The use of ultrasound maceration may offer benefits for the rapid extraction of phenolics, and also alternate compounds to drive alcoholic fermentation (e.g. hemicellulose breakdown). The trials conducted in this project provided mixed results and a more comprehensive focus on the technology is warranted for years with higher production pressure (e.g. vintage compression, low ripening). This examination might best be initially limited to varieties that offer a difficult phenolic extraction challenge or low colour.

Appendix 1: Communication

Media

1. Daily Wine News, 12 and 25 March 2015
2. ABC Rural Radio, 24 March 2015. Reporter Deb O'Callaghan

TRENTHAM turns it up - Testing out new cutting-edge technology

Trentham Estate recently participated in a trial using a new device that ultimately improves the quality of wine. The Pinot Noir fruit from Trentham's 2015 vintage played guinea pig to the invention, being the first time it has been trialled in a winery, and the results are promising! Angela Sparrow, a postgraduate from the University of Tasmania, approached Trentham's chief winemaker and director Anthony Murphy with the idea late last year. Excited by it all and never afraid of a little innovation, the Trentham founder was more than willing to test it out.

New technology uses giant blender to chop up wine grape skins to improves reds



Photo: Dr Angela Sparrow, of Tasmania University, and Trentham Estate winemaker Anthony Murphy trial her inline wine skin blender. (Supplied - Trentham Estate)

Map: Mildura 3500

A giant stick blender that chops wine grape skins into a pulp could be the next big thing in red wine making.

Dr Angela Sparrow, from the University of Tasmania, has been carrying her blender around in a suitcase, trialling it in Pinot Noir winemaking in Mildura, Adelaide, Canberra and soon Tasmania. She says her trial is showing by leaving the red grape skins in contact with the wine, it can improve flavour, colour and even reduce labour in the process.

Audio: New wine skin blending technology trialled in red wine making (ABC Rural)

"I think most of the winemakers just go 'wow'," she said.
"You can see it makes a difference already and I have said to them that the major difference you will see is in six months, then ultimately, as the years progress, five, ten years down the track."
Dr Sparrow said one of the key things winemakers wanted to maintain was the aroma and flavour and the best way to do this was to keep the fruit intact, but once it was crushed 'many of the good bits you want to keep' were lost, including colour.
"Normally with a Pinot Noir, the colour doesn't last. That's why normally you don't age a Pinot for 10 or 15 years, because the colour won't last," she said.
But she said with her mechanism, because more skin tannin came out as well as the colour, it would be more stable down the track.
Dr Sparrow has been working on the project for five years and an inline prototype of the blender is now being trialled in commercial wineries.
She said it had the potential to change some of the 100-year-old practices of the red wine industry.
When red wine is being fermented, wineries must try to keep the skins in contact with the wine, as not to lose flavours into the atmosphere.
"You have to punch down the fermenting pumice each day in order to keep the skins submerged under the liquid," she said.
So, with her device, the skins do not float as much, because they are chopped finer, but flavour and skin tannins are also released faster.
"If you can skip that step, that's a labour saving, and then if you can press it off early and retain your fruit flavours that way, your whole ferment takes up less space. And you can also close it, and retain your flavour and floral compounds," Dr Sparrow said.

3. University of Tasmania, Tasmanian Institute of Agriculture Newsletter March 2015



Rosy results for red wine trials

TIA's Angela Sparrow, based at Mt Pleasant, has been working on the science of improving your experience of sitting down for a nice glass of wine.
She has been working on trials at Trentham Estate in Gol Gol, NSW, using a device called an ACE (Accentuated Cut Edges) macerator, which helps improve wine quality. Trentham's 2015 Pinot Noir was the guinea pig. Essentially, the device macerates and cuts the grape skins to allow more thorough extraction of colour pigments and tannins. Ultimately, it builds the wine's intensity and stability and improves ageing potential. The Pinot Noir wine that resulted was judged to have an overall sensory score 35% better than the control wines. The trial was funded by the Australian Grape and Wine Authority, and trials have also been established in McLaren Vale, South Australia and Canberra. During late March and early April, the ACE maceration trial will be continued at four wineries in Tasmania.

4. Sunday Examiner, Launceston, Tasmania, 23 October 2016. Reporter: Hamish Geale

The Pinot Scientist: Could a simple stick mixer help combat the effects of climate change on the Australian wine industry

Posters

Carew, A. 2013. Perfecting Pinot Noir, Microwave maceration and yeast treatments. Posters 106, 108, 109. 15th AWITC Workshop 1.

Carew, A., Kerslake, F. 2015. TIA trials innovative maceration for Pinot Noir winemaking. Agfest.

Carew, A., Kerslake, F., Bindon, K., Smith, P., Dambergs, R., Close, D.C. 2016. Innovative maceration for fine, fast Pinot Noir wines. Poster 136. AWITC 2016.

Sparrow, A., Dambergs, R., Close, D. 2013. Cutting edge Pinot. http://www.utas.edu.au/__data/assets/pdf_file/0010/409159/Sparrow-101_Cutting-Edge-Pinot.pdf

Presentations

Carew, A., Sparrow, A., Dambergs, R., Glaetzer, S., Dineen, J. (2014). Distributed Industry Trials, oenological research design for changed winemaking practice. 2014. Crush Symposium 2014, Adelaide.

Carew, A., Dambergs, R. (2014). Rapid methods of phenolic extraction in reds. ASVO Inputs to Outputs: Is less more? AWITC symposium, Adelaide, 2014.

Carew, A., Dambergs, R. (2015). Winemaking style & red wine phenolics. Perfecting Pinot Noir Workshop, Mornington, 17 June 2015.

Carew, A. (2015). Impacts of innovative winemaking on Pinot Noir phenolics. Plumpton College Winery. 7th July 2015.

Carew, A., Kerslake, F., Dambergs, R., Close, D. Innovative maceration for fine, fast Pinot Noir wines. Crush Symposium, Adelaide 2016.

ACE Presentations 2015

Sparrow, A. (2015). Manipulation of red wine phenolic profiles during winemaking. Australian Wine Research Institute Roadshow' Hunter Valley NSW 19 May 2015

Attendance (40): 47% winemaking; 40% viticulture; 7% chemical suppliers; 6% contract packaging

Quote from the floor, (Hunter Valley Winemaker and consultant):

"Your process could save us a LOT of money!"

Sparrow, A. (2015). ACE maceration: An innovative processing technique to improve the ageing potential of Pinot Noir wine. Australian Wine Research Institute 'Perfecting Pinot Noir Workshop', Mornington Peninsula VIC 17 June 2015

Attendance (64): 88% viticulture and winemaking; 6% researchers; 3% marketing, media; 3% other

Quote from the floor (Mornington Peninsula Winemaker):

"You are suggesting a process that is completely different to our current methods"

Comment by East Gippsland winemaker following up on the presentation:

"I can see your process could be very useful for making rosé, so much faster and with better control over colour extraction"

Sparrow, A. (2015). ACE maceration delivers quality and space. Australian Society of Viticulture and Oenology seminar Adelaide, South Australia 19 November 2015.

Sparrow, A. (2015). ACE maceration: Is that any way to treat a wine grape? CRUSH 2015 The grape and wine science symposium, Adelaide, South Australia 20 November 2015.

Sparrow, A. ACE Manipulation of wine phenolics during winemaking. 9th International Cool Climate Wine Symposium, Brighton, England 26-28 May 2016.

Sparrow, A. ACE for quality and taste. Tasting workshop: "Innovations in Cool Climate Wine". 9th International Cool Climate Wine Symposium, Brighton, England 28 May 2016.

Online Publications

Carew, A.L. Lloyd, N.D.R. Close, D.C. Dambergs, R.G. (2013). Wine phenolic and aroma outcomes from the application of controlled phenolic release to Pinot Noir must**.** Proceedings of the 15th Australian Wine Industry Technical Conference, Sydney, New South Wales 13-18 July 2013. http://awitc.com.au/past_conferences/?proceedings_id=10626593

Carew, A. (2013). Here's a hot idea: Straight out of the microwave [online]. Australian and New Zealand Grapegrower and Winemaker, No. 598, Nov 2013: 58, 60, 62-63.

Carew, A. 2016. Innovative maceration for more options in the quest for perfect Pinot Noir [online]. Australian and New Zealand Grapegrower and Winemaker, No. 624, Jan 2016:70. *Original article*: [online] in Wine Australia, 2015. http://research.wineaustralia.com/innovative-maceration-for-more-options-in-the-quest-for-perfect-pinot-noir/

Controlled phenolic release – microwave maceration. Sally Easton. Winewisdom, [online] June 16, 2016. http://www.winewisdom.com/articles/techie/controlled-phenolic-release-microwave-maceration/.

Innovative maceration in the quest for perfect Pinot Noir. Grapegrower & Winemaker, February 2016. https://issuu.com/provincialpressgroup/docs/gw_currentissue_fceacf8266cb1c

Microwave for the vinification of Pinot Noir. Alessandra Biondi Bartolini. VQ – Vite, Vino & Qualità. July 2014. http://www.vigneviniequalita.it/microonde-per-la-vinificazione-del-pinot-nero/ *Original article*: Carew, A. Sparrow, ASM, Curtin, CD, Close, DC, Dambergs, RG. Microwave maceration of Pinot Noir grape must. Sanitation and extraction effects and wine phenolics outcomes. Food and Bioprocess Technology, April 2014, Volume 7, Issue 4, pp 954-963.

Shall I put your Pinot in the microwave? Sally Easton, Winesearcher, [online] 2013 https://www.wine-searcher.com/m/2013/08/shall-i-put-your-pinot-in-the-microwave

Factsheets

Carew, A. (2014). Cool climate Pinot Noir winemaking research. http://www.utas.edu.au/__data/assets/pdf_file/0006/991833/2014-fact-sheet_Pinot-noir_winemaking-research.pdf

Carew, A. (2017). A business case for adoption of microwave and ACE maceration in commercial red winemaking (in publication).

Hammond, S., Jones, J., Gracie, A., Kerslake, F., Dambergs, R. (2014). Does fruit light environment influence Pinot Noir quality? http://www.utas.edu.au/__data/assets/pdf_file/0008/742643/2015_SHammond_Ferment.pdf

Sparrow, A. Smart, R., Dambergs, R., Close, D. (2015). ACE maceration for improved extraction of skin tannin in Pinot Noir. http://www.utas.edu.au/__data/assets/pdf_file/0003/742638/2015_Angela_ACE_Ferment.pdf

Additional communications are listed in Appendix 5.

Appendix 2: Intellectual Property

The CPR process was a novel application of an existing technology, and hence did not represent new intellectual property. The commercialisation of the ACE maceration process represents a successful investment in research by Wine Australia, having generated a working novel maceration instrument which is still undergoing industry trials and evaluation.

Appendix 3: References

References in the report are listed at the end of each of the sections in which they are used.

Appendix 4: Staff, Industry Partners and Collaborators

Staff:

Dr Anna L Carew

Dr Angela Merry

A/Prof Dugald C Close

Dr Angela Sparrow

Dr Fiona Kerslake

Mrs Hanna Westmore

Dr Robert Dambergs

Dr Jenn Lavers

Acknowledgements:

Dr Paul Smith (AWRI)

Dr Keren Bindon (AWRI)

Dr Francesca Blefari, Australian Wine Research Institute, South Australia

Dr Leigh Francis, Australian Wine Research Institute, South Australia

Dr Wies Cynkar, Australian Wine Research Institute, South Australia

Dr Kai Knoerzer (CSIRO)

Mr Piotr Swiergon (CSIRO)

Mr Dylan McMahon (Seville Estate, VIC)

Mr Dan Buckle (Domain Chandon, VIC)

Mr Simon Black (Montalto, VIC)

Mr Franco D'Anna (Hoddles Creek Estate, VIC)

Mr Philip Moraghan (Curly Flat)

Mr Jeremy Dineen, Mr Ockie Myburgh and Mr Stewart Byrne (Josef Chromy, TAS)

Mr Brett McLean (Brown Brothers)

Ms Kellie Hoffmann (Treasury Wine Estates)

Mr Anthony Murphy, Mark Holm, Trentham Estates, Gol, New South Wales

Mr Frank Van de Loo, Mt Majura Vineyard, Majura, ACT.

Alain Rousseau, John Bown, Frogmore Creek Winery, Cambridge, Tasmania

Julian Alcorso, Winemaking Tasmania, Cambridge, Tasmania

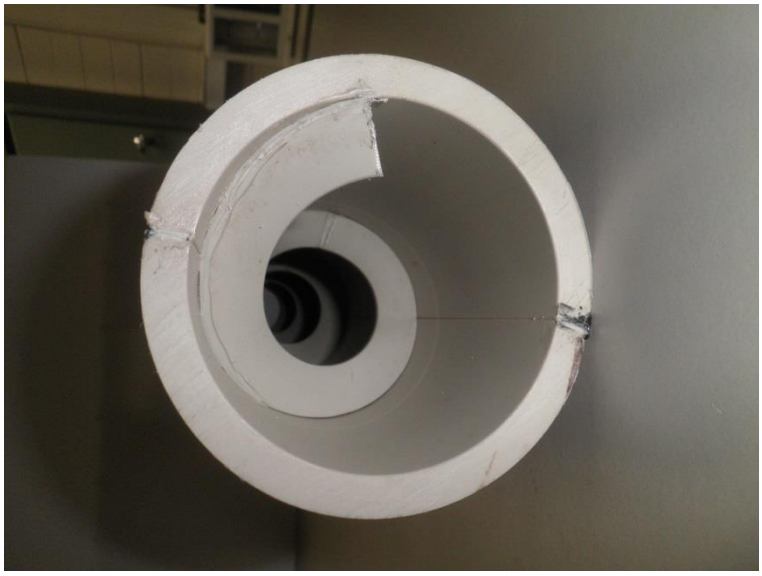
Appendix 5: Other Relevant Material

Appendix 5.1 Information about Grape Must Conveyor Prototype

Notes on prototype Grape Must Conveyor from Dr Roshan Dodanwela, CSIRO Clayton (14/01/2015) - We have completed welding the spiral sections into the 500mm section of pipe. I have attached a few photos. A few points to note:

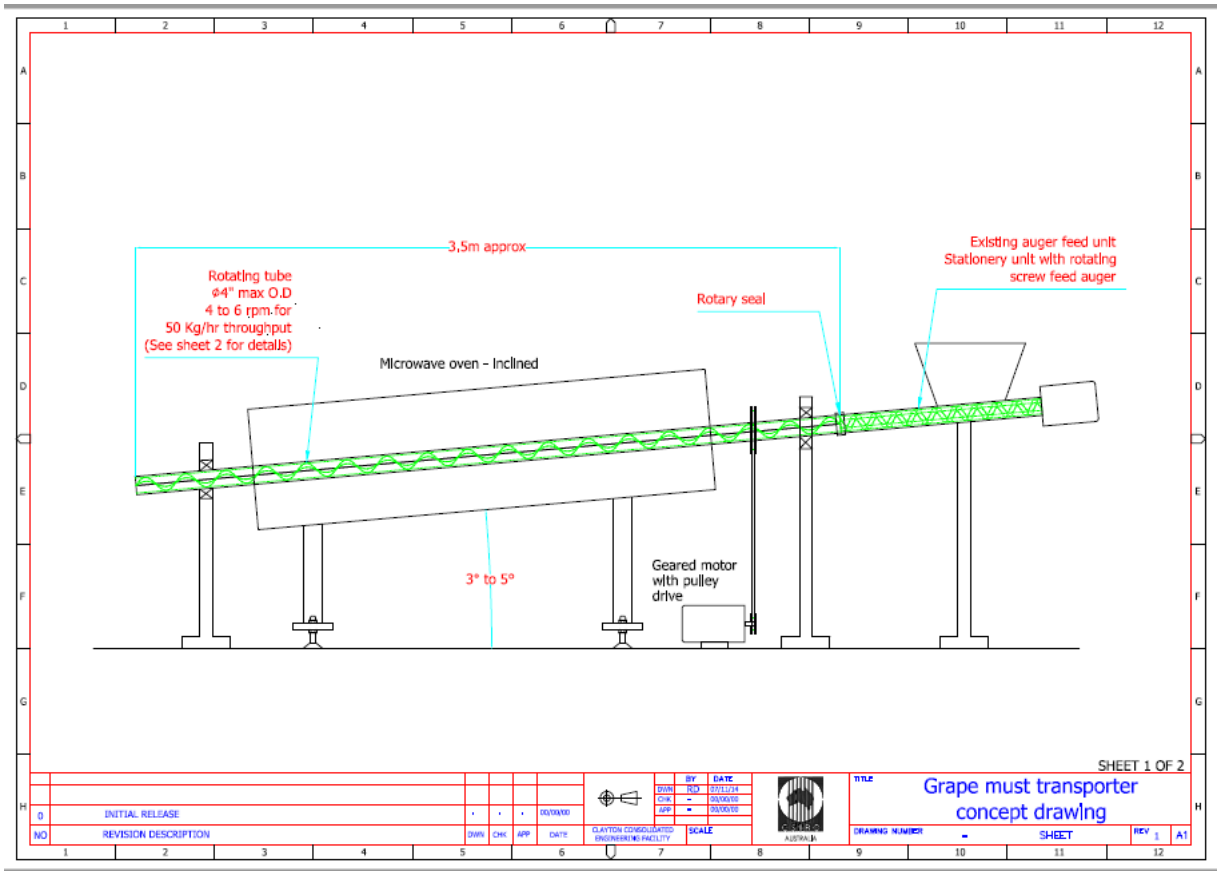
1. The plastic is food grade Polypropylene
2. The spiral sections are fused instead of welded – this was done to minimise heat distortion. The trade-off of fusing instead of fully welding is the strength. Fused bonds are not as strong as fully welded bonds. However, the fused spirals are quite rigid inside the tube and may well do the job.
3. There are a few burn marks on the outside of the tube which happened when we placed the tube in the oven. When the tube was split it opened out and we had to put it in an oven to get it back to its correct shape. The burn marks are as a result of playing around with the settings of the oven and attempting to reshape the pipe. It won't happen in the next version as we have worked out what requires to be done.

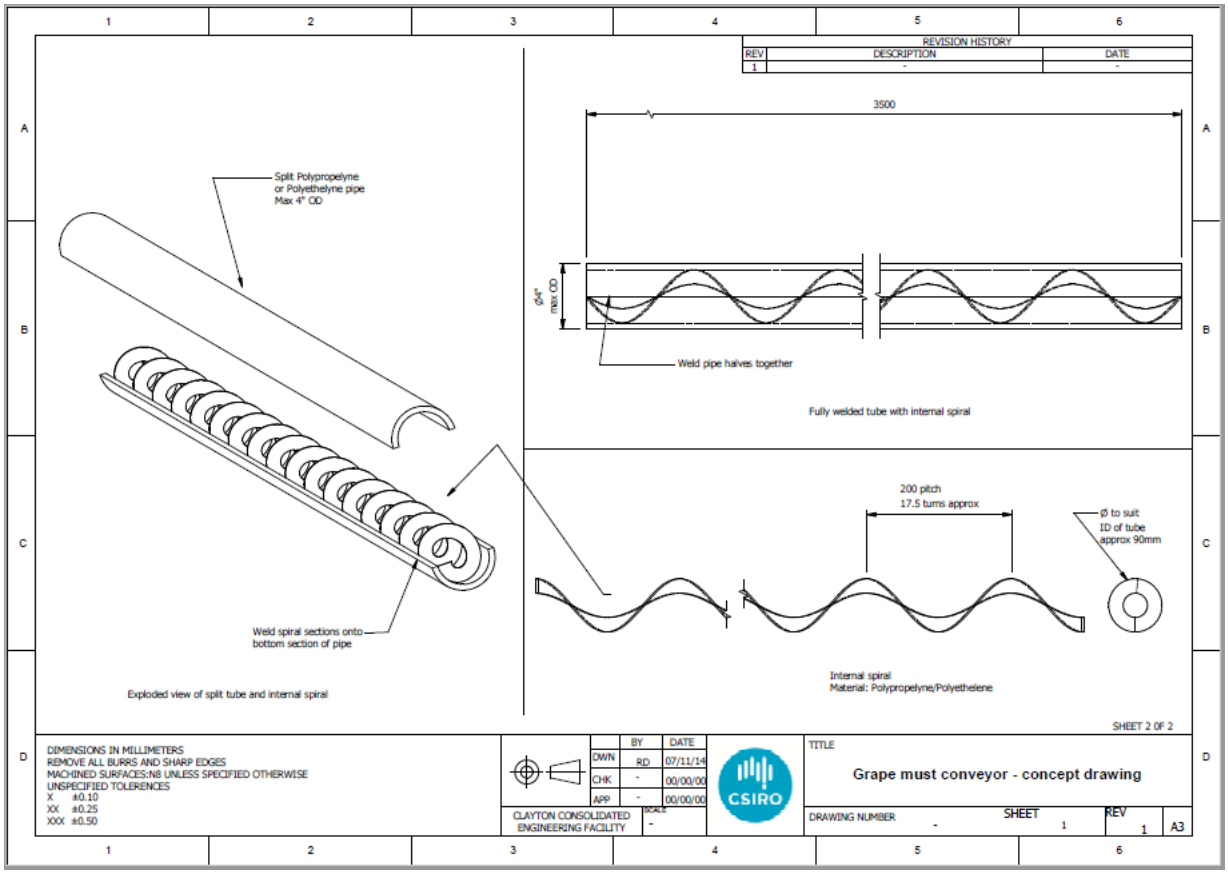
Please let me know if you want this section sent to Werribee or to Tasmania. We have another section of pipe and more spiral sections in the workshop and are ready to fabricate another 500mm section if required. If your trials indicate that the fused bonds are not strong enough we can fully weld the next section.





Concept drawings for Grape Must Conveyor provided by Dr Roshan Dodenwala, CSIRO Clayton (17/11/2014)




REVISION HISTORY
REV
DESCRIPTION
DATE
1
-
-
3500
Split Polypropelyne
or Polyethelyne pipe
Max 4" OD
Ø4"
max OD
Weld pipe halves together
Fully welded tube with internal spiral
200 pitch
17.5 turns approx
Ø to suit
ID of tube
approx 90mm
Weld spiral sections onto
bottom section of pipe
Exploded view of split tube and internal spiral
Internal spiral
Material: Polypropelyne/Polyethelene
SHEET 2 OF 2
DIMENSIONS IN MILLIMETERS
REMOVE ALL BURRS AND SHARP EDGES
MACHINED SURFACES:N8 UNLESS SPECIFIED OTHERWISE
UNSPECIFIED TOLERENCES
X ±0.10
XX ±0.25
XXX ±0.50
BY
DATE
DWN
RD
07/11/14
CHK
-
00/00/00
APP
-
00/00/00
CLAYTON CONSOLIDATED
ENGINEERING FACILITY
SCALE
-
CSIRO
TITLE
Grape must conveyor - concept drawing
DRAWING NUMBER
-
SHEET
1
REV
1
A3

Appendix 5.2: Phylloxera risk - Victorian Department of Environment and Primary Industries

APPLICATION FOR PERMIT TO TRANSPORT GRAPE MUST BETWEEN PHYLLOXERA ZONES 2014

PROJECT: Microwave maceration of Pinot Noir grape must

RESEARCH ORGANISATION: Tasmanian Institute for Agriculture, University of Tasmania

CONTACT:

Dr Anna Carew

165 Westbury Road, Prospect TAS 7250

anna.carew@utas.edu.au

Phone: +61 3 6336 5285

Mobile: +61 411894997

(Alternate contact: A/Prof Dugald Close dugald.close@utas.edu.au +61 3 6226 2776 +61 419386288)

PURPOSE: The Grape and Wine Research and Development Corporation has funded the University of Tasmania to collaborate with CSIRO Animal, Food and Health Sciences (Werribee VIC) to investigate the impact of microwave maceration of Pinot Noir grape must on the quality and production efficiency of Pinot Noir wines. Five volunteer winemakers (listed below) will contribute 1x400kg lot of destemmed Pinot Noir grapes to be transported to CSIRO Werribee. This 400kg lot will be microwave macerated. Following maceration, the 400kg lot of must will be transported back to the winery of origin.

PROPOSED TRANSPORTATION PLAN

1/ At the winery, fruit will be destemmed into non-vented 780 Nally IBCs (fruit picking bins) lined with 1000L capacity open top Sealed Air Entapac IBC liners (triple layer, food grade, barrier polymer).

2/ Excess bag capacity will be twisted and tied at two points with a large cable tie. Nally bins will be lidded and lids sealed around the perimeter with duct tape, and strapped down across two points (via fork lift channels) with heavy duty ratchet tie downs.

3/ Nally bins will be loaded to a one tonne ute with 2.4m wheel base and tied down to ute side and cargo bars with heavy duty ratchet tie downs.

4/ Nally bins will be transported on sealed roads from winery to Werribee (see attached proposed route).

5/ At CSIRO Werribee, grape solids will be hand sieved from juice, packed to 400 mL capacity containers and fed through the microwave processing unit. Liners will be discarded to a biohazard bin and fresh liners applied to Nally bins. Fruit will be returned to bins and sealed and loaded as per 2/ and 3/ above.

6/ Nally bins of fruit will be transported on sealed roads from Werribee to winery (as per 4/ above).

PROPOSED SPILL RESPONSE

The ute will be equipped with a Strataspill Spill Response Kit. In the event of a spill, Dr Carew will apply the spill kit to contain spilled grape must, and immediately contact the Metropolitan Fire Brigade (000) to request assistance for a biological hazard spill. Dr Carew will also notify DEPI VIC Regulation and Compliance about the spill on 03 8371 3500.

PROPOSED TRANSPORTATION PERIOD: 1st March 2015 to 10th April 2015

State Government Victoria Department of Primary Industries

PERMIT APPLICATION
PHYLLOXERA HOST MATERIALS

Exporter or Consignor details

Name (eg business name, individual etc): CURLY FLAT

Contact Name: PHILLIP MORAGHAN / ANNA CAREW

Postal Address: 263 COLLIVERS ROAD LANCEFIELD

VIC Post Code: 3435

Production Address (ie origin of plant material or machinery): AS ABOVE

Post Code:

Telephone: (03) 54291956 Mobile: 0417583387 Fax: phillip@curlyflat.com

Importer or Consignee details

Name (eg business name etc): CSIRO ANIMAL, FOOD & HEALTH SCIENCES

Contact Name: MR ROD SMITH / DR ANNA CAREW

Postal Address: 671 SNEYDES ROAD WERRIBEE VIC

Post Code: 3030

Receiver Address (eg Vineyard Address): AS ABOVE

Post Code: anna.carew@utas.edu.au

Telephone: (03) 9731 3208 Mobile: 0411894997 Fax: (03) 9731 3202

Consignment Type:

- ☐ grapevine cuttings
- ☐ grapevine rootlings
- ☑ whole grapes (fruit)
- ☐ samples - soil
- ☐ samples - leaves, berries, plant material
- ☐ agricultural equipment
- ☐ wine manufacturing equipment
- ☐ packages / bins
- ☐ other .....

Date To Be Exported: (ESTIMATED)

Item to be exported (incl variety, type, model etc)	Quantity	Vine Material Only Rootstock/Clones	Vine Material Only- District name & street address where planting
Pinot noir grape must	400kg		
(4) MUST WILL BE OF ORIGIN AT THE CSIRO, WERRIBEE		RETURNED TO WINERY AFTER TREATMENT	(4)

In the case of agricultural machinery, the business may obtain approval to sign Plant Health Declaration permitting the movement of each piece of agricultural equipment / bins. Name s of persons proposed as signing and issuing declarations -

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of Applicant: ANNA CAREW Signature: A Carew Date: 24/2/15

Please return form by fax to the nearest Plant Standards Officer in the region in which the consignment will be received:

Ballarat	Peter Clarke	(03) 5336 6859 (ph)	(03) 5336 6601 (fax)
Bendigo	Judy Pouliot	(03) 5430 4444 (ph)	(03) 5430 4533 (fax)
Irymple	Peter Dixon	(03) 5051 4500 (ph)	(03) 5051 4518 (fax)
Melbourne	Tanya Krause	(03) 8371 3500 (ph)	(03) 9687 6746 (fax)
Rutherglen	Chris Pollard	(02) 6030 4500 (ph)	(02) 6030 4600 (fax)
Tatura	Trish Lord	(03) 5833 5222 (ph)	(03) 5833 5299 (fax)

Department of Primary Industries

PERMIT APPLICATION
PHYLLOXERA HOST MATERIALS

Exporter or Consignor details

Name (eg business name, individual etc): DOMAIN CHANDON
Contact Name: DAN BUCKLE / ANNA CAREW
Postal Address: GREEN POINT, COLD STREAM VICTORIA
Post Code: 3770
Production Address (ie origin of plant material or machinery): AS ABOVE
Post Code:
Telephone: (03) 9738 9200 Mobile: 0417328142 Fax: dbuckle@domainechandon.com.au

Importer or Consignee details

Name (eg business name etc): CSIRO ANIMAL, FOOD & HEALTH SCIENCES
Contact Name: MR ROD SMITH / DR ANNA CAREW
Postal Address: 671 SNEYDES ROAD WERRIBEE VIC
Post Code: 3030
Receiver Address (eg Vineyard Address): AS ABOVE
Post Code: anna.carew@utas.edu.au
Telephone: (03) 9731 3208 Mobile: 0411894997 Fax: (03) 9731 3202

Consignment Type:

- ☐ grapevine cuttings
- ☐ samples - soil
- ☐ wine manufacturing equipment
- ☐ grapevine rootlings
- ☐ samples - leaves, berries, plant material
- ☐ packages / bins
- ☑ whole grapes (fruit)
- ☐ agricultural equipment
- ☐ other .....

Date To Be Exported: (ESTIMATED)

Item to be exported (incl variety, type, model etc)	Quantity	Vine Material Only Rootstock/Clones	Vine Material Only- District name & street address where planting
Pinot noir grape must	400kg		
(*) MUST WILL BE OF ORIGIN AT THE CSIRO, WERRIBEE		RETURNED TO WINERY AFTER TREATMENT (**)	

In the case of agricultural machinery, the business may obtain approval to sign Plant Health Declaration permitting the movement of each piece of agricultural equipment / bins. Name s of persons proposed as signing and issuing declarations -

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of Applicant: ANNA CAREW Signature: A Carew Date: 24/2/15

Please return form by fax to the nearest Plant Standards Officer in the region in which the consignment will be received:

Ballarat	Peter Clarke	(03) 5336 6859 (ph)	(03) 5336 6601 (fax)
Bendigo	Judy Pouliot	(03) 5430 4444 (ph)	(03) 5430 4533 (fax)
Irymple	Peter Dixon	(03) 5051 4500 (ph)	(03) 5051 4518 (fax)
Melbourne	Tanya Krause	(03) 8371 3500 (ph)	(03) 9687 6746 (fax)
Rutherglen	Chris Pollard	(02) 6030 4500 (ph)	(02) 6030 4600 (fax)
Tatura	Trish Lord	(03) 5833 5222 (ph)	(03) 5833 5299 (fax)

Department of Primary Industries

PERMIT APPLICATION
PHYLLOXERA HOST MATERIALS

Exporter or Consignor details

Name (eg business name, individual etc): SEVILLE ESTATE

Contact Name: DYLAN McMAHON / ANNA CAREW

Postal Address: 65 LINWOOD ROAD SEVILLE VIC

Post Code: 3139

Production Address (ie origin of plant material or machinery): AS ABOVE

Post Code:

Telephone: (03) 59642622 Mobile: 0407334 605 Fax: 03 5964 2833

Importer or Consignee details

Name (eg business name etc): CSIRO ANIMAL, FOOD & HEALTH SCIENCES

Contact Name: MR ROD SMITH / DR ANNA CAREW

Postal Address: 671 SNEYDES ROAD WERRIBEE VIC

Post Code: 3030

Receiver Address (eg Vineyard Address): AS ABOVE

Post Code: anna-carew@utas.edu.au

Telephone: (03) 9731 3208 Mobile: 0411894997 Fax: (03) 9731 3202

Consignment Type:

- ☐ grapevine cuttings
- ☐ samples - soil
- ☐ wine manufacturing equipment
- ☐ grapevine rootlings
- ☐ samples - leaves, berries, plant material
- ☐ packages / bins
- ☑ whole grapes (fruit)
- ☐ agricultural equipment
- ☐ other .....

Date To Be Exported: (ESTIMATED) .....

Item to be exported (incl variety, type, model etc)	Quantity	Vine Material Only Rootstock/Clones	Vine Material Only- District name & street address where planting
Pinot noir grape must	400kg		
(*) MUST WILL BE ORIGINAL AT THE CSIRO, WERRIBEE		RETURNED TO WINERY AFTER TREATMENT	(*)

In the case of agricultural machinery, the business may obtain approval to sign Plant Health Declaration permitting the movement of each piece of agricultural equipment / bins. Name s of persons proposed as signing and issuing declarations -

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of Applicant: ANNA CAREW Signature: A Carew Date: 24 2 15

Please return form by fax to the nearest Plant Standards Officer in the region in which the consignment will be received:

Ballarat	Peter Clarke	(03) 5336 6859 (ph)	(03) 5336 6601 (fax)
Bendigo	Judy Pouliot	(03) 5430 4444 (ph)	(03) 5430 4533 (fax)
Irymple	Peter Dixon	(03) 5051 4500 (ph)	(03) 5051 4518 (fax)
Melbourne	Tanya Krause	(03) 8371 3500 (ph)	(03) 9687 6746 (fax)
Rutherglen	Chris Pollard	(02) 6030 4500 (ph)	(02) 6030 4600 (fax)
Tatura	Trish Lord	(03) 5833 5222 (ph)	(03) 5833 5299 (fax)

Department of Primary Industries

PERMIT APPLICATION
PHYLLOXERA HOST MATERIALS

Exporter or Consignor details

Name (eg business name, individual etc): HODDLES CREEK WINERY
Contact Name: FRANCO D'ANNA / ANNA CAREW
Postal Address: 505 GEMBROOK RD HODDLES CREEK
VICTORIA Post Code: 3139
Production Address (ie origin of plant material or machinery):
Post Code:
Telephone: 03 5967 4692 Mobile: 0412 034 608 Fax: franco@hoddlescreekestate.com.au

Importer or Consignee details

Name (eg business name etc): CSIRO ANIMAL, FOOD & HEALTH SCIENCES
Contact Name: MR ROD SMITH / DR ANNA CAREW
Postal Address: 671 SNEYDES ROAD WERRIBEE VIC
Post Code: 3030
Receiver Address (eg Vineyard Address): AS ABOVE
Post Code: anna-carew@utas.edu.au
Telephone: (03) 9731 3208 Mobile: 0411894997 Fax: (03) 9731 3202

Consignment Type:

- ☐ grapevine cuttings
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- ☐ other .....

Date To Be Exported: (ESTIMATED)

Item to be exported (incl variety, type, model etc)	Quantity	Vine Material Only Rootstock/Clones	Vine Material Only- District name & street address where planting
Pinot noir grape must	400kg		
(A) MUST WILL BE OF ORIGIN AT THE CSIRO, WERRIBEE		RETURNED TO WINERY AFTER TREATMENT	(A)

In the case of agricultural machinery, the business may obtain approval to sign Plant Health Declaration permitting the movement of each piece of agricultural equipment / bins. Name s of persons proposed as signing and issuing declarations -

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of Applicant: ANNA CAREW Signature: A Carew Date: 24/2/15

Please return form by fax to the nearest Plant Standards Officer in the region in which the consignment will be received:

Ballarat	Peter Clarke	(03) 5336 6859 (ph)	(03) 5336 6601 (fax)
Bendigo	Judy Pouliot	(03) 5430 4444 (ph)	(03) 5430 4533 (fax)
Irymple	Peter Dixon	(03) 5051 4500 (ph)	(03) 5051 4518 (fax)
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Rutherglen	Chris Pollard	(02) 6030 4500 (ph)	(02) 6030 4600 (fax)
Tatura	Trish Lord	(03) 5833 5222 (ph)	(03) 5833 5299 (fax)

State Government Victoria — Department of Primary Industries

PERMIT APPLICATION
PHYLLOXERA HOST MATERIALS

Exporter or Consignor details

Name (eg business name, individual etc): MONTALTO WINERY
Contact Name: SIMON BLACK / ANNA CAREW
Postal Address: 33 REDHILL - SHOREHAM ROAD REDHILL VICTORIA Post Code: 3937
Production Address (ie origin of plant material or machinery): TURBARUBBA RD REDHILL, VIC Post Code: 3937
Telephone (03) 59898412 Mobile: 0458 209 970 Fax: simon@montalto.com.au

Importer or Consignee details

Name (eg business name etc): CSIRO ANIMAL, FOOD & HEALTH SCIENCES
Contact Name: MR ROD SMITH / DR ANNA CAREW
Postal Address: 671 SNEYDES ROAD WERRIBEE VIC Post Code: 3030
Receiver Address (eg Vineyard Address): AS ABOVE Post Code: anna.carew@utas.edu.au
Telephone: (03) 9731 3208 Mobile: 0411894997 Fax: (03) 9731 3202

Consignment Type:

- ☐ grapevine cuttings
- ☐ samples - soil
- ☐ wine manufacturing equipment
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- ☐ agricultural equipment
- ☐ other .....

Date To Be Exported: (ESTIMATED)

Item to be exported (incl variety, type, model etc)	Quantity	Vine Material Only Rootstock/Clones	Vine Material Only- District name & street address where planting
Pinot noir grape must	400 kg		
(4) MUST WILL BE OF ORIGIN AT THE CSIRO, WERRIBEE		RETURNED TO WINERY AFTER TREATMENT	(4)

In the case of agricultural machinery, the business may obtain approval to sign Plant Health Declaration permitting the movement of each piece of agricultural equipment / bins. Name s of persons proposed as signing and issuing declarations -

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of signatory: ..... Signature: ..... Date: ...../...../.....

Name of Applicant: ANNA CAREW Signature: A. Carew Date: 24 2 15

Please return form by fax to the nearest Plant Standards Officer in the region in which the consignment will be received:

Ballarat	Peter Clarke	(03) 5336 6859 (ph)	(03) 5336 6601 (fax)
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Appendix 5.3 Paper published in Food Chemistry (Bindon et al., 2016)

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Characterization of macromolecular complexes in red wine: Composition, molecular mass distribution and particle size

Keren A. Bindon [a,*], Anna L. Carew [b], Agnieszka Mierczynska-Vasilev [a], Stella Kassara [a], Fiona Kerslake [b], Paul A. Smith [a,c]

[a] The Australian Wine Research Institute, Hartley Grove, Urrbrae, 5064 Adelaide, SA, Australia
[b] Perennial Horticulture Centre, Tasmanian Institute of Agriculture, University of Tasmania, 165 Westbury Road, Prospect, Tasmania 7250, Australia
[c] Centre for Marine Bioproducts Development, Flinders University, Level 4, Health Sciences Building, Flinders University Registry Road, Bedford Park, SA 5042, Australia

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ABSTRACT

Precipitates were prepared from two compositionally different Pinot noir wines with addition of excess ethanol, and contained primarily polysaccharide, tannin and protein. The ethanol-soluble material was further fractionated into polymeric (tannin) and monomeric phenolics. Tannin associated with precipitates was of a higher molecular mass than that remaining in ethanolic solution. Wine fractions were reconstituted at the ratios of the original wine and analyzed using nanoparticle tracking analysis. The average particle size of the tannin fraction was 75–89 nm, and increased when combined with the precipitate (≅200 nm). Addition of the monomeric fraction to the tannin–precipitate complex increased both the incidence and concentration of smaller particles, reducing the average particle size. The formation of aggregates occurred in all fractions and only minor differences in particle size distribution were found between wines. Differences in particle concentration between wines appear to be due to differences in the total concentration of macromolecules rather than compositional differences.

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1. Introduction

Wine constitutes a complex matrix of volatile and nonvolatile compounds, which interact to confer the stability and sensory properties of a given wine. In red wines, the major classes of macromolecules are proanthocyanidins (condensed tannins), polysaccharides and proteins. The intrinsic nature of these types of macromolecules is their propensity to interact with one another (Le Bourvellec & Renard, 2012).

The interaction between tannin and protein has been well documented (Le Bourvellec & Renard, 2012), and in wines is understood to contribute to the formation of precipitates (Charlton et al., 2002), hazes (Siebert, Carrasco, & Lynn, 1996; Van Sluyter et al., 2015) and is the mechanism underpinning astringency perception (De Freitas & Mateus, 2012; McRae, Falconer, & Kennedy, 2010). In red wines, nonglycosylated proteins, including haze-forming pathogenesis-resistant proteins found in white wine, typically present a minor component or are absent (Ferreira, Piçarra-Pereira, Monteiro, Loureiro, & Teixeira, 2001; Rowe et al., 2010). It is speculated that these are lost due to precipitation, denaturation and/or proteolysis. As a result, the principle proteins in red wines are those associated as a minor component of a larger polysaccharide molecule, the grape-derived arabinogalactans, or yeast-derived mannoproteins (Ferreira et al., 2001; Rowe et al., 2010). These are thought to be retained due to their high resistance to proteolysis and low pH conditions, and possibly because they form stable colloids in wine. It is important to note that these glycosylated proteins (polysaccharides) typically demonstrate weaker interactions with tannin than observed for other protein types (Rowe et al., 2010; Watrelot, Le Bourvellec, Imberty, & Renard, 2014). Despite these weak associations, wine polysaccharides are thought to interact with tannins in solution (Aron & Kennedy, 2007; Diaz-Rubio & Saura-Calixto, 2006; Guadalupe & Ayestarán, 2008; Poncet-Legrand, Doco, Williams, & Vernhet, 2007; Rodrigues, Ricardo-Da-Silva, Lucas, & Laureano, 2012; Saura-Calixto & Diaz-Rubio, 2007), resulting in the formation of large macromolecular complexes. Key studies have highlighted that under wine-like conditions, a significant proportion of red wine polyphenols remain in a complexed form (Aron & Kennedy, 2007; Diaz-Rubio & Saura-Calixto, 2006; Saura-Calixto & Diaz-Rubio, 2007). However, as discussed previously, these interactions may be limited by polysaccharide composition and tertiary structure, as well as the molecular mass of the tannin itself

* Corresponding author.
E-mail address: keren.bindon@awri.com.au (K.A. Bindon).

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(Le Bourvellec, Watrelot, Ginies, Imberty, & Renard, 2012; Watrelot, Le Bourvellec, Imberty, & Renard, 2013; Watrelot et al., 2014). By inference, complex formation could have implications for sensory perception (Quijada-Morin, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailon, 2014; Vidal et al., 2004) (astringency, viscosity) and possibly the stability of wine in terms of haze or precipitate formation, (Poncet-Legrand et al., 2007; Riou, Vernhet, Doco, & Moutounet, 2002) yet the system remains poorly characterized.

The current study has aimed to provide preliminary data on the concentration, composition and size of macromolecular complexes formed in red wines. To do this, wines were compared which had differences in macromolecules yet otherwise similar composition, achieved through microwave and standard maceration procedures. Three wine fractions were prepared: monomeric phenolics, tannin and 'precipitate' (a component containing polysaccharide, protein and bound tannin prepared by precipitation of wine in excess ethanol). We aimed to determine the proportion of tannin which exists in a complexed form, and to infer the compositional differences in macromolecules which drive this, if any. Secondly, the role of macromolecule concentration and composition on particle size properties was studied on the three wine fractions using nanotracking analysis (NTA). NTA was selected in favor of dynamic light scattering (DLS) due to the high polydispersity index of wine fractions (Tomaszewska et al., 2013). NTA is a technique which combines laser light scattering microscopy with a CCD camera. This approach enables the visualization and recording of nanoparticles in solution and is useful for the assessment of particle size and concentration (Dragovic et al., 2011). This is the first report of NTA use in a wine system.

2. Materials and methods

2.1. Instrumentation

An Agilent model 1100 HPLC (Agilent Technologies Australia Pty Ltd., Melbourne, Australia) was used with Chemstation software for chromatographic analyses. Nanoparticle tracking analysis was performed using a NanoSight NS300 Instrument (NanoSight Ltd., Amesbury, Wiltshire, United Kingdom).

2.2. Grape samples and winemaking treatments

Vitis vinifera L. cv. Pinot noir fruit was obtained from Tasmania, Australia at 22.5 °Brix. Bunches were randomly allocated to 1 kg lots (12 replicate lots) for vinification. For each replicate, grapes were de-stemmed by hand and crushed using a custom-made bench-top crusher. The must was decanted to 1.5 L Bodum™ plunger coffee pots. To each must, 50 mg/L SO_2 was added in the form of potassium metabisulfite solution and four replicate lots were transferred immediately to a 27 °C temperature-controlled room (standard winemaking, control). Microwave maceration was performed after SO_2 addition on the remaining experimental replicates using a previously published method (Carew, Gill, Close, & Dambergs, 2014; Carew, Sparrow, Curtin, Close, & Dambergs, 2014) with the following modifications. Each pot was individually transferred a domestic 1150 W Sharp™ 'Carousel' R-480E microwave and irradiated at full wattage for three time periods of 2 min, 1 min and 15–40 s. Between each irradiation, samples were stirred and the temperature of must using recorded using a solid stem thermometer. The second temperature reading was used to determine the length of time applied for the final irradiation. Each pot reached a peak temperature of 70–71 °C and was held at this temperature by means of a thermal blanket for 1 h. Pots were then placed in an ice bath and stirred periodically, with must temperature in each pot reaching 30 °C in approximately 30 min. The microwave treatment pots were then moved to the 27 °C (±3 °C) constant temperature room. Each pot was inoculated with active dried yeast strain EC1118 (Lallemand Pty Ltd., Australia) that had been rehydrated according to the manufacturer's instructions and fermented at 28 °C for 7 days. The Bodum™ plunger was lowered to leave approximately 1 cm of wine above the plunger surface. The progress of fermentation was monitored daily by the reduction in ferment weight. On a daily basis the plunger was gently lifted to mix the wine, and thereafter re-submerged. After fermentation the plunger was lowered, the wines were decanted and thereafter cold-settled at 4 °C. Wines were not subjected to malolactic fermentation. All wines were stabilized at first racking with 80 mg/L SO_2 and bottled under CO_2 cover in 50 mL amber glassware with wadded polypropylene capping, and stored for 12 months before analysis.

2.3. Small-scale preparation of precipitates for statistical comparison of winemaking replicates

A 1 mL wine aliquot of each of the four treatment replicates was added to 5 mL of absolute ethanol and allowed to precipitate at 4 °C for 18 h. Samples were centrifuged at 8000 *g* for 5 min, the supernatant was discarded and the pellet allowed to air dry briefly to remove excess ethanol. The pellets were reconstituted in Milli Q water and lyophilized. For the respective winemaking treatments, the percentage standard deviation of the mean for the gravimetric recovery of pellets was 8.7% and 4.1% respectively. Pellet recovery was significantly higher for the microwave-macerated treatment in comparison with the control ($P = 0.002$ by *T*-test assuming unequal variance). The treatment replicates were therefore deemed to be sufficiently reproducible to enable wine pooling for subsequent fractionation and analysis. The wines are henceforth referred to as Wine 1 (standard maceration) and Wine 2 (microwave maceration).

2.4. Wine fractionation

A schematic representation of the procedures followed for the preparation of wine fractions and their subsequent analysis is shown in Fig. 1. Wines from the four treatment replicates were pooled in order to obtain sufficient material for fractionation and reconstitution experiments. Four volumes of 96% v/v ethanol were added to 40 mL of wine, and precipitation took place at 4 °C overnight. The sample was centrifuged at 10,000*g* for 20 min, and both supernatant and pellet were retained. The pellets were reconstituted in Milli Q water, lyophilized, recovered gravimetrically as dry powders, and are henceforth referred to as the precipitate fraction. The ethanolic supernatant was recovered, concentrated under reduced pressure at 35 °C and reconstituted in 13% v/v ethanol containing 0.05% v/v TFA, and made up to 40 mL. A 50 mL bed volume Toyopearl TSK HW 40-F (180 × 25 mm) in a glass column (Michel-Miller, Vineland, NJ, USA) was used. The column was equilibrated with 13% v/v ethanol, 0.05% v/v TFA/water containing 0.1% v/v formic acid and thereafter the reconstituted wine sample was applied using a peristaltic pump. The following solvent were applied successively, all containing 0.05% v/v TFA: 150 mL 13% v/v ethanol, 150 mL 50% v/v methanol and 100 mL 70% v/v acetone. The column was re-equilibrated with 13% v/v ethanol, 0.05% v/v TFA between samples. The 50% methanol and 70% acetone fractions were retained, and will henceforth be referred to as methanol and acetone fractions respectively. The eluted fractions were concentrated under reduced pressure at 35 °C. The methanol fraction was dried under a stream of nitrogen. The acetone fraction was frozen at −80 °C and then lyophilized to a dry powder. The gravimetric recovery of all fractions was recorded.

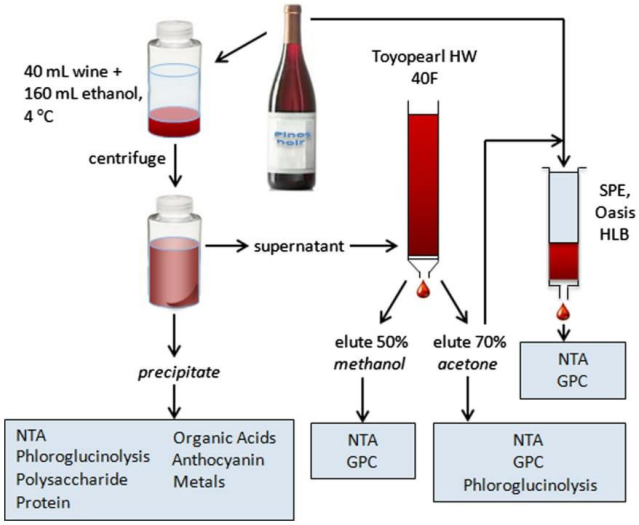


Fig. 1. Schematic diagram showing protocols for the preparation of wine fractions and analytical methods performed (NTA, nanoparticle tracking analysis; GPC, gel permeation chromatography; SPE, solid-phase extraction).

2.5. Solid phase extraction (SPE) of wines and wine fractions

Whole wines and the wine acetone fraction described above were prepared using SPE on Oasis HLB (3 mL, 60 mg, 30 μm) cartridges (Waters, Rydalmere, NSW, Australia). The method was a variation of published approach (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008) modified for the isolation of total tannin (Kassara & Kennedy, 2011). SPE isolates were reconstituted in methanol, and analyzed using gel permeation chromatography (GPC) as described below.

2.6. Tannin composition

Phloroglucinolysis (Kennedy & Jones, 2001) was used to determine tannin subunit composition and mean degree of polymerization (mDP). Purified tannin fractions reconstituted in methanol were analyzed using the high-throughput approach described previously (Bindon, Smith, & Kennedy, 2010). In addition, precipitates (5 mg) were extracted in 2 mL 70% v/v acetone overnight and allowed to precipitate at −20 °C. After centrifugation at 16,000*g*, the supernatant was concentrated under a stream of nitrogen, reconstituted in methanol and analyzed as described above. Dried precipitate samples and the corresponding reconstituted acetone extracts of the respective precipitates were analyzed using direct phloroglucinolysis, as described previously (Bindon, Smith, Holt, & Kennedy, 2010). The phloroglucinolysis reaction was carried out at 50 °C for 25 min, neutralized and analyzed by RP-HPLC according to the conditions outlined in a published method (Kennedy & Taylor, 2003) using (−)-epicatechin (Sigma Aldrich, St. Louis, MO, USA) as the quantitative standard. Based on data collected from the studied wines, the loss in phloroglucinolysis yield during wine aging was the same for acetone and precipitate fractions (data not shown). Therefore, the mass conversion (subunit yield by phloroglucinolysis per unit tannin dry mass as%) of the purified acetone fraction was assumed to be identical to the precipitate fraction. The mass conversion of the acetone fraction was used to derive a gravimetric estimate of tannin in precipitates.

The methanol and acetone fractions, as well as SPE isolates of whole wine and acetone fractions were analyzed by GPC to determine size distribution properties. Prior to analysis, samples in methanol were diluted with 4 volumes of mobile phase and the injection volume was 20 μL. The GPC approach was adapted from the original method (Kennedy & Taylor, 2003) to allow for increased size distribution resolution of high molecular mass material, as described previously (Bindon & Kennedy, 2011). Briefly, two PLgel columns were connected in series: 500 Å followed by 10^4 Å (both 300 × 7.5 mm, 5 μm) (Varian Inc., Mulgrave, Victoria, Australia) protected by a guard column (50 × 7.5 mm, 5 μm) containing the same material. The isocratic method used mobile phase of N,N-dimethylformamide containing 1% v/v glacial acetic acid, 5% v/v water and 0.15 M lithium chloride. The flow rate was maintained at 1 mL/min with a column temperature of 60 °C, where elution was monitored at 280 nm and 520 nm. The standards for calibration were those described previously (Bindon, Smith, & Kennedy, 2010). For calibration, a second order polynomial was fitted with the cumulative mass distribution at 50% for each standard. Values derived for the weight average molecular weight (M_w) and number average molecular weight (M_n) were used to determine the polydispersity index (D) for each fraction as:

$$D = \frac{M_w}{M_n}$$

2.7. Polysaccharide composition

Precipitates (5 mg) were reconstituted in 800 μL of Milli-Q water, transferred to a 1 mL Pur-a-Lyzer dialysis tube of molecular weight cut-off 3500 Da (Sigma–Aldrich, St. Louis, MO, USA) and dialyzed against 3 changes of Milli-Q water at 4 °C. After dialysis, each sample was transferred to a fresh screw-cap centrifuge tube, frozen at −80 °C and lyophilized. Lyophilized samples were reconstituted in 2 M TFA and were hydrolyzed at 100 °C for 3 h. Hydrolysates were cooled on ice, and thereafter concentrated under vacuum at 30 °C in a Heto vacuum centrifuge (Heto-Holten A/S,

Allerod, Denmark). For the analysis of hydrolytically-released monosaccharides an adaptation of a published method (Honda et al., 1989) was used with the following modifications. The dry TFA-hydrolysates were resuspended in Milli-Q water containing 0.3 M of ribose and deoxy-glucose as internal standards (Sigma–Aldrich, St. Louis, MO, USA). The derivatising reagent was 0.5 M of methanolic 1-phenyl-3-methyl-5-pyrazolone (PMP) (Sigma–Aldrich, St. Louis, MO, USA) in 1 M NH_4OH. For the derivatisation, 25 μL of sample was mixed with 96.2 μL of derivatising reagent and placed in a heating block at 70 °C for 1 h. After this step, the samples were cooled on ice and neutralized with 25 μL of 10 M formic acid. Then, the samples were extracted twice with dibutyl ether (Sigma–Aldrich, St. Louis, MO, USA) and the upper layer was discarded. The excess of dibutyl ether was dried under vacuum at room temperature. PMP-monosaccharide derivatives were quantified at 250 nm by HPLC using a C18 column (Kinetex, 2.6 μm, 100 Å, 100 × 3.0 mm) protected with a guard cartridge (KrudKatcher Ultra HPLC in-line filter, 0.5 μm) (Phenomenex, Lane Cove, NSW, Australia). The mobile phases were solvent A, 10% (v/v) acetonitrile in 40 mM aqueous ammonium acetate, and solvent B, 70% (v/v) acetonitrile in water. The following linear gradient was used: for solvent A (with solvent B making up the remainder) 92% at 0 min; 84% at 12 min; to 0% at 12.5 min; 0% at 14 min, then returning to the starting conditions at 14.5–18.5 min, 92%. A flow rate of 0.6 mL/min was used with a column temperature of 30 °C. The PMP-monosaccharide derivatives were identified using commercial standards (Sigma–Aldrich, St. Louis, MO, USA).

For analysis of polysaccharide molecular mass distribution, dried, dialyzed samples were resuspended in 0.1 M sodium nitrate and 25 μL was injected onto a BioSep Sec 2000 column (300 × 7.8 mm, Phenomenex, Lane Cove, NSW, Australia) fitted with a guard column of the same packing material. Analysis was performed by HPLC-SEC in 0.1 M sodium nitrate at a flow rate of 1 mL/min. Polysaccharide elution was monitored by refractive index detection over 14 min. Column calibration was carried out with Shodex P-82 Pullulan Standards (Phenomenex, Lane Cove, NSW, Australia) from ≅5 kDa to ≅800 kDa. A fourth order polynomial was fitted with the cumulative mass distribution at 50% for each standard in order to determine molecular mass. The polydispersity index (D) was calculated for the polysaccharide distribution was described above.

2.8. General compositional analysis of precipitates

All compositional analysis was performed on a minimum of duplicate samples. Anthocyanin in precipitates was determined using a modification of a high-throughput method for wine color analysis (Mercurio, Dambergs, Herderich, & Smith, 2007). Precipitates were prepared in 1 mL Milli Q water to the concentration found in wine and then diluted 50 times with 1 M HCl, mixed and left to stand for 1 h. Duplicate 300 μL aliquots were transferred into a 370 μL 96 well UV plate and the 520 nm absorbance measured using a SpectraMax M2 Microplate Reader (Molecular Devices, Australia). For organic acid analysis, precipitates were reconstituted in 1 M HCl, vortexed and extracted for 1 h on a suspension mixer. Samples were centrifuged at 16,000g for 5 min. Malic and tartaric acids in the supernatant were quantified by a published method (Frayne, 1986). For protein analysis, precipitates were analyzed using a modification of a published method for red wine (Smith, Penner, Bennett, & Bakalinsky, 2011). Precipitates were prepared in 70% v/v acetone and placed at −20 °C overnight, centrifuged at 16,000g for 10 min and the pellet washed in ice-cold (−20 °C) pure acetone. Samples were re-centrifuged and the pellet was air-dried. Samples were reconstituted in MilliQ water and quantified using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Gladesville, NSW, Australia) with Baker's yeast invertase from *Saccharomyces cerevisiae* (Sigma–Aldrich, St. Louis, MO, USA) as the quantitative standard. Further to protein quantification, 70% v/v acetone-purified pellets were analyzed by SDS–PAGE according to a published protocol (Marangon, Sauvage, Waters, & Vernhet, 2011). Metal analysis of precipitates was performed following digestion of 20 mg of precipitate in 4 mL of nitric acid at 100 °C for 1.5 h. A 2 mL aliquot of peroxide was then added and the sample heated at 100 °C for a further 2 h and diluted as required for analysis. Analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS) through an accredited laboratory (Flinders Analytical, Faculty of Science & Engineering, Flinders University, Adelaide SA, Australia).

2.9. Analysis of particle size

Dried, fractionated wine components were reconstituted to the concentrations which existed in the original wines based on gravimetric recovery in 13% v/v ethanol containing 0.01% v/v TFA. Each component was analyzed for particle size using a NanoSight NS300 (Malvern Instruments Ltd, Malvern, UK) nanoparticle tracking analysis (NTA) system equipped with a sample chamber with a 635 nm laser and a syringe pump. The NTA software identified and tracked individual nanoparticles moving under Brownian motion and related the movement to a particle size according to the following formula derived from the Stokes–Einstein equation:

$$\overline{(x,y)^2} = \frac{2k_BT}{3r_h\pi\eta}$$

where k_B is the Boltzmann constant and $\overline{(x,y)^2}$ is the mean-squared speed of a particle at a temperature T, in a medium of viscosity η, with a hydrodynamic radius of rh.

The wine samples were injected in the sample chamber with a sterile 1 mL syringe. Analysis was performed with a constant and controlled sample flow at room temperature. The software used for capturing and analyzing the data was the NanoSight NS300 NTA 2.3. The samples were measured for 60 s with manual shutter and gain adjustments. Measurements were repeated 5 times per sample. The "extended dynamic range mode" was used as recommended for polydisperse samples. NTA post-acquisition settings were optimized and kept constant between samples, and each video was then analyzed to give mean, mode, and median particle size together with an estimate of the concentration. Initial analysis revealed that wine-like concentration of the respective components was too dense to enable visualization of the particle properties. Therefore, samples were diluted 1:3 with 13% ethanol, 0.01% TFA, maintaining the original ratios present in wine. After each component was analyzed separately, the isolated wine components were recombined, maintaining the initial ratio in wine, and 1:3 dilution. This sequence of analysis was followed: precipitate + acetone fractions; precipitate + acetone + methanol fractions.

3. Results and discussion

3.1. Recovery of wine fractions

Data from the microwave method of winemaking has been previously published (Carew, Gill, et al., 2014; Carew, Sparrow, et al., 2014). The current dataset showed effects on phenolics which were consistent with these previous results, where microwave-macerated wines had significantly higher overall tannin concentration than the control (Supporting information S1). As discussed previously, based on the strong treatment differences which were consistent across the four treatment replicates, and in order to obtain sufficient material for reconstitution experiments, the winemaking replicates were pooled. Following wine fractionation in

duplicate, the precipitate, acetone and methanol fractions were recovered, and these values expressed (gravimetrically) as their respective concentrations in wine are shown in Table 1. Wine 2 had higher quantities of the precipitate and acetone fractions than Wine 1, supporting the original observations regarding tannin concentration (Supporting information S1). Recovery of the methanol fraction was similar between the two wines.

3.2. General analysis of wine fractions

A mass balance analysis of wine precipitates was carried out based on the expected composition (Table 1). A total of ≅70% of the precipitate was characterized quantitatively, which most likely reflects incomplete quantification of polysaccharide due to partial hydrolysis and methyl/acetyl groups not being assayed. The bulk of the precipitate was polysaccharide and tannin, with proportionally less protein. There were also significant amounts of tartrate and potassium associated with the precipitate, likely to be an artifact of the precipitation process which uses excess ethanol and low temperature, leading to the formation of insoluble salts which are not removed upon dialysis.

Table 1
Compositional analysis of wines expressed in terms of concentration and proportional (%, as italics) quantities.

	Wine 1	Wine 2
Wine composition		
Methanol fraction	556.5 ± 92.25[a]	562.0 ± 75.5
	16.6 ± 2.5[b]	*12.0 ± 1.5*
Acetone fraction	926.6 ± 77.9	1269.25 ± 38.0*[c]
	27.6 ± 1.9	*27.0 ± 1.0*
Precipitate	1872.5 ± 125	2865.0 ± 7.5*
	55.9 ± 4.5	*61.0 ± 0.5*
Precipitate composition		
Polysaccharide[d]	485.8 ± 10.7	547.3 ± 13.5*
	26.0 ± 1.2[b]	*19.1 ± 0.5*
Tannin[e]	417.1 ± 85.4	624.3 ± 54.3*
	22.3 ± 4.6	*21.8 ± 1.9*
Anthocyanin[f]	72.6 ± 11.5	99.3 ± 1.4*
	2.6 ± 0.7	*2.9 ± 0.1*
Protein[g]	76.7 ± 7.2	132.7 ± 3.9*
	4.1 ± 0.1	*5.6 ± 0.6*
Tartrate[h]	167.3 ± 20.5	257.9 ± 43.0
	9.0 ± 1.0	*9.0 ± 1.0*
Malate[h]	20.5 ± 0.5	43.0 ± 3.0*
	1.2 ± 0.1	*1.5 ± 0.1*
Potassium[i]	76.0 ± 6.9	107.7 ± 3.7*
	4.1 ± 0.1	*3.8 ± 0.1*
Calcium[i]	53.4 ± 3.7	59.0 ± 0.4
	2.9 ± 0.01	*2.1 ± 0.01*
Magnesium[i]	25.7 ± 2.6	37.8 ± 1.2*
	1.4 ± 0.05	*1.3 ± 0.04*
Trace metals[i]	1.9 ± 0.01	2.9 ± 0.28
	0.1 ± 0.01	*0.1 ± 0.01*

Data expressed as mean ± standard error of duplicate fractionation procedures of Pinot noir wines produced using standard winemaking (wine 1) and microwave maceration (wine 2).

[a] Concentration expressed as mg/L of wine.

[b] Data in italics is the estimated proportional contribution by mass (as %) to wine or dried ethanol-precipitated material respectively.

[c] Asterisk indicates a significant difference in concentration determined by a *T*-test assuming unequal variance, where $P < 0.05$.

[d] Polysaccharide determined as the sum of individual monosaccharides and uronic acids in dialysed precipitate samples.

[e] Tannin determined by direct phloroglucinolysis on precipitates with values adjusted using mass conversion data generated from purified tannin fractions (gravimetric).

[f] Anthocyanin estimated as the 520 nm absorbance of precipitate reconstituted in 1 M HCl.

[g] Protein determined colorimetrically using yeast invertase as the protein standard.

[h] Organic acids reconstituted from insoluble salts in 1 M HCl and analyzed by HPLC.

[i] Metals detected in precipitates determined using ICPMS showing the three major metals with the remaining Na, Al, Mn, Cu and Zn grouped as 'trace'.

In terms of wine macromolecules, Wine 2 had a 33% higher amount of tannin and 42% higher protein associated with the precipitate than Wine 1, with a less important increase in polysaccharide (Table 1). Analysis of proteins by SDS–PAGE showed an absence of the principal nonglycosylated wine proteins, the pathogenesis-resistant proteins (data not shown). However, this analysis does not necessarily indicate a lack of glycosylated proteins. This is because, as reported by others, glycosylated proteins do not react with the Coomassie Blue dye, and differ in their electrophoretic migration behavior from nonglycosylated proteins (Durchschlag & Jaenicke, 1997). Analysis of monosaccharide composition of the polysaccharides revealed similar total amounts of mannose, arabinose and galactose between the wines (Supporting information S2) indicating that the glycosylated fraction was probably not affected. This possibly indicates treatment effects on protein retention rather than differences in extraction from the grape (arabinogalactans) or synthesis by yeast (mannoprotein) and warrants further investigation. In terms of monosaccharide composition, polysaccharides from Wine 2 contained higher glucuronic acid, galacturonic acid, glucose, xylose and fucose than Wine 1 (Supporting information S2). Higher uronic acid content may point to increased pectin contribution within the polysaccharides of Wine 2, possibly due to enhanced breakdown of the grape cell wall by microwave treatment, but potentially also due to denaturation of endogenous pectolytic enzymes by heat. Although limited information on the composition of galacturonans can be inferred from the monosaccharide composition alone, speculatively, the lack of a difference in rhamnose or galactose between the wines indicates that the increase in pectin following microwave treatment was homogalacturonan rather than rhamnogalacturonan.

Some minor differences in polysaccharide size distribution were observed between treatments. Analysis by size exclusion chromatography showed that the polysaccharides from the Wine 2 had a smaller contribution of intermediate molecular mass polysaccharides than Wine 1 (Supporting information S3). The extent of this effect was clearer when the data was expressed in terms of molecular weight using Pullulan standards fitted to a 4th order polynomial (Supporting information S3). The precipitate from Wine 2 had an overall polysaccharide size distribution that was skewed toward a higher molecular mass than Wine 1. Due to the polydispersity of the respective polysaccharide samples, however, the molecular mass average calculated at 50% volume elution was equivalent, regardless of treatment, at ≈50 kDa (Supporting information S3).

3.3. Tannin composition

Compositional information on tannin in the acetone and precipitate fractions is shown in Table 2. The greater proportion of tannin was associated with the acetone fraction, and no significant differences were found between wines. The principle effect seen with the fractionation process was that the acetone fraction had a lower tannin mDP and estimated molecular mass than precipitate tannin, i.e. tannin in complexed form. Only minor differences in tannin composition were found between winemaking treatments, most notable being increases in extension (−)-epigallocatechin (acetone and precipitate fractions.) and terminal epicatechin-3-O-gallate (acetone fraction) in Wine 2.

Since the phloroglucinolysis method provides an estimate of average molecular mass of depolymerizable material only, GPC was also used to support the observation that tannin molecular mass was higher in the precipitate. In order to compare the molecular mass distribution of the acetone fraction with tannin that was

Table 2
Proportion and composition of tannin recovered in ethanolic supernatant and precipitates after addition of excess ethanol to wines.

Wine Component[a]	% of total wine tannin	MC[b] (%)	mDP[c]	MM[d] GPC	MM[d] Subunit	Extension subunits (%)[e] EGC[g]	C	E	ECG	Terminal subunits (%)[f] C	E	ECG
Acetone fraction[h]												
Wine 1	69.0 ± 5.8a	18.5 ± 0.1a	4.9 ± 0.1c	2421.2 ± 24.8	1467.3 ± 14.4c	14.1 ± 0.3c	5.7 ± 0.1a	75.1 ± 0.4a	5.0 ± 0.03b	67.9 ± 0.5	27.9 ± 0.5	4.3 ± 1.0b
Wine 2	67.0 ± 2.0a	25.0 ± 0.4b	4.4 ± 0.02c	2148.1 ± 143.7	1319.1 ± 7.5c	20.1 ± 0.3ab	5.5 ± 0.1a	68.0 ± 0.2b	6.3 ± 0.2a	62.3 ± 1.4	28.2 ± 0.4	9.8 ± 1.8a
Precipitate[i]												
Wine 1	31.0 ± 6.4b	na	8.2 ± 0.2a	na	2422.2 ± 68.3a	18.5 ± 1.1bc	3.7 ± 0.4b	73.8 ± 1.5a	4.0 ± 0.3c	65.5 ± 3.1	34.5 ± 3.1	nd
Wine 2	33.0 ± 2.9b	na	6.6 ± 0.5b	na	1964.6 ± 159.6b	23.4 ± 1.4a	3.6 ± 0.1b	68.3 ± 1.2b	4.7 ± 0.3bc	67.3 ± 0.7	32.7 ± 0.7	nd

Data expressed as mean ± standard error. na = not analyzed; nd = not detected.
[a] Wines and their respective fractions were compared by one-way ANOVA and a post hoc *T*-test where different letters after the mean within a column indicate a significant difference where $P < 0.01$.
[b] Mass conversion based on % estimate of proanthocyanidin by phloroglucinolysis per unit tannin by mass (as mg of purified tannin from acetone extraction).
[c] Mean degree of polymerization.
[d] Molecular mass determined at 50% volume elution by gel permeation chromatography (GPC) or from subunit composition by phloroglucinolysis.
[e] Percent composition of proanthocyanidin subunits (in mg) of extension subunits.
[f] Percent composition of proanthocyanidin subunits (in mg) of terminal subunits.
[g] Subunit abbreviations: EGC, (−)-epigallocatechin; C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-O-gallate.
[h] Supernatant recovered from addition excess ethanol to wine with centrifugation, whereafter tannin was purified by chromatography in 70% v/v acetone (acetone fraction).
[i] Precipitate recovered from addition of excess ethanol to wine and analyzed by direct phloroglucinolysis.

potentially bound in complexes, an SPE approach (Jeffery et al., 2008; Kassara & Kennedy, 2011) which uses HLB resin was used to purify tannin from whole wine. The same SPE method was used for the purified acetone fraction, representing the tannin component which remained in solution during precipitation. The SPE isolates were analyzed by GPC and compared with the original acetone fraction. From the GPC elution profiles (Fig. 2), it is evident that the molecular mass distribution of the acetone fraction was unchanged by the SPE protocol, and that a high molecular mass component present in the whole wine was absent in this fraction. It would be expected that wine tannin which might otherwise have remained bound in complexed form was desorbed from the HLB resin during the application of neat formic acid, followed by methanol (Jeffery et al., 2008). We therefore propose that the tannin fraction which is present in wine, but bound in complexes is of a higher molecular mass than the fraction which remains soluble in ethanol. This result would be expected based on studies in model systems (Carn et al., 2012; Le Bourvellec & Renard, 2012; Watrelot et al., 2013, 2014). To our knowledge, this is the first time that this has been characterized in a wine system.

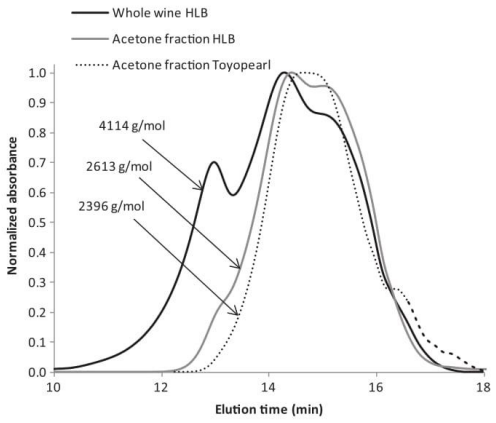


Fig. 2. Elution profiles of the acetone fraction from a Pinot noir wine (Wine 1) analyzed by gel permeation chromatography, in comparison with the same fraction and whole wine purified by solid-phase extraction on HLB resin. Annotated data indicate the molecular mass average of the fractions, determined at 50% elution.

The wine macromolecules which would be expected to form associations with tannin are protein and polysaccharide (Le Bourvellec & Renard, 2012). Since these were proportionally similar in both wines studied (Table 1), we were not able to speculate as to the key macromolecules involved in the formation of tannin complexes. Although nonglycosylated proteins are well known to complex with tannin, the point has been previously raised that glycosylated proteins demonstrate a markedly reduced interaction (Rowe et al., 2010; Watrelot et al., 2014). Nevertheless, the interaction of glycosylated proteins and tannin is defined by hydrogen bonding to a greater extent than has been observed for nonglycosylated proteins (Rowe et al., 2010). Hydrogen bonds between tannin and protein should not have been disrupted during the ethanol precipitation process, suggesting that protein may have mediated the formation of complexes under the experimental conditions.

On the other hand, soluble pectic polysaccharides are known to demonstrate a strong affinity for tannin (Le Bourvellec et al., 2012; Watrelot et al., 2013) but this effect appears to be limited by the polysaccharide structure. A comparison of published studies revealed that tannin interacts more strongly with polysaccharides of a complex tertiary structure (Le Bourvellec et al., 2012), with the strength of binding decreasing dramatically when polysaccharide sub-fractions are studied in isolation (Watrelot et al., 2013, 2014). An important observation in model studies is that the interaction between soluble polysaccharide and tannin is predominantly driven by hydrophobic interaction (Le Bourvellec et al., 2012; Watrelot et al., 2013, 2014), a phenomenon which would most likely have been disrupted in the presence of the high concentration of ethanol during the precipitation process.

It is therefore likely that under the conditions of this experiment, binding events between tannin and macromolecules in precipitates was primarily via hydrogen or covalent bonding. Extraction of precipitates in 70% v/v acetone, removed up to 94% of tannin from the precipitate complex, based on phloroglucinolysis analysis while the polysaccharide (and protein) remained in the acetone-extracted precipitate as confirmed by recovery experiments (data not shown). Since tannin binding to protein and/or polysaccharide was largely disrupted by acetone, we suggest that this excludes covalent associations within the bulk of the complexed materials.

3.4. Reconstitution of wine fractions and particle size analysis

The wine fractions were analyzed separately using NTA, and then reconstituted, all diluted 1:3 relative to their original

concentration in wine. As discussed previously, NTA was selected as the method of analysis because it overcomes some of the limitations for polydisperse samples encountered when using dynamic light scattering. All the wine fractions studied had polydispersity indices > 0.7 (Table 3), over which it is recommended that dynamic light scattering is not used. The particle size distribution of the acetone fractions estimated by NTA is shown in Table 3 and Fig. 3A, showing a particle size range from 20 to 200 nm, with an average (D50) of 88.5 and 75 for Wine 1 and Wine 2 respectively. As would be expected based on the higher tannin concentration in Wine 2 (Table 1, Supporting information S1) the total concentration of particles in the acetone fraction was higher for this wine (Table 3). For wine tannin fractions of similar mDP to the acetone fraction studied here, dimensions derived from small-angle X-ray scattering (SAXS) for individual tannin molecules are in the order of 4–6 nm (McRae, Kirby, Mertens, Kassara, & Smith, 2014). That work, as well as other studies indicate that in dilute ethanol, self-association of tannin molecules (Poncet-Legrand et al., 2007, 2010; Riou et al., 2002) is expected resulting in the formation of aggregates, and in some cases precipitates (Zanchi et al., 2007). The large particle size average, as well as the polydispersity of the particle size range probably indicates that aggregation of the acetone fractions occurred. By way of comparison, aggregation was also found for the low molecular mass (≃450 g/mol by GPC, data not shown) methanol fraction, and was significantly higher for Wine 2 with average particle size of 200 nm compared with 48 nm for Wine 1, and a corresponding lower particle number (Table 3, Supporting information S4). Although the concentration and molecular mass of the methanol fractions were similar between treatments, the different aggregation phenomena may indicate compositional differences in monomeric phenolics between the wines, but this was not investigated further.

Analysis of the precipitate fractions using NTA indicated larger particle sizes than the acetone fractions, and a lower particle concentration at wine-like ratios (Table 3, Fig. 3B). Addition of the acetone fraction to the precipitate had no effect on particle size average, and the general size distribution of the precipitate was maintained (Table 3, Fig. 3C). For both wines studied, combining the acetone and precipitate fractions increased the particle concentration from that observed for the precipitate analyzed alone. Conversely, a decrease in particle concentration was found for the acetone fraction analyzed alone. This is particularly evident when the distribution of the Wine 2 is compared from Fig. 3A–C, where a drop in particle concentration occurred concurrent with the shift to larger particle size. This provides evidence that association of the lower molecular mass tannin of the acetone fraction and the precipitate complex occurred.

Other studies using DLS to explore the effect of polysaccharide–tannin interactions have used model polysaccharides (Carn et al., 2012), or purified polysaccharide fractions from wine (Poncet-Legrand et al., 2007; Riou et al., 2002). Since the precipitate explored in the present study represents a crude isolate from wine, containing both protein and tannin in combination with compositionally heterogeneous polysaccharide, this limits comparison of our data with these studies. Studies which have looked at grape-derived seed tannins (Poncet-Legrand et al., 2007; Riou et al., 2002) at a similar mDP range to the wine tannins investigated here, have noted far higher particle sizes (500–2500 nm). The introduction of mannoproteins has been shown to reduce tannin aggregation, resulting in smaller particle sizes with averages ≃200 nm (Poncet-Legrand et al., 2007; Riou et al., 2002). Other wine-derived polysaccharides either enhance tannin aggregation as in the case of rhamnogalacturonan II, or have no effect, as in the case of arabinogalactan protein (Riou et al., 2002). Our results suggest that reconstitution of tannin and polysaccharide reduced tannin self-association, resulting in larger complexes of a similar particle size range to published reports for tannin–mannoprotein complexes (Poncet-Legrand et al., 2007; Riou et al., 2002).

The addition of the methanol fraction containing monomeric phenolics or small colored derivatives (Table 3, Fig. 3D), resulted in increased particle concentration, in particular in the intermediate particle size range 90–200 nm. This resulted in a significantly decreased particle size across the entire distribution. The skewing of the particle size distribution toward a lower average with addition of the methanol fraction may indicate that not all of the monomeric material became stably complexed to larger particles. While this result may indicate that monomeric material may have disrupted the interactions between other macromolecules resulting in the formation of smaller aggregates, it cannot be not excluded that self-association of low molecular mass phenolics occurred, as discussed previously (Supporting information S4).

In summary, when the respective wine fractions were reconstituted in the ratios present in wine, the formation of aggregates occurred and these were more abundant in Wine 2 compared with

Table 3
Polydispersity index, particle concentration and size in wine fractions and their respective combinations.

Wine component[a]	Polydispersity index[b] (*D*)	Particle concentration (e^8 particles/mL)	Particle size (nm)[c]		
			D10	D50	D90
Wine 1					
Methanol fraction	2.7 ± 0.09	11.9 ± 1.1d	28.0 ± 2.0h	48.0 ± 2.0g	93.0 ± 4.0g
Acetone fraction	2.1 ± 0.03	12.7 ± 0.8d	54.5 ± 1.5f	88.5 ± 1.5e	138.0 ± 8.0e
Precipitate	2.8 ± 0.03	4.8 ± 1.0e	107.0 ± 1.0b	193.0 ± 1.0b	382.0 ± 2.0a
Precipitate + acetone fraction		8.5 ± 2.2de	108.0 ± 2.0b	190.5 ± 1.5b	356.0 ± 2.0b
Precipitate + acetone fraction + methanol fraction		15.3 ± 2.6cd	100.0 ± 1.0d	174.0 ± 4.0c	317.0 ± 2.0d
Wine 2					
Methanol fraction	2.4 ± 0.09	3.1 ± 0.3e	119.0 ± 1.0a	200.0 ± 1.0a	329.0 ± 1.0c
Acetone fraction	1.8 ± 0.05	30.5 ± 2.1a	45.5 ± 0.5g	75.0 ± 1.0f	121.5 ± 1.5f
Precipitate	2.9 ± 0.06	13.7 ± 0.8cd	101.0 ± 1.0cd	202.5 ± 1.5a	355.5 ± 2.5b
Precipitate + acetone fraction		20.2 ± 5.3bc	105.5 ± 3.5bc	201.5 ± 2.5a	364.5 ± 0.5b
Precipitate + acetone fraction + methanol fraction		27.0 ± 1.3ab	85.0 ± 1.0cd	166.0 ± 1.0d	318.0 ± 2.0d

Data expressed as mean ± standard error of duplicate fractionation procedures, compared by one-way ANOVA ($P < 0.0001$) followed by post hoc *T*-test; statistically significant differences between wine fractions and winemaking treatments within a column are shown as different letters after the mean.

[a] Wines prepared using standard winemaking (wine 1) and microwave maceration (wine 2) were fractionated by precipitation in excess ethanol into sub-components and sequentially reconstituted in the ratios present in wine: methanol fraction = ethanol-soluble monomeric phenolics, acetone fraction = ethanol-soluble tannin, precipitate = ethanol-insoluble polysaccharide, protein and associated tannin.

[b] Polydispersity index calculated using size exclusion chromatography (termed gel permeation chromatography for phenolic fractions).

[c] Particle size in nm shown as showing average values at the 10th (D10), 50th (D50) and 90th (D90) percentiles of the distribution (i.e. at D90, 90% of particles are below this size).

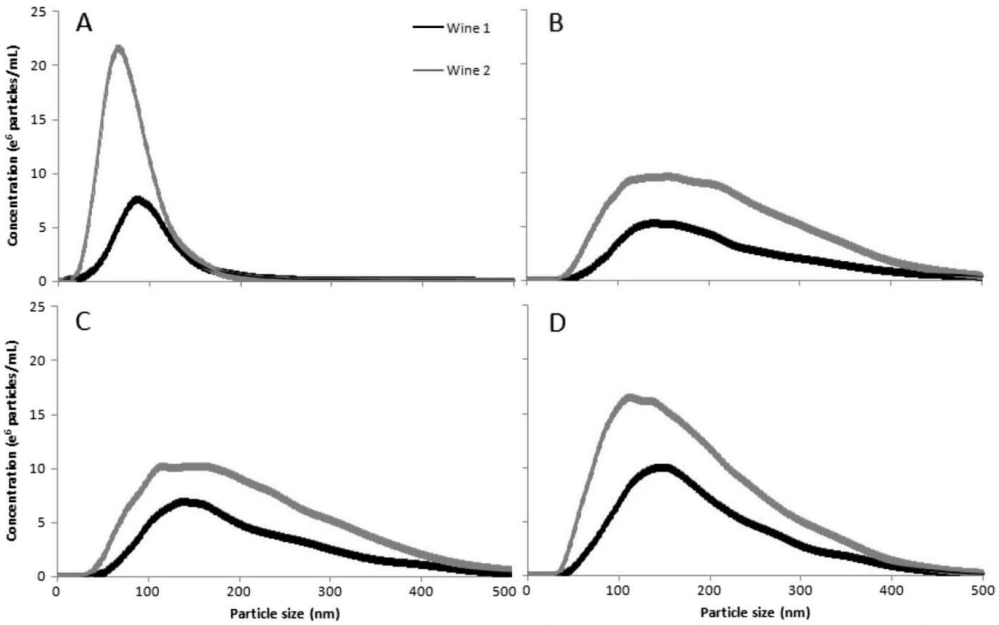


Fig. 3. Particle size and concentration of wine fractions isolated from two Pinot noir wines showing (A) acetone fraction in isolation; (B) precipitate in isolation; (C) acetone fraction + precipitate; (D) acetone fraction + methanol fraction + precipitate. Line thickness indicates the standard deviation of duplicate samples.

Wine 1. Although some minor compositional changes in precipitate composition were observed between the two wines studied, the ratios of the key classes of compounds which would be involved in intermolecular associations: polysaccharide, protein and tannin, were similar (Table 1). It is therefore likely that the factors contributing to the increase in particle number Wine 2 compared with Wine 1, were more strongly related to differences in concentration.

4. Conclusions

The current results provide preliminary evidence that microwave maceration during red winemaking can increase tannin concentration, together with small increases in polysaccharide and protein, resulting in complex formation. The primary effect of increasing macromolecule concentration in wine was to increase particle concentration, with only minor effects observed for particle size. This suggests that for only small changes in polysaccharide and protein concentration, larger changes in tannin concentration did not lead to a significant shift in particle size. The relatively low particle sizes <500 nm formed reflected that of stable tannin–polysaccharide (mannoprotein) complexes reported for model systems (Poncet-Legrand et al., 2007; Riou et al., 2002) which may show that the system was not rendered unbalanced by changes in macromolecule concentration. Ongoing work will aim to investigate the long-term stability of macromolecular complexes, to determine whether their concentration and/or size properties have implications haze or precipitate formation. Other implications of the manipulation of wine macromolecular profile may be in the modulation of wine astringency and viscosity due to impacts on the colloid state. While some key research has demonstrated the potential for tannin–polysaccharide interactions to impact wine mouthfeel (Quijada-Morin et al., 2014; Vidal et al., 2004), this remains a challenging research space. Based on the preliminary model reconstitution experiments, we propose that the measurement of wine particle size may be a promising new tool to measure wine colloids. In moving toward this objective, the current results help to generate a conceptual and experimental approach to the identification and characterization of complexes formed in red wine. Future experiments will aim to identify winemaking approaches which impact upon the formation of macromolecular complexes in wine, with special reference to wine colloidal stability and texture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.12.079.

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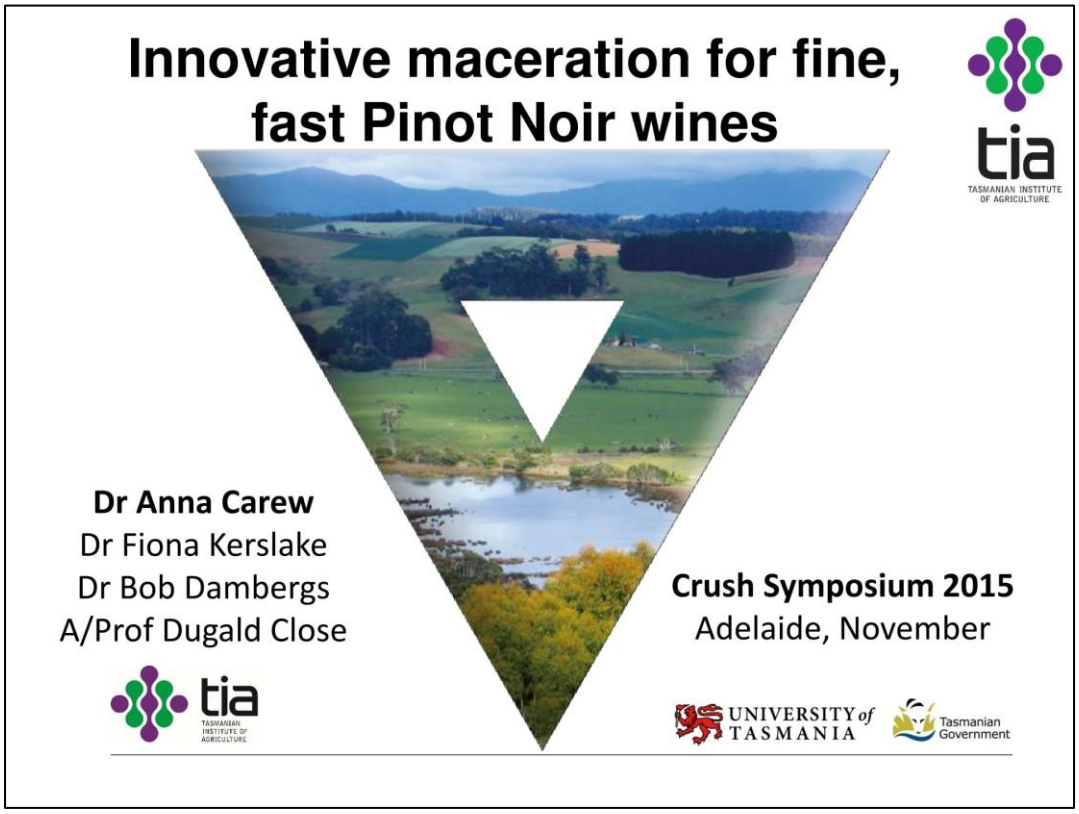
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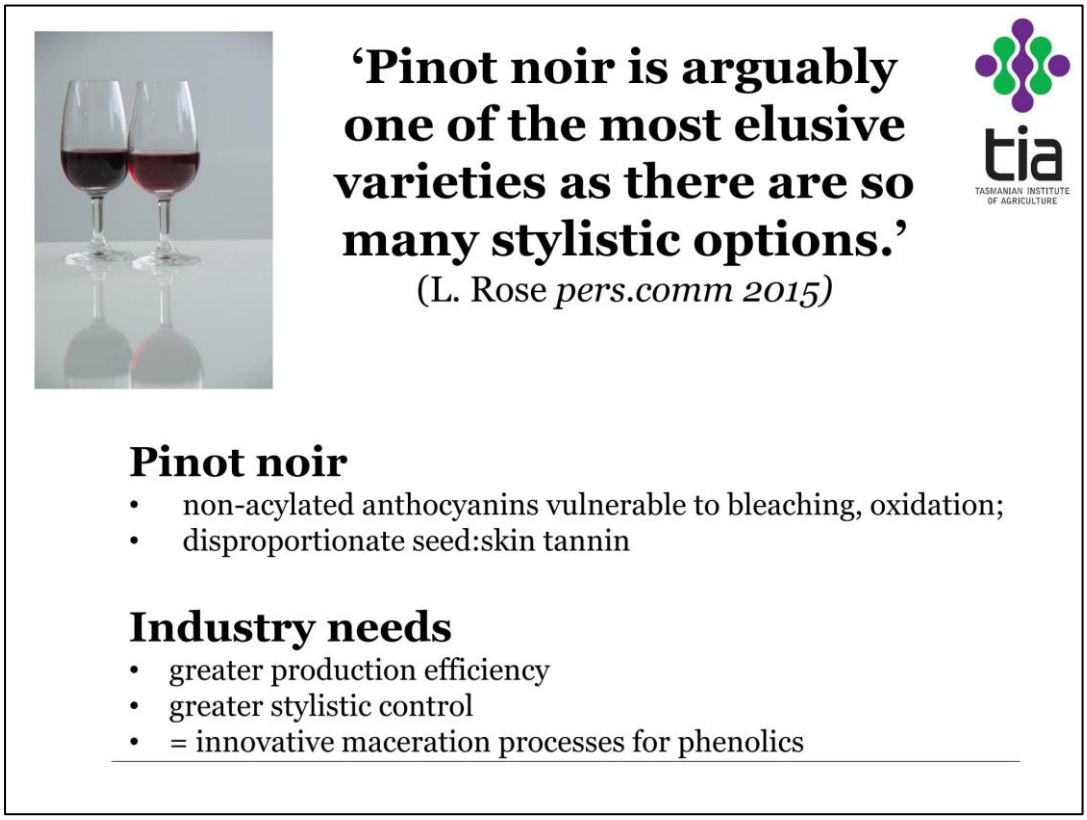
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Appendix 5.4 Novel approaches to red wine maceration

Crush 2016 PowerPoint presentation (Carew, Kerslake, Close and Dambergs 2016)





Maceration treatments

Control – 8 day fermentation on skins

Cold soak – 4°C for 4 days pre-inoculation

Extended maceration - wine left on skins for 4 days post-fermentation

CPR on skins - grape solids microwaved pre-alcoholic fermentation

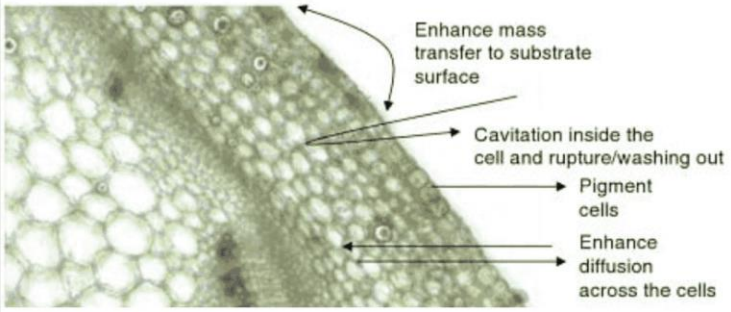
CPR early pressing - grape solids microwaved, press off at day 4

In-line CO_2 – must chilled to 10°C using CO_2 (dry ice), CS 4°C for 4 days

Ultrasound – grape solids softened/perforated with ultrasonics (cavitation)

Acknowledgment: Maceration trial 2015 – Carew, Kerslake, Dambergs, Close (TIA, AWRI)

Ultrasound





Acknowledgment: Maceration trial 2015 – Carew, Kerslake, Dambergs, Close (TIA, AWRI)
Mint stem cavitation slide TS 2000x (Vikhu et al. 2011)

‘In-line’ CO2 simulating ‘Boreal’





Acknowledgment: Maceration trial 2015 – Carew, Kerslake, Dambergs, Close (TIA, AWRI)

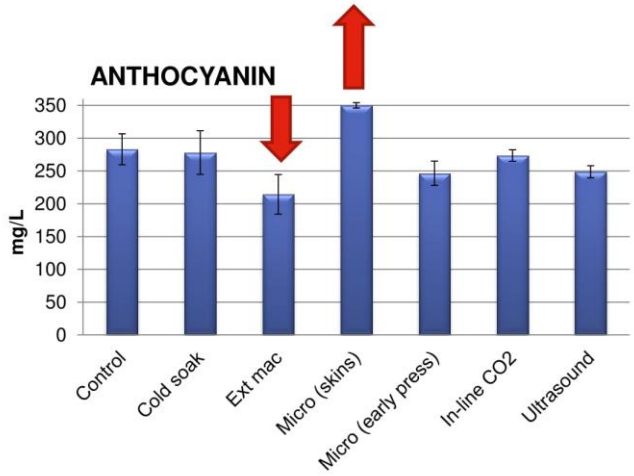
Analysis at 6 months

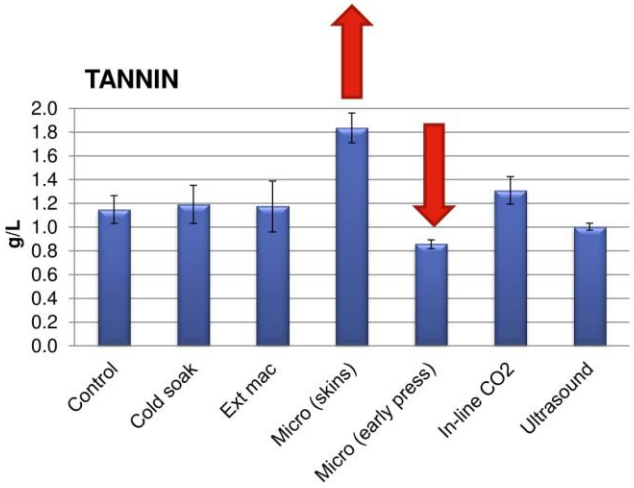


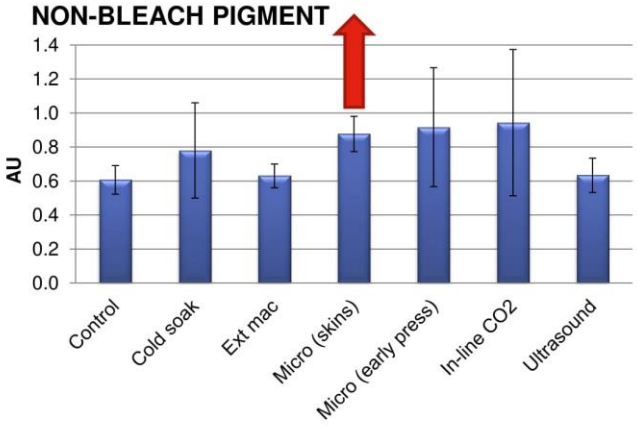
* basic composition (pH, TA, RS)
* phenolics by UV-vis Spectrophotometry

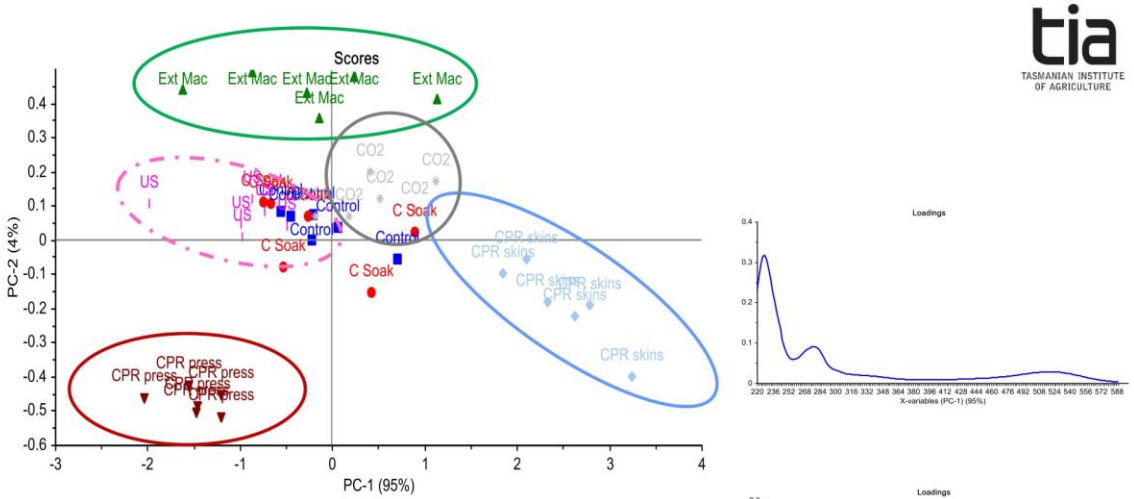


* informal sensory (winemakers)


ANTHOCYANIN
mg/L
350
300
250
200
150
100
50
0
Control
Cold soak
Ext mac
Micro (skins)
Micro (early press)
In-line CO2
Ultrasound

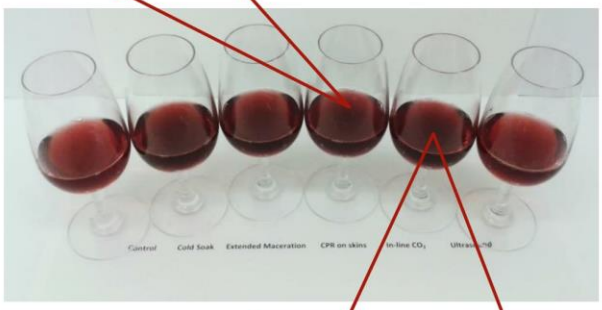

TANNIN
g/L
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
Cold soak
Ext mac
Micro (skins)
Micro (early press)
In-line CO2
Ultrasound


NON-BLEACH PIGMENT
AU
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control
Cold soak
Ext mac
Micro (skins)
Micro (early press)
In-line CO2
Ultrasound


Scores
Ext Mac
CO2
US
Control
C Soak
CPR press
CPR skins
PC-2 (4%)
PC-1 (95%)
Loadings
X-variables (PC-1) (95%)
X-variables (PC-2) (4%)

Winemaker comments

CPR on skins
'lifted dark plum, cherry and meaty aromas…crunchy bright tannins…a really good wine'



In-line CO_2
'black fruit, berry compote… generous non-dry tannin…I like that'

Take-home messages & the future of maceration….

- Innovative methods provide diverse Pinot noir wine styles
- Cost and practicality is an issue
- In-line CO_2 warrants further investigation

- If you would like to analyse these weird wines…please get in touch (anna.carew@utas.edu.au)

Thanks for your attention!
Acknowledgements!

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wineTQ

Wine quality through technical excellence

The Australian Wine Research Institute

BROWN BROTHERS
EST. 1889

Australian Government

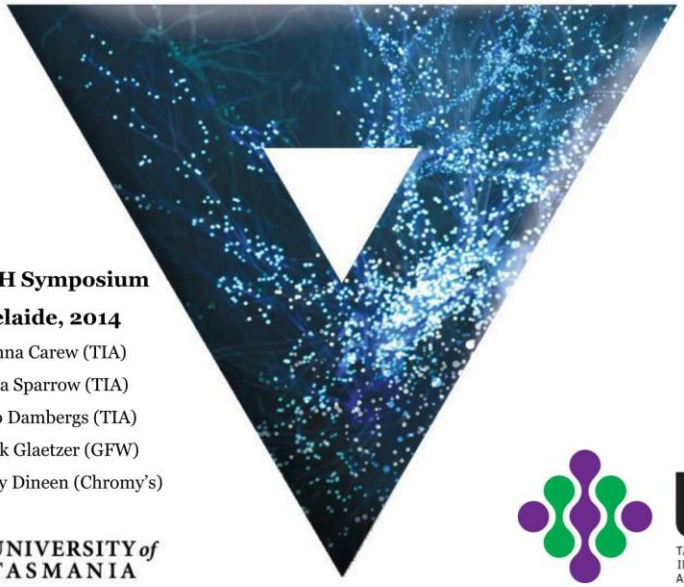
Australian Grape and Wine Authority

Appendix 5.5 Distributed Industry Trials

PowerPoints from Crush 2014 presentation on change oriented research (Carew, Sparrow, Dambergs, Glaetzer and Dineen 2014).

Distributed Industry Trials:

oenological research design for changed winemaking practice



CRUSH Symposium

Adelaide, 2014

Dr Anna Carew (TIA)

Angela Sparrow (TIA)

Dr Bob Dambergs (TIA)

Mr Nick Glaetzer (GFW)

Mr Jeremy Dineen (Chromy's)

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Acknowledgements

- Australian Grape and Wine Authority
- Josef Chromy Wines, Moorilla Estate, Brown Brothers
- Glaetzer-Dixon Family Winemakers
- Domain Chandon, Seville Estates, Hoddles Creek
- Australian Wine Research Institute
- Curly Flat – Phil Moraghan
- Moorooduc Estate – Richard McIntyre
- Dromana Estate – Duncan Buchanan
- Lethbridge Winery – Ray Nadeson
- Paradigm Hill – George Mihaly
- Ten Minutes by Tractor – Jeremy Magyar
- Yalumba

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Engineering Education & Transdisciplinarity Publications

Wickson, F and **Carew**, ALJ (in press) Quality criteria and indicators for responsible research and innovation: Learning from transdisciplinarity. *Journal of Responsible Innovation.*

Carew, A. L., & Wickson, F. (2010). The TD Wheel: A heuristic to shape, support and evaluate transdisciplinary research. Futures, 42(10), 1146-1155. doi: DOI 10.1016/j.futures.2010.04.025

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Symes, M and Ranmuthugala, D and **Carew**, A, (2012) "A sequential project based leaning programme designed to meet the graduate attributes of engineering students", Journal of Modern Education Review, 2 (1) pp. 30-37. ISSN 2155-7993 (2012) [Refereed Article]

Symes, M and Ranmuthugala, D and Chin, C and **Carew**, A, (2011) "An Integrated Delivery and Assessment Process to Address the Graduate Attribute Spectrum", US-China Education Review A, 1 (7) pp. 1-11. ISSN 2161-6248 (2011) [Refereed Article]

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Carew, A.L. Lefoe, G. Bell, M and Amour, L (2008) Elastic Practice in academic developers *International J. for Ac Development* 13(1), 51-66

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Wickson, F, Carew, A.L and Russell, A.W. (2006) Transdisciplinary Research: Characteristics, Quandaries and Quality. Futures 38, 1046-1059.

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What are DITs?

A **Distributed Industry Trial** (DIT) engages ≥3 wineries in a replicated experiment where each winery makes 1x control, 1x treatment wine at commercial scale.

Winemakers and scientists negotiate agreed winemaking protocols for control and treatment. For example:

- Volume of must & size of fermentation vessel
- Maceration & cap management processes,
- Inoculating yeast & malo strain
- Cooperage

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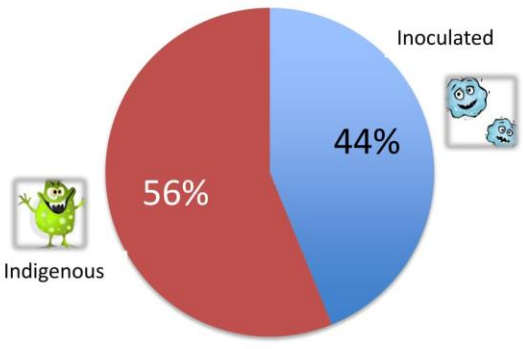
Why DITs?

- Working with industry - practical insight into “real-world” winemaking issues
- Winemaking on a commercial scale has more credence with industry
- Each winemaker performs one replicate = reduces cost and logistics to individuals, but robust replication
- Many collaborators reduces the risk of a “vintage disaster” affecting the trial
- Brain-storming with a large group to think of all the angles
- Uptake facilitated through initial, active industry participation

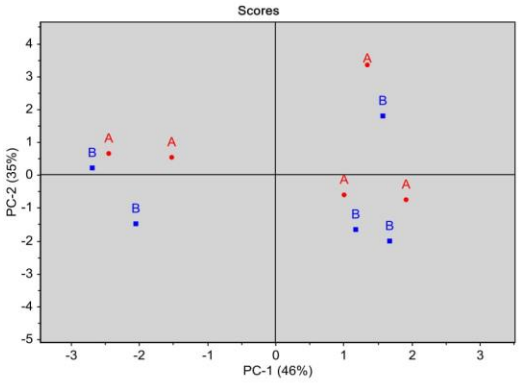
DITs Example 1 –

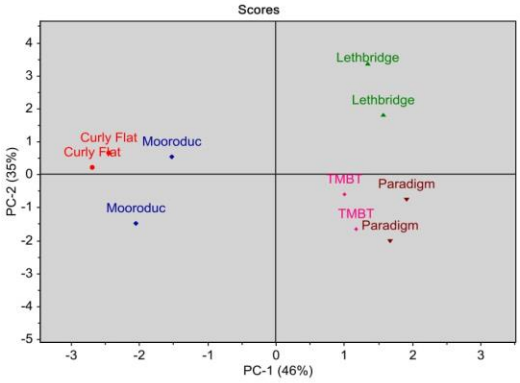
Delayed, indigenous malolactic fermentation on Pinot noir phenolics

- 5 Victorian wineries, 2011 vintage
- MV6 clone but own grapes, equipment and initial winemaking protocol
- Indigenous MLF was compared with wines inoculated with an MLB strain
- Wines tasted at the Victorian Pinot Noir Winemakers Workshop [VPNW]
- Phenolic analysis of wines
- Data presented at AWITC-15



- Indigenous = higher: colour density, non-bleachable pigment and % stabilised pigment
- Indigenous = lower: hue (more purple) and free anthocyanins





Cluster analysis (PCA) reveals differences between wineries but treatment caused similar shift

A= Indigenous B= Inoculated

Benefits: Industry view

'some [treatments] gave interesting characters in the wines'

'you…see the results of a trial on your own fruit and situation, lots of conclusions are drawn from trials [done elsewhere] but [trials on your own fruit] are more reliable'

'it feels like the right thing to do, I'm inclined to help the Uni of Tas out'

'might not be directly relevant or practical but it keeps you thinking about novel ways to do things…[you] look at your own operation differently'

'spend time interacting with people whose full time job is to think about wine science'

'the researcher [gets] to look at their research in a practical, industrial, real-world situation'

'learning about and having early access to new [yeasts, processes]'

'analysis from Bob on tannin and colour was useful…You can taste it but having the figures in front of you gives you a better picture of the wines'





Costs/difficulties: Industry view

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'I felt committed to participate…difficult if the fruit was down or not the way I wanted'

'the wine might be bad, which would be a risk for a small producer if a barrel was bad'

'practical things like trying to fit…a ½ tonne ferment, into a larger scale operation'

'more physical labour input as the large scale stuff is mostly automated but [with small scale trials] pressing by hand, plunging by hand, crushing separately…'

'keeping the ferments separate, not being able to put batches in the press together'

'the intellectual and management input for the small ferments is as much as large ones, [all up] with labour and intellectual input it might add several days worth of labour to participate in a [DITs]'

'when the trial is happening, even though there is lots of time [pre] planning, it happens at the peak of busyness…another element to manage in that time'





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Transdisciplinary research

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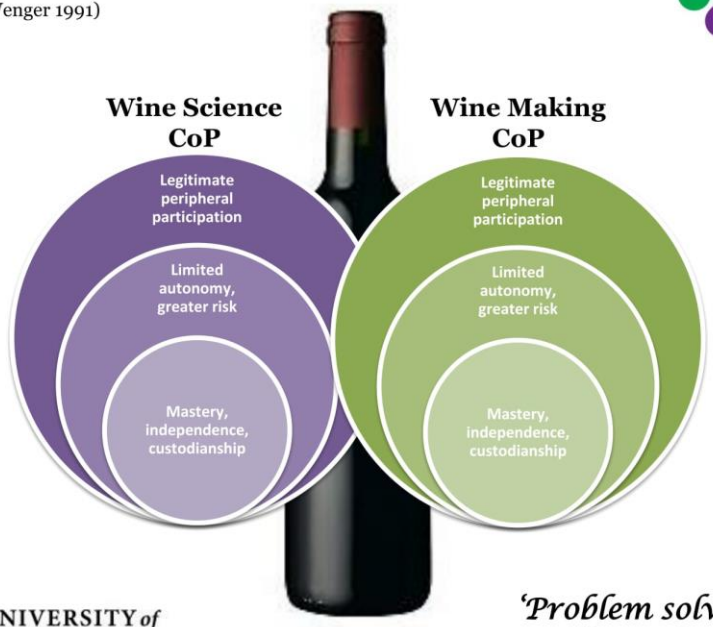
'Problem solving through the context-specific negotiation of knowledge'

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(Wickson, Carew and Russell 2006)

Communities of Practice & Situated Learning

(Lave and Wenger 1991)



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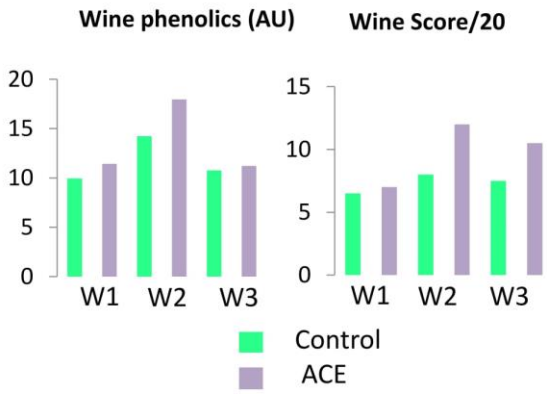
'Problem solving through the context-specific negotiation of knowledge'

(Wickson, Carew and Russell 2006)

DITs Example 2 – ACE maceration 2013

- Three winemakers confirm participation 2012
- Winery visit by scientist, protocol agreed by group emails Jan 2013
- Pinot Noir clone D5V12 available at each site
- Staggered harvest dates Apr-May help transfer of single ACE macerator
- Control and ACE ferment prepared at selected size: 250 kg, 330 kg and 1 tonne
- Fermentation on skins ~8 days prior to inoculation with PN4 MLF culture





- Wines transferred to single winery for bottling
- Chemical and statistical analysis
- Industry wide tastings conducted at 6 and 12 months bottle age = 34% improvement with ACE

Costs & benefits: Researcher view

BENEFITS

Bob "Although each winery has only done a single replicate, the overall trial replication produces robust results, it takes into account vineyard and winemaking variability"

Angela "Unique opportunity to realise vintage pressures, while testing validity of new techniques at commercial scale. Outcome is a tangible product for thorough chemical analyses and industry wide response"

Anna "A big bucket of cold water on impractical ideas"

COSTS/DIFFICULTIES

Bob "Industry trials can be interrupted by commercial vintage pressures but a distributed trial reduces that risk"

Angela "Plan carefully, then prepare for complete flexibility"

Anna "Lots and lots of communication and negotiation required, which can be really time consuming and taxing"





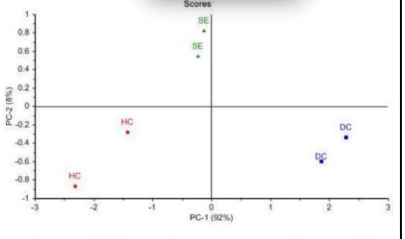


DITs Example 3

Controlled Phenolic Release

- Five winemakers recruited during 2013
- Negotiate agreed protocol (~4 mtgs)
- 2014 Yarra/Mornington yields down 50-80%
- Six 400kg lots of Pinot noir must
  - CPR x three lots (CSIRO Werribee)
  - Control x three lots
- Fermentation on skins ~8 days
- Press to barrel, inoc for malo
- Chemical and statistical analysis
- Tastings planned for October 2014







A word to the wise…

Researcher

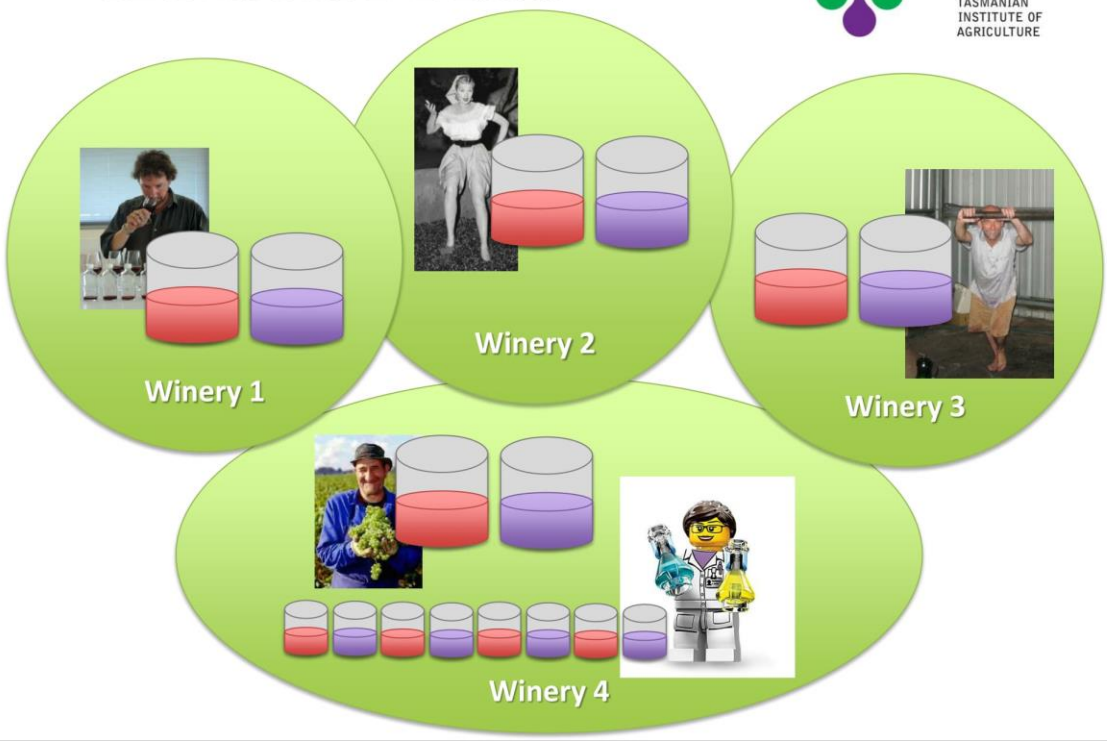
- Maintain active lines of communication
- Think ahead and be prepared- JIT will lead to stress and error
- Prepare a clear, practical protocol, agreed on by all the collaborators
- Ensure adequate feedback of results to collaborators

Winemaker

- Plan well, walk around the winery and show the researcher how you'd do it and how that differs from standard operations
- researcher and winemaker both have to think deeply about where the potential sources of variability are in an established industrial setting

- Embrace it! Read up on the literature related to the subject and get the most you can out of it. You can just be labour, but [its] better to feel part of it [and] contribute to the science.

An ideal DITs…



Thank you! Questions?

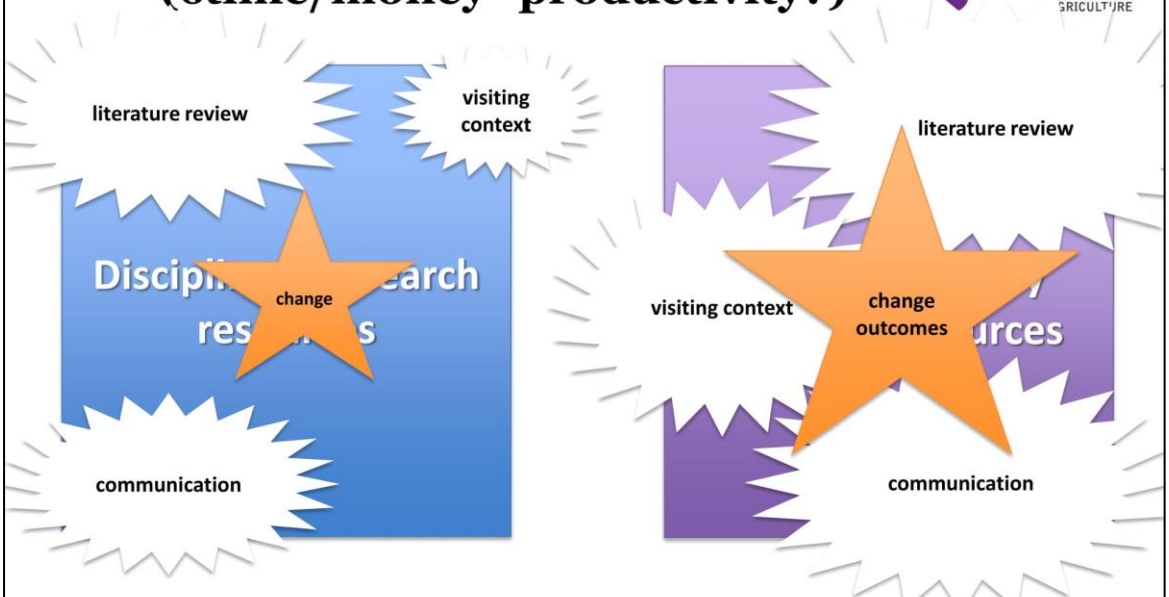
CONTACTS

- Dr Anna Carew anna.carew@utas.edu.au 0411 894997
- Angela Sparrow angela.sparrow@utas.edu.au 0437 752 775
- Dr Bob Dambergs bob@winetq.com.au 0408 895858





Research resources
(¿time/money~productivity?)



Transdisciplinary research as a 'meglomaniac endeavour'
(Pohl 2005)

Appendix 5.6 Yalumba wine descriptors

Terms used by the Yalumba winemaking team to describe control, microwave on skins, and microwave with early pressing wines in a blind tasting (eight tasters).

wine	descriptors
control	light-colour bright pink rose VA raspberry confection nice-tannin juicy kirsch blood-plum apparent-tannin pretty confection sweet delicate sandalwood rhubarb light-colour fresh citrus grapefruit ethyl-acetate isoamyl light-colour vibrant red light-colour red cordial confection floral alco-pop juicy fruit red strawberry raspberry bright red petal clean-finish fruit varietal bright light-colour juicy light-colour raspberry strawberry rose fine-tannin pink crimson ribena fine-tannin
mwv-press	intense bright garnet purple deep rose plum raspberry complex layered-tannin fruit cocoa acid spice apparent-tannin acid not-extracted caramel raspberry strawberry herbal sweet savoury less-acid mid-colour garnet purple red-jubes confection air-affected grippy-tannin persistent dark-colour plum violets floral grainy-tannin extracted structure fragrant complex confection dark-colour fruit-bomb dense even dark-colour stalky extracted medium-tannin apparent-tannin hollow-finish purple sulfidic rhubarb ribena chewy apparent-tannin bright fruit
mwv-skins	intense bright purple pink grainy-tannin caramel banana dusty stalky apparent-tannin blueberry herbal vanilla ice-cream caramel stalky angular-aggressive tight fresh mid-colour crimson magenta purple apricot dried-plums raspberry fine-tannin juicy persistent round-mouth flesh dark-colour burnt blueberry structure spice more-interest wine-gums lifted-fruit varietal dark-colour persistent even dark-colour red raspberry strawberry angular-aggressive persistent purple leather varietal rhubarb grippy-tannin ribena

AWRI Report

Analysis of wines from microwave treatment trial

Author: Neil Scrimgeour

9 May, 2017

PO Box 197 | Glen Osmond, SA 5064
T: +61 8 8303 6600 | F: +61 98 8303 6601; www.awri.com.au

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T: +61 8 8303 6600 | F: +61 98 8303 6601; www.awri.com.au

1. Introduction

AWRI Commercial Services was engaged to provide analytical services for wines that had been produced as part of a 2015 trial investigating the impact of microwave maceration. Four pairs of control and microwave treated wines were supplied through four different commercial wine producers, providing a total of eight samples for analysis.

Testing was carried out on bottled wine samples, as supplied, for basic chemical attributes as well as phenolics and colour profile. Sensory evaluation of the wines was included to evaluate the effect of the treatment on perceived aroma and flavour attributes in the wines.

2. Methodology

The following chemical analyses were performed on duplicate samples by AWRI Commercial Services' NATA accredited (ISO 17025 certified) wine laboratory:

- Basic chemical attributes, including % alcohol, titratable acidity, volatile acidity, glucose/fructose, specific gravity.
- Free and total sulfur dioxide (SO_2).
- Phenolics and colour profile, including colour density, hue, total phenolics, total pigments and total tannin.

Sensory evaluation of the wines was carried out using the AWRI's internal expert sensory panel. A compare and contrast method was used to evaluate each pair of wine samples (treatment vs control). The tasters provided detailed tasting notes and rated each wine using the 20-point quality scoring system as used in Australian wine show judging. All tasters' comments and scores are provided. The panel was also instructed to identify and rate the intensity of any perceived off-flavours or faults in the wines.

3. Results

3.1 Chemical analysis

The results of the chemical analysis of the four wines are presented below in Table 1. There are minor differences in the basic chemical attributes within each pair of wines. Free and total SO_2 concentrations in the Seville control sample were significantly higher than those in the treated wine, presumably due to a winemaking addition, either pre- or post-treatment.

In each of the four pairs of wines, the colour density was higher in the treated wine than in the control and hue was lower, indicating a higher absorbance at 520nm due to increased red pigmentation in the wines. In three out of the four pairs of wines, this is also reflected in higher total pigment concentration in the treated wines (the exception being the Seville wine). Total tannin and total phenolics were also significantly higher in the treated wine, in comparison with the control wine, in all four cases.

In three out of the four pairs of wines, the treated wines exhibited a higher concentration of free anthocyanins than the control wines (Seville again being the exception) and the pigmented tannin concentrations were higher in the treated wines in all four cases. These results all indicate an increased extraction of colour and phenolic compounds from the microwave maceration treatment.

PO Box 197 | Glen Osmond, SA 5064
T: +61 8 8303 6600 | F: +61 98 8303 6601; www.awri.com.au

Table 1: Chemical analysis results

Attribute	Chandon		Curly Flat		Montalto		Seville	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Alcohol	11.9	12.2	13.9	14.1	12.5	12.5	12.7	13.2
Glucose + Fructose	0.35	0.40	0.40	0.60	0.40	0.40	0.65	0.80
Malic acid	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40	<0.40
pH	3.61	3.69	3.65	3.63	3.44	3.44	3.73	3.42
Titratable acid pH 7.0	4.9	5.1	5.0	5.2	5.7	5.6	4.8	5.8
Titratable acid pH 8.2	5.4	5.7	5.6	5.7	6.2	6.2	5.3	6.3
Specific gravity	0.993	0.994	0.991	0.992	0.993	0.993	0.993	0.993
Volatile acidity as acetic acid	0.53	0.6	0.73	0.55	0.47	0.47	0.53	0.46
Sulfur Dioxide (free)	21.5	19.5	18.0	14.0	15.0	20.5	44.0	18.0
Sulfur Dioxide (total)	121	122	78.5	76	72	77	111	67.5
Colour Density	2.6	4.4	4.4	6.9	4.2	6.4	5.0	5.6
Hue	0.88	0.84	0.82	0.79	0.78	0.72	0.72	0.80
Free Anthocyanins	65.5	95.5	93	131	104	148	216	153
Total phenolics	23.0	35.0	36.5	48.5	27.0	38.0	33.0	38.0
Total pigment	4.2	6.3	6.4	9.2	6.55	9.6	11.9	9.3
Total tannin	0.18	0.73	0.86	1.55	0.31	0.81	0.40	0.75
Pigmented Tannin	0.52	0.91	1.06	1.62	0.82	1.32	0.70	1.00
% Pigmented tannin	12.4	14.4	16.5	17.6	12.6	13.8	5.8	10.7
Chemical Age 1	0.38	0.39	0.44	0.42	0.35	0.36	0.24	0.32
Chemical Age 2	0.13	0.15	0.17	0.18	0.13	0.14	0.06	0.11

3.2 Sensory analysis

The full tasting notes generated from the sensory evaluation can be found at the end of this report, along with individual taster comments on the perceived differences within each pair of wines. A summary of the average ratings awarded to each pair of wines, along with the standard error across nine tasters, is provided below.

	Control		Treatment	
Wine	Average	St Err	Average	St Err
Chandon	14.1	0.4	15.1	0.4
Curly Flat	14.6	0.4	15.3	0.3
Montalto	15.3	0.4	15.6	0.5
Seville	14.7	0.3	14.3	0.4

In three out of the four pairs of wines, the microwave treated wine was awarded a higher average rating than the control. The exception was the Seville wine, which showed a higher rating for the control. The Seville wine treated using microwave maceration exhibited the highest incidence of perceived taints (faults) across the tasters, potentially indicating issues experienced during winemaking.

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T: +61 8 8303 6600 | F: +61 98 8303 6601; www.awri.com.au

4. Summary

In all four pairs of wines evaluated, the wine treated using microwave maceration exhibited a greater degree of red pigmentation, tannin and phenolics than the control, indicating increased extraction during processing. This was reflected in the sensory evaluation ratings awarded to the wines, with a higher average rating for three out of the four treated wines, in comparison to the control. The Seville wine appears to be the exception to this, with a lower average rating for the treated wine, in comparison to the control, although this wine was considered to exhibit taints (faults) by five out of the nine tasters.

PO Box 197 | Glen Osmond, SA 5064
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Sensory Evaluation Technical Quality Report

Attention: **Neil Scrimgeour**

Sample received: 24/04/2017 Report date: 1/05/2017

Sample Description: **Eight wines for comparison and quality scores**
Analysis Report: **Wies Cynkar**

These wines were supplied to the AWRI for quality assessment by the Technical Quality sensory panel, to provide an indication of sensory characteristics and quality, and the judges were asked to compare the pairs of wines. The results of the assessment are provided in Tables 1, 2, 3 and 4.

Table 1a: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Seville Estate – Control AE 10987

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Light-mid brick red	Nose lifted but slightly bilgey/reductive, showing rubber and garlic notes alongside green olive/roasted capsicum/tomato fruit with herbal notes.	Supple fleshy fruit somewhat nondescript - strawberry, green plum, green olive - with subtle tannins slightly emery on finish.	13	4 - Reductive /rubber/garlic
2	Light red	Red berries, slightly smoky, savoury, slightly spicy	Round palate, some fruit, slightly drying, medium length	15	-
3	Clear, bright, pale garnet	Clean, medium intensity, complex, earthy, forest floor, floral- violet, spice, Developing	Dry, high alcohol, medium + acid, medium + bodied, low tannin powdery and sappy, medium astringent, medium flavour intensity, plum, spice, cherry, medium length	14.5	-
4	Dull pale red, brown tint	Quite developed, meaty, lacks vibrant fruit. Some strawberry.	Moderate acidity, quite hot, light body, low level cherry fruit.	14	-
5	Mid intensity orange/ garnet	Quite intense nose with sweet orange flower water initially followed by roast onion, mercaptan aromas	Quite intense flavours of sweet mandarin and cherry, medium acid with very fine-grain tannins; heavier palate weight with longish finish	15	3 - reduced
6	Light tawny with red hues	Quince jelly like with some resinous and spicy oak.	Low in primary fruit but had interesting strawberry jam and cinnamon like flavours. Also, had an attractive roundness and a soft tannic finish.	16	-
7	Light red with tawny, brick red hue	Dry grass, cooked red fruits,	Lean on flavour, quite acidic and tight, short palate	14.5	-

AWRI
The Australian Wine Research Institute Ltd
Corner of Hartley Grove & Paratoo Road
Urrbrae (Adelaide) SA 5064
PO Box 197 | Glen Osmond
SA 5064 | Australia

ABN 83 007 558 296
T +61 8 8313 6600
F +61 8 8313 6621
E commercialservices@awri.com.au
www.awri.com.au

8	Light/ medium red orange	Cherry, strawberry, charry, wet hessian, spice, potpourri, savoury, cooked vegetables	Acid bit low, viscous, low fruit, some bitterness	14.5	-
9	Light red, clear, brown /orange tinge	Medium aroma intensity, floral, stem, savoury, strawberries and other red fruits, slight burnt sugar note, complex	Medium bodied, lacking in fruit concentration, fresh and vibrant, but no stuffing. Short/medium length finish	15.5	-

The mean quality score assigned to 2016 Pinot Noir Seville Estate – Control was 14.7 with a standard error of 0.3. Two judges gave fault scores for reductive character.

Table 1b: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Seville Estate – Treatment AE 10990

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Mid ruby / garnet	Lifted ripe red/blue fruits showing fresh raspberry/blueberry/strawberry/plum, milk chocolate/carob notes, slight rubber/reduced character.	Bright fleshy red berry raspberry / cherry / strawberry / sour plum fruit up front, moderate supple tannins, quite (excessively) sour throughout and on finish in particular. Slightly bitter finish.	15	2 - Reduced / rubber
2	Mid red	Oaky, slightly leather, slightly smoky, slightly rubbery, slightly tobacco, some red berries	Slightly acid finish, slightly sour, short finish	13.5	2 - Reduced
3	Clear, bright, medium intensity ruby	Unclean (meaty, sweaty, cheesy, animal), medium intensity, complex tertiary aromas of rubber, bitumen, plum, leather. Developed	Dry, medium bodied, medium acid, low tannin- powdery, low intensity flavour, plum, cherry, low persistence	13	4 - Unclean - dirty
4	Deep red	Quite intense red berry, raspberry some VA?	Moderate intensity cherry fruit, quite acid, some VA. Low astringent finish.	14.5	2 - VA
5	Deepish crimson /garnet	Fairly intense secondary Mocha aromas	Fairly intense flavours of sour cherry; medium+ acid, low palate weight	14	-
6	Light to medium red	Red cherry with some stinky gas-like characters	Good lifted fruit, red berry flavours but the acid dominated as did some bitterness on the finish. Light drying astringency but the acid and hardness became prominent right at the end.	14	3 - Bitter
7	Light to mid red, with ruby hue	Subtle florals, rose, red fruits, moderate intensity	Vibrant, fleshy, primary red fruits, florals and estery flavours, moderate length	16.5	-
8	Medium red	Berries, cherry, floral, herbal notes, savoury notes	Acid bit flat, bitter finish, green harshness, some fruit and floral length	15	-
9	Bright red, clear, vibrant	Low/medium aromatic intensity, flinty reduction, red fruits, flowers,	Viscous and mouthfilling, bitterness takes over mid palate and continues on into the finish. Some fruit concentration but a bit swamped as it heads to the mid palate.	13.5	-

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PO Box 197 | Glen Osmond
SA 5064 | Australia

ABN 83 007 558 296
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F +61 8 8313 6621
E commercialservices@awri.com.au
www.awri.com.au

The mean quality score assigned to 2016 Pinot Noir Seville Estate – Treatment was 14.3 with a standard error of 0.4. Five judges gave fault scores, two for reductive character, one for unclean/dirty character, one for VA and one for bitter taste.

Taster comments on differences between the samples:

- Taster 1: Treatment shows riper more concentrated red/blue fresh fruits on nose and palate, more pronounced tannins (although still relatively modest) and more acid. Control shows more reductive characters, although also apparent in Treatment to a lesser extent.
- Taster 2: Control is cleaner on both nose and palate with more fruit intensity than Treatment
- Taster 3: Treatment was unclean, Control appears less so but similar descriptors
- Taster 4: Treatment much deeper colour, more intense fruit, higher astringency, higher VA.
- Taster 5: Colour: Control lighter and more orange hue; reduced aromas in Control vs developed secondary characters in Treatment, flavour descriptors differ too: Control more mandarin vs Treatment - sour cherry
- Taster 6: Treatment brighter and younger but had a roughness and coarseness to it. Control was very attractive drinking now with lots of sweet fruit and complexity but it would be a short-term proposition.
- Taster 7: Colour. More tawny brick red in Control. Treatment more red/pink. Treatment has more fruit, flavour, length. Control forward and looks a different wine almost
- Taster 8: Control is lighter and more orange in colour than Treatment. Control is more savoury and not so nice wet hessian aroma but still has quite a bit of fruit. Treatment has a less intense aroma but is more bitter on the palate than Control.
- Taster 9: Control much lighter in colour, along with a brown/orange tinge, likely from some whole bunch/stem influence, as the nose would also attest to. Treatment appears to be traditionally made in comparison, and is richer and fuller on the palate, however a bitterness envelops the wine through the mid palate and is unpleasant.

Table 2a: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Montalto – Control AE 10991

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Light - mid ruby / garnet	Lifted and fragrant floral/bubble-gum characters dominate nose with confection/jube/fruit pastille/blackcurrant notes also evident.	Mid-full weight fresh red/dark berry fruits - raspberry/strawberry/plum - with ripe chewy tannins, acid a little excessive throughout. Herbal/white pepper notes on finish	16	-
2	Mid red	Red berries, slightly strawberries, slightly lolly, slightly syrup	Plenty of fruit, slightly sour, slightly drying, medium length	14.5	-
3	Clear, bright, medium ruby	Clean, low intensity, primary fruit sour cherry, youthful, subtle animal notes	Dry, medium + bodied, medium + acid, medium+ alcohol, low intensity flavour- cherry, medium tannin - sappy and drying and unbalanced, low persistence	14	-
4	Bright pale-mid red	Moderate red berry, caramel	Quite acidic, moderate red berry fruit, soft finish. acid a little too high. some drying astringency	14.5	-
5	Light crimson	Red currant fruits with a hint of balsamic vinegar and some dried herb, reduced notes	Fairly intense red cherry flavours with high acid and some vinegar on the palate; alcohol heat; gritty tannins	14.5	-

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The Australian Wine Research Institute Ltd
Corner of Hartley Grove & Paratoo Road
Urrbrae (Adelaide) SA 5064
PO Box 197 | Glen Osmond
SA 5064 | Australia

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T +61 8 8313 6600
F +61 8 8313 6621
E commercialservices@awri.com.au
www.awri.com.au

6	Light red	Medium green plum and stalky character.	Medium fruit but the acid and some greenness, stalkiness came in late. Some sweeter fruit on the finish but overall it had an unripe and acidic character	14.5	-
7	Light to mid red with ruby pink hue	Moderate intensity raspberry, estery, floral notes	Good flavour, esters, rose, red fruits, with some mid palate richness, silky texture and some good length,	17.5	-
8	Light/ medium red	Intense blackcurrant and menthol cough lollies. Sweaty, towards cat pee	Strange acid, low flavour compare with the intense aroma but long.	15	
9	Vibrant bright red, clear	flinty, gun smoke - good reduction, but pushing the envelope (could go either way). Some stem, green notes, herbaceous, complex	Viscous, concentrated attack, vibrant, balanced, length, very fine tannin, very nice	17	-

The mean quality score assigned to sample 2016 Pinot Noir Montalto – Control was 15.3 with a standard error of 0.4.

AWRI
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Corner of Hartley Grove & Paratoo Road
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T +61 8 8313 6600
F +61 8 8313 6621
E commercialservices@awri.com.au
www.awri.com.au

Table 2b: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Montalto – Treatment AE 10993

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Mid ruby /garnet	Intense ripe fleshy red/dark berry fruits plum/strawberry/blackberry with milk chocolate notes.	Mid-full fleshy red berry fruits on front palate with integrated silky fine-grained tannins, acid slightly elevated and slightly warm finish	16.5	
2	Dark red	Smoky, slightly ashtray, some fruit, slightly volatile, slightly EA	Slightly hard, drying, very sour	13	2 – smoke taint
3	Clear, bright, medium ruby	Clean, medium intensity, primary fruit - cherry, secondary spice- five spice, youthful	Dry, full bodied, medium+ acid, medium tannin- fine, high alcohol, medium flavour intensity- Morello cherry, spice, medium length	15.5	-
4	Deep mid red	Moderate red berry/cherry	High acid, low-moderate fruit intensity, some VA? low level astringency	15	-
5	Deepish crimson	Red currant and with a hint of Morello cherry and a slight touch of marzipan	Intense cherry fruits and bitter almond flavours, medium+ acid with finer-grain tannins	15	-
6	Medium red	Really attractive aroma of ripe cherry with some chocolate notes. Some fleshiness reminiscent of carbonic maceration	Mid weight palate. Good genuine fruit sweetness in the "Cherry Ripe" spectrum. Soft tannins, balanced acid and with a long persistent finish. Reminds me a lot of a very good Dolcetto. Really nice drinking	17.5	-
7	Light to mid red with ruby pink hue	Moderate intensity raspberry, estery, floral notes	Good flavour, esters, rose, red fruits, with some mid palate richness, silky texture and some good length, more alcohol warmth?	17.5	-
8	Medium red	Blackcurrant, menthol & mint cough lollies, chocolate. Tobacco, towards cat pee, machine oil	acid reasonably crisp with some astringency. Low but long flavour.	15	
9	Dark red, clear, very dark for Pinot	Red and black fruit, herbs, low/med aromatic intensity	Concentrated attack, medium bodied, some vibrancy, length. Lacking a bit of excitement	15	-

The mean quality score assigned to 2016 Pinot Noir Montalto – Treatment was 15.6 with a standard error of 0.5. One judge gave a fault score for smoke taint.

Taster comments on differences between the samples:

- Taster 1: Treatment shows primary red/dark fruits on nose and palate, Control shows more aromatic bubblegum/jube/ester type characters. Treatment shows slightly more tannin, finer grained. Acid on Control slightly more apparent.
- Taster 2: Treatment has a strong smoke/ashtray smell with an extremely sour palate, Control is cleaner and fruitier
- Taster 3: Treatment slight deeper colour, more fruit on nose and cleaner, fuller bodied and richer flavour
- Taster 4: Treatment - deeper colour, more intense fruit, higher astringency, some VA
- Taster 5: Colour: Control lighter, darker berry fruit aromas in Treatment; coarser astringency in Control

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F +61 8 8313 6621
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- Taster 6: Treatment had a lots more fruit and fruit sweetness. Control was greener, more acidic and harder. Also, I would drink Treatment any day.
- Taster 7: Treatment slightly more colour depth, Aroma - little differences noted. Slightly warmer finish on Treatment, otherwise quite similar.
- Taster 8: Treatment is darker than Control. Control more sweaty than Treatment. Treatment less intense overall but with more complexity and more minty and machine oil notes. Not sure how to score them - not something that I have smelled before in PN.
- Taster 9: Control lighter in colour than Treatment. Control has a reductive note that Treatment lacks. Overall Treatment appears to be less vibrant and complex than Control. Control's reductive note will polarize, but to me lies in the realm of adding complexity, and is a positive

Table 3a: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Curly Flat – Treatment AE 10995

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Mid - deep ruby / brick red	Intense deep rich dark fruits cassis/blackberry/satsuma plum/dark cherry with dark chocolate notes	Intense ripe dark fruits on palate persistent throughout, chewy tannins persistent and ripe, long finish, slightly warm.	17	-
2	Dark red	Low intensity, some fruit, red berries, violets, dark berries	Slightly drying, red berries, slightly sour, medium length	14	-
3	Clear, bright, medium ruby	Clean, medium intensity, primary plum and cherry, secondary spicy oak - clove. Youthful	Dry, high acid but balanced, medium bodied, medium + alcohol, medium intensity fruit, spice, plum, raspberry, Medium persistence	16	-
4	Deep mid red	Quite intense dark cherry, not very varietal, slightly jammy	High acid, moderate fruit intensity, dark cherry. moderate-high drying tannin, a little excessive for the fruit	15.5	-
5	Deep crimson/ garnet	Mid intensity nose: Morello cherries	Mulberry and Morello cherry flavours, medium+ acid; fine-grain tannins, longish fruit finish	15	-
6	Medium red	Lots of estery notes here. But of the overripe banana confectionary type. Seems out of place in a red wine.	Very raspberry confectionary-like. Very good intensity but rather simple. Firm drying tannins and lots of alcohol warmth. Also, there was some bitterness and distinct hotness on the finish	15	-
7	Mid red, with pink hue	Stalky, whole bunch type notes, esters some florals,	Fuller palate, richer flavour with lots of tannin and grip, tannin is silky and ripe, and gives the wine some length	16.5	-
8	Medium dark red	Intense blackcurrant, menthol/eucalypt cough lollies and after dinner mints	Palate is much like the aroma, eucalyptus leaves.	14	-
9	Dark red, clear, very dark for Pinot	Medium aromatic intensity, dark and red fruits, mint and other herbs, floral notes.	Rich but medium bodied, highly concentrated flavours, extracted, feels a bit overworked. Powerful and tannic, a formidable wine but hard work on its own.	15	-

The mean quality score assigned to sample 2016 Pinot Noir Curly Flat – Treatment was 15.3 with a standard error of 0.3.

Table 3b: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Curly Flat – Control AE10997

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Light - mid ruby / brick red	Moderate intensity fleshy boiled lolly / strawberry / raspberry characters	Mid weight, fleshy red berry fruit freshy raspberry / cherry / strawberry primary fruit with low moderate papery / dusty tannins, herbal / white pepper notes on finish	15.5	-
2	Dark red	Slightly chemical, slightly plastic, slightly smoke, slightly bacon, some berries	light body, drying, sour	13	1 - plastic
3	Clear, bright, pale ruby	Clean, medium intensity, primary floral -violet, strawberry, raspberry, secondary spice- cinnamon, clove. Youthful	Dry, high acid, sour, light bodied, medium + alcohol, low level tannin-sappy, medium - intensity fruit-raspberry, strawberry, cherry. Low persistence. Bitter finish	15	-
4	pale mid red	dark cherry, caramel, quite intense, a little cooked/stewed	quite acid, fairly thin, low tannin. Acid too high for the fruit weight	14.5	-
5	Lightish crimson	Mid intensity nose: Morello cherries	Red currant and mulberry and Morello cherry and marzipan; medium acid; some bitterness on palate, longish finish	15.4	-
6	Medium red with some brick hues	Good complexity of sweet spices, stewed plums and some leathery development.	Palate was much thinner than the nose would suggest. The acid poked out immediately and while there was some developed fruit sweetness, the palate screamed thin and acidic.	14	-
7	Light red with brick red hue	Stalky, whole bunch type notes, esters some florals,	Fuller palate, spice, richer flavour with lots of tannin and grip, tannin is silky and ripe, and gives the wine some length	16.5	-
8	Medium red	Blackcurrant and menthol cough lollies. Sweaty, towards cat pee. Burnt fruit compote	Harsh finish, bitter and burnt flavour with green. Quite astringent	13.5	-
9	Dark red, clear, tinge of orange	Low/medium aroma intensity, red and blue fruits, sawdust character, resiny, almost VA like,	Viscous, lacking in vibrancy and intensity, hard acid but flabby. short, bitter finish	14	-

The mean quality score assigned to 2016 Pinot Noir Curly Flat – Control was 14.6 with a standard error of 0.4. One judge gave a fault score for plastic aroma.

Taster comments on differences between the samples:

- Taster 1: Treatment shows more intense dark fruits on nose and palate, more pronounced and riper tannins, greater overall intensity and length. Control shows more confectionary / boiled lolly notes, more simple and lower intensity overall on nose and palate, tannins more papery and showing more of a herbal/pepper character than Treatment.

AWRI
The Australian Wine Research Institute Ltd
Corner of Hartley Grove & Paratoo Road
Urrbrae (Adelaide) SA 5064
PO Box 197 | Glen Osmond
SA 5064 | Australia

ABN 83 007 558 296
T +61 8 8313 6600
F +61 8 8313 6621
E commercialservices@awri.com.au
www.awri.com.au

- Taster 2: Control has a slight plastic/chemical smell and a sour palate, Treatment has more fruit in both nose and palate
- Taster 3: Treatment - deeper colour, Control - redder fruit spectrum and unbalanced bitter acid palate.
- Taster 4: Treatment - much deeper colour, more intense fruit, much higher astringency
- Taster 5: Difference mainly in colour: Treatment darker
- Taster 6: Treatment was riper but also had a strong yeast influence. Control was more developed and thinner in fruit.
- Taster 7: Control - less colour, not as intense. Similar nose and palate, perhaps some more spice in Control but overall quite similar except for colour depth.
- Taster 8: Treatment is darker than Control. Treatment has more intense eucalyptus - aroma and palate. Control harsh finish.
- Taster 9: Treatment deeper in colour, more extracted and a bigger wine. Control has a strange sawdust character and lack the freshness of Treatment.

Table 4a: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Chandon – Control AE 10999

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Light - mid salmon with brick red hue	Fragrant and lifted strawberry/raspberry/musk primary fruit characters with earthy and white pepper notes	Light-mid weight silky fruit with moderate somewhat papery/chalky tannins, slightly sour finish	15	-
2	Salmon	Smoky, savoury, bacon	Thin, lacks fruit and freshness, no weight, short	12.5	-
3	Clear, dull, medium salmon	Clean, low intensity toward neutral. Youthful?	Dry, light bodied, medium tannin - chalky, medium alcohol, non-descript low level flavour, low persistence	14	-
4	Pale red, very light	low intensity red berry, some caramel	high acid, light, thin, lacks fruit intensity. quite soft finish	14	-
5	Very light orangish /crimson	Low aroma, some sweet cherry with a slightly ether solvent note	Dilute(d), low flavour, low body/palate weight	14	-
6	Very pale red with brick hues	Lots of the character that you see in Rose's made from juice runoff. Commercial apple juice with some perfumed notes. Weak.	Palate the same as nose. Just weak flavour and a nothingness about it except for some residual sweetness.	13	-
7	Light tawny, brick red	Quite low intensity red fruits, esters, lolly	Lean, developed red raspberry fruits, ok acid, but a little short, and grippy on finish	14	-
8	Light/medium red orange	Cherry, strawberry, charry, wet hessian, spice potpourri, savoury, pencil shavings. Solvent/paint*	Low acid, bitterness on finish, odd cooked oil taste	14	3 – solvent/paint
9	Clear, light, red, could be rose	Medium aroma intensity. very pretty aromas: strawberries, floral notes, forest floor, candied/burnt sugar note.	Vibrant, mouthfilling, balanced, with good fruit concentration, sweet fruited. Good persistence. Not profound, but good Pinot.	16.5	-

The mean quality score assigned to 2016 Pinot Noir Chandon – Control was 14.1 with a standard error of 0.4. One judge gave a fault score for solvent/paint.

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Table 4b: Tasting notes and scores from nine judges for sample 2016 Pinot Noir Chandon – Treatment AE 11002

Taster	Colour and Condition	Nose	Palate	Score /20	Taint/ Faults /9
1	Mid garnet / crimson	Rich primary red/blue fruits, ripe strawberry/raspberry/blueberry/plum with milk chocolate/herbal complexing notes	Mid weight fleshy primary red fruits up front, moderately intense ripe fruit throughout with ripe velvet tannins nicely integrated, good length	16.5	-
2	Light red	Medium intensity, red berries, smoky, slightly savoury	Juicy palate, plenty of fruit, round/smooth	15.5	-
3	Clear, bright, medium ruby	Clean, medium intensity, primary fruit-cherry, florals- violets, secondary spice- nutmeg. Developing	Dry, medium bodied, medium alcohol, medium acid, medium flavour, cherry, plum, low level persistence. Low level simple tannin	15	-
4	Moderate mid red	Fairly aromatic red berry/caramel	Moderate acidity, moderate red berry flavour, lacks mid-palate, moderate drying tannin, some VA (acetic)?	15	2 - VA
5	Deepish garnet/crimson	Low aroma, sweet cherry	Dilute(d), low flavour, low body/palate weight; slightly more rough tannins	14	-
6	Light to mid red.	Low intensity fleshy skinsy notes.	Lolly water, but it has some yeast estery influence but the fruit was hard to find. Maybe some lemon flavours on the finish	13.5	-
7	Dull, mid red	Low intensity red fruits, esters, lolly	Low intensity red fruits, some development, ok acid, slight grip on finish.	14	-
8	Medium red	Strawberry jam, cherry, floral, herbal notes, savoury notes, pencil shavings	Acid flat, some astringency, low flavour.	15.5	-
9	Dark red, clear, plum tinge	Medium aroma intensity, strawberries, flowers, burnt sugar note.	Mouthfilling, medium bodied, firm structure, fine tannin, some length	16.5	-

The mean quality score assigned to 2016 Pinot Noir Chandon – Treatment was 15.1 with a standard error of 0.4. One judge gave a fault score for VA.

Taster comments on differences between the samples:

- Taster 1: Treatment darker, shows significantly riper and more intense fruit characters on nose and palate, riper more intense and more integrated tannins. Control less characterful and leaner fruit, tannins more chalky and papery and less intense / shorter palate.
- Taster 2: Control does not taste like wine, Treatment has a good nose and palate with plenty of fruit and smoky characters.
- Taster 3: Treatment brighter, slightly richer on the nose and palate than Control.
- Taster 4: Treatment much deeper colour, more intense fruit, much higher astringency, more VA
- Taster 5: Colour mainly, Control lighter; low aroma in both and watery flavours in both.
- Taster 6: Treatment had more fresh fruit character but overall both were pretty poor.

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- Taster 7: Colour - more depth and different hue (more red) in Treatment. Control more tawny, brick red. Aroma fairly similar, with Control being lower in intensity, Control less flavour with more development.
- Taster 8: Control is lighter and more orange in colour than Treatment. Control is more savoury and has a solvent note plus wet hessian aroma.
- Taster 9: Control much lighter in colour than Treatment. Aromatics are very similar between the two. Treatment has more structure and has some firmness to the palate, with fine grained tannin, while Control is softer and less serious. Both are about equal from a quality standpoint, just different.



Leigh Francis
Research Manager - Sensory

Notes:
The samples have been assessed under blind tasting conditions using standardised sensory evaluation procedures. The samples were identified with a three-digit random code and the tasters were informed of the vintage and variety.

The samples (30 mL) were presented in a randomized order to experienced tasters in a coded, covered XL5 (ISO standard) glass at 22-24 °C. The test was carried out in the AWRI's purpose built sensory facility in isolated, temperature controlled tasting booths under constant daylight type lighting. The tasters were instructed to comment in detail regarding the appearance, aroma and palate attributes of the wine, and to score the wine using the 20 point method of quality scoring as used in major Australian wine show judging. Tasters were also asked to score any taint/off flavour from 0 to 9, where 1 corresponds to just detectable, 5 to a moderate intensity, and 9 to a very strong intensity.

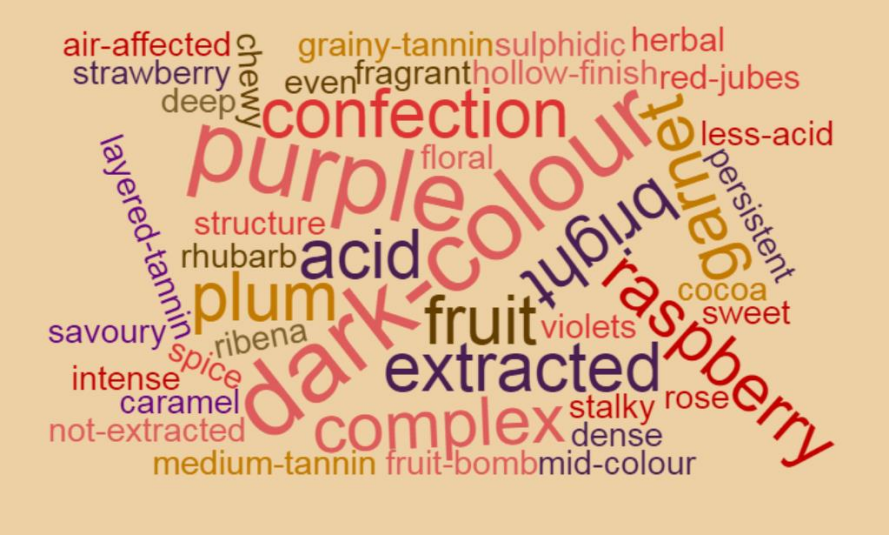
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Control



Mwv-press



MWV-skins

