A Novel Process for Pinot Noir Wine Making

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STATEMENTS AND DECLARATIONS

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Chapters two through eight are co-authored papers or manuscripts. The following colleagues are listed as co-authors on these papers or manuscripts, and their relative contribution to each paper or manuscript is noted on the frontispiece to each chapter. The percentages following each colleague listed below are indicative of their overall contribution to the body of work presented in this thesis:

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We the undersigned agree with the stated proportion of work undertaken by the primary and co-authors of chapters two through eight of this thesis, as detailed in the frontispiece to each of those chapters:

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ABSTRACT

Pinot noir grapes offer a challenge for wine makers due to the variety's unusual tannin distribution and non-acylated (colourless) anthocyanins. Initially, two scoping studies using replicated 'French Press' microvinification of Pinot noir must were undertaken and wines were evaluated for seven phenolic measures quantified by modified Somers analysis using UV-visible spectrophotometry and chemometrics (total phenolics, total pigment, anthocyanin, tannin, non-bleachable pigment, colour density, hue). The scoping studies established that yeast strain choice and maceration approach could significantly influence the concentration of phenolics in Pinot noir wine.

A novel maceration process, microwave maceration, which was included in the scoping study was associated with significantly higher concentration of total phenolics, anthocyanin, tannin and non-bleachable pigment in wines at 18 months bottle age, compared with control wines that had been fermented on skins for a comparable period (usually seven or eight days). Microwave maceration involved heating Pinot noir must to 70°C using a domestic 1.5 kW microwave oven. Must was held at that temperature for a 'hold time' period to allow for time/temperature mediated phenolic extraction, before being cooled to ~27°C for inoculation and fermentation under controlled conditions.

The rapid and effective phenolic extraction from microwave maceration of Pinot noir grape must allowed several novel approaches to winemaking. For example, microwave macerated must with one hour of hold time was pressed off skins and fermented as an 'enriched juice'. Early press-off microwave maceration wines were equivalent to control wine that had been fermented on skins for most phenolic parameters, but were significantly higher in fourteen out of sixteen aroma

compounds examined by GCMS. For example, the microwave treatment wines were generally four to six-fold higher for the acetates examined, and two-fold higher for most of the ethyl esters examined. Microwave maceration was effective for sanitation of Pinot noir grape must and reduced background yeast to ≤100 cfu/mL by plating to Wallerstein Laboratories Nutrient agar, which was more effective than standard must sanitation by application of 50 mg/L sulphur dioxide in the form of potassium metabisulfite solution ($2x10^4$ cfu/mL).

Due to the sanitation effect of microwave maceration, must did not require application of sulphur dioxide at crushing to suppress background yeast and bacteria. Sulfur dioxide and high fermentation temperatures are stressors which have been shown to inhibit malo-lactic fermentation in red wine fermentation. The rapid extraction of phenolics by microwave maceration prior to alcoholic fermentation, offset the need for high fermentation temperature (eg. 30°C) and this, coupled with the absence of sulphur dioxide, allowed co-inoculation of yeast and malo-lactic bacterial cultures for simultaneous alcoholic and malo-lactic fermentation. Early press-off microwave macerated Pinot noir must inoculated in this way finished alcoholic and malo-lactic fermentation within 17 days demonstrating the possibility of highly efficient red wine making from this novel process.

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Chapter 1. INTRODUCTION

The work in this thesis draws from several bodies of literature and theory to propose a novel method for the rapid and effective extraction of grape phenolics into grape juice during red wine production. The focal grape variety is (*Vitis vinifera* L.) Pinot noir and the founding bodies of knowledge are: red wine phenolic chemistry, contemporary maceration processes in red wine making, and microwave-mediated phytoextraction. These founding bodies of knowledge are reviewed in the immediate sections, and then their relationship to Pinot noir winemaking is explored.

Red wine phenolic chemistry

The visual and sensory appeal of red wine depends to a large extent on phenolic compounds extracted from grape solids into juice, and stabilised in the wine matrix. These compounds are constituted from phenolic subunits (hydroxylated benzene, Figure 1) and fall into two main classes, non-flavonoids and flavonoids.

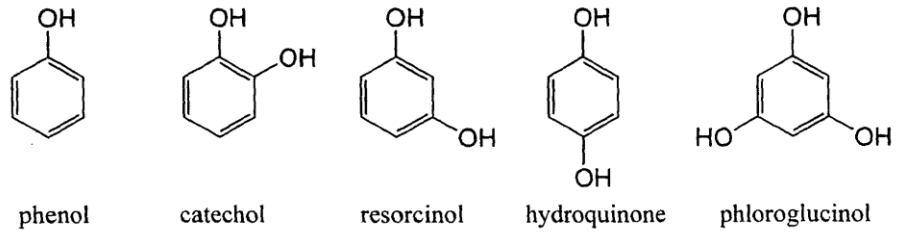

Figure 1. Wine phenolics are constituted from hydroxylated benzene subunits (‘phenol’).

Non-flavonoids

Non-flavonoids are constituted of one or two aromatic rings, and are differentiated by their attached constituent elements and molecules. For example, Figure 2 shows the stilbene

resveratrol which is made up of two hydroxylated benzene rings. The grape-derived non-flavonoids include the stilbenes, hydroxycinamates and gallic acid, and have not been associated with direct sensory impact in red wines (Cheynier et al., 2006). The non-flavonoids have however been a focus of wine research due to their purported health properties (Stockley & Hoj, 2005). For example, a study of four *Saccharomyces cerevisiae* strains fermenting Shiraz must demonstrated significant yeast strain effects on the concentration of a range of resveratrol isomers (Clare, Skurray, & Shalliker, 2005). A range of vinification techniques have been examined for their capacity to increase stilbene (mostly resveratrol) concentration in red wines (Atanackovic et al., 2012; Kostadinovic et al., 2012). The hydroxycinnamates, which derive primarily from grape pulp, have been associated with astringent mouthfeel effects in white wine (Boselli, Minardi, Giomo, & Frega, 2006), and may play a role in the oxidative browning of white wines (Kallithraka, Salacha, & Tzourou, 2009), but are not currently considered important to the aesthetic or sensory appeal of red wines. The role of oak-derived non-flavonoids in red wine, however, is of greater note. Vanillin is an oak-derived non-flavonoid (Figure 3) which in its volatile phenol form imparts a vanilla aroma. Vanilla aroma has been identified by Gas Chromatography – Olfactometry Analysis as an important odorant in Pinot noir (Fang & Qian, 2005).

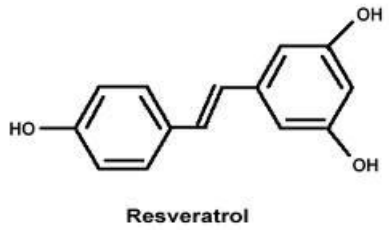

Figure 2. Resveratrol, a non-flavonoid phenolic found in red wine.

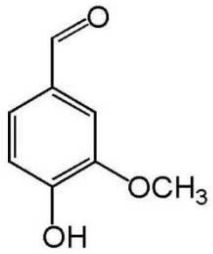

Figure 3. Vanillin, an oak-derived non-flavonoid which is an important Pinot noir odour compound.

Flavonoids

The flavonoids are flavan structures constituting three aromatic rings. These compounds are of substantial importance for red wine sensory appeal, and can be particularly challenging for Pinot noir winemakers due to the grape's relatively unique flavonoid distribution and concentration (Kennedy, 2008). Two classes of flavonoid are important in red wine: anthocyanins and flavonols.

The anthocyanins and anthocyanidins contribute colour to red wine. Anthocyanidins are the aglycone ('sugarless') species of anthocyanins. The five forms of anthocyanidin found in red grapes are delphinidin, cyanidin, peonidin, petunidin and malvidin (Cheynier et al., 2006). The glucosylated derivative of malvidin, malvidin-3-glucoside, is the most abundant form of anthocyanin in red wine grapes (Somers 1998) (Figure 4). Anthocyanidins can be vulnerable to discolouration in wine, depending on their acylation state. Acylation is linking of an acid moiety at the second aromatic ring, non-acylated forms are hydrated at this point. Non-acylated forms of anthocyanin can be colourless at normal wine pH and are vulnerable to oxidation and bisulfite bleaching (Cheynier et al., 2006). Pinot noir grapes offer a challenge in terms of wine colour

stability because they have only non-acylated forms of anthocyanin: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside (Somers, 1998; Cheynier et al., 2006). Acetic, caffeic and coumaric acids found in grape juice and wine can increase the colour stability of anthocyanidins through acylation.

The flavonols in red grapes occur as monomers, oligomers and polymers of catechin (Figure 5) and its derivatives (epicatechin, gallocatechin, epigallocatechin, epicatechin 3-gallate, epigallocatechin 3-gallate; Cheynier et al., 2006). This class of compounds are referred to as 'tannins' and also 'proanthocyanidins', and are available from grape skin, seed and stalks (Figure 9). The term 'tannins' also encompasses hydrolyzable tannins which are oak-derived non-flavonoid ellagitannins (Michel et al., 2011), and are a minor component of red wine. Of greater importance, are the 'condensed tannins' which are proanthocyanidin complexes made up of multiple tannin subunits. The proportion of various catechin derivatives in grape skin differs from the proportion in grape seed and stalk (Cheynier et al., 2006). The trihydroxylated form of catechin, epigallocatechin is more prevalent in grape skin, and the dihydroxylated form, epicatechin 3-gallate more prevalent in seed (Hayasaka, Waters, Cheynier, Herderich, & Vidal, 2003). These two forms have therefore been proposed as markers of wine tannin origin (Cheynier et al., 2006). Polymerization of tannin monomers into condensed tannins has been associated with moderation of the sensory impact of these compounds in wine (Hayasaka & Kennedy, 2003; McRae & Kennedy, 2011). Formation of condensed tannins is either via direct chemical condensation or via bridging by co-factors such as acetaldehyde, pyruvate. Figure 6 shows several proanthocyanidin complexes and their associated visible colour in wine (He, Pan, Shi, & Duan, 2008). Research has suggested that large, water soluble condensed tannins have a more astringent mouthfeel in wine than smaller, less water soluble condensed tannins (McRae,

Schulkin, Kassara, Holt, & Smith, 2013). Sensory research has shown red wines tends to become less perceptibly astringent with age (McRae, Falconer, & Kennedy, 2010) which has been attributed to large condensed tannins settling out of the wine matrix but is more likely due to condensed tannins taking an increasingly folded form with less available protein binding sites to trigger an astringency response in human tastebuds (McRae et al., 2012; McRae et al., 2013).

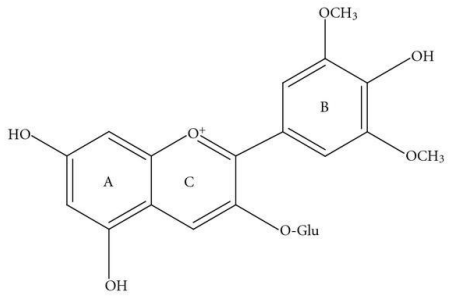

Figure 4. The anthocyanin malvidin-3-glucoside is most abundant in red grapes.

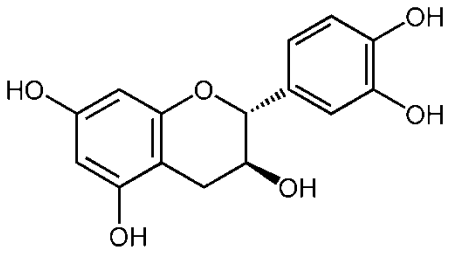

Figure 5. Catechin is a flavonoid of seed origin which has been reported as ‘bitter’ in red wine.

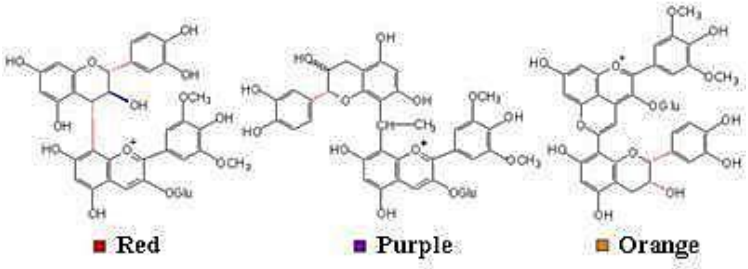

Figure 6. Proanthocyanidin complexes and their associated wine colour (He et al., 2008)

Anthocyanin stabilisation, co-pigmentation and pigmented polymer formation

Red wine colour changes as wine ages. These changes are due to the ongoing formation and degradation of various phenolic compounds and complexes (Somers 1998). These include stabilisation of anthocyanins in coloured and colourless forms, formation of association complexes involving anthocyanins and tannins (co-pigmentation) and formation of pigmented polymers.

Anthocyanin stabilisation can occur through reaction of an anthocyanidin molecule with a yeast metabolite (eg. pyruvate, acetaldehyde). These stabilised forms are important for red wine colour as they can be colourless or coloured (Cheynier et al., 2006). Pyranoanthocyanins or 'vitisins' contribute orange colour to red wine and form through reaction between malvidin-3-glucoside and pyruvic acid (vitisin A) or acetaldehyde (vitisin B). These two yeast metabolites contribute a pyran ring to the anthocyanin (Figure 7) thereby limiting the opportunity for hydration of the anthocyanin to the hemiketal (colourless form) as wine pH rises with aging, or for bisulfite addition and associated bleaching. A second mechanism for stabilisation of anthocyanins is enzymatic oxidation. For example, formation of the red coloured caftaric acid-anthocyanin adduct. As wine ages, the contribution of pyranoanthocyanins to visible colour can become significant as free anthocyanin concentration declines. After one year's bottle aging of port wines, vitisin A was 3-4 fold higher than anthocyanidin-3-glucosides (Mateus & de Freitas, 2001).

Co-pigmentation refers mainly to the phenomena of association between anthocyanins, or between anthocyanins and flavanols to form dimers, many of which are coloured (Boulton, 2001; Cheynier et al., 2006; He et al., 2008). Co-pigmentation occurs by two main mechanisms. The first is direct chemical condensation and the second is via bridging between flavanol and

anthocyanin moieties by yeast metabolites (eg. pyruvate, acetaldehyde, ethanol). Bridging has been identified as the faster and more prevalent process of co-pigment formation in wine (Hayasaka, Birse, Eglinton, & Herderich, 2007; Timberlake & Bridle, 1976).

Pigmented polymers are built from tannins and anthocyanins; they contribute to the development of colour stability and mouthfeel in red wines. While the chemical structure and formation of pigmented polymers in red wine have been reasonably well studied, research continues into the relationship between these polyphenolic compounds and wine mouthfeel (McRae et al., 2010; McRae et al., 2013), and into the contribution of these compounds to mouthfeel changes as wines age. 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 than anthocyanin after the first two years' bottle aging, and that anthocyanin did not contribute to visible colour in wines by four years bottle age (McRae et al., 2012). Figure 8 shows that the stability and number of subunits in the polymer depends on anthocyanin subunit addition. Anthocyanins are termed 'terminal subunits' as they have only one reaction point for bridging with the tannin moieties in red wine, hence, when anthocyanin is attached to each end of a pigmented polymer it is considered 'stable' (Figure 8).

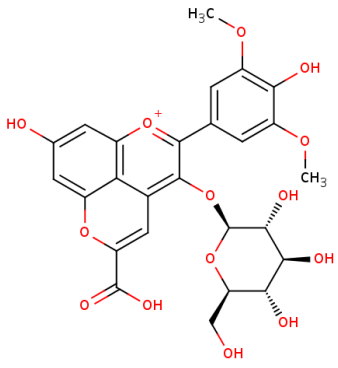

Figure 7. Vitisin A from reaction between malvidin-3-glucoside and pyruvic acid.

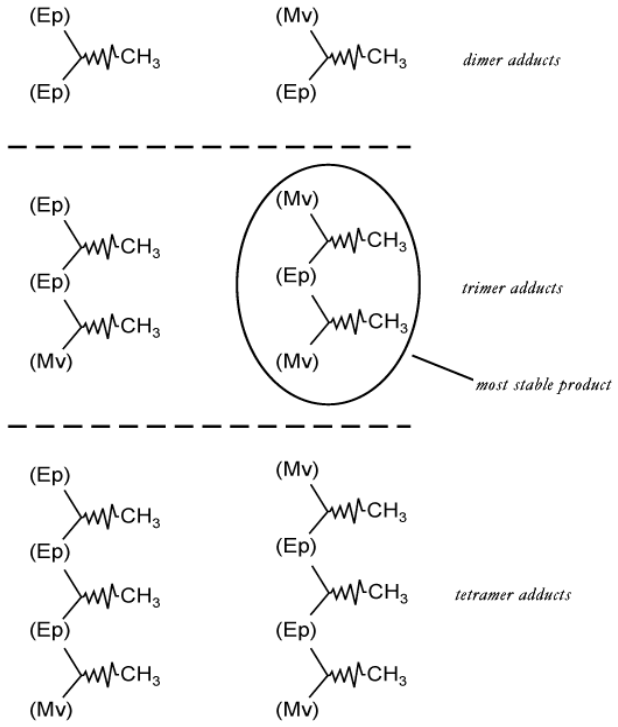

Figure 8. Acetaldehyde-mediated pigmented polymers formed from epicatechin (Ep) and malvidin (Mv) subunits.

Maceration and red wine phenolics

Maceration is the process by which winemakers affect mass transfer of phenolic and other compounds (eg. polysaccharides, amino acids) from grape solids, into grape juice. The phenolic compounds of relevance to red wine occur in the grape skin (anthocyanins, tannins, stilbenes), seed (tannins) and stalk tannins (Figure 9).

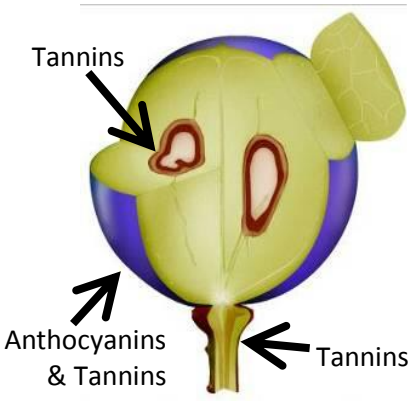

Figure 9. Grape solids sources of phenolic compounds of relevance to red wine.

Research into the relative effectiveness of various maceration processes for phenolic extraction has returned mixed findings. A 2005 review of research on the impact on red wine phenolics of various winemaking techniques concluded that: fermentation temperature, thermovinification, must freezing, saignée (bleeding off), pectolytic enzyme treatment and extended maceration increased wine phenolics, that high sulphur dioxide levels and cold soaking were ineffective or decreased phenolics, and that carbonic maceration, yeast selection and various forms of skin and juice mixing produced variable results (Sacchi, Bisson, & Adams, 2005).

There are several challenges to drawing firm conclusions about the impact of maceration technique on red wine phenolic concentration. These relate to poor experimental design (eg. lack of replication, variation in time on skins), lack of acknowledgement of changes in the relative impact of maceration treatments with bottle age (eg. variation in the rate and extent of development of pigmented polymers associated with different maceration treatments), and the methods used to quantify red wine phenolics.

As is apparent from the preceding section on red wine phenolic chemistry, there is a wide array of compounds which contribute to the 'phenolic quality' of red wine. There is still no consensus,

however, on which of these compounds might be an effective indicator of red wine quality. Until recently, available methods tended to favour particular phenolic compounds. For example, a comparison of three analytical methods which are used to quantify grape skin tannin (protein precipitation, methyl cellulose precipitation, HPLC) concluded that each method measured a different tannin fraction (Seddon and Downey, 2008). A comparison of five current methods for analysis of red wine colour compounds found good correlation (R^2=0.992) between HPLC and the original Somers method for quantifying red wine polymeric pigment (Versari et al., 2008). These methods each provide insight into red wine phenolics, but have proven difficult to relate directly to phenolic quality (sensory appeal). Research is therefore continuing into more effective methods to quantify phenolic compounds in red wine and relate those directly to positive consumer sensory experiences (Cozzolino et al., 2008; Dambergs et al., 2011; Dambergs et al., 2012a; Harbertson et al., 2003; Herderich et al., 2006; Mercurio et al., 2007; Sarneckis et al., 2006; Seddon and Downey, 2008; Skogerson et al., 2007; Versari et al., 2008).

The lack of objective and agreed methods to quantify red wine phenolic quality means that studies into the impact of various maceration methods on phenolic concentration have been limited in their capacity to comment on the relative value of each method. Studies employing limited and relatively crude measures of phenolics, like free anthocyanin or total phenolics provide little insight into the range of compounds of relevance and their interaction. The concentration of free anthocyanin in must begins to decline approximately four days after the onset of fermentation when the rate of their conversion to more complex polymers begins to exceed the rate of extraction (Somers 1998). Some methods used to quantify free anthocyanin do not quantify anthocyanin in polymeric pigments and so may ‘undervalue’ a maceration process that is highly effective for both anthocyanin extraction and precocious formation of pigmented

polymers. For example, cold soak was described ineffective or detrimental for phenolic concentration, compared with conventional fermentation (Sacchi, et al., 2005). A replicated trial assessing the impact on Shiraz wine anthocyanin concentration of cold soak and cold soak with extended maceration concluded that control wines at bottling were significantly lower in anthocyanin concentration than the cold soak and extended maceration treatments (370 mg/L, 416 mg/L, 444 mg/L respectively) (Reynolds, et al., 2001). However polymeric pigment concentration was not reported and the impact of bottle aging was not examined.

Pectolytic enzyme treatment was identified as effective for increased phenolics concentration in red wine (Sacchi, et al., 2005) but others have reported that the effectiveness of enzyme treatment vary by grape variety (Busse-Valverde, et al., 2010) and by the enzyme preparation used (Wightman, Price, Watson, & Wrolstad, 1997). The use of maceration enzymes in Pinot noir wine making was examined over 18 months of bottle aging, and shown to have a significant impact on the concentration of anthocyanin and polymeric pigment in wines. Anthocyanin concentration was low in enzyme treated wines, and polymeric pigment high, suggesting the enzyme used in that study was associated with precocious conversion of free anthocyanin to the stable polymeric form (Parley, Vanhanen, & Heatherbell, 2001).

Total phenolics is a ‘catch-all’ measure which does not distinguish between anthocyanin, tannin and low molecular weight, non-pigmented phenolic compounds. Either anthocyanin or tannin concentration can be a rate limiting factor for pigmented polymer formation (Boulton 2010), hence total phenolics is likely to be a poor indicator of ‘phenolic quality’ as it has little relevance for the rate and extent of pigmented polymer formation, and the allied effects of colour stabilisation and mouthfeel moderation. It may also be a poor measure of red wine balance; a wine with high total phenolics may be disproportionately endowed with either anthocyanin

(fleeting colour and limited length in the mouth) or tannin (potentially low in colour and overly astringent). Wine total phenolic content (TPC; gallic acid equivalents) was reported in a study of maceration techniques applied to four different varieties of red grape must (Atanackovic et al., 2012). The authors concluded thermovinification was an effective method for phenolic extraction as that treatment was associated with wines with high TPC. Research correlating Pinot noir show judging results with a range of phenolic measures, however, concluded that over-extracted wines (eg. high total phenolics, high tannin) tended to perform poorly compared with Pinot noir wines that had intermediate tannin extraction and high total pigment concentration (Dambergs et al., 2007); intermediate tannin and high total pigment concentrations were associated with gold or silver medal show results. Similar results have been generated for Shiraz wines (Smith et al, 2010).

The use of must freezing as a maceration method was compared with cold soaking, dry ice maceration and enzyme treatment for Cabernet Sauvignon and Shiraz winemaking, and all pre-fermentation maceration techniques were concluded effective for colour extraction (Gil-Munoz, et al., 2009). In this study however, the researchers pressed wine off at the conclusion of alcoholic fermentation, so the five treatments ranged between 12 and 28 days skin contact time. This means observed effects that were attributed to the maceration treatment may simply have been due to greater or lesser opportunity for diffusion, rather than the treatments applied.

In summary, limitations in the range and effectiveness of analytical methods for quantifying red wine phenolics, and the nascent state of research linking instrumental measures with sensory impact, mean that the impact of alternate maceration techniques on red wine quality remains a somewhat open question.

Microwave heating

Heat transfer mechanisms

Heat transfer is the movement of energy in the form of heat from one point to another and is generally driven by a difference in temperature. Three heat transfer mechanisms operate in conventional food processing systems – convection, conduction and radiation (Toledo, 2007). Radiant heating occurs when electromagnetic waves in the light frequency (~400-800 nm) intercept a surface and are absorbed. A common application of radiant heat for food processing is grilling of food by the use of overhead elements. Conductive heating is the direct transfer of heat by contact between adjacent molecules. For example, transfer of heat from a heating element to the food being heated, usually via an intermediary conducting container (eg. a metal saucepan). The third form of heat transfer in conventional food processing is convection which is upwelling of heated liquids or gasses, and their mixing into the bulk lot, causing increase in mean temperature across the bulk lot of liquid or gas being heated. Convective heating still relies on transfer of heat by contact between different-temperature molecules. Conduction and convection often operate together in food processing for point-source heated liquid or in stirred multi-phase food processing (Toledo, 2007).

Microwave heating is based primarily on the heat transfer mechanism of radiation and the use of electomagnetism, but longer wavelengths are employed (~1 mm – 1 m). In a microwave heating system, a magnetron is used to rapidly switch the magnetic field within the microwave cavity (dipole switching). As the field is switched, free electrons within the food in the microwave unit realign (move away from the negative charge and towards the positive charge) in a phenomenon known as ‘molecular dipole rotation’. The realignment of electrons at this rapid rate causes

friction at a molecular level, and that friction is released in the form of heat. In this way, microwave creates heat at a distributed and molecular level within the foods being heated. Heat that has been generated in foodstuffs by microwave is governed by the heat transfer mechanisms discussed above, hence heat within microwaved foods disperses from its molecular point of origin within the food matrix by conduction (solid, multiphase and liquid foods) and by convection (liquid foods, food-bound gasses). The difference with microwaved foods is that heat conduction and convection processes initiate from more widely distributed origins (dispersed throughout the food) than point source heating (eg. saucepan, tubular walls of heat exchanger).

Microwave processing of food

Microwave-assisted extraction has been shown to be a time and energy efficient option for extraction of plant compounds and for food pasteurisation or sterilisation, but has not been examined for red wine making. Microwave has been demonstrated to be efficient in terms of both rate of extraction and effectiveness of extraction for a range of plant compounds (Casazza, Aliakbarian, Mantegna, Cravotto, & Perego, 2010; Liu, Wen, Zhang, & Ma, 2009; Trendafilova & Todorova, 2008). Mandal and others have speculated that the diffuse or distributed, internal origins of heat under microwave conditions may aid mass transfer of target compounds (eg. use of microwave for phytoextraction) because the heat transfer and mass transfer are operating in the same direction (Mandal, Dewanjee, & Mandal, 2009). In conventional food heating for extraction, heat and mass transfer operate in opposing directions. There is no experimental evidence to support Mandal and others' hypothesis but further investigation of the potential extraction benefits of synchronous heat and mass transfer may be warranted. Microwave has also been shown as effective for pasteurisation or sterilisation of various foods, beverages and other items (Mima et al., 2008; Tajchakavit, Ramaswamy, & Fustier, 1998), although uneven heat

distribution remains a problem for microwave sterilisation of multi-phase solutions (Kumar, Coronel, Simunovic, & Sandeep, 2007). Research from food and water science into the mechanism of microwave microbial sterilisation suggests cellular disruption may result from both thermal and enhanced-thermal effects (Hong, Park, & Lee, 2004; Tajchakavit et al., 1998). This infers that microwave may offer effects beyond standard thermal treatment of must. Microwave might also support novel research into yeast strain effects in winemaking as an alternative to currently used chemical or physical sterilisation techniques like dimethyldicarbonate dosing (Cavazza, Poznanski, & Guzzon, 2011) and high hydrostatic pressure processing (Takush & Osborne, 2011).

An additional opportunity offered by microwave maceration is possible inactivation of heat labile enzymes in grape must. Microwaving has proven more effective than thermal processing for enzyme inactivation in other food products (Keying, Changzhong, & Zaigui, 2009; Matsui, Gut, de Oliveira, & Tadini, 2008), and the consequence of rapid heating using microwave may alleviate the problem of enzyme stimulation during thermovinification when a must transitions through 45-50°C (Rankine, 2004).

Based on current use of microwave in other applications, investigation of microwave for thermal treatment of grape must offers two main possibilities as an alternative to standard maceration: increased phenolic extraction from pomace during the primary ferment (phytoextraction), and the potential for SO_2-substitution at crushing via heat-mediated suppression of grape-associated microflora in grape must (pasteurisation, sterilisation).

Pinot noir phenolics are challenging for wine makers

Pinot noir has been termed 'the heartbreak grape' (de Villiers, 1994) with its relatively high cost of production and unique phenolic profile. Pinot noir grapes can be challenging for the winemaker as they are typically low in anthocyanin and have an unusual tannin distribution. Free anthocyanin concentration in commercial Pinot noir wine has been reported at around 60mg/L compared with around 110mg/L for Merlot and 125 mg/L for Cabernet Sauvignon (Cliff, King, & Schlosser, 2007). Also problematic for wine makers is the form of anthocyanin found in Pinot noir grapes; all Pinot noir anthocyanin is of the 'non-acylated' form (Heazlewood, Wilson, Clark, & Gracie, 2006; Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999) which is colourless at wine pH. The formation of stable, visible colour in Pinot noir wine is achieved through reaction between anthocyanin and tannins to form polyphenols (eg. pigmented tannins) (Broulliard, Chassaing, & Fougerousse, 2003) which means that timely and adequate anthocyanin and tannin extraction are important to achieve visually appealing wine.

Tannin extraction is important for wine astringency and palate weight. In Pinot noir grapes, an unusually high proportion of tannin is found in the seed. Kennedy estimated 89% of Pinot noir tannin to be seed-bound and 11% to be located in grape skin (Kennedy, 2009). Perhaps associated with this disproportionate allocation of tannin to seed, final tannin concentration in Pinot noir wine can be low (Harbertson et al., 2008) and many Pinot noir wine makers choose to extend pomace contact time after the AF to obtain sufficient tannin (Haeger, 2008; Joscelyne, 2009). Extended maceration can be risky and adds to the cost of Pinot noir production as it; occupies fermenter space, requires maintenance of anaerobic conditions in the tank and increases the risk that wines will become oxidised.

Pinot noir wine making practices are diverse

'New world methods of making Pinot Noir vary even more wildly than those of Burgundy' (Halliday and Johnson 2007, pg 153).

Pinot noir winemaking does not follow a single, prescribed process. Halliday and Johnson (2007) describe the production of Pinot noir as a series of choices and consequences. These authors list 17 decision points for the Pinot noir winemaker including: method of picking, crushing options, maceration options, choice of yeast, fermentation temperature and fining approach. Perhaps the most comprehensive survey of New World Pinot noir winemaking was undertaken by John Winthrop Haeger (Haeger, 2008) who interviewed 216 of the 238 most recognised Pinot noir wine producers in Oregon and California, USA. At that time, Oregon and California produced over 95% of the USA's Pinot noir (Haeger, 2008). Table 1 lists some of the practices in winemaking that Haeger surveyed and the percentage of participating winemakers who reported using that practice. Table 1 reinforces Halliday and Johnson's (2007) view of Pinot noir winemaking as highly varied in the New World.

Table 1. Pinot noir winemaking practices in Oregon and California, USA (Haeger, 2008)

Winemaking practice	Winemakers using the practice
Use of all whole clusters in primary ferment	3%
Complete destemming of fruit	57%
Use of pre-ferment maceration ('cold soak')	92%
Complete reliance on resident yeast for primary	26%

fermentation[a]	
Complete reliance on inoculated yeast for primary fermentation	37%
Use of post-ferment maceration ('extended maceration')	25%

[a] 'resident' yeast ferments are elsewhere called 'wild', 'spontaneous' or 'natural' ferments

Approximately one quarter of winemakers participating in Haegar's study relied entirely on resident yeast to carry primary fermentation (Haeger, 2008), and those participating winemakers who used inoculated fermentation favoured two strains for fermentation of Pinot noir: RC212 and Assmanshausen (Haeger, 2008). This picture of winemaker practices may appear conservative, however a 1983 survey of red winemaking practices in California found 14 out of the 18 winemakers surveyed were inoculating their red ferments with the same yeast strain they used in white winemaking, only one used a specialised yeast (Assmanshausen) for Pinot noir ferments and only one relied on resident yeast (Cooke & Berg, 1983). Anecdotal evidence suggests Australian Pinot noir makers who inoculate are similarly conservative in their yeast strain choice for Pinot noir, with three strains - RC212, Burgundy (BGY), EC1118 - most commonly identified.

Ninety two percent (92%) of the USA winemakers interviewed by Haeger reported using pre-ferment maceration (cold soak) (Table 1), the duration of which ranged from 1 day to more than 7 days, and winemakers reported diverse cold regimes (eg. dry ice maceration, refrigeration temperatures ranging from ~4°C to ~15°C) (Haeger, 2008). The high percentage of winemakers reporting use of pre-ferment maceration (cold soak) in the USA study contrasts with survey research on Australian Pinot noir makers' use of cold soak which concluded that only 39%

employed pre-ferment maceration (cold soak) (Joscelyne, 2009). A recent informal survey of the practices of 60 Australian Pinot noir winemakers suggested a much higher incidence of cold soak (86%) (Dr Bob Dambergs AWRI *pers comm.*). Post-ferment maceration (extended maceration) practices also appeared to vary between Australian Pinot noir makers and their USA counterparts, with only 16% of surveyed Australian Pinot noir winemakers reporting its use (Joscelyne, 2009) compared with 25% in the Haeger study (Haeger, 2008).

The diversity of Pinot noir winemaking practices in the New World suggests there is ample scope for clear research outcomes to influence yeast strain choice and maceration practices in the winery. The most important question being whether specific yeast strains and maceration practices can offer consistent and reliable phenolic outcomes for Pinot noir winemakers.

Fermentation yeast strain can influence Pinot noir wine phenolics

Yeast metabolism influences wine aroma via volatiles (Swiegers & Pretorius, 2005; Walsh, Heinrich, & Skurry, 2006), colour via interaction with phenolics (Medina, Boido, Dellacassa, & Carrau, 2005), and mouthfeel through metabolism of alcohol, glycerol and the action of yeast polysaccharides (Escot, Feuillat, Dulau, & Charpentier, 2001; Fleet, 2003). Production of metabolites by yeast during primary fermentation varies by species and strain of yeast (Fleet, 2003). Grape variety is also a factor influencing the metabolite profiles that are produced by yeast strains whilst fermenting grape musts. Hence, specific commercial yeast strains are recommended by manufacturers to achieve desired sensory characteristics in finished wines of various varieties (Lallemand; Mauri Yeast Australia). The limited literature on Pinot noir winemaking practices (Halliday and Johnson 2007) (Cooke & Berg, 1983; Escot, et al., 2001; Haeger, 2008; Jeandet, et al., 1995; Joscelyne, 2009) and anecdotal evidence suggests New

World Pinot noir wine makers who inoculate continue to favour a limited number of strains – AMH, BGY, RC212 and EC1118. However, research into the relative impact of such strains on phenolic outcomes in Pinot noir wine has returned variable findings (Escot, et al., 2001; Girard, Yuksel, Cliff, Delaquis, & Reynolds, 2001; Lorenzini, 2001; Mazza, et al., 1999). For example, Girard and others concluded that yeast strain effects on Pinot noir were mediated by both maceration approach and fermentation temperature, although Principal Component Analysis scores plots appeared to show consistent separation associated with yeast treatment in that study (Girard, et al., 2001). A comparison of Pinot noir wines made with Burgundy and RC212 yeast strains showed the RC212 treatment was associated with significantly higher anthocyanin concentration in wine directly after alcoholic fermentation, but this effect was reversed following malolactic fermentation (Escot, et al., 2001). A trial of eight yeast strains in Pinot noir must over two vintages concluded that some yeast strains produced noticeable variation in phenolic concentration between treatments, with the Wädenswil 27 strain being associated with lower colour density and phenolic content than other yeast treatments (Mazza, et al., 1999).

Mannoproteins and polysaccharides influence complexing of red wine phenolics during alcoholic fermentation and wine aging (Z. Guadalupe & Ayestaran, 2008; Zenaida Guadalupe, Martínez, & Ayestarán, 2010). Polysaccharide release from yeast has been shown to depend on both yeast strain (Rosi et al. 1998), and must turbidity (Escot, et al., 2001). A microvinification comparison of two yeast strains (RC212; BRG) in Pinot noir must demonstrated each strain liberated similar quantities of polysaccharide during primary fermentation. The RC212 treatments, however, showed significantly lower levels of these liberated polysaccharides bound to phenolic compounds (Escot, et al., 2001), inferring less colour stability effect from RC212. The researchers applied mannoproteins liberated by RC212 during alcoholic fermentation to a red

wine and observed these to be less effective in reducing wine astringency than those released by BRG and another yeast strain (BM45)(Escot, et al., 2001). These findings showed polysaccharide/polyphenol complexing during alcoholic fermentation was yeast strain-mediated, and emphasised the importance of strain selection as a mechanism for winemakers seeking to reduce overly bitter or astringent qualities in wine.

Maceration processes can influence Pinot noir wine phenolics

The extent of phenolics extraction during red wine alcoholic fermentation has been estimated at 30% to 40% of the total quantity available (Boulton, 2001). As described above, six maceration practices were identified in a review as effective for increasing phenolic concentration in red wine (Sacchi, Bisson, & Adams, 2005): fermentation temperature, thermovinification, must freezing, saignee ('bleeding off'), pectolytic enzyme treatment and extended maceration.

Several studies have concluded thermovinification is effective for enhancing phenolic extraction in red winemaking (Clare, Skurray, & Shalliker, 2004; Fischer, Strasser, & Gutzler, 2000; Fretté, Hansen, Raasthøj, Broe, & Christensen, 2012; Netzel, et al., 2003; Sacchi, Bisson, & Adams, 2005) and this appears consistant in Pinot noir winemaking. For example, a recent study compared thermovinification with standard winemaking across four red grape varieties, including Pinot noir, and showed thermovinification consistently associated with higher total phenolic content in the resulting wines (Atanackovic, et al., 2012). While this study showed a consistent trend across the varieties trialled, the findings were undermined by use of single replicates with multiple assays conducted on the same wine sample (pseudoreplication) to support claims of significant difference (Atanackovic, et al., 2012). Tannin extraction by thermovinification is of particular interest in the context of Pinot noir. A review concluded that

heat damage to berries that occurs during thermal treatment did not increase tannin concentration in grape must (Sacchi, et al., 2005), however skin contact time after heating has been identified by several authors as a way to increase must tannin concentration (Netzel, et al., 2003; Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006). It is not clear from the literature whether an increase in tannin concentration depends simply on skin contact after heating, or if must needs to be held at peak temperature over an extended period to achieve increased tannin extraction.

Extended maceration is used in industry to enhance phenolic extraction in red wine making (Haeger, 2008; Halliday & Johnson, 2007) and research has shown this approach to be effective (Casassa, et al., 2013; Puertas, Guerrero, Jurado, Jimenez, & Cantos-Villar, 2008). However, extended maceration of Pinot noir may lead to an unbalanced or overly bitter wine. Investigation of extended maceration winemaking for Cabernet Sauvignon wines showed a 30 day extended maceration regime produced wine with significantly higher tannin concentration than a 10 day maceration control (~1200 mg/L versus ~900 mg/L catechin equivalents, respectively), but that the origin of tannins in the extended maceration wine was different to the control wines (Casassa, et al., 2013). Casassa and others showed the proportion of skin-origin tannin in extended maceration wine was lower than control wine (28% and 45%, respectively) and proportion of seed-origin tannin higher (72% and 55%). While the relationship between instrumental measures of wine composition and sensory effects is still somewhat unclear (Ebeler & Thorngate, 2009; Gardner, Zoecklein, & Mallikarjunan, 2011), seed tannin has been associated with bitterness in red wine (McRae & Kennedy, 2011). In a replicated study of Pinot noir wine examined over an 18 day alcoholic fermentation, the concentration of seed-origin compounds (eg. flavan-3-ols, gallic acid) increased markedly in the latter half of the alcoholic ferment (Zou, Kilmartin, Inglis, & Frost, 2002), and a comparison of control versus extended maceration Pinot noir wines

showed the extended maceration treatment was associated with significant increase in flavan-3-ols, compared with control maceration treatment (Joscelyne, 2009). The Joscelyne study included sensory analysis of extended maceration Pinot noir wines and these were judged by the sensory panel to be significantly more bitter than control wines (Joscelyne, 2009).

Aims

The initial aim of this research was to examine yeast strain and maceration effects on phenolic outcomes in Pinot noir wine, with a view to advising industry on reliable means to improve the phenolic structure of Pinot noir wine. As the research progressed, a novel maceration process was identified and the aim of the research shifted to elucidating the novel process, and examining the interaction between the novel process and several important winemaking parameters (eg. yeast strain, must nutrient status, wine aroma, viticultural interventions).

Thesis structure

The University of Tasmania encourages doctoral candidates to present doctoral research in the style of 'thesis by publication'. Hence, subsequent chapters in this thesis are presented in the form of stand-alone scientific journal papers. Each chapter is preceded by a brief account of the journal or forum to which the paper was submitted, publication status at the time the thesis was submitted, and relative contribution of each named co-author. For chapters where additional pertinent data was generated but not published, or published in a separate form (eg. conference poster, conference extended abstract, technical magazine article), the frontispiece for each chapter directs the reader to the appropriate appendices.

Figure 1 is a thesis structure diagram which provides an indication of the main question addressed by each chapter, and how findings from earlier chapters supported the development of research questions for subsequent chapters.

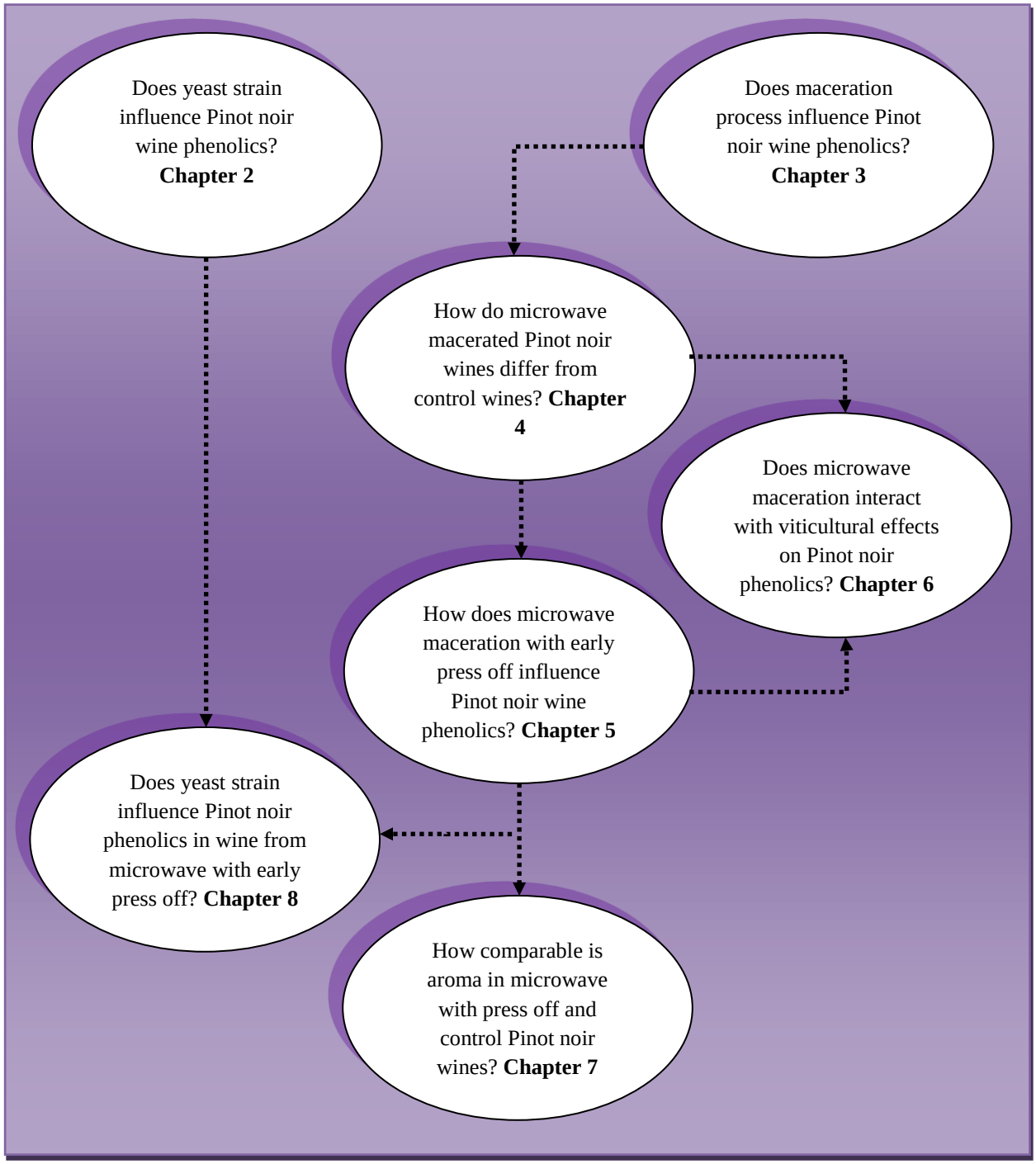

Figure 1. Flow chart of thesis structure for 'A Novel Process for Pinot Noir Wine Making'.

The body of research presented in this thesis had its beginning in two scoping studies that examined yeast strain and maceration impacts on Pinot noir wine phenolics (Chapters 2 and 3). The research then narrowed to focus on microwave maceration (Chapter 4), and testing diverse applications of microwave maceration (Chapters 5 and 6). The final tranche of work considered the impact of microwave maceration beyond wine phenolics (eg. impact on wine aroma; Chapter 7) and, drawing on the findings presented in Chapter 3, the impact of yeast strain on wine phenolics within the novel conditions of microwave macerated must (Chapter 8). In brief, the following chapters will cover:

Chapter 2 – The impact of yeast strain on Pinot noir phenolics was investigated. Five yeast treatments were trialled in replicated Pinot noir microvinification and wines were analysed for phenolics by UV-visible spectrophotometry, and for tannin composition using gel permeation chromatography and depolymerisation with phloroglucinol. Wine from the yeast strain *Saccharomyces cerevisiae* RC212 was higher in phenolics than wine from *Saccharomyces cerevisiae* AWRI1176. Yeast strain treatments were also associated with significant difference in wine tannin composition. This study demonstrated yeast strain choice was important for managing Pinot noir wine phenolic outcomes, particularly the concentration and composition of tannin in finished wine.

Chapter 3 – A wide range of maceration processes are used by Pinot noir makers to enhance phenolic extraction. Six maceration processes were trialled in replicated microvinification of Pinot noir must and resulting wines were analysed for treatment impacts on pH and phenolics (by UV-visible spectrophotometry). Phenolics were quantified at six and 30 months bottle age.

The relative impact of maceration treatment on wine phenolics changed as wines aged. Young cold soak wine had non-bleachable pigment concentration equivalent to control, whereas cold soak wines at 30 months bottle age had a significantly greater concentration of non-bleachable pigment. Freeze treatment wines remained high in tannin at 30 months bottle age and microwave treatment wine was largely equivalent for phenolic profile to the three extractive maceration treatments (cold soak, freeze, extended maceration). The novel microwave maceration treatment examined in this study was deemed sufficiently effective to warrant further investigation.

Chapter 4 – Microwave maceration had shown potential as an effective method to increase phenolic concentration in Pinot noir wines, and was adapted and trialled against control microvinification. The sanitation effects of microwave maceration were examined by plate culture on Wallerstein Laboratories Nutrient agar (Oxoid) and wine phenolics were examined by UV-visible spectrophotometry. Microwave maceration produced wines that had greater concentration than control wines of . Phenolic concentration in microwave macerated must was examined prior to inoculation for alcoholic fermentation (immediately post-maceration), and was observed to be similar to phenolic concentration in finished control wine. This, combined with the effective sanitation of must by microwave, suggested microwave maceration might support the development of several novel forms of wine making (see Chapters 5 and 6).

Chapter 5 – Microwave maceration of red grape must is a new process for rapid extraction of phenolic compounds in red wine making. Microwave maceration with early press off was compared to control microvinification on pomace for seven days and the two treatments were

found to produce wines that were comparable in terms of phenolic parameters. We also compared wines from microwave maceration with early press off, to wines from heat maceration of Pinot noir must with early press off. Some phenolic differences were observed between juices for the microwave and heat maceration treatments, but those differences were not sustained with bottle aging of wines. Juice yield, intra-cellular damage of grape skins and fermentation kinetics differed between microwave and heat maceration treatments. The results suggested microwave maceration with early press off may offer process efficiencies for red wine making that are novel, including the option of alcoholic fermentation off skins, rapid fermentation kinetics and potentially more effective phenolic extraction than obtained from standard alcoholic fermentation of red grape must.

Chapter 6 – Pinot noir wine quality is influenced by phenolic concentration. The effects of viticultural and winemaking interventions on Pinot noir phenolics concentration were examined with leaf removal (LR) and no leaf removal (noLR) treatments applied at two vineyard sites. LR was associated with higher total phenolics (7%) and total tannin (7%) in fruit, compared with noLR. Fruit from each treatment was vinified using control or microwave winemaking, and resulting microwave wines were higher in total phenolics (30%) and total tannin (50%) than control wines. Viticultural and winemaking treatment effects were additive for total phenolics and total tannin in wine. The study concluded that leaf removal and microwave maceration were both effective interventions with potential to improve Pinot noir wine quality.

Chapter 7 – Red wine is a complex sensory pleasure. Phenolic compounds contribute visual and mouthfeel effects in red wine, but aroma has a crucial role to play in ensuring red wine serves its

purpose. The impact of microwave maceration with early press off on Pinot noir aroma was examined, in comparison with aroma outcomes from control microvinification. Wines were examined by UV visible spectrophotometry for phenolics, and by GCMS for response ration to 16 wine aroma compounds. Compared with control wine, microwave maceration with early press off was largely equivalent for phenolics concentration but there were substantial apparent aroma differences, with early press off wines significantly higher for most ethyl ester and acetate compounds examined..

Chapter 8 – This study examined yeast strain effects from the application of microwave maceration with early press off, and co-inoculation of yeast and malolactic bacteria for simultaneous alcoholic and malolactic fermentation. Yeast treatments (*Saccharomyces cervisiae* RC212 and EC1118, and *Saccharomyces bayanus* AWRI1176) were co-inoculated with *Oenococcus oeni* PN4 into juice immediately after must had been microwave macerated and pressed off. Alcoholic and malolactic fermentation were complete 17 days post-inoculation for all three yeast treatments. At 16 months bottle age, AWRI1176 treatment wines had approximately twice the concentration of non-bleachable pigment and colour density of EC1118 and RC212 wines. There was no apparent inhibition between the yeast strains and malolactic strain applied in this study. The novel process delivered strong phenolic extraction coupled with potential production efficiencies (rapid malolactic fermentation; no cap management required) but yeast strain influenced phenolic development with bottle age, and hence yeast strain choice will be an important consideration for industry uptake of the novel winemaking process described in this thesis.

Chapter 9 – The findings of the preceding eight chapters are synthesised and considered in light of the aims identified at the outset of the thesis. Scientific and industry implications are examined, and additional research questions are identified which would necessarily precede the development and uptake of the novel maceration approach on an industry scale.

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Chapter 2. Yeast effects on Pinot noir wine phenolics, color and tannin composition.

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Appendix A is a Powerpoint™ presentation from an oral presentation of some of this research at the 8th International Cool Climate Symposium in Hobart, Tasmania, February 2012.

Yeast effects on Pinot noir wine phenolics, color and tannin composition.

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Abstract

Extraction and stabilisation of wine phenolics can be challenging for wine makers. This study examined how yeast choice impacted phenolic outcomes in Pinot noir wine. Five yeast treatments were applied in replicated microvinification, and wines analysed by UV visible spectrophotometry. At bottling, yeast treatment *Saccharomyces cerevisiae* RC212 wine had significantly higher concentration of total pigment, free anthocyanin, non-bleachable pigment and total tannin, and showed high color density. Some phenolic effects were retained at six months' bottle age, and RC212 and *S. cerevisae* EC1118 wines showed increased mean non-bleachable pigment concentration. Wine tannin composition analysis showed three treatments were associated with a higher percentage of tri-hydroxylated subunits (skin tannin indicator). A high degree of tannin polymerisation was observed in wines made with RC212 and *Torulaspora delbruekii*, while tannin size by gel permeation chromatography was higher only in the RC212 wines. The results emphasise the importance of yeast strain choice for optimising Pinot noir wine phenolics.

Key words

wild fermentation, sequential inoculation, anthocyanin, pigmented tannin

INTRODUCTION

Phenolic compounds are important to the aesthetic, flavour and mouthfeel qualities of red wine[1] but the extraction and stabilisation of phenolics can be a particular challenge for Pinot noir wine makers[2]. Red wine color depends on anthocyanin extraction from grape skin and its stabilisation in wine in a colored form. Compared with other red wine grape varieties, Pinot noir grapes have low anthocyanin content and what anthocyanin is present is of the less-stable non-acylated form [3-4]. Stabilisation of anthocyanins occurs through reaction between anthocyanins and tannins to form pigmented tannins[5] and through copigmentation of anthocyanins[6]. For this reason, extraction of both anthocyanin and tannin is important for achieving stable color in red wine. In addition to being low in anthocyanin concentration, Pinot noir grapes have a low skin-to-seed tannin ratio compared with many other red wine grape varieties. A recent review by Kennedy concluded that only 11% of total Pinot noir grape tannin was of skin origin[7], however seed tannin is more difficult to extract than skin tannin. Consequently, to achieve sufficient color stability and wine astringency, Pinot noir winemakers need to optimise tannin extraction during the alcoholic fermentation and maintain extracted tannin in the liquid phase during alcoholic fermentation and subsequent wine aging.

One option available to winemakers for managing phenolics in wine is choice of yeast strain. Wine color is influenced by direct yeast interaction with phenolics[8-9], and by enhancement of phenolic reactions by reactive yeast metabolites and by-products of fermentation[10]. Furthermore, wine mouthfeel is influenced through yeast-mediated biosynthesis of alcohol, glycerol and polysaccharides[11-12]. Research describing the impact of yeast strain on phenolic extraction and retention in red wine has returned variable findings[8, 10, 13-17]. It has been suggested that poor experimental design has contributed to uncertainty over yeast strain impacts on red wine

phenolics[18] and experimental design problems, and measures applied to address them, have continued to make objective assessment of yeast strain effects on red wine phenolics challenging[3, 19-22]. A further confounding factor has been the difficulty of comparing between studies, given the wide range of analytical techniques used to determine phenolic concentration and composition[19, 23-25], and concerns about the robustness of some measures[26-27].

Research specifically focussed on Pinot noir has also returned variable findings regarding the effect of yeast strain on wine phenolics[3, 11, 28-29]. Girard and others concluded that yeast strain effects on Pinot noir were mediated by both maceration approach and fermentation temperature, although Principal Component Analysis scores plots appeared to show consistent separation associated with yeast treatment in that study[28]. A comparison of Pinot noir wines made with Burgundy and RC212 yeast strains showed the RC212 treatment was associated with significantly higher anthocyanin concentration in wine directly after alcoholic fermentation, but this effect was reversed following malolactic fermentation[11]. A trial of eight yeast strains in Pinot noir must over two vintages concluded that some yeast strains produced noticeable variation in phenolic concentration between treatments, with the Wädenswil 27 strain being associated with lower color density and phenolic content than other yeast treatments[3]. Wines in that trial were reported to have been pressed off pomace 'at dryness' which appeared to undermine the finding of strain-attributable difference due to potential effects of non-uniform pomace contact time[18], however, Wädenswil 27 was reported to have 'fermented at a slower rate' (pg. 4016 [3]) and produced the lowest phenolic outcomes. The lowest phenolic outcome treatment - Wädenswil 27 – appeared to have had the longest pomace contact time.

In summary, research into yeast strain phenolic effects in red wine has delivered mixed findings. There has been little attention to the impact of yeast strain on tannin concentration and tannin

composition. Insight into tannin concentration and composition are important for Pinot noir as this is a variety where a well balanced wine tannin profile can be difficult to achieve, and long-term color stabilisation can be challenging. The aims of this study were to:

1. Assess yeast treatment impacts on phenolic concentration and composition in Pinot noir wine made under controlled conditions; and

2. Assess the impact of yeast treatment on changes in the phenolic concentration and composition with bottle aging of Pinot noir wine.

METHODS

2.1 Microvinification and yeast treatments

Pinot noir grapes were harvested from a vineyard in Northern Tasmania, Australia in 2011 at 12.5 °Baume, pH 3.27 and titratable acidity 8.39. Grapes were vinified on the day of harvest following a modified version of the 'French Press' method [30]). Grape bunches were randomised to 1.1kg batches and each batch was allocated to one of five yeast treatments (Table 1) in four replicates (n=20). For each batch, grapes were crushed using a custom-made bench-top crusher and destemmed by hand before the resulting must was decanted to a 1.5L Bodum™ 'Kenya' plunger coffee pot. The coffee pots acted as pilot-scale submerged cap fermenters. Twenty mg/L free sulphur dioxide (SO_2) in the form of potassium metabisulfite solution was applied to each pot; 20mg/L was the recommended maximum SO_2 dose rate for one of the yeast treatments. All pots were moved to a 27°C (±3°C) constant temperature room after application of SO_2 and, after two hours, were inoculated according to Table 1. All yeast strains were commercially available active dried yeast cultures that were rehydrated according to the manufacturers'

instructions. Haemocytometer counts showed inoculation resulted in between $1.9x10^6$ and $6.0x10^6$ cells of inoculating strain added per milligram of must.

Table 1. Yeast treatments.

Treatment	Inoculation strategy	Yeast inoculated at day 0	Yeast inoculated at day 3
RC	Single strain	*Saccharomyces cerevisiae* RC212 (Lallemand)	-
EC	Single strain	*S. cerevisiae* EC1118 (Lallemand)	-
WD	Sequential	-	*S.cerevisiae* EC1118 (Lallemand)
AW	Single strain	*S.cerevisiae* AWRI1176 (Maurivin)	-
TD	Sequential	*Torulaspora delbruekii* (from Level2™ TD Lallemand)	*S.cerevisiae* EC1118 (Lallemand)

A diverse set of yeast treatments was chosen to represent current and novel practices in Pinot noir winemaking. The strain EC1118 (EC) is widely used in winemaking and wine research [3, 14, 31-32] and was selected as a 'control' strain. Two practices commonly used in New World Pinot noir winemaking were applied as treatments; inoculation with RC212 (RC) and wild-initiated fermentation sequentially inoculated with EC1118 (WD)[2]. Two novel yeast treatments were included; inoculation with the *S.bayanus* strain AWRI1176 (AW), and *T.delbrueckii*-initiation followed by sequential inoculation with EC1118 (TD). Sequential inoculation (as in the case of

WD and TD treatments) represents a common practice in Pinot noir winemaking[2] and ensured all treatments completed fermentation at around the same time, ensuring equivalent skin contact time[18].

Following inoculation, all pots were incubated at 27°C (±3°C)[2, 33] and fermented for seven days on skins. Pots were weighed daily and weight loss through evolution of carbon dioxide was used as an indicator of fermentation kinetics. Sixty mg/L of yeast assimilable nitrogen in a 20% di-ammonium phosphate solution was applied at day three of the ferment. Pots were all pressed off at day seven by hand pressing down the Bodum filter screen with 10 seconds hold at full pressure. Wines were cold settled for 14 days at 4°C, prior to first racking, and were not inoculated for malolactic fermentation. Cold settled wines were assessed for residual reducing sugar using $CuSO_4$-NaOH tablets (Clinitest™, Bayer) and all wines were ≤5 g/L for residual sugar. This threshold was slightly above the ≤2.5 g/L customary in red wine research, but was within the range of reported residual sugar for commercial red wines[34]. A slightly higher residual sugar threshold was selected due to the variable fermentation rates associated with the yeast treatments applied, and the need to control for skin contact time. Due to the slightly higher residual sugar threshold and as wines were not filtered, wines were stabilised by application of 0.5 mL of a 20% potassium metabisulphite solution resulting in a concentration of 140 mg/L SO_2 in wines at first racking. This concentration of SO_2 would be predicted to slow wine maturation, but relative treatment effects would be the same as all treatments received equivalent application. Wines were stored for one month's further settling prior to bottling in amber glassware with polypropylene screw cap closures. Bottled wines were stored at 14°C. Wines were analysed for phenolics at bottling and 6 months after bottling. Wines were analysed for tannin composition 8 month after bottling. Due to the small volumes produced, it was not

possible to perform formal sensory analysis of the wines. A fresh bottle of wine from each replicate was opened for phenolics and tannin composition analysis.

UV-Visible Spectrophotometry

Samples at bottling (n=20) and wines that had been stored for 6 months (n=20) were analysed using UV-Visible spectrophotometry in HCl, acetaldehyde and metabisulphite buffers to quantify: total phenolics, total pigment, anthocyanins, total tannin, non-bleachable pigment, color density and hue with the modified Somers method[35] and spectral tannin method[36]. The acetaldehyde buffer used in the modified Somers method negates SO_2 effects on color.

Total phenolics in red wine consist predominately of colored and non-colored tannin and anthocyanin, plus low molecular weight, non-pigmented phenolic compounds. Total pigment is a measure of total red color in the sample, including free anthocyanins and pigmented tannins. Total tannin includes both pigmented and non-pigmented tannin. Non-bleachable pigment results from reactions between anthocyanins and tannins, and has been correlated with concentration of pigmented tannin[37]. Color density is a measure of wine saturation with visible color compounds. Hue gives an indication of wine shade (eg. garnet, purple) with values around 0.7 more purple and values around 0.8 more garnet and values above 0.9 in the brick color range, indicative of more 'developed' wines.

2.3 Tannin Composition

For each wine at 8 months bottle age, a 4ml sample was loaded onto a solid phase extraction (SPE) cartridge and total tannin isolated following the method of Kassara and Kennedy[38]. For treatment RC, 4ml of sample overloaded the SPE cartridge so findings for this treatment were confirmed via analysis of 2ml samples. Isolated tannins were subjected to acid catalysed

depolymerisation in the presence or phloroglucinol[39]. Four tannin composition measures were calculated from total tannin isolated from wine samples: % tri-hydroxylated subunits (an indicator of the proportion of skin tannin in wine[38]); % galloylated subunits (an indicator of the proportion of seed tannin in wine[38]); mean degree of polymerisation (mdp), % conversion yield; and molecular size at 50% elution by gel permeation chromatography (GPC). GPC was used as an indicator of the median size of tannin polymers in wine, mdp provided a measure of the mean number of polyphenol subunits in wine tannin polymers, and percent conversion yield indicated the proportion of total tannin that was depolymerisable. Tannin with a high % conversion yield indicates a higher proportion of unmodified, grape-like tannins. The measures mdp, % trihydroxylation and % galloylation can only be applied to converted tannins and hence are interpreted relative to % conversion yield.

Statistical Analysis

Mean and standard error (SE) were calculated in Excel™ for: weight loss during alcoholic fermentation, the five phenolic and two color indicators for wines at bottling and 6 months bottle age, and the four tannin composition measures at 8 months bottle age. R (GNU General Public License) two-factor ANOVA was applied to determine phenolic and color effects at bottling and at 6 months bottle age, and compare concentrations between the two sampling periods. Single factor ANOVA in R was used to identify between-treatment tannin species effects at 8 months bottle age. Post-hoc analysis in R using Tukey's test identified significant differences between specific treatments for phenolic, color and tannin composition measures (95% confidence interval).

RESULTS

Fermentation kinetics

Figure 1 shows cumulative percentage weight loss, for each treatment over the seven days of alcoholic fermentation. Fermentation kinetics varied by yeast treatment with RC, EC and AW following a normal fermentation pattern[40] with rapid weight loss to approximately 75% of mean total final weight loss by day three of the ferment. Fermentation was slow to initiate in the TD and WD treatments which did not reach 75% mean total weight loss until day five or six. At day three, WD and TD pots were inoculated with a log phase culture of EC1118 which did not appear to effect fermentation rate for TD treatment but coincided with a fermentation rate increase in WD treatment pots. Figure 1 shows that all experimental ferments finished alcoholic fermentation prior to pressing off at day seven and this provided equivalent pomace contact time for all treatments.

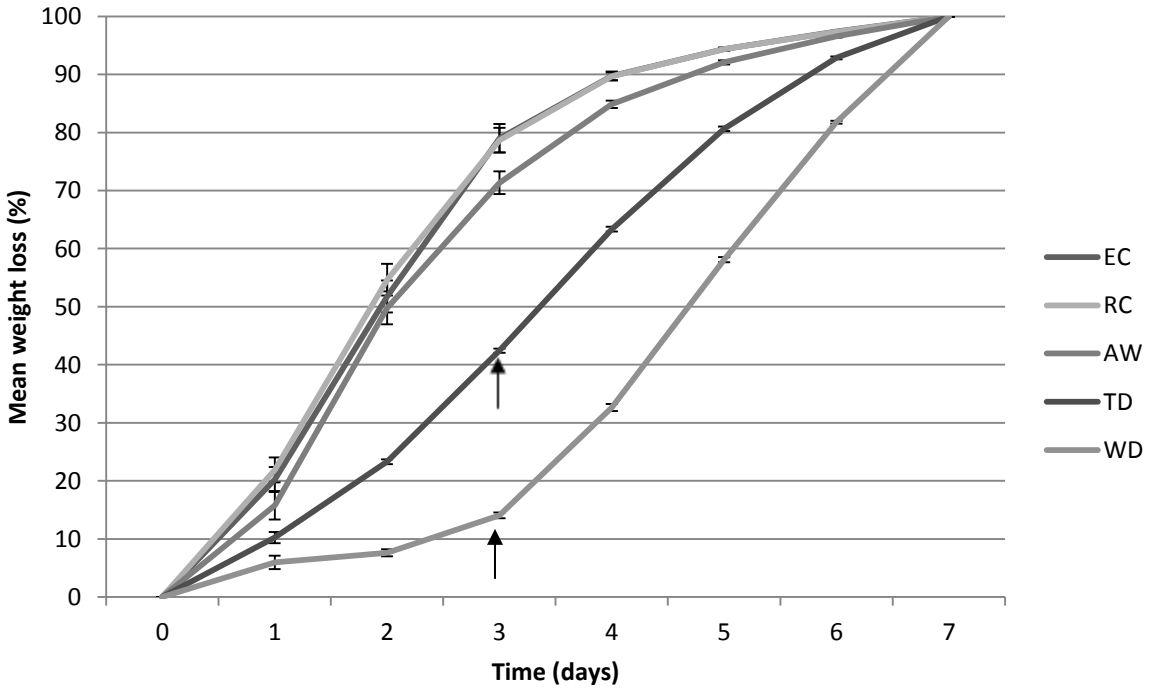

Figure 1. Fermentation kinetics for Pinot noir wine made with five yeast treatments (SE)(arrows indicate sequential inoculation with EC1118).

Phenolic and color measures

Significant differences were observed amongst yeast treatments for all measures related to color intensity (Table 2). Treatment effects were discernable at bottling for total pigment, anthocyanin and color density. RC treatment was associated with significantly higher mean total pigment at bottling compared with treatments AW and WD. RC was also significantly higher at bottling in mean anthocyanin than AW, and had significantly higher mean color density than the AW, TD and WD treatments. The effect of RC treatment on color-related measures was maintained after six months in bottle, with RC wines significantly higher in mean total pigment and anthocyanin at six months bottle age than AW and WD. By six months bottle age, RC treatment mean color density was between 16% and 30% higher than all other yeast treatments. RC was also the only treatment associated with a significant increase in color density with bottle age ($P<0.001$), increasing in color density by 14% between bottling and 6 months bottle age.

Yeast treatment also impacted on hue development with bottle age. All but the TD treatment showed significant change in wine hue with age ($P<0.001$) away from younger blue-purple hue values (0.71-0.74) and towards a more garnet, ruby hue values (0.80-0.81). The TD treatment showed no change in hue value between bottling and 6 months in bottle ($P>0.05$).

Yeast treatment impacted on formation of stable color as shown by significant differences in non-bleachable pigment concentration between the experimental wines (Table 2). Analysis of wines at six months bottle age showed RC and EC yeast treatments were associated, respectively with 37% and 21% higher non-bleachable pigment than the TD treatment. Treatments RC and

EC also showed significant increase in mean concentration of non-bleachable pigment between bottling and 6 months bottle age ($P<0.001$) whereas change in mean non-bleachable pigment concentration for the three remaining yeast treatments was non-significant ($P>0.05$).

Table 2 shows yeast treatment was associated with significant difference in the total tannin concentration in wine at bottling and the patterns of difference were maintained at 6 months bottle age. Compared with the RC treatment, the *S.bayanus* (AW) and WD treatments were significantly lower in mean total tannin concentration. In the most extreme case, WD at six months had a mean total tannin concentration 70% lower than RC treatment wine.

Table 2. Concentration of phenolic and color indicators in Pinot noir wine at bottling and six months bottle age (SE)

Phenolic/ Color measure	Bottle age	Yeast treatment				
		RC	EC	AW	TD	WD
Total phenolics (AU)	bottling	34.3±1.3	30.3±1.7	28.3±0.8	29.0±1.4	27.8±1.2
	6 months	36.6±1.5a	32.7±1.6ab	30.4±2.3ab	30.8±0.9ab	28.4±2.3b
Total pigment (AU)	bottling	11.8±0.4a	10.3±0.5ab	9.4±0.2b	11.0±0.5ab	10.1±0.2b
	6 months	11.4±0.04a	10.0±0.3ab	9.1±0.4b	10.0±0.3ab	8.7±0.7b
Anthocyanin (mg/L)	bottling	221±8.5a	193±10.5ab	176±3.1b	209±9.7ab	190±4.7ab
	6 months	211±0.9a	187±4.9ab	171±8.4b	188±6.4ab	162±13.6b
Tannin (g/L)	bottling	0.54±0.04a	0.34±0.06ab	0.23±0.03b	0.32±0.05ab	0.23±0.05b
	6 months	0.48±0.06a	0.30±0.05ab	0.19±0.01b	0.23±0.03ab	0.14±0.07b
Pigmented tannin (AU)	bottling	0.43±0.01a	0.34±0.01ab	0.35±0.00ab	0.31±0.02b	0.34±0.02ab
	6 months	0.49±0.02a	0.39±0.01a	0.34±0.01ab	0.31±0.01b	0.34±0.02ab
Color density (AU)	bottling	3.2±0.06a	2.9±0.01ab	2.7±0.06bc	2.5±0.08cd	2.4±0.06d
	6 months	3.7±0.07a	3.1±0.10b	2.7±0.07c	2.7±0.05c	2.6±0.08c
Hue	bottling	0.73±0.01	0.71±0.01	0.71±0.004	0.75±0.005	0.74±0.02
	6 months	0.81±0.01a	0.80±0.004a	0.80±0.01a	0.76±0.01b	0.80±0.01a

Lowercase letters denote significant difference amongst treatments at specified bottle age (Tukey's Test $P<0.05$).

Tannin composition

In this study, yeast treatment effected wine tannin composition (Table 3). Significant differences were observed between yeast treatments for molecular size at 50% elution by GPC (Table 3), with the RC treatment yielding wine tannin polymers 15% greater in size than AW or WD treatments. The higher conversion yield associated with tannins isolated from EC and TD treatments (~60%) compared to those from RC, AW and WD treatments (~50%), suggested EC and TD wines had undergone less tannin modification than RC, AW and WD wines. Comparing those treatments with similar conversion ratios, mdp results showed RC treatment wine tannin had a depolymerisable portion 1.7 subunits longer than that of the AW treatment, and 0.9 subunits longer than in the WD treatment. Similarly, depolymerisable TD wine tannin was 0.9 subunits longer than in the EC treatment.

Table 3 shows there were yeast treatment effects on the relative representation of the two seed and skin tannin indicators: % galloylation (epicatechin gallate) and % trihydroxylation (epigallocatechin). Wines with similar percent conversion yield showed differences in % galloylation, for example RC wine had a higher percentage of galloylated tannin subunits than AW wine, and EC wine was significantly higher in galloylated subunits than TD wine. RC and WD treatments were associated with significantly higher % trihydroxylated subunits, compared with AW wine, and TD wine was higher in trihydroxylated subunits than EC wine.

Table 3. Tannin size indicators and subunit composition for Pinot noir wine at eight months bottle age (SE)

	Yeast treatment				
Tannin size indicator	RC	EC	AW	TD	WD
Molecular size at 50% elution by GPC (g/mol)	1223±14 a	1127±14 b	1020±16 c	1084±16 b	1010±9 c
% conversion yield	50% a	62% b	49% a	60% b	49% a
mean degree of polymerisation (mdp)	6.2±0.1 a	5.3±0.2 b	4.5±0.2 b	6.2±0.4 a	5.3±0.3 b
% galloylation	2.8±0.26 a	2.3±0.17 ab	2.2±0.06 b	1.9±0.03 b	2.3±0.16 ab
% trihydroxylation	24±0.5 a	22±0.7 b	21±0.6 b	25±0.6 a	25±0.3 a

DISCUSSION

A limited range of yeast strains have tended to be employed by New World Pinot noir wine makers[2] and more research into yeast strain effects on Pinot noir phenolics is needed to assist winemakers to optimise phenolics in this variety[3, 11, 28-29]. This study assessed the impact of yeast treatment on Pinot noir phenolics under controlled conditions using a range of phenolic and color measures to quantify differences at bottling and after aging in bottle. The results reported here are considered in relation to two mechanisms that have been proposed for yeast strain mediated variation in wine phenolics: fining of phenolics from wine by differential adsorption or adhesion

to yeast cell walls, and rapid stable pigment formation from anthocyanin and tannin condensation by yeast metabolites, particularly acetaldehyde [15, 41].

Yeast treatment and phenolics

Yeast treatment had significant impact on wine phenolics, color, and both tannin concentration and composition. One yeast treatment - RC212 - was consistently associated with high concentration of wine phenolics. For example, RC wines were significantly higher in anthocyanin concentration at six months than AW and WD wines. Differential fining of phenolics via adsorption to yeast cell walls has been demonstrated elsewhere(Mazauric and Salmon 2006; Caridi, Sidari et al. 2007; Sidari, Postorino et al. 2007). In a study of five *Saccharomyces cerevisae* strains used to make Graciano wines, strain-related variation in anthocyanin adsorption percentages ranged from 1.6% to 5.9% [8]. While anthocyanin adsorption was not quantified in our study, color variation between treatments was visible in yeast lees following cold settling (data not shown). This observation suggested that differential adsorption of visible color compounds likely contributed to differences shown by instrumental analysis of wines (Table 2). Further research would be required to comment on the relative contribution of yeast adsorption to strain-related differences in wine phenolics observed in this study. Such research would require quantification of yeast population development over time, estimation of yeast surface area and strain adsorption capacity, and the development of reliable methods to extract or detach phenolic compounds from yeast lees. The RC212 treatment was associated with significantly higher total tannin in wine at bottling and 6 months bottle age compared with two other treatments (AW, WD), and the magnitude of difference was substantial; wine from the

wild-initiated ferment at six months had a mean total tannin concentration 70% lower than the wine made by treatment with RC212. A yeast-attributable difference of this magnitude is practically important given the difficulty some winemakers face in extracting and retaining sufficient tannin in Pinot noir wine. High tannin concentration in Shiraz and Cabernet Sauvignon has been correlated with higher wine grade[42], which suggests yeast choice may influence red wine market value. Our results demonstrated that choice of yeast strain may greatly assist winemaker's efforts to enhance tannin in Pinot noir wine.

While the WD treatment wine was significantly lower in total tannin than RC wine, WD and TD wines both had high percentages of trihydroxylated tannin subunits compared with AW and EC yeast treatments, respectively (Table 3). This suggested that WD, TD and RC wines had a high relative proportion of grape skin tannin. Grape skin tannin has been associated with positive mouthfeel qualities in wine[43]. The RC treatment wines were also high in relative representation of galloylated tannin. This suggested that RC wines may have had a high proportion of seed tannin, which has been associated with mouthfeel coarseness[43].

The measure 'total tannin' has been correlated with high wine grade[42]. Our findings showed that both tannin concentration and composition can vary as a result of yeast treatment. Variation in tannin composition was observed in relation to the ratio of trihydroxylated and galloylated subunit tannins in wines, and the extent of tannin polymerisation (mdp, GPC). The implications of yeast-mediated variation in tannin composition are, as yet, poorly understood and need to be further investigated via formal sensory evaluation of wines with known tannin compositions.

Tannins and anthocyanins are extracted during pomace contact and their concentration steadily declines in wine from pressing[44-46]. It is anticipated that the decline in anthocyanins and tannins

in wine during aging, translates into creation of stable color (non-bleachable pigment). In this trial, there was no significant decline in mean anthocyanin or mean tannin for any yeast treatment between racking and 6 months bottle age, but RC and EC treatments showed a significant increase in mean non-bleachable pigment over that period. Two pathways have been described for formation of stable color in red wine. Direct chemical condensation between anthocyanin and tannins, and acetaldehyde-mediated dimer formation via an ethyl-bridge (most commonly between malvidin-3-glucoside and catechin)[41, 47]. The latter pathway is more rapid, and depends on acetaldehyde production. Acetaldehyde is primarily produced by yeast as an intermediate product in alcoholic fermentation, and production has been shown to vary by yeast strain, fermentation conditions and grape variety[15, 48-49]. It is possible that differences observed in non-bleachable pigment concentration for wines made with RC and EC strains may have been due to their production of greater quantities of acetaldehyde, thereby contributing to faster and more effective color stabilisation. The non-bleachable pigment results demonstrated that yeast treatment had the capacity to impact both the quantity of stable color in wine, and the rate of its development.

The yeast strain AWRI1176 (AW) was associated with low concentration of wine phenolic indicators and low color density (Table 2), shorter tannin polymers and a low percentage of tri-hydroxylated tannin (Table 3). Consistent with the findings of our study, two additional replicated trials during 2011 showed significantly lower mean tannin in Pinot noir wines made using AWRI1176 compared with EC1118 control wine (data not shown). The performance of AWRI1176 in these trials highlights yeast strain and grape variety as important variables effecting phenolic outcomes in red wine. Previous research on a closely related strain concluded that *S.bayanus* strains might offer positive outcomes for color stabilisation in red wines;

compared with a control strain, the *S.bayanus* strain AWRI1375 was found to optimise pigmented polymer formation in Cabernet Sauvignon wine [15]. Our results contrast with this finding, and emphasise the importance of better understanding yeast strain effects by grape variety.

Fermentation kinetics and phenolics

Novel strategies like use of non-*cerevisiae* and non-*Saccharomyces* strains, and co- or sequential-inoculation have been investigated as a way to diversify wine styles and build complexity [12, 50-51]. The slower fermentation kinetics associated with the novel yeast treatments employed in this study suggest Pinot noir ferments initiated with *T.delbruekii* (TD) or wild-initiation of ferments (WD) may be vulnerable to colonisation by undesirable fermentation strains, or aerobic spoilage yeast and bacteria during the first days of fermentation (Figure 1). This is of particular concern for TD as the manufacturers recommend low sulphite application (20mg/L) to reduce inhibition of the yeast inoculum. This may limit application of the TD strain trialled in this study to fruit of the best condition.

There has been debate in the literature over whether tannin extraction is mainly ethanol-mediated [52] or more strongly dependent on the physical breakdown of grape solids[53-54]. Figure 1 showed that EC, RC and AW had largely completed alcoholic fermentation by day five which meant pomace in those treatments was in contact with a relatively high ethanol environment for three days prior to pressing off. Under the ethanol-mediated extraction of tannin hypothesis, EC, RC and AW wines would be expected to have similar mean total tannin concentration, and be higher in tannin than TD and WD wines. Mean total tannin concentration in AW wine was, however, lower than RC but equivalent to WD wines (Table 2) which had slower development of ethanol

(Figure 1). These results suggest the relationship between tannin concentration, ethanol concentration and pomace contact time is a more complex one than suggested by the ethanol-mediated extraction of tannin hypothesis. Alternate explanations include; tannin extraction was influenced by the physical breakdown of grape solids[53], there was differential yeast fining of tannin from the liquid phase of wine[9, 19, 55], or there was differential expression by yeast of extracellular enzymes contributing to the release of tannins from the grape matrix (eg. beta-glucosidase, pectinase, proteolitic enzymes)[56-58]. The mechanisms by which yeast mediate tannin concentration and composition in wine require further research.

The findings presented here suggest that informed selection of fermentation yeast might assist winemakers to produce longer-lived, more aesthetically pleasing Pinot noir wine. In a first for Pinot noir research, we demonstrated that yeast strain significantly impacted both the concentration and composition of Pinot noir wine tannin. We showed strain-associated effects on the relative representation of seed and skin tannin indicators, and differences in the extent of tannin polymerisation in wine at eight months bottle age. While the importance of seed-to-skin tannin ratio, and of tannin polymerisation, to the sensory qualities of wine is the subject of ongoing research, understanding yeast strain effects on these parameters will position the industry to better manage Pinot noir wine phenolics through judicious choice of fermentation strain or strategy.

Abbreviations

AF – alcoholic fermentation

ANOVA – analysis of variance

AU – absorbance units

AW – yeast treatment AWRI1176

EC – yeast treatment EC1118

GPC - gel permeation chromatography

Mdp – mean degree of polymerisation

RC – yeast treatment RC212

SE – standard error

SPE – solid phase extraction

TD – yeast treatment *Torulaspora delbruekii* and sequential inoculation with EC1118

WD – yeast treatment wild-initiated and sequential inoculation with EC1118

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Chapter 3. Maceration treatment effects on Pinot noir wine phenolics

This chapter has been drafted for submission as a journal paper. It has not yet been submitted.

Author contributions to-date were: Carew 70%, Sparrow 20%, Close 5%, Dambergs 5%

Appendix B is a poster associated with this research that was presented at the 8th International Cool Climate Symposium in Hobart, Tasmania, February 2012. Chapter 4 is based on analysis of phenolics in microwave maceration musts and wines from this trial at inoculation, and 6 and 18 months bottle age.

Maceration treatment affects Pinot noir wine phenolics

Carew, A.L., Sparrow, A.M., Close, D.C., and Dambergs, R.G.

ABSTRACT

Pinot noir wine can be low in phenolic compounds which are an important contributor to the quality of red wine. One option available to Pinot noir winemakers for enhancing the extraction and stabilisation of phenolic compounds is varying the maceration approach used during vinification. Six maceration treatments were applied in a replicated microvinification trial: control, enzyme, microwave, cold soak, must freezing and extended maceration. Analysis of the wines at six and thirty months bottle age showed that wine phenolics of the enzyme treatment were not significantly different from control treatment wines. At six months bottle age, cold soak treatment wines were no different to control but by thirty months bottle age cold soak wines had significantly greater non-bleachable pigment concentration than control wines. Phenolic effects changed as wines aged with extended maceration wines at six months significantly higher than all other treatments for non-bleachable pigment and significantly lower than all other treatments for free anthocyanin, suggesting precocious colour stabilisation. However, by thirty months bottle age, extended maceration wine non-bleachable pigment concentration had declined and was equivalent to all other treatments apart from control, which was the lowest of all treatments for this parameter. Freeze treatment wines were high in tannin at thirty months bottle age and all treatments but enzyme were associated with some phenolic difference to control wines at thirty months bottle age. The study demonstrated that maceration is an important tool for winemakers managing phenolic character in Pinot noir wine, and that the polymerisation of anthocyanin and

tannin to form non-bleachable pigment proceeds at different rates depending on maceration approach. This means bottle age at analysis is an important factor to consider when judging the merit of various maceration approaches. Microwave maceration yielded wine most similar to extended maceration, but with a shorter skin contact time and, by implication, less risk of wine oxidation or microbial spoilage due to time on skins post-fermentation.

INTRODUCTION

The extraction and stabilisation of phenolic compounds is central to making good quality red wine (Peynaud, 1984). Phenolics fall into two broad classes – anthocyanins which are important for red wine colour, and tannins which are important for wine mouthfeel and complex with anthocyanins to produce stable colour (polyphenols). The concentration of anthocyanin and tannin in wine has been correlated with measures of red wine quality like wine scores at judging, and retail price (Cozzolino, et al. 2008, Holt, et al. 2008, Kassara and Kennedy 2011).

Pinot noir grapes tend to be low in anthocyanin concentration, and the five types of anthocyanin found in Pinot noir are of an unstable, non-acylated form (Heazlewood, et al. 2006, Mazza, et al. 1999). Non-acylated anthocyanins tend to be colourless at normal wine pH. While the total quantity of tannin in Pinot noir grapes is similar to that of other well studied varietals like Cabernet Sauvignon, Merlot and Shiraz, the tannin in Pinot noir is disproportionately distributed (high in seeds, low in skins) (Mattivi, et al. 2009, Kennedy 2008, Downey, et al. 2003). Seed tannin can be difficult to extract which may explain why Pinot noir wines are often low in tannin concentration, compared with other red varietals (Harbertson, et al. 2008). For example, analysis of tannin concentration in 1350 commercial red wines found Pinot noir to have approximately half the concentration of tannin (catechin equivalents) compared with Cabernet Sauvignon and

Merlot (Harbertson, et al., 2008). The unique phenolic composition and distribution in Pinot noir grapes means that Pinot noir winemakers need winemaking options for increasing extraction.

One option available to winemakers is to vary the maceration approach applied before, during or immediately after alcoholic fermentation. Maceration processes are applied to grape must by winemakers to control the release of phenolics from berries into juice. A range of maceration approaches is used in industry including: **cold soaking** which is intended to enhance colour diffusion while simultaneously hindering the onset of fermentation; treatment with **enzymes** to degrade grape cellular elements (eg. pectinases, hemicellulases, glucanases, glycosidases); **thermal maceration** of must to degrade cell elements and hasten phenolic diffusion; and post-fermentation **extended maceration** to enhance extraction (particularly, tannin extraction).

Six maceration practices were identified in a review as effective for increasing phenolic concentration in red wine (Sacchi, Bisson, & Adams, 2005): fermentation temperature, thermovinification, must freezing, saignee ('bleeding off'), pectolytic enzyme treatment and extended maceration. While most research confirms the effectiveness of thermovinification and extended maceration for enhancing phenolic extraction (Sacchi, Bisson, & Adams, 2005), findings related to the use of freezing, enzyme and cold soak maceration treatments have been mixed. Cold soak has been described as having no effect, little lasting effect or a detrimental effect on wine colour, compared with conventional fermentation (Joscelyne, 2009; Sacchi, et al., 2005). Others, however, have concluded the method increased the concentration of some phenolic compounds in red wine (Busse-Valverde, et al., 2010; Gil-Munoz, et al., 2009; Reynolds, Cliff, Girard, & Kopp, 2001). A replicated trial assessing the impact on Shiraz wine anthocyanin concentration of cold soak and cold soak with extended maceration concluded that control wines at bottling were significantly lower in anthocyanin concentration than the cold

soak and extended maceration treatments (370 mg/L, 416 mg/L, 444 mg/L respectively) (Reynolds, et al., 2001). However the cold soak applied in this study was relatively long at 10 days, polymeric pigment concentration was not reported and the impact of bottle aging was not examined. The use of maceration enzymes in Pinot noir wine making was shown to have a significant impact on the concentration of anthocyanin and polymeric pigment in wines; anthocyanin was low in enzyme treated wines and polymeric pigment high, and those effects were maintained to 18 months bottle age (Parley, Vanhanen, & Heatherbell, 2001). Others have reported that the effectiveness of maceration processes like cold soaking and enzyme vary by grape variety (Busse-Valverde, et al., 2010) and by the enzyme preparation used (Wightman, Price, Watson, & Wrolstad, 1997). The use of must freezing as a maceration method was compared with cold soaking, dry ice maceration and enzyme treatment for Cabernet Sauvignon and Shiraz winemaking, and all pre-fermentation maceration techniques were concluded effective for colour extraction (Gil-Munoz, et al., 2009). In this study however, the researchers pressed wine off at the conclusion of alcoholic fermentation, so the five treatments ranged between 12 and 28 days skin contact time. This means observed effects may simply have been due to greater or lesser opportunity for diffusion, rather than the treatments applied.

The aim of our research was to compare the effect of six maceration treatments on Pinot noir wine phenolics, and to examine if relative phenolic effects from maceration treatments changed during bottle aging. We selected one bottle age time to represent the time at which winemakers would potentially make blending and price point decisions about a wine (6 months bottle age), and a second bottle age time to represent when consumers might drink that wine and judging its phenolic quality (30 months bottle age).

MATERIALS & METHODS

Maceration & Microvinification

Thirty 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 characterization and then bunches were randomly allocated into 24 replicates, each approximately 1.1 kg. Four replicates were allocated to each of six maceration treatments (Table 1). Prior to application of the maceration treatments, grapes from each replicate were crushed using a custom-made bench-top crusher, and de-stemmed by hand before the resulting must was decanted to a 1.5L Bodum™ 'Kenya' plunger coffee pot for fermentation following the 'French Press' method (Dambergs & Sparrow, 2011; Dambergs, Sparrow, et al., 2012). All pots were treated with 50mg/L sulphur dioxide (SO_2) in the form of potassium metabisulfite solution. Control and extended maceration treatment pots were moved to a 27°C (±3°C) constant temperature room in preparation for inoculation. Enzyme maceration treatment pots had 40 mg/L Lafase HE Pectolytic Enzyme (Laffort) stirred through must prior to being moved to the 27°C (±3°C) constant temperature room. Must in the microwave treatment was microwave macerated, which involved heating to 70°C by microwave, holding at that temperature for one hour and cooling in an icebath for ~30 min (Carew, Sparrow, Curtin, Close, & Dambergs, 2013). Cold soak treatment pots were covered with plastic film (Glad Wrap™) and stored at 4°C for 4 days prior to removal from the fridge, 4 hours of warming at ambient (20°C) and 2 hours warming at 27°C (±3°C) in preparation for inoculation. Freeze and thaw treatment pots were covered with plastic film (Glad Wrap™) and stored at -20°C for 3.5 days prior to 20 hours of thawing at ambient (20°C) and 2 hours warming at 27°C (±3°C).

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

TREATMENT	Skin contact time (days)	Day inoculated	Maceration process applied
control	8	1	Nil.
enzyme	8	1	40 mg/L Lafase HE Pectolytic Enzyme (Laffort) added to must at day 1 of skin contact time.
microwave	8	1	Must microwave macerated to 70°C with one hour hold time at day 1 of skin contact time.
cold soak	12	4	Must stored at 4°C prior to warming and inoculation on day 4 of skin contact time.
freeze and thaw	12	4	Must stored at -20C prior to thawing and inoculation on day 4 of skin contact time.
extended maceration	12	1	Wine held on skins for 4 days post-fermentation.

Approximately 3 hours after control, enzyme, microwave and extended maceration treatments were applied, those pots were inoculated with the active dried yeast strain *Saccharomyces cerevisiae* EC1118 (Lallemand, Australia) that had been rehydrated according to the manufacturer's instructions. Cold soak and freeze treatment pots were inoculated in the same way at day 4 of the experiment. This meant that control, enzyme, and microwave treatments

entailed 8 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.

All pots were incubated at 27°C (±3°C) and weighed regularly over the course of the 8 or 12 day ferment to track CO_2 (an indicator of the progress of fermentation). At day 3 after yeast inoculation, 60 mg/L of yeast assimilable nitrogen was added to each pot in the form of diammonium phosphate solution. Control, enzyme and microwave treatment pots were pressed off at day 8 and cold soak, freeze and extended maceration treatment pots were pressed off at day 12. Pressing off was by firm downward depression of the Bodum™ coffee pot plunger for 10 seconds, after which wine was poured into screw cap 375 mL glass bottles 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 cold settled wine was tested for residual sugar using Clinitest™ tablets; all wines were found to be dry (≤2.5 g/L residual sugar). Wine was racked to 250 mL Schott™ bottles under CO_2 cover and stabilised by the addition of 80 mg/L sulphur dioxide (SO_2) in the form of potassium metabisulfite solution. After two weeks, wines were bottled to polypropylene lidded 25 mL amber glass bottles under CO_2 cover and stored for bottle aging. Five bottles of wine was made from each replicate which allowed a fresh bottle to be opened at each bottle age analysis time period.

UV-Visible spectrophotometry

Wines were examined for phenolics concentration at 6 and 30 months bottle age using a modified Somers method (Dambergs, Mercurio, Kassara, Cozzolino, & Smith, 2012; Mercurio, Dambergs, Herderich, & 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 measures at 6 and 30 months bottle age, and for pH at 30 months bottle age. One-way and two-way ANOVA in Genstat™ 14th Edition were used to identify phenolics treatment effects at 6 and 30 months bottle age, and Tukey's test was used for post-hoc analysis. In the following section, one-way ANOVA results are reported separately for each bottle age period as the periods represent two distinct points at which the phenolic quality of wines would be judged independently – 6 months bottle age was selected as indicative of the time that winemakers would be evaluating wines for blending and bottle price point decisions, and 30 months bottle age was selected to represent when a wine buyer would potentially open wine for consumption. Interactions based on two-way ANOVA are reported after one-way ANOVA results in the following section as they are indicative of the relationship between the winemakers' decision point and consumption of the wine.

RESULTS

The maceration treatments applied in this study were associated with significant differences in phenolic concentration in wines, and the pattern of difference changed between the two bottle age periods that were examined.

At 6 months bottle age, enzyme and cold soak treatment wines were statistically indistinguishable from control wines on the basis of the seven phenolic measures examined (Table 2). Freeze and microwave treatment wines had two-fold higher tannin concentration than control wines, and were also higher than control wines for total phenolics concentration, suggesting those two treatments had been very effective for extraction. The extended maceration treatment wine was also higher than control wine for mean tannin concentration, but significantly lower in tannin than freeze and microwave treatment wines. Microwave treatment wine showed the highest concentration of anthocyanin of all treatments, and extended maceration showed the lowest anthocyanin concentration. Control treatment had the highest hue value (indicative of ruby coloured wine). The extended maceration treatment showed the lowest hue value (blue-purple coloured wine).

At thirty months bottle age, control and enzyme treatment wines were indistinguishable from each other on the basis of phenolics concentration. Freeze treatment wines had a higher concentration of total phenolics and tannin than control wines, but had no greater concentration of non-bleachable pigment than the control. Cold soak and extended maceration wines were significantly higher than control wines in non-bleachable pigment, and microwave wines were significantly higher in concentration than control wines for all phenolic parameters examined. Examination of hue value showed that control, enzyme and freeze thaw wines had relatively high

hue values, approaching the garnet-brick colour range. Microwave and cold soak wines were in the garnet range, and extended maceration wine showed a 'younger' hue value indicating the wine was ruby-garnet coloured.

Interactions were observed between maceration treatment and bottle age for all seven of the phenolic parameters examined in this study (total phenolics $P=0.003$; total pigment $P<0.001$; anthocyanin $P<0.001$; non-bleachable pigment $P<0.001$; tannin $P=0.009$; colour density $P<0.001$; hue $P=0.002$). Table 2 shows that the cold soak treatment was indistinguishable from control at six months bottle age, but had greater non-bleachable pigment concentration than control wine at thirty months bottle age. The extended maceration treatment was fast to develop non-bleachable pigment being significantly higher for this measure that all other treatments at six months bottle age, but was the only treatment in which non-bleachable pigment concentration declined by thirty months bottle age. At six months bottle age, microwave and freeze treatments showed significantly greater tannin concentration than all other treatments whereas at thirty months, only the freeze treatment had retained a significantly higher concentration of tannin compared with control wine.

The wines in this study were made from fruit harvested at pH 3.28, no acid adjustment was made during microvinification and wines were not subjected to malolactic fermentation. Maceration treatment was associated with significant differences in wine pH at 30 months bottle age (Table 2). Enzyme treatment had the lowest pH at 30 months bottle age. The extended maceration treatment was significantly higher in pH than control and enzyme treatment wines, but still within the normal pH range for red table wine (Peynaud, 1984).

Table 2. Pinot noir wine phenolics at 240 and 960 days post-inoculation (±SD) and Pinot noir wine pH at 960 days post-inoculation (±SD) for six maceration treatments: control, pre-fermentation enzyme addition, pre-fermentation microwave maceration, pre-fermentation cold soak, pre-fermentation freezing and thawing, and post-fermentation extended maceration. Different lowercase letters indicate significant differences in concentration for each parameter within the corresponding bottle age period (Tukey's Test P≤ 0.05). ND = below detection limit for the assay.

	Wine at 240 days post-inoculation (6 months bottle age)							**Wine at 960 days post-inoculation (30 months bottle age)**							
	Total phenolics (AU)	Total pigment (AU)	Anthocyanin (mg/L)	Non-bleachable pigment (AU)	Tannin (g/L)	Colour density (AU)	Hue	Total phenolics (AU)	Total pigment (AU)	Anthocyanin (mg/L)	Non-bleachable pigment (AU)	Tannin (g/L)	Colour density (AU)	Hue	pH
control	28.16 ±2.26 **a**	7.66 ±1.50 **ab**	133 ±29 **b**	0.60 ±0.05 **a**	0.38 ±0.05 a	2.91 ±0.31 **a**	0.86 ±0.03 **a**	18.72 ±1.77 **a**	1.47 ±0.25 **a**	1.16 ±0.65	0.85 ±0.13 **a**	0.15 ±0.07 **a**	2.58 ±0.40 **a**	1.39 ±0.07 **a**	3.23 ±0.04 **ab**
enzyme	30.21 ±0.58 **a**	7.71 ±0.21 **b**	131 ±5 **b**	0.69 ±0.24 **a**	0.51 ±0.03 **ab**	3.23 ±0.23 **a**	0.80 ±0.04 **ab**	20.80 ±1.00 **ab**	1.85 ±0.27 **ab**	ND	1.14 ±0.24 **ab**	0.32 ±0.06 **ab**	2.83 ±0.14 **ab**	1.10 ±0.26 **abc**	3.17 ±0.10 **a**
microwave	41.12 ±1.48 **b**	13.90 ±0.74 **c**	252 ±19 **c**	0.79 ±0.14 **a**	0.88 ±0.09 **c**	4.31 ±0.39 **b**	0.75 ±0.06 **bc**	25.47 ±3.19 **c**	2.42 ±0.26 **b**	ND	1.46 ±0.11 **b**	0.43 ±0.11 **bc**	3.71 ±0.61 **b**	1.04 ±0.13 **bc**	3.46 ±0.24 **bc**
cold soak	29.75 ±2.27 **a**	7.82 ±0.88 **b**	138 ±15 **b**	0.57 ±0.11 **a**	0.47 ±0.10 **ab**	4.58 ±0.45 **a**	0.81 ±0.06 **ab**	20.02 ±1.22 **a**	1.93 ±0.25 **ab**	ND	1.28 ±0.16 **b**	0.33 ±0.08 **ab**	2.82 ±0.14 **ab**	0.91 ±0.07 **bc**	3.48 ±0.04 **bc**
freeze and thaw	40.07 ±2.31 **b**	9.43 ±1.17 **b**	169 ±23 **b**	0.59 ±0.15 **a**	0.94 ±0.10 **c**	3.07 ±0.56 **a**	0.83 ±0.02 **ab**	25.02 ±2.61 **bc**	1.90 ±0.34 **ab**	ND	1.19 ±0.21 **ab**	0.56 ±0.13 **c**	3.08 ±0.59 **ab**	1.16 ±0.12 **ab**	3.49 ±0.09 **bc**
extended maceration	30.14 ±2.60 **a**	5.61 ±0.42 **a**	52 ±5.0 **a**	1.80 ±0.20 **b**	0.61 ±0.13 **b**	4.58 ±0.20 **b**	0.69 ±0.01 **c**	21.06 ±1.72 **abc**	2.04 ±0.18 **ab**	ND	1.29 ±0.07 **b**	0.41 ±0.10 **bc**	2.71 ±0.14 **a**	0.85 ±0.02 **c**	3.63 ±0.06 **c**

DISCUSSION

Maceration effects on wine phenolics

This study demonstrated that maceration treatment is an important tool for winemakers in managing the phenolic profile of Pinot noir wine. The bottle age period at which wine was evaluated for phenolic quality, was demonstrated to be of critical importance to the appraisal of maceration effects because the relative phenolic quality (as indicated by the phenolic measures examined in this study) of wines from several of the maceration treatments shifted between six and 30 months bottle age. For example, cold soaking appeared to have conferred no benefit compared with control treatment at six months bottle age, but cold soak wines showed greater stable colour development (non-bleachable pigment) than control wines at 30 months bottle age. The difference in non-bleachable pigment for those two treatments was 0.43 AU which would be discernible in the visual appearance of the wine. Conversely, the extended maceration treatment appeared advantageous at six months bottle age, as extended maceration treatment wines were significantly higher in non-bleachable pigment than all other treatments at that time, however by 30 months bottle age, non-bleachable pigment concentration in extended maceration treatment wines had declined and was not significantly different from any treatment apart from control. Analysis of the species of phenolics that extract from grape solids into juice over the course of red wine fermentation has shown that skin-associated phenolics extract in the early part of maceration and alcoholic fermentation, and seed-associated phenolics extract in the latter part of maceration and alcoholic fermentation (Koyama, Goto-Yamamoto, & Hashizume, 2007). Cold soaking arguably extends the early part of maceration, whereas extended maceration lengthens the latter part of the extraction phase. This suggests that cold soak may offer greater extraction of skin-associated phenolics, and extended maceration greater extraction of seed associated

phenolics. The results reported here suggest that seed associated phenolics may contribute faster but less permanent colour stability, whereas skin associated phenolics may be slower to polymerise but provide more enduring wine colour. However this is speculation and needs targeted research to be confirmed in Pinot noir.

The freeze treatment at 30 months bottle age had high tannin but no detectable anthocyanin, and this suggests the wine may have lacked sufficient anthocyanin to capitalise on the high remaining tannin concentration for complexing those two elements to create additional non-bleachable pigment. Co-pigmentation of anthocyanin has been proposed as an important contributor to colour in young red wines (Boulton, 2001) and has been associated with a 'bathochromic shift' to more 'young', blue-purple hue in wine (Boulton, 2001). The decline in non-bleachable pigment concentration observed in the extended maceration treatment wines from six to 30 months bottle age, and the maintenance of a relatively low hue value for those wines at 30 months bottle age would suggest some of the non-bleachable pigment may have dissociated and formed into anthocyanin co-pigmentation complexes. However the low anthocyanin concentration (not detected; Table 2) suggests these complexes were either in very low abundance or not able to be detected by the analytical method used in this study.

Maceration effects on wine pH

Red wine pH tends to increase as wine ages and free hydrogen ions are bound by phenolic compounds, SO_2 or in oxidation reactions. In the case of Pinot noir wine, a low pH confers mouthfeel freshness and length in wines that are low in tannin, whereas low pH tends to enhance the astringency and bitterness of tannin in wine. This means that a high tannin, low pH Pinot noir wine may be perceived by a consumer as out of balance (Peynaud cited in Kennedy et al., 2006).

Formal sensory appraisal would be required to judge the relative palatability of the tannin-pH value combinations that resulted at 30 months bottle age for the maceration treatments applied in this study (Table 2) however, the high tannin and moderate pH values for the freeze treatment wines suggest that wine may be perceived as unbalanced (overly astringent). Non-bleachable pigment formation has been reported to modify wine astringency (Kennedy, Saucier, & Glories, 2006), so the concentration of non-bleachable pigment in the freeze treatment wines at 30 months also supports the idea that these wines may be perceived as quite astringent. In contrast to the freeze treatment wines, the relatively low pH value for the enzyme treatment wines may confer mouthfeel length that this wine could be lacking due to its relatively low tannin concentration.

Industry implications

In practice, the decision to use a particular maceration process in winemaking will be guided by evaluation of the costs (eg. time, winery capacity, inputs) versus the benefits (eg. greater phenolic concentration in wine, more balanced phenolic profile, reduced risk). Four of the six maceration treatments examined in this study conferred greater phenolic concentration on resulting Pinot noir wines but each treatment had cost implications. The application of enzyme in winemaking was the lowest cost option in terms of inputs and use of fermenter tank (8 days) however, this treatment was shown to confer no phenolic advantage. The cold soak treatment used energy and cool store space but conferred greater non-bleachable pigment in aged wine. The freeze treatment was relatively high in energy cost and required access to freezer capacity and produced a highly tannic wine. This option may be appealing for winemakers seeking a small batch of highly tannic Pinot noir wine for blending. The extended maceration process consumed fermentation tank space for 12 days, and extended maceration wines risk of over-

extraction (particularly of seed tannin) (Joscelyne, 2009), wine oxidation, and contamination by the aerobic, volatile acid-producing *Acetobacter* bacterium. While the energy and capital expense of microwave maceration was high, the wines at 30 months showed the highest stable colour concentration of all wines, and moderate pH and tannin values, compared with freeze and extended maceration treatments. The microwave maceration treatment wines had finished fermentation after eight days skin contact time, representing a 'saving' of four days fermentation tank time, compared with extended maceration.

CONCLUSION

This study demonstrated that the use of different maceration processes is an important part of the Pinot noir winemaker's toolkit for managing extraction and stabilisation of phenolics in this somewhat challenging variety. The occasionally maligned cold soak maceration treatment was demonstrated to have value for long term colour stability, which may justify the use of this process in the face of time and fermenter space pressures during vintage. The freeze treatment results suggest this method may be effective for producing very tannic wines, which may offer winemakers a blending option during vintages where tannin is low in grapes or winery capacity constraints mean limited opportunity for on-skins time during alcoholic fermentation. Further research is needed to understand why extended maceration treatment wines declined in non-bleachable pigment concentration by 30 months bottle age. For example, whether the source of tannin (seed or skin) or tannin composition in the three highly extractive maceration treatments differed from each other, and if this difference contributed to the decline observed. For practical purposes, the decline in stable colour observed in extended maceration wines may not matter given those wines were significantly higher than control wines in stable colour at both bottle age time periods. An important point is that the 30 month bottle age results show relative

equivalence in non-bleachable pigment and tannin concentration for microwave and extended maceration treatments. This suggests that the novel maceration approach using microwave may offer a time-saving alternative to extended maceration winemaking, but at substantial capital cost for microwave equipment.

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Chapter 4. Microwave maceration of Pinot noir grape must: sanitation and extraction effects, and wine phenolics outcomes.

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There are no appendices associated with this chapter.

Microwave maceration of Pinot noir grape must: sanitation and extraction effects, and wine phenolics outcomes.

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Abstract

Pinot noir grapes have a unique phenolic profile which can impinge on the extraction and stabilisation of compounds such as anthocyanins and tannins which contribute to the colour and mouthfeel of red wine. This study examined the concentration of phenolic compounds in Pinot noir grape must and wine following application of a novel microwave maceration treatment for red grape must. Microwave maceration was shown to be highly effective for extraction of phenolics from grape solids into grape juice. When juices were fermented to wine, UV-Visible spectrophotometry showed microwave maceration was associated with significantly higher concentrations of total phenolics, anthocyanin, tannin and pigmented tannin in wine at 18 months' bottle age, compared with control wine. Mean tannin concentration in microwave treatment wine was 0.60 g/L at 18 months, compared with 0.14 g/L in control wine. The microwave treatment was also associated with a substantial and rapid decrease in the grape-associated yeast population, compared with control maceration, and a shorter lag phase at the outset of alcoholic fermentation. Based on this study, microwave maceration warrants further investigation as a potential industrial-scale application in red winemaking.

Keywords

tannin, anthocyanin, phenolics, extraction, maceration, pasteurisation

Abbreviations

AU – absorbance units

AF – alcoholic fermentation

cfu – colony forming units

ctl – control

mwv – microwave

PCA – principal component analysis

PC1 – principle component 1

PC2 – principal component 2

SE – standard error

WLN – Wallerstein Laboratories Nutrient agar

1. Introduction

Alcoholic fermentation of red wine generally involves the partial crushing of grapes followed by yeast-mediated transformation of grape sugars and other compounds into ethanol and other secondary metabolites. Colour and mouthfeel in finished red wine are strongly influenced by phenolics (eg. tannins, anthocyanins) which are extracted from grape skins, pulp and seeds during 'contact time' which coincides with the alcoholic ferment. Phenolic extraction based on passive diffusion during contact time is slow, typically lasting five to ten days. This consumes winery tank space thereby limiting winery throughput. There are also costs associated with

management of the must during contact time (eg. application of sulphite to inhibit mould growth; staff time, energy and equipment to plunge the pomace cap). Rapid extraction of phenolics could improve the efficiency of winery operations.

Compared with other red wine grape varieties, Pinot noir is considered difficult to vinify because of its unique phenolic characteristics. Pinot noir grapes have low anthocyanin content, all of which is in the less-stable non-acylated form (Heazlewood et al., 2006; Mazza et al., 1999). Tannin is important for stabilizing colour in Pinot noir wine but Pinot noir grapes have a low skin-to-seed tannin ratio compared with other red wine grape varieties (Mattivi et al., 2009) and translation of grape tannin to wine tannin has been shown to be low and quite variable for Pinot noir compared with other red wine varieties (Harbertson et al., 2002).

Six maceration practices have been identified as effective for optimising phenolic extraction in red wine making (Sacchi et al., 2005) and two of these are commonly used by Pinot noir winemakers: extended maceration and high peak fermentation temperature (Haeger, 2008; Joscelyne, 2009). During extended maceration, wine is held in contact with pomace for an extended period, beyond the completion of alcoholic fermentation. The disadvantages of extended maceration include: over-extraction, particularly of seed tannin (Joscelyne, 2009); wine oxidation or contamination by aerobic spoilagee microorganisms; and reduction in effective winery capacity through extended use of fermenter space. High peak fermentation temperature (>30°C) has been shown to be effective for phenolic extraction but can volatilise compounds that contribute 'Pinot-like' aroma characteristics to finished wine (eg. plum, cherry). The disadvantages of extended maceration and high peak fermentation temperature suggest the need for more effective, rapid methods for phenolic extraction from Pinot noir grape must. Heat

treatment of red grape must (thermovinification) was identified as being effective for phenolic extraction (Clare et al., 2004; Netzel et al., 2003; Sacchi et al., 2005) but is not widely used for New World (eg. USA, Australia, South Africa) winemaking (Haeger, 2008; Halliday and Johnson, 2007). Heat treatment as a pasteurisation process is used for white grape musts (Rankine, 2004), but must pasteurisation has not been examined in the context of red winemaking. Rather, sulphur dioxide (SO_2) is commonly added to must at crushing to reduce the risk of oxidation and for inhibition of grape-associated microflora (Ugliano and Henschke, 2009). The wine yeast *Saccharomyces cerevisiae*, however, is susceptible to SO_2 and the additive has been shown to slow the onset of log phase fermentation by inoculated *S.cerevisiae* (Cocolin and Mills, 2003). Limiting early SO_2 addition also offers winemakers greater latitude for control of post-fermentation spoilage organisms like SO_2 resistant *Brettanomyces* (Curtin et al., 2012), excess residual SO_2 can inhibit malolactic fermentation (Wells and Osborne, 2011), and the addition of SO_2 to wine is unappealing to some wine consumers (Jackowetz and de Orduna, 2012). For these reasons, there is value in examining thermal maceration approaches that might offset the need for SO_2 addition.

Microwave-assisted extraction has been shown to be a time and energy efficient option for extraction of plant compounds and for food pasteurisation or sterilisation, but has not been examined for red wine making. Microwave has been demonstrated to be efficient in terms of both *rate* and *effectiveness* of extraction for a range of plant compounds (Casazza et al., 2010; Liu et al., 2009; Routray and Orsat, 2012; Trendafilova and Todorova, 2008). Microwave has also been shown as effective for pasteurisation or sterilisation of various foods, beverages and other items (Mima et al., 2008; Salazar-González et al., 2012; Tajchakavit et al., 1998), although

uneven heat distribution remains a problem for microwave sterilisation of multi-phase solutions (Kumar et al., 2007; Vadivambal and Jayas, 2010).

The objective of the study reported here was to evaluate a novel maceration treatment for Pinot noir winemaking: microwave maceration. Based on current use of microwave in other applications, investigation of microwave for thermal treatment of grape must offers two possibilities as an alternative to standard maceration: increased phenolic extraction from pomace during the primary ferment, and the potential for SO_2-substitution at crushing via heat-mediated suppression of grape-associated microflora in grape must (eg. pasteurisation, sterilisation). The study compared phenolic and colour outcomes from control and microwave maceration treatments at the outset of an seven-day Pinot noir primary ferment (inoculation) and following 6 and 18 months of bottle aging. The impact on the grape-associated yeast population in the microwave and control treatments was also compared and fermentation kinetics were monitored.

2. Methods

2.1 Microvinification

Pinot noir grapes were harvested from a vineyard in Northern Tasmania, Australia at 12.5°Baume. Approximately 1 kg of grape bunches was randomly allocated to one of the two treatments (control, microwave), with four replicates per treatment (n=8). For each replicate, grapes were crushed using a custom-made bench-top crusher and de-stemmed by hand and the must decanted to a 1.5 L Bodum™ 'Kenya' plunger coffee pot. The use of coffee plungers facilitates small-scale submerged cap, red wine alcoholic fermentation (AF) with high reproducibility and efficient phenolic extraction (Dambergs and Sparrow, 2011; Dambergs et al.,

2012b). To each must 50 mg/L sulphur dioxide (SO_2) was added, in the form of potassium metabisulfite solution and control treatment pots were moved to a 27°C (±3°C) constant temperature room. Must in the microwave treatment pots was microwave macerated (see 2.2) after SO_2 addition. Three hours after maceration treatment, each pot was inoculated with active dried yeast strain EC1118 (supplied by Lallemand Australia Pty Ltd) that had been rehydrated according to the manufacturer's instructions. All pots were incubated at 27°C (±3°C) and fermented for seven days. Pots were weighed regularly over the course of the ferment to track CO_2 loss as an indicator of fermentation kinetics. At day two of the ferment, 60 mg/L of yeast assimilable nitrogen was added to each pot in the form of diammonium phosphate solution. Pots were pressed off at day seven, racked into bottles and incubated at 27°C (±3°C) for 12 hours to ensure completion of AF. Wines were cold settled for two weeks at 4°C and tested for residual sugar using Dextrocheck™ tablets; all eight wines were found to be dry (≤2.5 g/L residual sugar). Wine was racked under CO_2 cover and stabilised by the addition of 80mg/L sulphur dioxide (SO_2) in the form of potassium metabisulfite solution. After two weeks, wines were bottled under CO_2 cover to 100 mL and 28 mL amber glassware. For each replicate, four 28 mL bottles of wine were stored for bottle aging analysis, allowing a fresh bottle of wine to be opened for analysis at each time period.

2.2 Microwave maceration

The microwave treatment pots were microwave macerated immediately after the crush and de-stem operation using a domestic 1150W Sharp™ 'Carousel' R-480E microwave oven. Each pot was microwaved at full wattage for three time increments with intervening stirring and temperature evaluation of must using a solid stem thermometer. The three increments were: 2

minutes, 1 minute, and 15-40 seconds (final increment was varied according to previous temperature reading). Each pot reached a peak temperature of 70-71°C and was held at this temperature by means of a thermal blanket for 10 minutes. Pots were then placed in an ice bath and stirred periodically, with must temperature in each pot reaching 30°C in approximately 30 minutes. The microwave treatment pots were then moved to the 27°C (±3°C) constant temperature room for sampling, yeast inoculation and fermentation (as per section 2.1).

2.3 Yeast counts

The impact of SO_2 addition (control) and microwave maceration on grape-associated yeast populations was evaluated in a separate trial. Four batches of Pinot noir grapes were dosed with SO_2 and two of the batches were microwave macerated. Must from each batch was sampled for total grape-associated yeast population at three time intervals: prior to SO_2 dosing and microwave maceration, and 1.5 hours and 3.0 hours post-maceration (n=12). Yeast population sampling finished at 3.0 hours, just prior to the time that must would generally be inoculated for fermentation (eg. as per section 2.1). At each time interval, a 1 mL sample of must from each pot was serially diluted in sterile 0.01% bacteriological peptone water (Oxoid LP0037) and 0.1 mL aliquots were spread plated in duplicate on Wallerstein Laboratory Nutrient (WLN) agar (Oxoid CM0309). WLN plates were incubated at 24°C for four days and then colonies were counted for those dilutions that yielded between 30 and 300 colonies per plate (Fugelsang and Edwards, 2007). Yeast colony counts were averaged to attain an estimate of colony forming units per millilitre of grape must (cfu/mL) for each treatment at each sampling time (pre-maceration, 1.5 hours, 3.0 hours).

2.4 UV-Visible spectrophotometry

Research is continuing into methods to quantify phenolic compounds in red wine (Cozzolino et al., 2008; Dambergs et al., 2011; Dambergs et al., 2012a; Harbertson et al., 2003; Herderich et al., 2006; Mercurio et al., 2007; Sarneckis et al., 2006; Seddon and Downey, 2008; Skogerson et al., 2007; Versari et al., 2008). Until recently, available methods tended to favour particular phenolic compounds. For example, a comparison of three analytical methods for grape skin tannin (protein precipitation, methyl cellulose precipitation, HPLC) concluded that each method measured a different tannin fraction (Seddon and Downey, 2008). Spectrophotometric methods have been shown to correlate well with a range of more complex, targeted methods for quantifying a range of red wine phenolics. A comparison of five current methods for analysis of red wine colour compounds found good correlation (R^2=0.992) between HPLC and the original Somers method for quantifying red wine polymeric pigment (Versari et al., 2008). In a different study, the methylcellulose precipitation method for wine tannin was shown to correlate well with the reverse-phase HPLC method (Sarneckis et al., 2006). The modified Somers method employed in this study was validated against HPLC and methyl cellulose precipitate methods, and showed a strong correlation with those methods (Mercurio et al., 2007)

A UV-visible spectrophotometric method coupled with a chemometric calculator was employed in this study for quantification of total tannin. This method had been calibrated and validated against the methylcellulose precipitation method for tannin using 392 red wines, of varying age, variety and origin, and found to be robust for quantifying tannin in fermenting and finished red wine (Dambergs et al., 2012a).

All eight wines were analysed for phenolics and colour at three time periods: inoculation, 6 months in bottle and 18 months in bottle (n=24). Wine samples were evaluated for absorbance in three buffers.

HCl buffer – 200 μL of sample pipetted to 10 mL of 1M hydrochloric acid in a 10 mL centrifuge tube, capped, mixed and left for three hours in the dark.

Acetaldehyde buffer – 330 μL of sample was pipetted to 3.0 mL acetaldehyde buffer (12% v/v ethanol solution saturated with potassium hydrogen-tartrate + 0.1% acetaldehyde) in a 10 mL centrifuge tube. The tube was capped, mixed and left for one hour in the dark.

Metabisulphite buffer – 330 μL of sample was pipetted to 3.0 mL metabisulphate buffer (12% v/v ethanol solution saturated with potassium hydrogen-tartrate + 0.375% sodium metabisulphite) in a 10 mL centrifuge tube. The tube was capped, mixed and left for one hour in the dark.

UV-Visible spectrophotometric analysis was conducted using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Samples were scanned in 10 mm quartz cuvettes, at 2 nm intervals for the wavelength range 200-600 nm against a buffer 'blank'. Resulting absorbance data for each sample was exported as ASCII files to Excel 2007 spreadsheets for subsequent data analysis.

2.5 Spectral data analysis

Spectral data from analysis of samples in 1 M HCl was imported from Excel 2007 files into *The Unscrambler* (Camo, Norway) for Principal Component Analysis (PCA). The NIPALS algorithm was used for PCA, with mean centering of data and "one-out" cross validation. PCA scores plots for absorbance between 220 and 590 nm were generated in *The Unscrambler*. *The Unscrambler* was also used for Linear Discriminant Analysis to generate confusion matrices for

must at inoculation and wines at bottling, 6 months and 18 months bottle age and provided a quantitative measure of clustering.

The 1 M HCl spectra were used to calculate total tannin and total phenolics using absorbance values at 250, 270, 280, 290, 315 and 520 nm (Dambergs et al., 2011; Dambergs et al., 2012a; Mercurio et al., 2007). Total phenolics in red wine consist predominately of coloured and non-coloured tannin and anthocyanin, plus low molecular weight, non-pigmented phenolic compounds. Total tannin includes both pigmented and non-pigmented tannin. Absorbances at 520 nm in 1 M HCl and in metabisulphite buffer were used to calculate free anthocyanin (Mercurio et al, 2007). Free anthocyanin is a measure of unbound or copigmented anthocyanin, and represents the unstable form of colour in young red wines. Absorbance at 520 nm in metabisulphite buffer provided a measure of pigmented tannin in wine samples (Mercurio et al, 2007). Pigmented tannin results from complexing of anthocyanins and tannins, has mouthfeel effects in red wine, and is presented here as an indicator of stable colour. The colour qualities of the wines – colour density and hue – were quantified using absorbance in acetaldehyde buffer at 420 and 520 nm. Colour density describes the degree of pigment saturation of the wine and hue indicates the nature of red wine colour with higher hue values being more ruby or garnet and lower hue values appearing more blue-purple (Somers and Evans, 1977).

2.6 Statistical analysis

Mean and standard deviation for microbial sanitation, fermentation kinetics and phenolic and colour measures were calculated in Excel 2007. Microbial sanitation differences were examined using paired two-tailed Student's T-test (Excel 2007). Two-factor ANOVA in the statistics package R (version 2.15.1) was used to establish whether there were significant differences

between treatments within and between sampling periods for phenolic and colour measures, and Tukey's test in R was used for post-hoc analysis. The results reported in this paper were from a single trial, however consistent results were observed in six additional independent replicated trials conducted by the researchers during the 2011 and 2012 vintages (Carew, unpublished data).

3. Results and Discussion

The aims of the study were to evaluate phenolic composition and colour differences resulting from microwave versus standard maceration of Pinot noir must. Additionally, microwave maceration was compared with standard maceration to establish its efficacy for sanitising grape must of grape-associated yeast, compared with the widely used practice of SO_2 addition.

3.1 Sanitation effects

Must samples were plated to WLN agar and Table 1 shows yeast population counts pre-maceration and at 1.5 and 3.0 hours post-maceration for control and microwave treatments. Paired two-tail Student's t-test showed no significant difference between mean yeast counts for the control and microwave treatments prior to maceration (P=0.96). While the result was not statistically verifiable due to the detection limit of the assay, at 1.5 hours after maceration, mean yeast count for the control appeared substantially different to the mean yeast population in the microwave treatment. Microwave maceration was associated with a yeast population decline of greater than three orders of magnitude. Three hours after maceration, the grape-associated yeast population in the control treatment had declined, but still appeared higher than microwave treatment yeast counts.

Table 1. Total yeast counts on WLN agar for control and microwave macerated Pinot noir must at three time intervals after maceration. NB: detection limit of assay was 100cfu/mL.

	Control - cfu/mL (SE)	Microwave - cfu/mL (SE)
pre-maceration	$3.0x10^5(3.7x10^4)$	$3.3x10^5$ $(8.5x10^4)$
1.5 hours post-maceration	$8.2x10^4$ $(1.0x10^4)$	<100
3.0 hours post-maceration	$2.1x10^4$ $(3.1x10^3)$	<100

The total yeast count results showed that microwave maceration was substantially more effective than SO_2 treatment for suppressing grape-associated yeast flora in grape must. In winemaking, the use of SO_2 to achieve around a two log reduction in grape-associated yeast flora to ~10^4 cfu/mL was shown to be sufficient sanitation to allow inoculated yeast to dominate a wine ferment (Cavazza et al., 2011). From that perspective, the yeast count of less than 100 cfu/mL in must that had been microwaved represented very effective must sanitation and was a novel outcome from the point of view of winemaking. Due to its role as an antioxidant, SO_2 addition is unlikely to be replaced by microwave maceration, however, microwaving may offer winemakers the option of reducing their use of SO_2 during crushing. Reduced SO_2 addition at crushing could alleviate the lag phase following inoculation that is commonly seen in commercial ferments. An effectively sanitised microwaved must could improve winemakers' success in inoculation and earlier initiation of AF with a preferred fermentation strain.

Research from food and water science into microwave microbial sterilisation has shown microwave to be highly effective for fluid foods and may offer effects beyond standard thermal treatment (Salazar-González et al., 2012). The mechanism of microwave sterilisation appears to be microbial cellular disruption which may result from thermal and so called 'enhanced-thermal' effects (Hong et al., 2004; Tajchakavit et al., 1998), and possibly from electrical disruption to microbial cell membrane function. Microwave might support novel research into yeast strain effects in winemaking as an alternative to currently used chemical or physical sterilisation

techniques like dimethyldicarbonate dosing (Cavazza et al., 2011) and high hydrostatic pressure processing (Takush and Osborne, 2011). An additional opportunity offered by microwave maceration is possible inactivation of heat labile enzymes in grape must. Microwaving has proven more effective than thermal processing for enzyme inactivation in other food products (Keying et al., 2009; Matsui et al., 2008; Salazar-González et al., 2012), and the rapidity of heating by microwave may alleviate the problem of enzyme stimulation during thermovinification when a must transitions through 45-50°C (Rankine, 2004).

3.2 Fermentation kinetics

Weight loss due to evolution of CO_2 during alcoholic fermentation was monitored as an indicator of fermentation kinetics. Figure 1 shows that the control ferment followed a fermentation profile typically observed for wine (Peynaud, 1984), and that both treatments were 'dry' on the seventh day of the ferment with mean percentage weight loss of approximately 6%. Notable in Figure 1 is a shorter lag phase associated with the microwave treatment. The cause of shorter lag phase remains speculative (eg. early liberation of grape nutrients, lack of SO_2 inhibition of yeast activity). A short lag phase, however, implies rapid consumption by inoculated yeast of available oxygen, and this would be expected to limit the proliferation of detrimental aerobic microbes during the initiation of the alcoholic ferment.

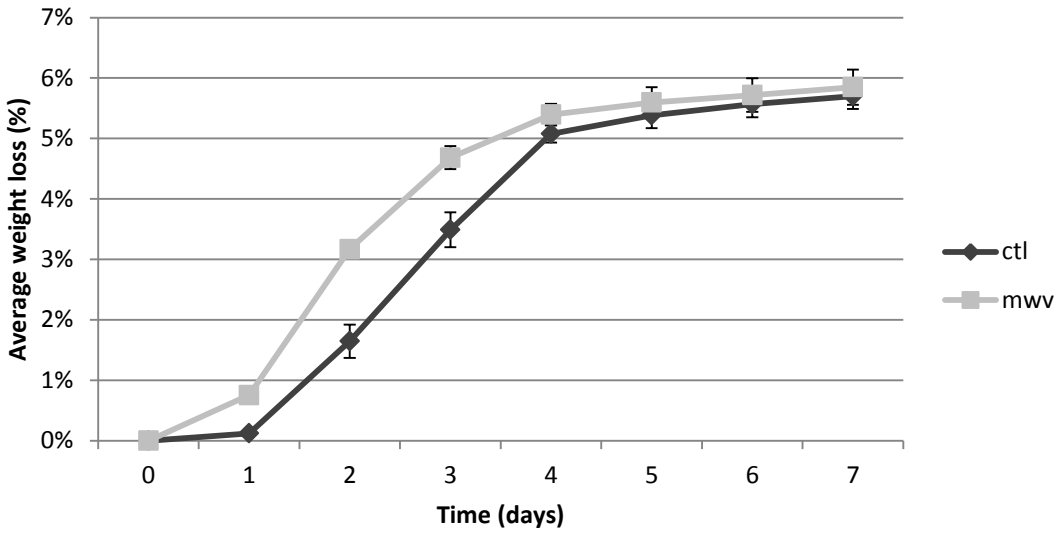

Figure 1. Alcoholic fermentation kinetics as indicated by mean pot weight loss for control (ctl) and microwave-macerated (mwv) Pinot noir must (SE).

3.3 Spectral, phenolic and colour analysis of musts

In the spectral fingerprint of wine, the UV-Visible spectral region is dominated by phenolic compounds such as tannin and anthocyanins (Dambergs et al., 2012a; Mercurio et al., 2010). Principal component analysis (PCA) of the UV-Visible spectra for must at inoculation showed treatment effects associated with the musts' phenolic profiles. Principal component analysis of musts at inoculation, showed clear separation between control and microwave treatments, and demonstrated that microwave maceration followed by 10 minutes hold time at 70°C was associated with distinct phenolic extraction outcomes. Linear discriminant analysis (LDA) for spectral data at inoculation correctly placed all eight samples in their respective treatments, which provided a quantitative confirmation of the clustering apparent in PCA plots (data not shown). Separation between treatments was explained by the first principal component (PC1 = 100%) and loadings analysis of PC1 showed that replicate positioning on this axis was strongly

influenced by absorbance at wavelengths which have been associated with red wine relevant phenolics (eg. 280 nm, 520 nm, 314 nm) (Dambergs et al., 2012a; Mercurio et al., 2010). The loadings analysis indicated there was greater extraction of phenolic compounds in microwave maceration replicates than control replicates.

Quantification of colour and phenolic indicators in must based on absorbance at specific wavelengths showed microwave maceration was associated with significantly higher mean concentrations of total phenolics and anthocyanin, and significant difference in mean colour density and hue, compared with control must (Table 2). Statistical analysis of difference was not possible in the case of tannin concentration in musts because tannin concentration in the control replicates was below the detection limit for the assay. The difference in mean concentration of pigmented tannin between control and microwave treatment musts was non-significant, which was unsurprising given that the formation of pigmented tannin has been associated with bottle aging of red wine (Somers, 1966).

Table 2. Colour and phenolic indicators for control and microwave-macerated Pinot noir must immediately prior to inoculation for alcoholic fermentation (±SD). Different lowercase letters indicate significant differences among samples (Tukey's Test $p < 0.05$). ND = not detected, below assay detection limit (0.05 g/L).

	Control	Microwave
Total Phenolics (AU)	6.7 (±0.52) **a**	22.3 (±8.39) **b**
Anthocyanin (mg/L)	19 (±5.48) **a**	250 (±146) **b**
Tannin (g/L)	ND	0.06 (±0.24)
Pigmented tannin (AU)	0.11 (±0.005)	0.25 (±0.23)
Colour density (AU)	0.48 (±0.02) **a**	2.69 (±0.79) **b**
Hue	1.25 (±0.10) **a**	0.65 (±0.23) **b**

The efficient and effective early extraction of phenolics into must from microwave maceration showed that this technology may have promise as a novel maceration approach for Pinot noir winemaking. Recent research into microwave extraction compared with thermal extraction of compounds from plant materials has shown microwave to be effective and outperform equivalent-temperature thermal extraction (Mandal et al., 2009; Routray and Orsat, 2012). The effectiveness of microwave, particularly in multi-phase or non-mixed fluids, is due to generation of volumetric heat within materials, as opposed to conventional heating which relies on heat conduction from external surfaces (Vadivambal and Jayas, 2010). The heating of foods by microwave has been successfully modeled using Maxwell's equations which are based on the properties of the microwave system, the interface between the microwave cavity space and material to be heated, and the dielectric properties of the material to be heated (Campañone et al., 2012; Routray and Orsat, 2012). Mass transfer of compounds in phytoextraction has been modeled using Fick's second law (Gekas et al., 2002; Ziaedini et al., 2010), which assumes time and temperature-driven diffusion as the main mechanism of mass transfer. Routray and Orsat (2012), however, identified three additional variables which may influence mass transfer in microwave extraction; dielectric properties of the solvent, microwave power level, and solvent-plant material contact surface area. Modeling the relationship between microwave heating and the extraction of phenolic compounds from grape solids into grape juice would require integration of these factors with Maxwell's and Fick's models.

There is a good deal of debate as to how microwave phytoextraction works. Several mechanisms have been proposed, for example: synergistic mass and heat transfer (Mandal et al., 2009); simplified mass transfer (Spigno and De Faveri, 2009) and change in dielectric properties and internal pressure gradients resulting from intra-cellular phase change (Dincov et al., 2004). It is

possible that all of these mechanisms could be contributing to the effects observed for Pinot noir must in this study. Additionally, release of phenolics in microwave maceration may have been influenced by heat-mediated electrical disruption to plant cellular components, for example, disruption of cell wall proteins; degradation of hydrogen bonding within cell membranes; and distortion of intra- and inter-cellular pores which mediate semi-permeability (Gekas et al., 2002). Regardless of the mechanism of extraction, microwave maceration resulted in strong phenolic and colour extraction into must. This would justify further investigation of microwave maceration for rapid and effective phenolic extraction from Pinot noir grape must, and possibly other red grape varieties.

3.4 Spectral, phenolic and colour analysis of wines

Spectral analysis of control and microwave wines after 6 and 18 months' bottle aging showed that the distinct phenolic effects observed in musts were maintained throughout bottle aging. Linear Discriminant Analysis offered quantitative confirmation of clustering; all four replicates were correctly allocated for each treatment at both time periods. The PCA scores plots for wines at 6 months and 18 months both showed separation that was strongly associated with PC1 and loadings analysis of wines at 6 months and 18 months indicated that microwave wines showed greater absorbance at those wavelengths that are associated with red wine phenolics (data not shown).

Quantification of specific wine phenolic and colour indicators in wine at 6 and 18 months bottle age revealed substantial and enduring effects associated with microwave maceration of Pinot noir must.

Table 3. Wine phenolic indicators at 6 months and 18 months bottle age for control and microwave macerated Pinot noir wines (±SE). Different lowercase letters indicate significant differences among samples (Tukey's Test $p < 0.05$).

	6 months		18 months	
	Control	Microwave	Control	Microwave
Total phenolics (AU)	28.2±2.26 **a**	41.2±1.47 **b**	22.2±1.65 **c**	34.3±1.62 **d**
Total pigment (AU)	7.7±1.50 **a**	13.9±0.74 **b**	2.0±0.23 **c**	5.7±0.99 **d**
Anthocyanin (mg/L)	133±29 **a**	252±19 **b**	8±3 **c**	68±35 **d**
Tannin (g/L)	0.18±0.06 **a**	0.70±0.10 **b**	0.14±0.04 **a**	0.60±0.05 **c**
Pigmented tannin (AU)	0.60±0.05 **a**	0.79±0.14 **ab**	0.95±0.10 **bc**	1.35±0.45 **c**
Colour density (AU)	2.91±0.31 **a**	4.31±0.39 **b**	3.10±0.36 **a**	4.56±0.64 **b**
Hue	0.86±0.03 **a**	0.75±0.06 **a**	1.32±0.05 **b**	0.82±0.07 **a**

There was a significant difference in mean total phenolic concentration attributable to maceration treatment (P=0) and time period (P=0), but no interaction between maceration and time (P=0.62). Microwave macerated wines were around 20 AU higher in total phenolics than control wines, and total phenolic concentration for both wines decreased by around 6 AU during bottle aging (Table 3).

Table 3 shows wine anthocyanin concentration followed a similar pattern to total phenolics with maceration and time period both highly significant factors (maceration P=0; time P=0). Microwave maceration generated wine with twice the anthocyanin concentration of control at 6 months bottle age, and six times the concentration of control wine at 18 months bottle age. An interaction was observed between maceration and time period (P=0.04) with the concentration of anthocyanin in microwave macerated wine declining at a more rapid rate than control wine although microwave wine started this decline from a comparatively high concentration.

Mean total tannin in wines was strongly affected by maceration (P=0) with microwave wine tannin concentration at 6 and 18 months nearly 0.5 g/L higher than control wine (Table 3). This represented a four-fold increase in wine tannin attributable to microwave maceration. Tannin concentration declined significantly over the bottle aging time period in the microwave treatment

(P=0.01) but there was no interaction between maceration treatments and time for tannin concentration (P=0.1) which suggests wine tannins had aged normally, regardless of maceration treatment.

The development of stable colour, in the form of pigmented tannin, was not significantly affected by maceration treatment (P=0.03) and there was no interaction between the two factors (P=0.38), but time in bottle was significant (P=0.01). At 6 months bottle age, mean pigmented tannin concentration in control and microwave wines was around 0.7 AU and at 18 months, the increase in pigmented tannin concentration that was observed for both control and microwave wines was found to be significant (Table 3). Table 3 shows that microwave treated wines were around 0.6 AU higher in pigmented tannin at 18 months compared with 6 months, and control wines were around 0.4 AU higher at 18 months compared with 6 months bottle age.

Maceration method had a significant impact on mean colour density (P=0) with microwave wines higher in colour density than control wines at both time periods tested. There was no interaction between maceration method and bottle aging period on colour density (P=0.9), and no change in mean colour density over time for either treatment (P=0.32) with microwave maceration wines maintaining a high colour density out to 18 months' bottle age (Table 3).

Analysis of wine hue suggested microwave maceration may have conferred resistance to oxidation. Table 3 shows no hue difference between treatments at six months but a significant increase in hue value for control wine at 18 months. Control wine replicates at 18 months all appeared to have an oxidative aroma and were coloured tawny brown, compared with microwave wine replicates which appeared to have a more purple colour and wine-like aroma at 18 months.

These observations are consistent with the hue values measured, with a lower hue representing purple colours and a higher hue brown and garnet colours.

Spectral, phenolic and colour analyses of wines showed microwave maceration was associated with significantly higher colour density and significantly higher concentration of total phenolics, anthocyanin and tannin in Pinot noir wines at 6 months bottle age, compared with control wines. These early differences were stable and translated into aged microwave wines at 18 months with strong phenolic profile and appealing colour.

The loss of visible colour in Pinot noir wine can detract from its visual appeal and, therefore, marketability. In this study, we documented a decline in total phenolics and anthocyanin concentration during bottle aging of Pinot noir wines. A decline in anthocyanin is typical of red wine aging (Monagas et al., 2006) and has been attributed to stabilisation of anthocyanins through complexing with tannins to form stable pigmented tannins (Somers, 1966) and anthocyanin oxidation. By 18 months bottle age, early differences in anthocyanin and tannin concentration observed in the microwave macerated wine appeared to have translated into high pigmented tannin concentration in control and microwave wines. The pigmented tannin concentration of microwave wines was 33% higher at 18 months bottle age than at 6 months bottle age. This is an important outcome as pigmented tannin contributes to the visual appeal, sensory qualities and marketability of red wine.

Tannin lends astringency and palate length to red wine. Microwave maceration was associated with significantly higher wine tannin concentration at the two bottle age periods investigated, compared with control wine. Tannin extraction can be particularly challenging for Pinot noir winemakers and current practices either extend time in tank (eg. extended maceration, cold soak)

or risk volatilisation of aromatic compounds (high fermentation temperature in late AF). The results reported here suggest microwave maceration could reduce or eliminate the need for current Pinot noir tannin optimization methods which can increase production costs, reduce winery throughput or risk wine spoilage. One concern, given the high tannin concentration observed in microwave macerated wines, is that of over extraction of tannins. Too high a concentration of tannin in Pinot noir can impart bitterness and over-astringency to wine, but informal tasting of the microwave and control wines by one experienced winemaker and one wine judge found both wines to be free of faults, and the microwave wines to have a fuller, softer mouth-feel and greater palate length than the control wines. In future, greater volumes of wine will be produced to allow for formal sensory analysis.

4. Conclusion

The results presented here support the contention that microwaving Pinot noir grape must has potential to solve several problems currently faced by winemakers working with this expensive grape variety. Microwaving Pinot noir must was highly effective for sanitization, potentially reducing the need for sulfur dioxide addition at crushing. A truncated AF lag phase was observed which could speed winery operations and reduce the opportunity for colonization of musts by aerobic spoilage microorganisms. Microwave wines were higher in total phenolic, anthocyanin and tannin concentration, and showed high colour density, compared with control wines. Particularly significant for Pinot noir winemaking was the finding that microwave maceration resulted in a four-fold increase in wine tannin that was stable to 18 months' bottle age.

Further research into adjusting microwave duration or hold time may allow for more control over extraction of phenolic compounds. The high phenolic extraction observed, particularly tannin, from microwave maceration may open the way for pressing microwaved must off early (ie. immediately after hold time) and conserving winery tank space through elimination of pomace (~40% of total must volume). It would also be useful to investigate how effectively existing heat and mass transfer models (eg. Maxwell's equations; Fick's second law) explain the observed heating and extraction outcomes, and development of the technology would benefit from greater insight into the mode of action of microwave treatment. For example, discriminating thermal effects from microwave-specific effects ('non-thermal' or 'enhanced thermal'), and clarifying cellular disruption mechanisms and their correlation with phenolic extraction and must sanitation effects. Testing of continuous microwave treatment (as opposed to incremental heating and stirring to achieve target temperature) would provide information on the sensitivity of must to uneven temperature distribution and could assist future upscaling to industrial microwave systems compatible with continuous processing, which is common in industrial-scale winemaking.

Microwave maceration for red wine making requires validation on a larger scale, but the dramatic results reported in this study offer encouragement for a paradigm shift in red winemaking methodology to include microwave maceration.

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Chapter 5. Microwave maceration and early press off improve phenolic extraction and fermentation kinetics in Pinot noir winemaking

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Appendix C is a draft of Methods and Results sections for a manuscript describing the effect of extended hold time after microwave maceration on phenolics diffusion in must, and wine resulting from 3 and 6 hour must hold times. This draft will be prepared for publication as a journal paper. Appendix D is a Powerpoint™ presentation delivered to the Australian Wine Research Institute on 30th May, 2012 which provided an overview of the PhD research including comparison of heat and microwave maceration outcomes.

Microwave maceration with early press off improves phenolics and fermentation kinetics in Pinot noir

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ABSTRACT

Microwave maceration with early press off was applied to Pinot noir must and produced wine with faster fermentation kinetics and significantly higher yeast assimilable nitrogen than control must, and 10% greater juice yield than heat macerated must. UV-visible spectrophotometry showed microwave maceration wine as equivalent to, or greater than, control wine for: total pigment, anthocyanin, tannin and non-bleachable pigment concentration. Microwave maceration was compared with heat maceration, and phenolic differences apparent in juice were not sustained in bottle-aged wines. Histological examination of grape skins showed more substantial intra-cellular damage in microwave macerated skins than heat macerated and control skins. The results suggest microwave maceration with early press off warrants serious consideration as an efficient production process for phenolic-rich Pinot noir wine.

INTERPRETIVE ABSTRACT

Microwave maceration of red grape must is a new process for rapid extraction of phenolic compounds. Previous research showed microwave maceration extracted phenolics effectively so that must could potentially be pressed off prior to inoculation for alcoholic fermentation (AF). Pinot noir wines from microwave maceration with early press off were compared to control wines fermented on pomace for seven days; resulting wines were comparable in phenolic concentration. Microwave maceration with early press off wines were compared to heat maceration with early press off wines; some phenolic differences were observed between juices from those treatments, but differences were not sustained with bottle age. Differences were observed between microwave and heat maceration for juice yield, intra-cellular damage of grape

skins and fermentation kinetics. The results suggest microwave maceration with early press off may offer important process efficiencies for red wine making.

KEYWORDS

thermovinification, red wine, grape histology, phenolics

INTRODUCTION

Pinot noir winemaking can be challenging as this variety is typically low in anthocyanin (Mazza et al. 1999) and has an unusual tannin distribution (Kennedy 2008). Anthocyanin concentration was examined in 173 commercial red wines representing four varieties and seven vintages; Pinot noir anthocyanin concentration was 60 mg/L, Merlot 110 mg/L and Cabernet Sauvignon 125 mg/L (Cliff et al. 2007). Many Pinot noir winemakers apply a pre-ferment 'cold soak' to aid anthocyanin extraction (Haeger 2008, Joscelyne 2009). Cold soak however, reduces effective winery throughput which adds to production costs. The anthocyanin in Pinot noir grapes is also problematic; all Pinot noir anthocyanin is of the less stable 'non-acylated' form (Mazza et al. 1999, Heazlewood et al. 2006). Stable color formation is achieved through reaction between anthocyanin and tannins to form polyphenols (eg. pigmented tannins) (Hayasaka and Kennedy 2003) which means timely and adequate anthocyanin and tannin extraction are important for visually appealing Pinot noir wine.

Pinot noir wines can be low in tannin (Harbertson et al. 2008). Mean tannin concentration was examined in 1325 red wines and Pinot noir, at 348 mg/L catechin equivalents, was significantly lower than the five other red varieties examined (Harbertson et al. 2008). An unusually high

proportion of Pinot noir tannin is found in the seed but seed tannins tend to extract slowly and mostly in the latter half of the AF (Kennedy, 2008). Consequently, many winemakers choose to extend pomace contact time after AF (Haeger 2008, Joscelyne 2009). Extended maceration adds to production costs through use of fermenter space, need for anaerobic tank conditions and increased risk of wine oxidation (Ribereau-Gayon et al. 2006b).

Thermovinification can be effective for phenolic extraction (Sacchi et al. 2005, Atanackovic et al. 2012). Microwave maceration is a novel thermovinification method which resulted in higher phenolic concentration in finished Pinot noir wines, compared with wines from a control treatment (Carew et al. 2013). The mechanisms of grape phenolic extraction from microwave maceration have not yet been explained and it is currently uncertain whether extraction effects differ from traditional heat-mediated thermovinification. Analysis of phenolic concentration in Pinot noir juice immediately after microwave maceration showed equivalent phenolic extraction to seven days AF on skins (Carew et al. 2013) and suggested 'early press off' as an option for Pinot noir winemaking. 'Early press off' entails microwave maceration then pressing juice off pomace prior to inoculation for AF. Early press off wine has approximately three hours total skin contact time.

The aim of this study was to compare microwave maceration with early press off, with control and heat maceration winemaking.

MATERIALS & METHODS

Microvinification

Two parcels of Pinot noir grapes were harvested in April 2012 from two blocks at a vineyard in Northern Tasmania, Australia. Trial A grapes were clone G5V15 harvested at 22.3 Brix (4488-

E08 hand-held digital refractometer, Atago, Tokyo, Japan), pH 3.15 and TA 8.48 (pH-matic 23, Crison, Barcelona, Spain). Trial B grapes were clone MV6 harvested at 22.7 Brix, pH 3.12 and TA 8.34. This allowed us to assess the impact of wine making treatments across different parcels of fruit. In each trial, between seven and ten grape bunches were randomly allocated to 1 kg lots. Random allocation of bunches reduced the effect of bunch-to-bunch and vine-to-vine variation in phenolic concentration and distribution, as did replication. Four 1 kg lots were allocated to each treatment (control, heat, microwave)(n=12). Grape lots were crushed using a custom-made bench-top crusher, destemmed by hand and decanted to 1.5 L Bodum™ (Lucerne, Switzerland) 'Kenya' plunger coffee pots. All musts were dosed with 50 mg/L sulfur dioxide (SO_2) in a potassium metabisulphite ($K_2S_2O_5$) solution and control treatment pots were moved to a 27°C (±3°C) constant temperature room. Microwave and heat maceration are described below. Controls were vinified by the French Press method which mimics submerged cap fermentation via coffee plunger screens submerging the cap approximately 1 cm below the juice surface (Dambergs and Sparrow 2011). Plungers were lifted daily to release trapped CO_2 and redistribute the cap.

Three hours post-maceration, each pot was sampled for phenolics and then inoculated with yeast strain EC1118 (Lallemand, Montreal, Canada) rehydrated according to the manufacturer's instructions and applied at the recommended rate (25 g/hL). All pots were incubated at 27°C (±3°C) for seven days and weighed daily to track CO_2 loss. Heat and microwave treatment replicates were loosely capped with Schott bottle lids until day three when airlocks were applied. At day three, 60 mg/L of yeast assimilable nitrogen was added to all replicates in a diammonium phosphate ($(NH_4)_2HPO_4$) solution. At day seven, control pots were pressed off by firm depression of the Bodum screen held down for 10 s, and wines were left at 27°C (±3°C)

overnight to finalize fermentation. Wines were tested for residual sugar (Clinitest™, Bayer, Leverkusen, Germany) and found to be dry (⩽2.5 g/L residual sugar). Wines were not inoculated for malolactic fermentation or subjected to filtration but were cold-settled at 4°C for two weeks, racked and stabilized with 80 mg/L SO_2 solution, and stored for two weeks prior to bottling under gas cover and aging at 14°C for six months (216 days post-inoculation).

Heat maceration

Heat treatment musts were macerated using a custom lab-scale heat exchanger. The exchanger relied on conductive heat and via a double-boiler with approximately 450 cm^2 of a lidded stainless steel parabola nested in a 10 L pot of simmering water (95°C). Must was poured into the parabola, lidded, stirred periodically and temperature monitored with a digital instant read thermometer. Each replicate attained a peak temperature of 71°C (±2°C) in 6 min (±2 min). Musts were decanted to 2 L beakers, held for 1 hour in a 70°C waterbath, and pressed off using a custom-made basket press. Resulting juice was decanted to 500 mL Schott bottles which were chilled to ~27°C and then moved to the 27°C (±3°C) constant temperature room for AF as described above.

Microwave maceration

Microwave treatment musts were macerated as previously described (Carew 2013). Briefly, each pot was microwaved for four cycles of approximately one minute to a peak temperature of 71°C (±2°C) and held for 1 hour in a 70°C waterbath. Musts were pressed off to 500 mL Schott bottles and fermented like heat treatment musts as described above. Microwave replicates yielded ~10% more juice than heat treatment replicates.

Yeast assimilable nitrogen

Heat and microwave maceration impact on must nutrient status was evaluated separately from Trials A and B, in two replicated trials which generated consistent outcomes. Pinot noir fruit was randomly allocated to eight replicates, crushed and destemmed, then evaluated using enzymatic analysis kits for ammonia (AN) (product number 4A120; Vintessential Laboratories, Melbourne, Australia) and primary amino acid nitrogen (PAAN) (product number 4A110; Vintessential Laboratories). Four replicates were heat macerated, and four microwave macerated (n=8). Musts were pressed off and cooled before nutrient status was evaluated.

Grape skin histology

The impact of heat and microwave maceration on Pinot noir grape skin cellular integrity was evaluated separately from Trials A and B. Fruit was harvested and destemmed, and berries randomly allocated to four 100 g replicates. Each replicate was crushed by hand and grape skin sampled immediately (control). Replicates were heat or microwave macerated and skin samples were refrigerated for three days at 4°C then fixed under vacuum in 2.5% gluteraldehyde in 0.1M phosphate buffer at pH 7.2 for 12 hr at 4°C. Following two buffer washes, samples were dehydrated by ascending acetone series (20% increments), three changes of 100% acetone, and two changes of propylene oxide. Samples were infiltrated with Spurr's resin (ProSciTech, Brisbane, Australia) and polymerized for 18 hr at 70°C. Semi-thick sections (4-5μm) were cut with a glass knife on a Reichert OmU2 ultramicrotome (New York, USA). Sections were expanded on distilled water on microscope slides and gently heat-fixed. Slides were immersed in 1% (w/v) Toluidine Blue O in 0.1M acetate buffer for 30 seconds, rinsed in 1% (w/v) sodium borate solution for 30 seconds, rinsed in distilled water, decolorized in 70% ethanol for 30

seconds, rinsed in distilled water and air dried. The sections were mounted in Euparal (Australian Entomological Supplies, Sydney, Australia) beneath a coverslip and heat cured. Slides were examined under a compound light microscope (Leica, Wetzlar, Germany). Phenolic material, previously distinguished in grape material using Toluidine Blue O staining (Ribereau-Gayon et al. 2006a, Cadot et al. 2011), showed as dark green-brown cellular inclusions.

The cellular integrity of skins was quantified by a new method adapted from the Allred Immunohistochemistry (IHC) Score (Allred et al. 1998). Cadot and others previously described a method to quantify phenolic development in grape berries which differentiated between intra-cellular structure types (Cadot et al. 2011) but the method described here enabled quantification of the integrity of intracellular structures, regardless of structure type.

Four grape skin sections for each treatment (n=12) were photographed at 400x magnification and a grid applied to divide images into 60 μm^2 segments, 14 of which were randomly selected for quantitative analysis. Analysis was restricted to the three outermost skin cell layers (exocarp). We recorded: number of cells; number of cells with visible intracellular material (eg. vacuoles, nucleus); mean integrity of intracellular material (1= material scattered and translucent; 2=areas of opacity; 3=entire and opaque); number of cells with brown (phenolic) intracellular material; and mean integrity rating of brown intracellular material (1= material pale, translucent; 2=areas of opacity, granular; 3=mostly entire, opaque). Two indicators of grape skin integrity were devized: **cellular integrity**, mean percentage of cells per segment with visible intracellular material multiplied by mean integrity rating for intracellular material; and **phenolic integrity**, mean percentage of cells per segment with visible brown intracellular material multiplied by mean integrity rating for brown intracellular material.

Ethanol concentration

Wine ethanol concentration was examined by NIR Spectroscopy ('Alcolyzer', Anton Paar, Graz, Austria) at the technical laboratory of Winemaking Tasmania, Australia.

UV-Visible spectrophotometry

Juices were sampled prior to inoculation, and wines at 216 days post-inoculation (six months' bottle age). Phenolics were quantified by a modified Somers method using a 10S UV-Vis Spectrophotometer (Genesys™, Thermo Scientific, Waltham, USA) as previously described (Mercurio et al. 2007, Dambergs et al. 2012, Carew et al. 2013). Briefly, samples were diluted in three solutions (1M hydrochloric acid, acetaldehyde solution, metabisulphite solution), scanned in 10 mm quartz cuvettes, at 2 nm intervals for the wavelength range 200-600 nm. Seven phenolics were quantified: total phenolics, total pigment, total tannin, free anthocyanin, non-bleachable pigment, color density and hue.

Statistical Analysis

Means and standard errors for fermentation kinetics, YAN, histology indicators, ethanol and phenolic data were calculated in Excel 2007. The rate of weight loss during AF was normalized between treatments by dividing mean daily weight loss per treatment by total mean weight loss for that treatment. Daily percentages were summed for cumulative percentage weight lost. The statistical package R (version 2.15.1) was used for single factor ANOVA within and amongst treatments and post-hoc analyses by Tukey's test ($P \leq 0.05$).

RESULTS & DISCUSSION

Grape skin histology

Microwave treatment was associated with significant degradation of cellular and phenolic integrity, and heat treated grape skin occupied an intermediate position between control and microwave treatments (Table 1). These results may explain why microwaved must yielded ~10% higher juice volume at pressing than heated must. Transverse sections of Pinot noir grape skin (Figure 1) illustrate the loss of cellular integrity and degraded phenolic intra-cellular material associated with microwave treatment (Plate c.), compared with control (Plate a.) and heat (Plate b.).

Table 1. YAN (±SE) in Pinot noir must, cellular and phenolic integrity (±SE) in Pinot noir grape skin with three maceration treatments: control (ctl), heat (heat), microwave (mwv). Different lowercase letters indicate significant difference (Tukey's Test $P \leq 0.05$).

	ctl	**heat**	**mwv**
Ammonia Nitrogen (mg/L)	28.7±0.5	30.0±0.7	31.2±0.4
Primary Amino Acid Nitrogen (mg/L)	173±19 **a**	185±23 **ab**	211±19 **b**
Total YAN (mg/L)	202±18 **a**	215±23 **ab**	242±19 **b**
Mean cellular integrity	1.48±0.10 **a**	1.54±0.09 **a**	0.65±0.06 **b**
Mean phenolic integrity	0.59±0.12 **a**	0.35±0.07 **ab**	0.11±0.04 **b**

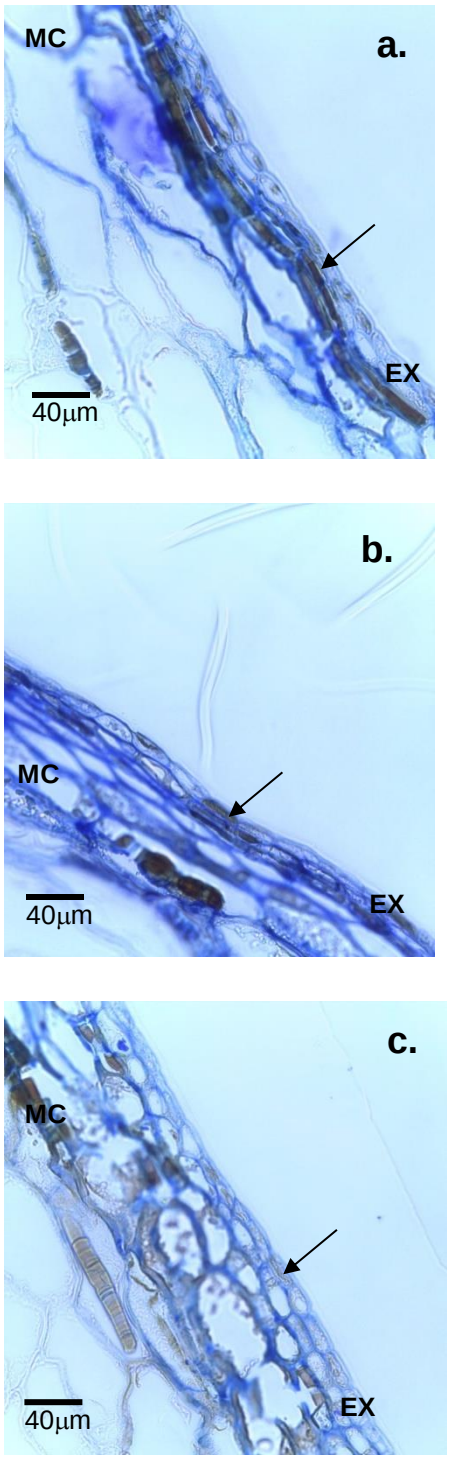

Figure 1. Pinot noir grape skin transverse section (400x magnification; EX=exocarp; MC=mesocarp ; black arrow indicate examples of difference between samples in phenolic integrity in the exocarp). Plate a. control. Plate b. heat. Plate c. microwave.

Yeast assimilable nitrogen

Microwaved must was 40 mg/L higher in YAN than control, and the difference in YAN was associated with a significant increase in PAAN (Table 1). Heat treated must trended between the other two treatments for PAAN. Yeast metabolism is influenced by the nutrient status of grape must (Bell and Henschke 2005), timing of nutrient addition (Adams and van Vuuren 2010) and nitrogen source. A comparison of amino acid and ammonium-based supplement treatments showed significant differences in production of glycerol by yeast, and that yeast grown on the amino acid supplemented media showed higher specific growth rates (Albers et al. 1996). Hence, the higher PAAN in microwaved must could have influenced fermentation rate, alcohol concentration or yeast metabolite production.

Fermentation kinetics

Microwaved must was consistently faster to initiate and finish fermentation than control must, whereas heated must kinetics varied between trials. Microwave must lag phase was shorter than for control (Figure 2), and microwave completed fermentation early (~day five) compared with control (day seven) and heat treatment (~day six). Heat and microwave treatments delivered an abrupt end to fermentation whereas control AF showed a more typical slow finish. The reasons for consistent rapid initiation of fermentation in microwaved must are speculative. Microwave maceration was more effective SO_2 for inhibiting background yeast population (Carew et al. 2013) so absence of microbial inhibition or competition (Fugelsang and Edwards 2007) might have influenced lag phase. Microwave maceration was associated with physical disruption at the grape cellular level (Table 1) and release of cellular components that enable AF (Table 2) which would be expected to prompt fast initiation and rapid exponential phase fermentation.

Table 2. Mean ethanol concentration in Pinot noir wines from three maceration treatments: control (ctl), heat (heat) and microwave (mwv). Different lowercase letters indicate significant difference (Tukey's Test $P \leq 0.05$).

	Trial A			Trial B		
	ctl	**heat**	**mwv**	**ctl**	**heat**	**mwv**
Ethanol (% v/v)	12.4±0.2 **a**	13.2±0.1 **b**	13.0±0.3 **ab**	12.8±0.2 **a**	13.8±0.1 **b**	13.7±0.1 **b**

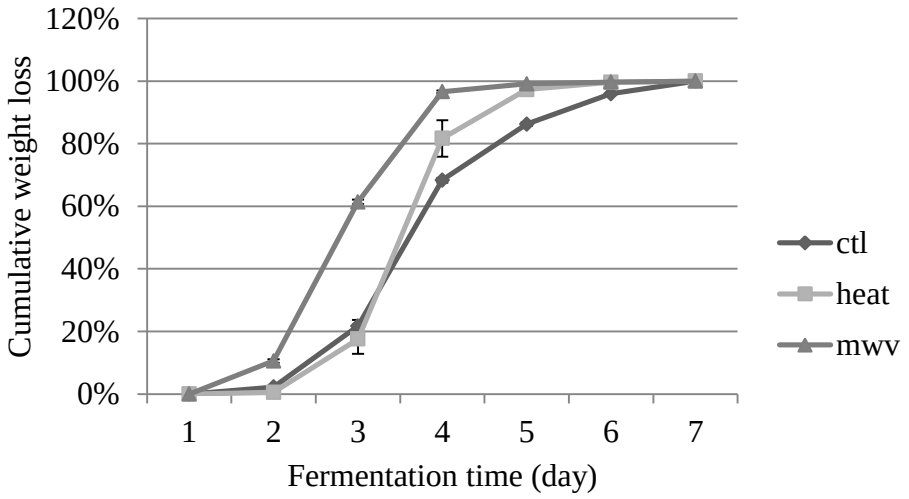

Plate a.

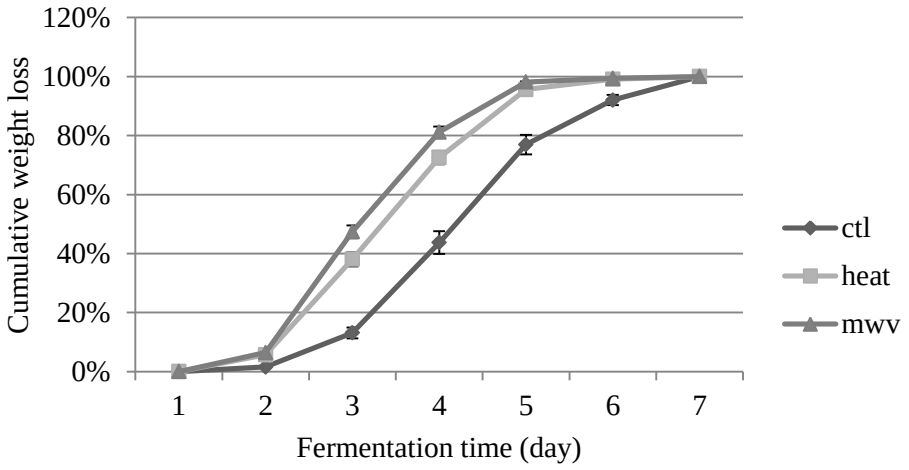

Plate b.

Figure 2. Mean cumulative weight loss (±SE) during fermentation of Pinot noir must with three maceration treatments: control (ctl), heat (heat), microwave (mwv). Plate a:Trial A. Plate b:Trial B.

During AF, pomace hinders free circulation of liquid (Ribereau-Gayon et al. 2006a) which may impact AF rate and completion. In this study, heat and microwave replicates had no pomace and flocs were observed circulating in those replicates during AF. Generation of CO_2 within settled particulate matter apparently caused flocs to rise from the base of the vessel, then sink as CO_2 was released into the vessel headspace, rendering these treatments 'self-mixing'. Ough and Groat observed increased fermentation rate associated with grape juice stirring, addition of grape solids and floc formation (Ough and Groat 1978). Our microwave and heat replicates inadvertently recreated Ough and Groat's factors. In contrast to microwave and heat treatments, control pots could be characterized as diffusion limited, static systems with a substantial trapped CO_2 load.

<u>Ethanol concentration</u>

Higher ethanol outcomes from heat and microwave maceration (Table 2) may indicate heat lysis of grape polysaccharides increased sugar availability in heated musts (Doco et al. 2007). Alternately, yeast metabolism may have been influenced by grape must nitrogen availability or nitrogen composition (ie. the ratio of AN to PAAN) (Bach et al. 2009) or heat and microwave wines may have been 'drier' than control wines. A high ethanol outcome is counter to current consumer and industry trends favouring lower alcohol wines.

Phenolics at inoculation

For both trials, phenolic concentration in control must was significantly lower than concentration in either heat or microwave macerated musts (Table 3). Differences in efficiency of extraction were observed between heat and microwave treatments with a significantly higher concentration of total phenolics and tannin in microwave macerated must for Trial A (Table 3), but differences in total phenolic and tannin concentration between heat and microwave treatments in Trial B were non-significant. This demonstrated extraction outcomes varied between the two parcels of fruit used. Trial A provided the first evidence that microwave may offer more effective phenolic extraction than traditional thermovinification. This warrants further investigation for optimising tannin extraction in Pinot noir winemaking, particularly investigation of the impact on stable colour formation of synchronising tannin and anthocyanin extraction (Boulton 2001).

Table 3. Pinot noir phenolic and colour indicators at inoculation (must) and 216 days post-inoculation (wine) (±SE) for three maceration treatments control (ctl), heat (heat) and microwave (mwv), applied to two parcels of fruit (Trial A, Trial B). Different lowercase letters indicate significant differences in phenolic parameter concentration within Trial and bottle age period (Tukey's Test $P \leq 0.05$). ND = below detection limit for the assay.

		Trial A			Trial B		
		ctl	**heat**	**mwv**	**ctl**	**heat**	**mwv**
At inoculation	Total phenolics (AU)	10.7±0.8 **a**	40.4±0.9 **b**	44.6±0.5 **c**	9.9±0.4 **a**	46.8±2.1 **b**	49.0±2.4 **b**
	Total pigment (AU)	4.3±0.8 **a**	27.8±1.0 **b**	29.9±0.8 **b**	3.3±0.2 **a**	27.5±0.6 **b**	28.7±0.4 **b**
	Anthocyanin (mg/L)	85±15 **a**	552±20 **b**	591±15 **b**	64±5 **a**	542±11 **b**	567±8 **b**
	Non-bleachable pigment (AU)	0.05±0.003 **a**	0.13±0.01 **b**	0.17±0.01 **b**	0.05±0.004 **a**	0.21±0.01 **b**	0.20±0.03 **b**
	Tannin (g/L)	ND	0.67±0.02 **a**	0.89±0.02 **b**	ND	1.08±0.13	1.15±0.14
	Colour density (AU)	0.77±0.11 **a**	4.80±0.37 **b**	5.61±0.15 **b**	0.69±0.03 **a**	4.85±0.17 **b**	4.68±0.45 **b**
	Hue	0.59±0.03 **a**	0.52±0.01 **ab**	0.51±0.01 **b**	0.68±0.03 **a**	0.53±0.01 **b**	0.53±0.004 **b**
Post-inoculation (216 days)	Total phenolics (AU)	27.5±2.3	29.1±0.8	33.4±0.9	24.3±2.4**a**	38.0±0.6**b**	35.0±2.6**b**
	Total pigment (AU)	10.6±0.7**a**	12.5±0.2**ab**	14.2±1.1**b**	8.5±1.2**a**	12.7±0.2**b**	11.7±0.6**ab**
	Anthocyanin (mg/L)	190±12**a**	228±3**ab**	260±23**b**	153±23**a**	228±5**b**	201±12**ab**
	Non-bleachable pigment (AU)	0.65±0.03	0.64±0.03	0.73±0.01	0.32±0.02**a**	0.77±0.02**ab**	0.98±0.21**b**
	Tannin (g/L)	0.30±0.09	0.32±0.03	0.51±0.01	0.21 ±0.08**a**	0.84±0.04**b**	0.69±0.11**b**
	Colour density (AU)	4.24±0.20	4.62±0.20	5.15±0.08	4.26±0.25**a**	5.43±0.13**ab**	6.19±0.69**b**
	Hue	0.73±0.01**a**	0.66±0.01**b**	0.66±0.006**b**	0.68±0.01	0.66±0.002	0.67±0.009

Phenolics at six months

In trial A, treatments were equivalent for total phenolics, non-bleachable pigment, tannin and color density (Table 3). Microwave wine was significantly higher than control for total pigment and anthocyanin, suggesting greater potential for color development. Heat treatment phenolic profile was equivalent to control for all parameters apart from hue.

Trial B demonstrated phenolic outcomes from heat and microwave treatment could vary between fruit with heat and microwave wines significantly higher in total phenolics and tannin, than control (Table 3). Microwave wines showed approximately three times the concentration of non-bleachable pigment (an important indicator of early stable color) than control wines. Contrary to Trial A, the Trial B heat maceration wines were significantly higher in potential color (anthocyanin, total pigment) than control wines, whereas differences were not significant for microwave wines. Trial B wines had also aged differently to Trial A; hue differences were non-significant in Trial B.

CONCLUSIONS

In this study, microwave maceration with early press off was demonstrated as a promising alternative to fermentation of Pinot noir on pomace for seven days. Differences in juice phenolics, fermentation kinetics and juice yield were documented between microwave and heat maceration treatments, but phenolic differences between wines from these two treatments were not consistent between two parcels of fruit, and did not persist with bottle age.

The consistent fast AF observed in microwave macerated must could offer substantial cost savings for Pinot noir winemakers, and perhaps red winemakers more generally, although high

ethanol outcomes observed would need to be considered in a business case for microwave maceration.

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Chapter 6. Viticultural and winemaking interventions increase phenolic concentration in Pinot noir wine.

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Author contributions to this version: Carew 43%, Kerslake 43%, Close 10%, Dambergs 4%

There are no appendices associated with this chapter.

Viticultural and winemaking interventions increase phenolic concentration in Pinot noir wine

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Keywords

viticulture, controlled phenolic release, Pinot noir, leaf removal, microwave

Abstract

Background and Aims: The effects of viticultural and winemaking interventions on Pinot noir phenolics were examined. Methods and Results: Viticultural treatments (no leaf removal 'noLR'; leaf removal 'LR') were applied at two vineyard sites. LR was associated with higher total phenolics (7%) and total tannin (7%) in fruit, compared with noLR. Fruit from each treatment was vinified using control or microwave winemaking . Fruit effects translated to wine with control wines from LR significantly higher than noLR for total phenolics (8%) and total tannin (13%). Microwave wines were higher in total phenolics (30%) and total tannin (50%) than control wines. Viticultural and winemaking treatment effects were additive for total phenolics and total tannin. Conclusions and Significance: While there were site differences, leaf removal and microwave maceration increased total phenolic and tannin concentration in wines. Wine quality is influenced by phenolic concentration, so the interventions trialled in this study could potentially improve Pinot noir wine quality.

Introduction

The concentration of phenolic compounds such as anthocyanin and tannin have been correlated with perceived red wine quality, wine scores at judging and retail price (Cozzolino, et al. 2008, Holt, et al. 2008, Kassara and Kennedy 2011). Pinot noir can offer some unique challenges in the way of phenolics as the variety is low in anthocyanin concentration and the five types of anthocyanin found in Pinot noir are of the unstable non-acylated form (Heazlewood, et al. 2006, Mazza, et al. 1999). In Pinot noir, the tannin distribution (skin to seed ratio) is different to other, well reviewed varieties like Cabernet Sauvignon, Merlot and Shiraz (Mattivi, et al. 2009,

Kennedy 2008, Downey, et al. 2003) and this contributes to Pinot noir wines often being low in tannin concentration, compared with other red varietals (Harbertson, et al. 2008).

Viticultural management techniques, such as leaf removal, are often applied to red grape varieties to increase berry colour and phenols (Diago et al 2009, Koblet 1986, Smart and Robinson 1991, Smart 2004). Trials investigating shading and exposing Pinot Noir bunches have shown a reduction in flavonols but little effect on anthocyanins, as a result of shading (Cortell and Kennedy, 2006, Price et al, 1995). However a recent trend towards early leaf removal has been examined in a range of varieties including Pinot noir and been associated with a positive anthocyanin response (Lee & Skinkis, 2013). Pinot noir leaf removal trials for sparkling wine production have demonstrated that the anthocyanin response is highly site sensitive, with an anthocyanin increase at one site from early leaf removal and an anthocyanin decrease at another (Kerslake et al, 2013). Pinot noir grape composition responses to leaf removal treatments have been shown to not always translate through to wines (Sternad Lemut et al, 2013).

Six winemaking interventions have been demonstrated as effective for increasing phenolics in red wine (Sacchi, et al. 2005). Two of these are commonly used by Pinot noir winemakers: extended maceration and high peak fermentation temperature (Haeger 2008, Joscelyne 2009). 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). A new method for Pinot noir maceration called Controlled Phenolic Release (CPR) has proven effective for increasing the concentration of colour and tannin compounds in finished wine (Carew, et al. 2013). 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 vine-to-wine impacts on red wine phenolics, particularly whether phenolic effects from viticultural intervention are additive with phenolic effects attributable to winemaking.

This study examined the impact on phenolics of two viticultural treatments and two winemaking treatments using Pinot noir fruit grown at two sites. A key objective was to determine if winemaking treatment effects were independent of leaf removal treatment effects.

Materials and Methods

Vines and viticulture

The trial was carried out in the 2012/2013 season in two commercial vineyards in northern Tasmania, Australia. Own rooted, *Vitis vinifera* L. cv. Pinot noir clone MV6 vines trained to Scott Henry trellises were used at both sites. Vines were cane pruned to 2 arms per vine, 10 nodes per arm (total of 20 nodes per vine). Vines at site A were 13 years old with vine spacing of 2.25 m x 1.5 m (inter- and intra-row respectively) and received approximately 1.0 ML/ha drip irrigation. Vines at site B were 8 years old with vine spacing of 2.75 m x 1.50 m (inter- and intra-row respectively) and received approximately 1.5 ML/ha of drip irrigation. Soils at site A had predominantly bleached sandy topsoil over grey mottled clayey subsoil, whereas soils at site B had well drained brown clay loam soils over basalt.

Two leaf removal treatments were applied to vines at both sites. No leaves were removed (noLR) or leaves were mechanically removed post-flowering and pre-veraison (LR). For LR, at site A, leaves were removed on 26 December, 2012 and 31 January, 2013 using ERO Leaf Stripper and at site B on 21 December, 2012 and 21 January, 2013 using a Pellenc Leaf Remover. At both sites, the barrel depth was set to 380 mm to cover the fruiting zone.

The statistical design for the leaf removal trial was a randomised complete block design, with two treatments and four replicates. Each block was a separate row in the vineyard. Treatments were applied along the entire vineyard row with vines 8 and 12 used as measurement vines. For each plot, all results were taken as the mean per vine calculated from measurement vines. Fruit was harvested on 19 March, 2013 at site A and 3 April, 2013 at site B, aiming for 22.5 °Brix. Site B experienced a significant amount of rain before harvest (29.2 mm on 22 March, 2013).

Bunches infected with *Botrytis cinerea* were counted and then discarded during harvest. Bunch number per vine was recorded and yield per vine (with no *Botrytis* infected fruit) taken using scales accurate to 0.01 kg (A&D Co. Ltd., Korea, SK-20K). In the laboratory, 10 bunches were sub-sampled per plot and all berries removed, counted and weighed for mean berry weight and then 100 berries sub-sampled for fresh fruit composition and another 100 berries sub-sampled and frozen at -20 °C for later phenolic analysis.

Fruit and winemaking

Fruit bunches were randomly allocated to 1 kg lots for vinification. Fruit from each site (A,B) and from each viticultural treatment (LR, noLR) was allocated to two winemaking treatments (CTL, MWV). There were four 1 kg replicates for each combination of treatments (n=32). 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 7 days in Bodum™ coffee plungers, followed by press-off and cold settling at 4°C. Replicates of fruit for microwave treatment wines were macerated following a previously published method (Carew, et al. 2013), but hold time for the current experiment was 1 hour rather than the 10 minutes previously described. Wines were not subjected to malo-lactic fermentation. All 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.

Fruit, wine and statistical analysis

Must was analysed for pH and titratable acidity (TA) (Crison, pH-Matic 23, Italy), and total soluble solids was measured by digital hand-held pocket refractometer Pocket PAL-87S (Atago, Japan). Fruit phenolics were quantified by UV-visible spectrophotometry (Iland 2000). Frozen samples were thawed overnight prior to analysis and a Waring 8610EG blender (Waring Commercial, USA) was used to homogenise the berry samples for 30 seconds. Homogenates were weighed using FX300i laboratory scales, accurate to 0.001 g (A&D Co. Ltd., Korea). Ethanol extracts were prepared as per Iland (2000), centrifuged (Centurion Scientific EB Series E25B5 [Centurion Scientific, United Kingdom]), diluted 1 in 20 with 1M hydrochloric acid (Australian Wine Research Institute 2009) and sample absorbance read (Metertech UV/Vis SP8001 spectrophotometer [Metertech, Taiwan]). Analysis of wine phenolics was also by UV-visible spectrophotometry as previously described (Dambergs, et al. 2012, Mercurio, et al. 2007).

Data were analysed by two way and three-way ANOVA, and post-hoc analyses were undertaken by Fisher's protected least significant difference test (confidence limit 95%) in Genstat Version 15.1.0.8035.

Results

Site and viticultural effects on fruit composition and phenolics

Site was a main effect and had a significant impact on fruit yield and total soluble solids (TSS), but not pH and titratable acidity (TA). Yield was 56% lower at site A than site B (Table 1). Site

A had 31% lower total bunch number per vine (27.6) than site B (40.2),36% lower mean bunch weight (73.86 g) compared to site B (115.37 g) and 13% lower mean berry weight (0.86 g) than site B (0.99 g). The mean total phenolic and anthocyanin concentration of the fruit was 9% and 24% higher respectively at site A, compared with site B. Mean total tannin concentration, however was not significantly different between the sites.

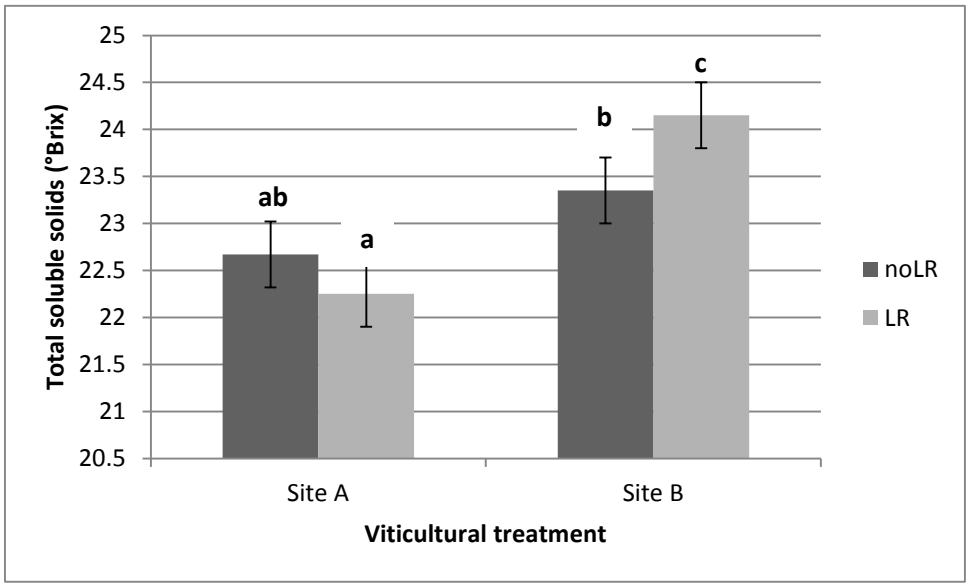
Viticultural treatment was a main effect and associated with significant differences in fruit yield, and phenolic and tannin concentrations (Table 1). Yield per vine for LR was 18% lower compared to noLR and mean bunch weight was 13% higher for the LR (101.08 g) treatment compared to noLR (88.15 g) with no difference in bunch number. Total phenolic and tannin concentration were both 7% higher for the LR treatment compared to the noLR treatment.

Analysis of variance revealed an interaction between site and viticultural treatment for TSS (Figure 1). At site A there was no significant change in TSS associated with removing leaves, but at site B TSS was 3% higher with LR compared to the noLR treatment.

Table 1. Main effects in Pinot noir fruit from two sites (A;B) subjected to two viticultural treatments (no leaves removed = noLR; leaves removed = LR). Different lower case letter denotes significant difference within treatments by Fisher's protected least significant difference test with p-value denoted by asterisks (*** <0.001; **<0.01; *≤0.05).

Treatment	Yield (kg/vine)	Titratable acidity (g/L)	pH	Total phenolics (AU/g)	Total anthocyanins (mg/g)	Total tannin (mg/g)
site A	2.02 a	8.34	3.28	1.35 b	0.86 b	7.81
site B	4.63 b	8.64	3.30	1.23 a	0.65 a	7.41
significance	***	ns	ns	*	**	ns
noLR	3.66 b	8.60	3.30	1.24 a	0.72	7.31 a
LR	2.99 a	8.39	3.27	1.34 b	0.79	7.92 b
significance	*	ns	ns	*	ns	*

Figure 1. Interaction between viticultural treatments (no leaves removed = noLR; leaves removed = LR) for total soluble solids in Pinot noir fruit grown at two sites (A;B).



The 2-fold yield increase at site B compared to site A may have been due to a combination of relatively fertile soils that increased vine capacity and the relatively wet flowering period at site A, that can hamper flower fertilisation (Vasconcelos et al 2009). The high yield at site B was associated with decreased phenolic production, perhaps due to a decreased leaf area to fruit weight ratio and delayed ripening/photosynthate source limitation.

The interactive effect between site and viticultural treatment that was observed for total soluble solids, suggested that compensatory photosynthesis may have been effective at the relatively fertile site B. The decrease in yield when leaves were removed and subsequent increase in fruit phenolics and tannin may have been due to the early timing of the first leaf removal allowing for early UV exposure in concert with compensatory photosynthesis in the remaining leaves.

Wine phenolics

Site A produced wines with significantly higher total phenolic (20%) and tannin (25%) concentration than site B (Table 2). LR was associated with an 8% increase in wine total phenolics and a 13% increase in wine total tannin, compared with noLR. Total phenolic and tannin concentrations were 30% and 50% higher in microwave wines, respectively, than control wines.

Table 2. Main phenolic effects in Pinot noir wine made using fruit from two sites (A;B) subjected to two viticultural treatments (no leaves removed = noLR; leaves removed = LR) and two winemaking treatments (control = CTL; microwave = MWV). Different lower case letter denotes significant difference within treatments by Fisher's protected least significant difference test with p-value denoted by asterisks (*** <0.001; **<0.01; *≤0.05).

Treatment	Total phenolics (AU)	Total tannin (g/L)
site A	58.42 a	1.85 a
site B	47.50 b	1.48 b
significance	***	***
noLR	50.96 a	1.57 a
LR	54.96 b	1.76 b
significance	**	*
CTL	45.77 a	1.28 a
MWV	60.16 b	2.05 b
significance	***	***

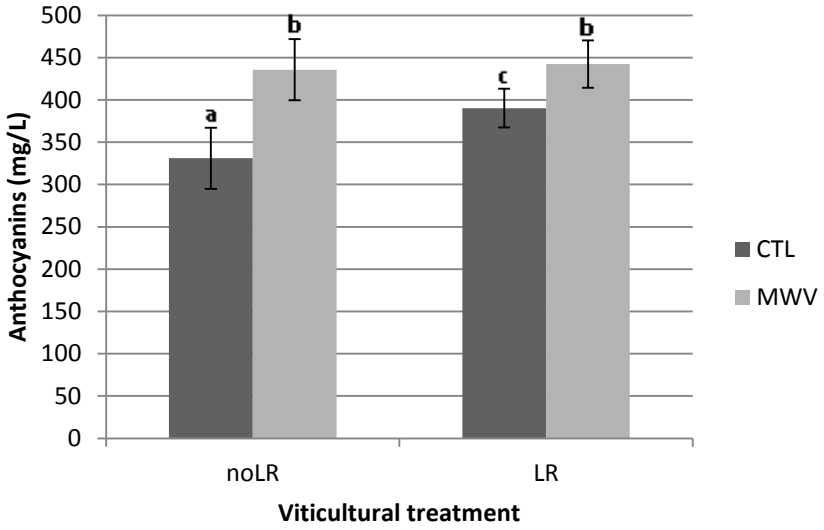
ANOVA identified interaction effects for five of the phenolic measures examined. Similar to the fruit TSS findings, mean concentration of total pigment, anthocyanin, non-bleachable pigment and colour density in wine were higher with LR at site B, but viticultural treatment at site A showed no significant effect (Table 3). At site B, wines from the LR treatment showed around 20% greater mean concentration for these four colour measures than wines made from noLR fruit.

With control winemaking, LR was associated with greater mean concentration of total pigment (17%), anthocyanins (19%), non-bleachable pigment (15%) and colour density (18%) in wine, compared with the noLR treatment (Table 3). Microwave treatment wines were significantly higher for these five measures than control wines, regardless of viticultural treatment but the concentration gain from microwave winemaking was smaller in the case of LR fruit (Figure 2).

Table 3. Interaction effects on phenolics in Pinot noir wine made using fruit from two sites (A;B) subjected to two viticultural treatments (no leaves removed = noLR; leaves removed = LR) and two winemaking treatments (control = CTL; microwave = MWV). Different lower case letter denotes significant difference within treatments by Fisher's protected least significant difference test with p-value denoted by asterisks (*** <0.001; **<0.01; *≤0.05).

Interaction	Total pigment (AU)	Anthocyanins (mg/L)	Non-bleachable pigment (AU)	Colour density (AU)	Hue
site A x noLR	25.32 a	475.1 a	0.94 a	6.77 a	0.65
site A x LR	25.26 a	475.7 a	0.89 a	6.72 a	0.64
site B x noLR	15.51 b	291.6 b	0.56 b	4.48 b	0.66
site B x LR	18.95 c	356.8 c	0.67 c	5.42 c	0.64
significance	**	**	*	**	ns
noLR x CTL	17.52 a	331.0 a	0.59 a	4.57 a	0.65 a
LR x CTL	20.66 b	390.3 b	0.69 b	5.44 b	0.62 b
noLR x MWV	23.31 c	435.7 c	0.91 c	6.68 c	0.66 a
LR x MWV	23.55 c	442.2 c	0.86 c	6.69 c	0.66 a
significance	**	*	*	*	*

Figure 2. Interaction between viticultural treatments (no leaves removed = noLR; leaves removed = LR) and anthocyanin concentration (±SE) in Pinot noir wine from two winemaking treatments (control =CTL; microwave = MWV). Different lowercase letters denote significant difference by Fisher's protected least significant difference test (P≤0.05).



Microwave maceration of Pinot noir grape must has previously been demonstrated to be highly effective for increasing the concentration of phenolics in wine (Carew, et al. 2013), and the findings reported in this paper concur. Microwave mediated extraction of compounds from plant material has been described as a time-temperature dependent process. Microwave maceration negated the impact of leaf removal for colour compound concentration in wines, suggesting that either a saturation point was reached where no more colour compounds diffused from the grape solid material, or that the difference between LR and noLR treatment fruit was one of cellular integrity or resilience rather than differential colour compound production. The observation that viticultural and winemaking effects may have been additive in the case of Pinot noir wine tannin is important for improving wine quality (Harbertson, et al. 2008) and retail price.Our

findings suggest that leaf removal may generate some increase in wine tannin, microwave maceration may also increase Pinot noir wine tannin concentration, and those two treatments in combination may produce wines with tannin concentration similar to those observed in Old World Pinot noir (around 2.0 g/L; Dambergs *personal communication*).

In summary, this study demonstrated that Pinot noir wine phenolics could be influenced in the vineyard through viticultural intervention, and in the winery through the application of a highly extractive winemaking process, but that these effects differed amongst the seven phenolic parameters examined. Pinot noir wine is often low in colour and tannin (Haeger 2008, Harbertson, et al. 2008). In this study, we demonstrated that leaf removal and microwave winemaking were both associated with increased wine tannin concentration (Table 2) and that the effects may be additive, but that the application of microwave maceration negated the impact of viticultural intervention on wine colour parameters (Table 3). This suggests leaf removal may be an economical way to increase Pinot noir wine colour for fruit that was destined for normal winemaking processes, but that leaf removal to increase wine colour would be costly and unnecessary for parcels of fruit to be vinified using a highly extractive winemaking treatment like microwave maceration.

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Chapter 7. Wine phenolic and aroma outcomes from the application of Controlled Phenolic Release to Pinot noir must

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A poster associated with this work, which won Best Student Poster (Oenology) at AWITC 2013, is attached as Appendix E. A Powerpoint™ presentation associated with this work, which won Best Presentation (Oenology) in the Fresh Research section of AWITC 2013, is attached as Appendix F.

Wine phenolic and aroma outcomes from the application of Controlled Phenolic Release to Pinot noir must

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Abstract

Approximately 40% of Pinot noir grape must is grape solids which are pressed off as marc, post-fermentation. Rapid phenolic extraction by Controlled Phenolic Release (CPR) offers an alternative to alcoholic fermentation (AF) of Pinot noir on pomace. In this independently replicated trial, 1kg lots of Pinot noir grape must were subjected to CPR and pressed off after approximately three hours total skin contact time. CPR juice was inoculated for AF and compared with control wine that was fermented on pomace for 7 days. Analysis of wines by UV-visible spectrophotometry at 210 days post-harvest (6 months bottle age) showed that CPR wines were equivalent to control wines for mean concentration of: total phenolics, total pigment, anthocyanin, total tannin, colour density and pigmented tannin. Non-targeted profiling analysis of volatile aroma compounds was carried out by GCMS at 320 days post-harvest (10 months bottle age). Control and CPR wines were distinct from each other for 12 out of 16 aroma compounds identified, with CPR wines generally four to six-fold higher for the acetates, and two-fold higher for most of the ethyl esters. We showed that microwave maceration may reduce constraints on winery capacity by eliminating pomace during fermentation, provide greater control over red wine phenolics, and that CPR may generate wines with distinct aroma qualities.

Introduction

Phenolic concentration and composition is central to red wine quality. Phenolic compounds contribute visual appeal in the form of colour (eg. anthocyanin, non-bleachable pigments), mouth feel qualities like astringency (eg. tannins) and red wine aroma in the form of volatile phenols. The concentration of phenolic compounds in red wine has been correlated with subjective measures of wine quality (Cozzolino et al., 2008; Mercurio et al., 2010). For example, analysis of 1,643 Cabernet Sauvignon and Shiraz wines showed that concentration of total phenolics and total tannin in wines was positively correlated with wine grade (Mercurio et al, 2010).

Pinot noir grapes are generally low in anthocyanin concentration (Cliff et al., 2007) and Pinot noir anthocyanins are of the non-acylated form (Heazlewood 2006) which is unstable at normal wine pH. Pinot noir grapes have an unusual tannin distribution, with a disproportionate amount of the total grape tannin bound up in the seed (Kennedy, 2008). Seed tannin can be difficult to extract and this may explain why Pinot noir wines are often tannin poor. Analysis by protein

precipitation of tannin concentration in 1,325 red wines showed Pinot noir and Shiraz wines were the lowest in tannin of the red varietals examined which included Cabernet Sauvignon, Zinfandel and Merlot (Harbertson et al., 2008). Tannin is important for stable long term colour in red wine. Stable colour results from polymerisation between anthocyanins and tannins (Hayasaka and Kennedy, 2003). Routine red wine making processes extract approximately 40% of available grape phenolics (Boulton, 2001; Stockley and Hoj, 2005). So for varieties with a challenging phenolics profile, like Pinot noir, winemakers need maceration options which allow them to achieve optimal phenolic extraction.

Thermal maceration has been identified as effective for optimising phenolic extraction in red wine making (Sacchi and Bisson et al, 2005). For example, thermal maceration of Merlot, Cabernet Sauvignon and Pinot noir musts under two different regimes (60°C for 1 hour; 80°C for 3 min) was associated with significantly higher concentration of total phenolics compared with control in wines from all varieties trialled except Merlot under the 80°C for 3 min treatment (Atanackovic et al., 2012). The Atanackovic study confounded two variables (two peak temperatures; two hold times) and so it was not possible to discern if the observed phenolic effects were attributable to peak temperature, duration of hold time, or the combination of both variables. Flash Détente (also called Flash Release) is a thermal treatment that has proven effective for extraction of phenolic compounds. This process involves heating must to approximately 95°C, applying vacuum to simultaneously rupture grape cell walls and vacuolar membranes, and cool the must (Doco et al., 2007; Morel-Salmi et al., 2006). Flash Détente was applied to Grenache, Mourvedre and Carignan musts over two vintages and Total Polyphenolic Index (TPI) in wines was shown to be higher in Flash Détente treatment wines for all varieties over both vintages, compared with control wines (Morel-Salmi et al., 2006). TPI does not distinguish between anthocyanins and tannins, however, and anthocyanins tend to extract readily so it is possible the high TPI result was dominated by anthocyanin extraction.

A newly developed thermal maceration process called Controlled Phenolic Release (CPR) also has the capacity to optimise phenolic extraction in red winemaking. CPR involves microwave heating of must to 70°C, followed by managed hold time at that temperature to allow for diffusion of phenolic compounds from grape solids into juice (Carew et al., 2013a; Carew et al., submitted). Application of CPR to Pinot noir must generated significant differences in wine

phenolic concentration when compared with control wines fermented on skins, for example, mean total tannin at 18 months bottle age was 0.60 mg/L for CPR wines and 0.14 mg/L for control wines (Carew et al., 2013a).

Both Flash Détente and CPR have been trialled for rapid phenolic extraction as a precursor to fermenting extracted red grape juice in the liquid phase (ie. pressed off pomace prior to alcoholic fermentation). Flash Détente with early press off generated wines with significantly lower Total Polyphenolic Index than control wines (Morel-Salmi et al., 2006). In contrast, CPR with early press off generated Pinot noir wines equivalent to, or greater than, control wine for mean concentration of: total pigment, anthocyanin, total tannin and non-bleachable pigment (Carew et al., submitted). Direct comparison of Flash Détente and CPR has not been undertaken, and hold times differed in the early press off studies described above – Flash Détente hold time was 6 min (Morel-Salmi et al., 2006), CPR hold time was 1 hour (Carew et al., submitted) – which may account for the differences in phenolic outcome between the two trials. Red wine making processes involving thermal phenolic extraction and press off prior to alcoholic fermentation (AF) are worthy of further research as they offer potential efficiencies in red wine production. Pomace occupies approximately 40% of tank space and requires active management over the life of a red wine AF. The impact of thermal treatments like CPR on red wine aroma, however, requires further research.

Wine aroma occurs when odour-active compounds in wine volatilise and are perceived by a wine consumer. Many important odour-active compounds in wine are metabolic by-products of yeast fermentation, like acetate esters, ethyl esters and higher alcohols (Swiegers et al., 2005; Varela et al., 2009). The concentration of aroma compounds in finished wines is influenced both by the chemical, and physical conditions in fermenting must. Yeast metabolism can be influenced by chemical conditions like variation in glucose concentration, availability of aroma compound precursors and must nutrient status (Swiegers et al., 2009; Ugliano et al., 2009; Vilanova et al., 2012). Physical conditions which can influence yeast metabolism, and hence aroma compound concentration, include fermentation temperature, degree of must oxygenation and the rate of CO_2 evolution from must (Albanese et al., 2013; Girard et al., 1997; Morakul et al.; Zhang et al., 2007). Few researchers have related the chemical and physical impact of thermal maceration processes on red grape must to red wine aroma outcomes (Chai et al., 2011; Fischer et al., 2000).

A pilot-scale study compared aroma outcomes in wines from standard wine making, with those from thermovinification of must at 75°C for 20 min followed by press off immediately after hold time and AF without pomace. Control and thermovinification wine making processes were applied to Dornfelder, Pinot noir and Portugieser musts, and resulting thermovinified wines were significantly higher in ester compounds, and displayed 'fruity' character (Fischer et al., 2000). Given the role of esters in Pinot noir wine aroma (Fang and Qian, 2005), investigating the impact of novel thermal wine making processes on aroma compounds like esters is important for this variety.

Our study compared the phenolic and aroma outcomes in Pinot noir wines made using a control microvinification process (CTL), with Pinot noir wines made by Controlled Phenolic Release with early press off (CPR). The CPR treatment involved approximately three hours' total skin contact time before must was pressed off and enriched juice fermented in the liquid phase. We report on the impact of these wine making treatments on wine phenolics concentration at six months bottle age (220 days post-harvest), and response ratio for 16 wine aroma compounds at ten months bottle age (320 days post-harvest).

Materials and Methods

Fruit, maceration and microvinification

Pinot noir fruit at 13°Baume and pH 3.3 was harvested from a vineyard in Northern Tasmania, Australia during April 2012. Fruit was randomly allocated to eight 1.1 Kg replicates and each was crushed and destemmed using a custom-made crusher. Each must replicate was treated with 50 mg/L sulphur dioxide in the form of a potassium metabisulphite solution, and four replicates allocated to the control treatment were transferred to a 1.5 L Bodum™ coffee plunger and moved to a 28±3 °C constant temperature room for vinification according to the 'French Press' method (Carew et al., 2013a; Dambergs and Sparrow, 2011).

Four replicates were subjected to the Controlled Phenolic Release process which entailed heating must to 70°C in a domestic 1150W Sharp™ 'Carousel' R-480E microwave oven followed by a 1 hour hold time in a 70°C waterbath. Replicates were pressed off immediately after the 1 hour hold time at 70°C, enriched juice was transferred to 500 mL Schott bottles and cooled to 28°C by

immersion in an icebath. CPR replicates were then loosely lidded with a Schott bottle cap and moved to a 28±3°C constant temperature room for yeast inoculation and fermentation.

All replicates were inoculated with the yeast strain *Saccharomyces cerevisiae* EC1118 (Lallemande) which had been rehydrated according to the manufacturer's instructions. Fermentation kinetics were monitored by daily weighing of fermentation vessels to record evolution of CO_2. At day three of the ferment, 60 mg/L of yeast assimilable nitrogen was added to each replicate in the form of diammonium phosphate solution. Alcoholic fermentation was complete by day seven and wine was tested for residual sugar using Clinitest™ tablets (Bayer) and all wines were found to be dry with ≤2.5g/L residual sugar. CTL wines which were fermented on skins were pressed off, racked into 375 mL bottles and cold settled for two weeks at 4°C. CPR wines were racked directly to 375 mL bottles and cold settled for two weeks at 4°C. All wines were then racked under CO_2 cover to 250 mL Schott bottles and stabilised by the addition of 80 mg/L sulphur dioxide in the form of potassium metabisulfite solution, and settled for an additional two weeks. Wines were bottled under CO_2 cover to 100 mL and 28 mL amber glassware with wadded polypropylene capping. A new 28 mL bottle of each wine was opened for each analysis – phenolics at six months bottle age and volatile aroma compounds at eight months bottle age.

Phenolics by UV-Visible Spectrophotometry

Wines were analysed for concentration of seven red wine phenolic measures at six months bottle age. Analysis was undertaken using a modified Somers method and chemometric calculator, both of which have been validated and are described in full elsewhere (Dambergs et al., 2011; Dambergs et al., 2012; Mercurio et al., 2007). In brief, wine samples were diluted in each of three solutions (1M hydrochloric acid, metabisulfite solution and acetaldehyde solution), and scanned in 10 mm quartz cuvettes at 2nm intervals for the wavelength range 200-600 nm using a Thermo Genesys™ 10S UV-Vis Spectrophotometer. Resulting absorbance data for each sample was exported to Excel 2007 spreadsheets and selected absorbance data was entered into the chemometric calculator to quantify wine tannin, total phenolics, total pigment, free anthocyanin, non-bleachable pigment, colour density and hue.

Aroma by GCMS

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 (crossbond carbowax polyethyleneglycol) 0.18 □m film thickness that has a 5 m x 0.18 mm retention gap. Helium was used as the carrier gas with flow-rate 0.8mL/min in constant flow mode. The oven temperature started at 33 ºC, held at this temperature for 4 mins, then heated to 60 ºC at 4 ºC/min, further heated to 100 ºC at 16 ºC/min, then heated 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 andinjected into a Gerstel PVT (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 were analysed concurrently with the wine samples and each sample was spiked with deuterated internal standard.

Statistical analysis

Means and standard deviation for phenolic measures and aroma compound response ratios were calculated in Excel 2007. Independent samples T-test was used to establish where there were significant differences between treatments ($P \leq 0.05$).

Results and Discussion

Wine phenolics

Statistical examination for differences between the CTL and CPR treatments in mean concentration of the seven phenolic indicators examined at six months bottle age showed no significant difference for total phenolics, total pigment, free anthocyanin, tannin, non-bleachable pigment or colour density (Table 1). This demonstrates that CTL and CPR wines could be termed ‘phenolically equivalent’ according to six out of the seven measures used in this study. Wines from the CPR treatment were significantly different from CTL wines for hue, however,

with CPR wines showing a more garnet hue, compared with CTL wines which were more blue-purple at six months bottle age.

The phenolic results presented here concur with our previous findings that CPR treatment involving microwave maceration to 70°C and one hour hold time, followed by AF off pomace delivers Pinot noir wine which is similar in phenolic concentration to wine fermented on pomace for seven days (Carew et al., 2013a; Carew et al. submitted). Similar results were recorded in a small-scale comparison in Shiraz must of CTL and CPR with early press off, however, that variety required a three hour hold time to produce CPR wine equivalent in phenolic profile to the CTL treatment (Carew et al. 2013b). The difference between treatments in hue value that was observed in this trial (Table 1) suggests that the CPR wines may have matured at a faster rate than CTL wines, although if this were the case, a significant difference in non-bleachable pigment value might have been expected. Alternately, the CPR wines may have suffered greater oxidation (oxidative browning) due to the lack of protective CO_2 layer (pomace) during AF, or poor management of the final days of AF; CPR wines were largely dry by day five, whereas CTL wines did not finish fermentation until day seven.

Table 1. Mean concentration of phenolics (±SD) in Pinot noir wine from control (CTL) and controlled phenolic release (CPR) maceration treatments at six months bottle age (220 days post-harvest). Results in bold typeface are significantly different to each other according to independent samples t-Test (P≤0.05).

	CTL	CPR	P -value
Total phenolics (AU)	20.2±2.0	21.2±3.2	0.25
Total pigment (AU)	10.0±0.7	9.1±0.3	0.32
Anthocyanin (mg/L)	163±12	147±4	0.07
Non-bleachable pigment (AU)	1.08±0.07	1.07±0.11	0.69
Tannin (g/L)	0.09±0.08	0.16±0.16	0.15
Colour density (AU)	5.07±0.37	4.95±0.41	0.35
Hue	0.68±0.01	0.74±0.03	0.04

Wine volatiles

There were significant differences between the CTL and CPR treatment wines for 12 of the 16 aroma compounds analysed, with CPR wines generally higher in these compounds than CTL wines (Table 2). Differences in aroma profile varied between the three classes of aroma compounds identified. The level of butanol was significantly different between treatments, with CPR slightly higher than CTL for this compound. Butanol can be perceived as fruity at low concentrations in wine, and as fusel or spirituous at higher concentrations. In contrast to the results for higher alcohols, differences between treatments were comparatively high for the three acetate compounds examined, with response ratios four to six times higher in CPR wines than CTL wines. For example, 2 and 3-methylbutyl acetate which are known for their fruity and

banana characters was six times higher in CPR wines compared to CTL wines. The ethyl esters examined were also consistently higher in CPR wines than CTL wines, with the exception of ethyl 3-methylbutanoate. Ethyl octanoate and ethyl decanoate have been identified as key odorants in the varietal aroma of Pinot noir wine (Fang and Qian, 2005) and these compounds were two-fold higher in the CPR wines than the CTL wines.

The aroma compound differences observed between CTL and CPR wines may have resulted from chemical, biological or physical differences in musts due to the different maceration regimes applied in this study. The treatments applied may have differentially influenced the availability of volatile aroma precursors, the viability of enzymes and transferases which act on aroma compounds, or must parameters which impact on yeast metabolism. Such changes to the must environment would likely influence the production of aroma compounds by yeast. For example, previous research has shown that CPR liberates around 16% greater yeast assimilable nitrogen than is liberated in control musts (Carew et al., 2013a), and yeast metabolism has been shown to be directly affected by not only must nutrient status but also by the type of nitrogen available (ie. ammonia nitrogen, primary amino acid nitrogen) (Bell and Henschke, 2005; Ugliano et al., 2008; Vilanova et al., 2007).

Pinot noir wine has at least 37 known aroma active compounds (Fang and Qian, 2005) and the sensory threshold for each of these compounds may differ. Pinot noir aroma is also influenced by aroma compound synergies, where different proportions of various aroma compounds generate perceived odour differences (Fang and Qian 2005). This means the aroma data reported here does not provide a clear indication of how the human sensory response may differ between wines from the treatments applied in this study. The data presented here do, however, provide a clear conclusion that the concentration of aroma active compounds differed by treatment. Formal sensory appraisal of these wines would be required to establish if the differences revealed by GCMS translate into different aroma experiences for consumers of CPR wines.

Table 2. Mean aroma compound response ratio (±SD) in Pinot noir wine from control (CTL) and controlled phenolic release (CPR) maceration treatments at ten months bottle age (320 days post-harvest). Results in bold typeface are significantly different to each other according to independent samples T-Test ($P \leq 0.05$). Aroma descriptors are drawn from several references (Fang and Qian, 2005; Siebert et al., 2005) and several descriptors are offered because the perception of an aroma compound may vary depending on compound concentration and human perceptual threshold.

	CTL	CPR	p-value	Aroma Descriptor
Ethyl Esters				
ethyl acetate	1.37±0.03	2.30±0.21	<0.01	sweet, tart, volatile acid, nail polish
ethyl propanoate	3.56±0.12	3.90±0.08	<0.01	fruity
ethyl 2-methylpropanoate	3.19±0.22	4.19±0.15	<0.01	fruity, sweet, apple
ethyl butanoate	1.06±0.04	1.47±0.08	<0.01	fruity, peach
ethyl 2-methylbutanoate	0.34±0.02	0.42±0.01	<0.01	sweet, fruit, honey
ethyl 3-methylbutanoate	0.25±0.04	0.26±0.02	0.47	berry, fruity
ethyl hexanoate	1.94±0.06	2.85±0.09	<0.01	green apple, fruity, wine
ethyl octanoate	1.67±0.05	3.67±0.15	<0.01	red cherry, raspberry, cooked fruit
ethyl decanoate	0.29±0.04	0.65±0.10	<0.01	fruity, black cherry, chocolate, barnyard

Table 2 cont…

Acetates				
2-methylpropyl acetate	0.012±0.001	0.046±0.006	<0.01	banana, fruity, floral
2&3-methylbutyl acetate	0.053±0.006	0.297±0.16	<0.01	banana, fruity
hexyl acetate	0.009±0.000	0.039±0.003	<0.01	sweet, perfume, floral
Alcohols				
2-methylpropanol	30.6±1.3	32.3±1.2	0.09	fusel, spirituous, nail polish
butanol	0.55±0.04	0.66±0.05	0.02	fruity, fusel, spirituous
2&3-methylbutanol	48.1±2.2	51.3±1.8	0.06	nail polish
hexanol	0.058±0.007	0.050±0.004	0.10	grape juice, green grass

Wine making differences

In this study, we compared two different wine making processes and reported their impact on wine phenolics and aroma compounds. Three variables were confounded in this experiment. The CPR process differed from control wine making in that: must was microwave macerated, enriched juice was fermented in the absence of pomace, and CPR juice was fermented in a 'semi-closed' fermentation system (loosely lidded 500 mL Schott bottles). Each of these factors may have contributed to the results observed. Preliminary research (data not shown) informed the design of the CPR treatment process and the parameters of peak temperature and hold time were managed to ensure CPR and CTL wines would be approximately equivalent for phenolics (Table 1). This ensured that microwave maceration did not contribute significant differences for phenolics, and the trial demonstrated the capacity of CPR to deliver production efficiencies (AF without pomace, no cap management required).

The distinct differences in aroma compound response ratios seen between CPR and CTL treatments in this study (Table 2) and similar aromatic differences observed in an earlier comparison of control and thermovinification wines (Fischer et al., 2000), need to be interpreted with the confounded variables in mind. Seven hypotheses can be advanced to explain why aroma differences have been observed between thermovinified and standard wines.

1. Liberation of grape aromas and aroma precursors – Aroma compounds may have been heat-mediated products from precursors in grape juice, or heat may have liberated aroma precursor compounds which were subsequently available as yeast metabolites.
2. Fermentation temperature differences – Fischer and others employed a lower fermentation temperature with thermovinified must because high fermentation temperature, which is often used to enhance phenolic extraction in red wine making (Haeger, 2008; Peynaud, 1984), has been imputed in volatilisation of red wine aroma compounds during fermentation. Our CPR replicates were fermented at the same temperature as CTL replicates and still showed significantly greater response ratio for most aroma compounds examined, however there were marked differences in the scale of difference between our trial and Fischer and others' trial. Fischer and others reporting 20-50 times greater hexyl acetate in thermovinified Pinot noir compared with control (Fischer et al., 2000), whereas we recorded only four times greater hexyl acetate for CPR, compared with CTL wines.
3. Slower CO_2 evolution rate – Fischer and other suggest a slower CO_2 evolution rate may account for greater preservation of volatiles in wine (Fischer et al., 2000), however model system research examining gas-liquid partitioning in wine fermentation suggested must composition and fermentation temperature, not CO_2 evolution rate, where key drivers of aroma loss (Morakul et al., 2011). We have previously reported faster fermentation kinetics for CPR with early press off than for control fermentation (Carew et al., submitted), and the aroma results reported in this paper support the conclusions of Morakul and others'.
4. Volatilisation of aroma compounds during cap management (Fischer et al., 2000).
5. Heat inactivation of aroma degrading enzymes and transferases (Fischer et al., 2000).

6. The presence of pomace – pomace may contribute aroma precursors as it degrades and as chemical conditions in the fermenting must change (ie. hydrophobic aroma precursors may liberate more readily as ethanol concentration increases). Visual observation of fermenting must also suggests that pomace can act as a trap which slows CO_2 release. CO_2 has been identified as an 'aroma scrubber' with differential effects on various wine aroma species. Recent research demonstrated that around 50% of ethyl hexanoate produced in a model red wine fermentation was stripped away with CO_2 gas emissions (Morakul et al., 2013). An earlier study identified ethyl decanoate as particularly susceptible to CO_2 scrubbing (Ferriera et al., 1996). Coincidentally, wines fermented in the semi-open fermentation system in our study (CTL) were approximately 50% lower in ethyl hexanoate and ethyl decanoate than wines from the semi-closed fermentation system (CPR) (Table 2). These two compounds are key odorants for Pinot noir wine (Fang and Qian, 2005). This hypothesis may account for variation between aroma compound differences as the volatility and hydrophobicity of individual wine aroma compounds influences their capacity to be stripped out in CO_2 emissions (Morakul et al., 2010).
7. Use of semi-open and semi-closed fermentation systems – Wine aroma differences may have resulted from differences in transfer dynamics between the two fermentation systems. In the semi-open system, gas-phase or volatilised aroma compounds could readily exit the system, whereas those compounds may well have remained trapped in the semi-closed system. Boulton has highlighted diffusion equilibrium between solid and liquid phases in grape must as potentially influencing phenolics extraction (Boulton, 2001), we propose similar diffusion equilibrium conditions may govern exchanges between the gas (headspace) and liquid (fermenting juice) phases in the semi-closed CPR fermentation system.

Conclusion

CPR treatment for making Pinot noir wine was demonstrated as efficient, with pomace pressed off after three hours skin contact time, and resulting wines equivalent to control wines for phenolics. The CPR treatment wines were, however, quite different from control wines for 12 out of 16 aroma compounds analysed. CPR wines showed particularly high levels of ethyl esters

and acetate compounds which have been associated with fruity and floral aromas in wine. The study was not able to identify which of the three variables distinguishing CPR from CTL vinification was responsible for the marked differences observed for aroma profile, but seven hypotheses were offered which warrant further investigation. The CPR process may offer efficient production of wines with highly fruity or floral bouquet, and further research on the mechanisms driving aroma differences may offer insights of more general value to wine making.

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Chapter 8. Yeast strain affects phenolic outcomes in Pinot noir wines from simultaneous alcoholic and malolactic fermentation of microwave macerated grape must

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Appendix G is a poster associated with this research which was presented at the 15th Australian Wine Industry Technical Conference (July 2013, Sydney, Australia).

Yeast strain affects phenolics concentration in Pinot noir wines made by microwave maceration with early pressing

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ABSTRACT

Aims: This study examined yeast strain effects in a novel wine making process that had been designed to optimise phenolics extraction and improve production efficiency for Pinot noir wine making.

Methods and Results: Microwave maceration with early pressing and co-inoculation of yeast and malolactic bacteria for simultaneous alcoholic and malolactic fermentation was investigated. Yeast treatments (*Saccharomyces cervisiae* RC212 and EC1118, and *Saccharomyces bayanus* AWRI1176) were co-inoculated with *Oenococcus oeni* PN4 immediately after must microwave maceration. Alcoholic and malolactic fermentation were complete 17 days post-inoculation for all three yeast treatments. At 16 months bottle age, AWRI1176 treatment wines had approximately twice the non-bleachable pigment and colour density of wines fermented by EC1118 and RC212.

Conclusions: The novel winemaking process produced Pinot noir wine that was stable 37 days after fruit had been harvested and yeast strain choice significantly impacted the stability and phenolic character of wine.

Significance and Impact of Study: There was no apparent inhibition between the yeast strains and malolactic strain under the novel winemaking conditions applied, and yeast strain influenced phenolic development. Further investigation would be required to assess strain effects on wine aroma, mouth feel and taste, if the novel process is to transition from the laboratory to industry application.

KEYWORDS

anthocyanin, tannin, pigmented polymers, thermovinification, microwave-assisted extraction (MAE), alcoholic fermentation, malolactic fermentation

INTRODUCTION

Microwave maceration with early pressing is a novel red wine maceration process that has shown potential to enhance phenolic extraction from Pinot noir grape solids into juice prior to alcoholic fermentation (AF)(Carew *et al* 2014). Phenolics, including the anthocyanins and tannins, are important to red wine quality (Cozzolino *et al* 2008; Mercurio *et al* 2010; Kassara and Kennedy 2011) so enhanced phenolic extraction offers potential to improve Pinot noir wine quality and production efficiency (Carew *et al* 2013a; Carew *et al* 2014). Anthocyanins contribute red colour to wine and Pinot noir wine is typically low in anthocyanin. A survey of 173 commercial red wines showed Pinot noir anthocyanin concentration to be the lowest at 60 mg/L, with Merlot at 110 mg/L and Cabernet Sauvignon at 125 mg/L (Cliff *et al* 2007). Furthermore, the anthocyanins found in Pinot noir grapes are non-acylated, which may reduce their stability (Mazza *et al* 1999; Heazlewood *et al* 2006). Colour stability in red wine can be enhanced through complexing between anthocyanins and tannins (Peng *et al* 2002; Hayasaka and Kennedy 2003) however, Pinot noir wine is typically low in tannin; analysis of 1350 commercial red wines found Pinot noir to have approximately half the concentration of tannin (catechin equivalents) of Cabernet Sauvignon and Merlot (Harbertson *et al* 2008). Because of its challenging phenolic profile, Pinot noir wine makers could benefit from new options like microwave maceration with early pressing to improve the efficiency and effectiveness of phenolic extraction (Carew *et al* 2014). Currently however, little is known about how different yeast strains might perform under the novel conditions of microwave maceration with early pressing.

Choice of yeast strain is one option available to winemakers for optimising phenolics in wine. Research into the impact of yeast strain on phenolic extraction and retention in Pinot noir wine made under normal wine maceration and fermentation conditions has confirmed strain-related

effects (Mazza *et al* 1999; Escot *et al* 2001; Girard *et al* 2001; Lorenzini 2001; Carew *et al* 2013b). A trial of eight yeast strains in Pinot noir must over two vintages concluded that some yeast strains were associated with noticeable variation in phenolic concentration, with the *Saccharomyces cerevisiae* Wädenswil 27 treatment wine showing lower colour density and phenolic content than other yeast treatments (Mazza *et al* 1999). A replicated microvinification trial of five yeast strain treatments in Pinot noir must showed significant strain-associated differences in the concentration of seven phenolic indicators in the wines at six months bottle age, and five tannin composition measures in wines at eight months bottle age (Carew *et al* 2013b). For example, the concentration of tannin (0.48 g/L) and anthocyanin (211 mg/L) was significantly higher in *S.cerevisiae* RC212 treatment wines at six months, compared with wines from *Saccharomyces bayanus* AWRI1176 treatment (tannin 0.19 g/L, anthocyanin 177 mg/L). One of the difficulties of translating yeast strain research outcomes for industry application is that strain-related effects may vary with winemaking approach, and during the course of wine maturation. Girard and others investigated yeast strain impacts in Pinot noir vinification and concluded that effects were mediated by maceration approach and fermentation temperature (Girard et al., 2001). A comparison of Pinot noir wines made with the yeast strains *S.cerevisiae* 'Burgundy' and RC212, showed the RC212 treatment was associated with significantly higher anthocyanin concentration in wine directly after AF, but this effect was reversed following malolactic fermentation (Escot *et al* 2001). These studies demonstrated that yeast strains applied in a novel wine making process may generate unexpected or unwanted phenolic outcomes in red wine.

Red wines are routinely subjected to malolactic fermentation (MLF) after AF to confer microbial stability (Ribereau-Gayon *et al* 2006; Fugelsang and Edwards 2007). Pinot noir is a cool climate

variety and hence a secondary advantage of MLF is the moderation of perceived acidity. MLF of wine impacts on production efficiency because it can be difficult to initiate and slow to finish. Five parameters influence initiation and rate of MLF: temperature, pH, SO_2 concentration, ethanol content and the presence of phenolic compounds, which may have antibacterial effects (Davis *et al* 1985; Versari *et al* 1999; Ribereau-Gayon *et al* 2006). In a complex system like wine, interactions between stressors determine the level of inhibition of various microorganisms (Fugelsang and Edwards 2007). The novel microwave maceration process which is the focus of this study extracts phenolics prior to AF, and obviates the need for the high temperature AF conditions usually applied to extract phenolics during Pinot noir vinification (25-30°C). The process also eliminates the need for addition of SO_2 to must at crushing for sanitation as the microwave treatment has been shown to reduce background yeast population to ≤100 cfu/mL (Carew *et al* 2013a). This suggests that MLF of Pinot noir wine could be hastened through application of microwave maceration with early pressing and co-inoculation of malolactic bacteria with yeast for simultaneous AF and MLF.

We examined the capacity of three yeast strain treatments (*S.cerevisiae* EC1118 and RC212, and *S.bayanus* AWRI1176) in combination with a single malolactic bacterial strain (*Oenococcus oeni* PN4) to complete fermentation in microwave macerated Pinot noir must that was pressed off after a total of two hours skin contact time. We report on the success of AF and MLF, and yeast strain treatment effects on the development of wine phenolics over a 16 month bottle aging period.

MATERIALS and METHODS

Fruit and preparation

Pinot noir grapes were harvested at commercial ripeness (13°Baume, pH 3.3) from a vineyard in Northern Tasmania, Australia on 3rd April, 2012. Approximately 8 kg of grape bunches were randomly allocated to one of five batches (~1.6 kg/batch). Grapes were then crushed using a custom-made bench-top crusher and destemmed by hand before the resulting must was decanted to a 2 L beaker for microwave maceration. No sulphur dioxide was added to must at crushing. Beakers of must were microwaved in a domestic 1150W Sharp™ 'Carousel' R-480E microwave oven. Each beaker was microwaved at full power for four time increments with intervening stirring and temperature evaluation of must using a digital instant read thermometer. Each pot reached a peak temperature in the range of 68°C to 73°C and was then held for 1 hour in a 70°C waterbath. Musts were pressed off in a custom-made basket press and the resulting juice from each batch was distributed evenly amongst 12x500 mL sterilised Schott bottles (n=12). This method of juice preparation resulted in a highly standardised experimental system with little variation between replicates (data not shown). This approach to juice preparation would constitute pseudoreplication if the research were focussed on viticultural impacts on wine quality but provided a strong basis for this research focussed on yeast impacts because it reduced juice variation between replicates. This approach to juice preparation countered a problem common to small-scale Pinot noir winemaking research where high bunch-to-bunch phenolic variation can result in substantial variance between replicates. Following juice distribution, bottles were capped and placed in an ice bath for approximately 30 minutes by which time they had cooled to approximately 23°C.

Cooled bottles of juice were inoculated with one of three yeast treatments, *Saccharomyces cerevisiae* EC1118 (Lallemand Australia Pty Ltd), *S.cerevisiae* RC212 (Lallemand Australia Pty Ltd), *Saccharomyces bayanus* AWRI1176 (Lallemand Australia Pty Ltd), and simultaneously

inoculated with a malolactic fermentation culture *Oenococcus oeni* PN4 (Lallemand Australia Pty Ltd). Two of the yeast treatments, EC1118 and RC212, are commonly used in Pinot noir winemaking (Haeger 2008) and one is a novel strain, AWRI1176. Yeast and malolactic cultures were rehydrated according to the manufacturer's instructions and applied at the recommended dosage rate of 25 g/hL. The inoculated bottles were loosely capped with Schott bottle lids to allow release of CO_2 and were incubated for 12 hours at 22°C (±2°C) to establish active fermentation, prior to being transferred to a 19°C (±2°C) controlled temperature room for the remainder of the fermentation period. Bottles were weighed regularly over the course of the ferment to track CO_2 loss as an indicator of fermentation kinetics. On day two of the ferment, 60 mg/L of yeast assimilable nitrogen was added to each bottle in the form of a 20% diammonium phosphate solution and airlocks were applied. At day 13, all wines were tested with an L-Malic Acid Enzymatic Analysis Kit (Vintessential Laboratories, Australia), and residual sugar was assessed using Clinitest™ tablets. Those wines that had not completed AF by day 13 were tested again at day 17. At day 17, all wines were transferred under CO_2 cover to 250 mL Schott bottles, stabilised through addition of 80 mg/L sulphur dioxide solution in the form of a 20% potassium metabisulfite solution and cold settled at 4°C for two weeks. Stabilised, settled wines were sampled and then bottled under CO_2 cover on 10th May, 2012 and stored at 14°C for bottle aging. Fresh bottles of wine were opened for analysis at six and 16 months post-bottling (215 and 435 days post-harvest, respectively).

UV Visible Spectrophotometry

Wines were analysed at bottling and after bottle aging for seven phenolic measures using a modified Somers method (Mercurio *et al* 2007) and tannin UV-Visible chemometric calculator

(Dambergs *et al* 2012). Absorbance was examined by UV-Visible spectrophotometry (Thermo Genesys™ 10S UV-Vis Spectrophotometer) as previously described (Carew *et al* 2013a). The phenolic measures were: total phenolics (predominately coloured and non-coloured tannin and anthocyanin, and low molecular weight, non-pigmented phenolic compounds); total tannin (pigmented and non-pigmented tannin); free anthocyanin (unbound or co-pigmented anthocyanin); non-bleachable pigment (stable colour resulting from formation of pyranoanthocyanins and complexing of anthocyanins and tannins); colour density (pigment saturation), hue (higher values being ruby or garnet, and lower values more purple) and hue-SO_2 (hue in the presence of high SO_2 concentrations, with lower values indicating non-bleachable pigment of a more purple hue; note that this was not described in the original Modified Somers method, but was calculated from absorbance at 420 nm and 520 nm of wine diluted in the same high-SO_2 buffer used to measure non-bleachable pigment in the Modified Somers method).

Statistical analyses

Means and standard errors for fermentation kinetics, malolactic fermentation and phenolic data were calculated in Excel 2007, and Genstat 14th edition was used to identify main effects and interactions within and between yeast treatments and bottle age time periods (two-way ANOVA), and for post-hoc analyses to identify significant treatment effects (Tukey's Test $P \leq 0.05$).

RESULTS

Fermentation kinetics and wine stability

All three treatments exhibited similar initiation of AF (Figure 1). While AF was conducted at a low temperature (19±2°C) relative to the industry standard for red wine (25-30°C), ferments were between 40% and 60% complete by day 5 after inoculation. The AWRI1176 treatment

showed a slower exponential phase than EC1118 and RC212 treatments, and was slower to finish compared with EC1118. An apparent small increase in fermentation rate after day 13 coincided with fermentation vessels being opened for residual sugar and L-malic acid sampling. This may infer wines were slow to complete due to a lack of oxygen; airlocks were applied at day 2.

Analysis of mean concentration of residual sugar in wines concurred with the fermentation kinetic data (Figure 1). Treatment EC1118 had ≤2.5g/L of residual sugar at day 13, RC212 ≤6g/L and AWRI1176 ≤5g/L. At day 17, RC212 treatment had mean residual sugar ≤2.5g/L and AWRI1176 ≤3.5g/L. A decision was made to conclude fermentation at day 17 because EC1118 treatment had been largely dry for four days and risked spoilage.

Mean concentration of L-malic acid in juice at inoculation and wine at day 13 of fermentation are shown in Table 1. All replicates across all three yeast treatments had reached the target L-malic concentration of ≤0.15 g/L by day 13 of AF. This demonstrated that under the fermentation conditions of this study the inoculated lactic acid bacterial strain PN4 was compatible with the three yeast strains trialled. Malolactic fermentation was completed ahead of AF for two of the yeast treatments (RC212 and AWRI1176) and mean L-malic acid concentration at day 13 for the RC treatment was significantly lower than for EC1118 and AWRI1176.

Wine phenolics

Wines were analysed for phenolics at bottling, 6 months (215 days post-harvest) and 16 months (435 days post-harvest). Strain-related effects were observed, and some effects persisted to 16 months bottle age (Table 2). There were no differences amongst yeast treatments for total

phenolics or total tannin, and concentration of both followed a similar pattern of decline for all treatments with bottle age (Figures 2a, 2b).

Yeast treatment and bottle age were both main effects for anthocyanin concentration (Table 2). As is generally observed in red wine (McRae *et al* 2012), free anthocyanin concentration declined with bottle age (Figure 2a). RC212 treatment wines were consistently higher in anthocyanin concentration than AWRI1176 treatment wines, and by 16 months bottle age, that difference was approximately 40 mg/L. EC1118 treatment wines were also significantly higher in anthocyanin concentration than AWRI1176 by 16 months bottle age, however this was not a result that could have been predicted by the anthocyanin concentration observed for that treatment at bottling or six months bottle age (Figure 2c).

Interaction between yeast treatment and bottle age was observed for four of the phenolic parameters, non-bleachable pigment, colour density, hue and SO_2 corrected hue (Table 2). The development of non-bleachable pigment during bottle aging in the AWRI1176 treatment wines followed a distinct pattern from that of EC1118 and RC212 wines (Figure 2d). AWRI1176 wines were significantly higher in non-bleachable pigment concentration at each of the time periods sampled, however by 16 months bottle age the difference was substantial, with AWRI1176 wines around two-fold higher in non-bleachable pigment concentration than wines from the other yeast treatments. The development of colour density over time appears to show a similar pattern to that of non-bleachable pigment, with AWRI1176 treatment wines nearly two-fold the colour density of RC212 and EC1118 wines at 16 months bottle age (Figure 2e). The non-bleachable pigment and colour density results suggested that AWRI1176 wines may have matured at a faster rate than RC212 and EC1118 wines however, hue results showed AWRI1176 wines had a significantly more purple (young) hue than EC1118 and R212 wines at 16 months bottle age

(Figure 2f). The hue SO_2 value for AW treatment was significantly lower than that of EC and RC treatments at all bottle ages and by 16 months bottle age, the difference was substantial (approximately 0.5 AU). This result suggests that the young purple hue of AW wine was due to a greater proportion of that treatment's non-bleachable pigment being of a purple hue, as opposed to the more customary garnet hue of pigmented polymers.

DISCUSSION

Yeast and wine phenolics

Yeast treatment effects were documented for five of the seven phenolic measures examined in this study. Wine phenolic composition can be influenced by yeast through direct interactions (eg. absorption to yeast cell walls, yeast enzyme mediated hydrolysis) (Manzanares *et al* 2000; Caridi *et al* 2004; Morata *et al* 2005; Mazauric and Salmon 2006), and indirectly by the enhancement of phenolic reactions by yeast primary and secondary metabolites, yeast breakdown products and by-products of fermentation (e.g. yeast mannoproteins and polysaccharides, acetaldehyde, pyruvic acid) (Caridi *et al* 2004; Medina *et al* 2005; Caridi 2006). Wine is a complex medium and the fermentation approach applied in this study was novel in several ways, hence numerous factors may have contributed to the significant differences in phenolic concentration observed between yeast treatments. For example, yeast strain effects on anthocyanin concentration may have been due to differential fining of anthocyanin via adsorption to yeast cell walls. Adsorption of wine colour compounds has been shown to be a heritable trait in yeast (Caridi *et al* 2007). In a study of five *S.cerevisae* strains used to make Graciano wines, strain-related variation in anthocyanin adsorption percentages ranged from 1.6% to 5.9% (Morata *et al* 2005).

A second factor which may have influenced phenolic development in the wines was the production by yeast of primary and secondary metabolites that play a role in pyranoanthocyanin and polymer formation. The development of non-bleachable pigment in AWRI1176 treatment wines suggested yeast-mediated polymer formation may have been an important factor in our study. Two mechanisms have been documented for the early formation of stable colour in red wine: direct chemical condensation between anthocyanin and tannins, and acetaldehyde-mediated dimer formation (most commonly via an ethyl-bridge between malvidin-3-glucoside and catechin) (Monagas *et al* 2005). Dimer formation is dependent on acetaldehyde and acetaldehyde is produced by yeast as an intermediate product in AF. The rate and concentration of acetaldehyde production during AF has been shown to vary by yeast strain, fermentation conditions and grape variety (Liu and Pilone 2000; Hayasaka *et al* 2007). It is possible that yeast treatment AWRI1176 was associated with greater production or liberation of acetaldehyde than treatments RC212 and EC1118, under the conditions of our trial. The propensity for AWRI1176 to produce more acetaldehyde than *S.cerevisiae* strains has been demonstrated in Chardonnay fermentation (Eglinton *et al* 2000) and research by Hayasaka and others on *S.bayanus* AWRI1375 concluded that *S.bayanus* strains might offer positive outcomes for colour stabilisation in Cabernet Sauvignon wines (Hayasaka *et al* 2007). However, we have previously reported poor wine phenolic outcomes and no significant difference for non-bleachable pigment concentration in a comparison of the *S.bayanus* strain AW1176 with *S.cerevisiae* strains RC212 and EC1118 in a Pinot noir microvinification trial using the 'French Press' method (seven days AF on-pomace with submerged cap)(Carew *et al* 2013b). In the current study however, Pinot noir wines were made by microwave maceration with early pressing and the *S.bayanus* treatment AW1176 was associated with higher non-bleachable pigment formation, and the relative

concentration of non-bleachable pigment in AWRI1176 treatment wines increased substantially between six and 16 months bottle age (Figure 2d).

The hue SO_2 results (Figure 2g) showed a substantial proportion of the AW1176 treatment non-bleachable pigment was of a 'young' purple hue, rather than the aged garnet hue generally observed for pigmented polymers (Dambergs *et al* 2012). Further research would be required to identify the pigmentation complexes or polymers responsible for this purple hue, however copigmentation between anthocyanins, (self-association), with co-factors, and between anthocyanins and tannins has been associated with a bathochromic or 'blue' shift in red wine (Boulton 2001). Some of these stabilised forms of colour may be less desirable than others as copigmented anthocyanins formed by hydrogen-bonded self-association or with other low molecular weight phenolics tend to be sensitive to SO_2 bleaching. The ethyl-bridged flavanol-anthocyanin and anthocyanin-anthocyanin dimers have been describe as purple in colour and the vinyl-bridged flavanol-anthocyanin dimer ('portisin') as blue coloured (Cheynier *et al* 2006). Our findings may mean that *S.bayanus* yeast strain AW1176 acetaldehyde production or release led to more prolific ethyl-bridged dimer formation. Alternately, AW1176 may have overproduced or released a greater concentration of pyruvate catalysing the formation of portisins (Mateus *et al* 2004).

Notwithstanding differences in phenolic concentration between yeast treatments, all of the wines in this study appeared to have aged normally. There was significant and relatively uniform decline in mean total phenolics, total tannin and anthocyanin concentration between bottling and sixteen months bottle age (Figures 2a, 2b and 2c). This pattern of decline during early bottle aging is normal in red wine (McRae *et al* 2012). Informal sensory appraisal of wines from this trial by an experienced wine scientist and an experienced wine judge concluded that wine from

all three yeast treatments were without faults and were 'very varietal', however the tasters observed that yeast treatment was associated with different wine aroma and mouthfeel effects. Wines would need to be subjected to formal sensory appraisal to verify these apparent strain-related sensory effects.

Simultaneous AF and MLF

The Pinot noir fruit used in this study was harvested on 3rd April, 2012 and wine was bottled on 10th May, 2012 (37 days from harvest to bottling) demonstrating that co-inoculation for simultaneous AF and MLF of enriched juice from the novel microwave maceration process supported rapid red wine production. Beaujolais wines are bottled in a similarly rapid timeframe, but Beaujolais is a fruit-driven wine style with little tannin that is generally not subjected to MLF (Halliday and Johnson 2007). The novel winemaking approach described herein offers potential for efficient production of more tannic-styles of Pinot noir wine.

There was no apparent inhibition of MLF by any of the three yeast strains trialled. Previous research into co-inoculation at initiation of AF has returned mixed findings, with some studies reporting successful simultaneous AF and MLF (Massera *et al* 2009, Abrahamse and Bartowsky 2012) and others reporting yeast inhibition of malolactic bacteria (Comitini and Ciani 2007) through for example, competition for resources (eg. glucose, amino acids) (Ribereau-Gayon *et al* 2006) or production of differential SO_2 binding compounds, toxic metabolites or inhibitory proteins (Osborne and Edwards 2006; Osborne and Edwards 2007; Wells and Osborne 2011). The use of two commonly available and widely used yeast strains (EC1118, RC212) for successful simultaneous AF and MLF with PN4 may have application for red wines beyond Pinot noir. Should the lack of inhibition observed be a robust result, their use in simultaneous

fermentation could increase product turnover rate via earlier stabilisation of red wines (Jussier *et al* 2006).

The successful MLF observed in this study may have been due to alleviation by microwave maceration of multiple stressors that inhibit MLF under normal winemaking conditions. For example, as alcohol concentration increases, the thermal tolerance of malolactic bacteria declines (Ribereau-Gayon *et al* 2006). So, although lactic acid bacteria can be cultured at 37°C on neutral media (Ribereau-Gayon *et al* 2006), the optimal temperature range for MLF in red table wine is 20-25°C (Peynaud 1984). MLF can therefore be inhibited during the AF of Pinot noir because this grape must is generally subjected to fermentation temperatures exceeding 25°C to optimise phenolic extraction (Haeger 2008; Peynaud 1984). The microwave maceration process we used extracted phenolic compounds prior to inoculation for AF and MLF thereby eliminating the need for high fermentation temperature. Early, rapid extraction of phenolics via the microwave method allowed AF to proceed at a temperature suited to the malolactic culture (20-25°C) (Peynaud 1984) and the malolactic culture added at the outset of AF in our study also had the opportunity to establish MLF prior to the development of a potentially inhibitory ethanol concentration.

Free- and bound-SO_2 in wine can inhibit malolactic bacteria, especially at low pH (Ribereau-Gayon *et al* 2006; Osborne *et al* 2006). As microwave maceration is effective for sanitising must (Carew *et al* 2013a), application of SO_2 was omitted at crushing. The absence of added SO_2 in this expermental system may have enabled more rapid growth and less metabolic inhibition of the MLF inoculum. Malolactic bacteria play multiple roles in red wine fermentation (Liu 2002). One of these roles is the degradation of free- and SO_2 bound-acetaldehyde (Osborne *et al* 2006).

A high concentration of acetaldehyde in red wine is considered a fault (Peynaud 1984), however the compound plays an important role in early colour stabilisation (Boulton 2001). This suggests that further investigation may be warranted on the impact on acetaldehyde metabolism of low levels of SO_2 in microwave macerated musts.

Malolactic fermentation often takes place after AF and can add 40 or more days to the duration of red wine vinification (Abrahamse and Bartowsky 2012). In this study, the low fermentation temperature that was used to aid MLF extended the fermentation period by approximately 10 days compared with previous experimental ferments (Carew *et al* 2013a; Carew *et al* 2013b), however this enabled simultaneous AF and MLF to take place with wines finishing by day 17 after inoculation.

In conclusion, we demonstrated that yeast strain choice is important to long term phenolic character in wines made under the novel winemaking process based on microwave maceration and early pressing of Pinot noir grape must. Yeast strain AWRI1176 was associated with significantly higher concentration of stable colour at 16 months bottle age and more purple hued polymeric pigments compared with two other yeast strain treatments. Our finding concurs with the idea that strain-related phenolic effects are contingent on fermentation conditions (Girard *et al* 2001), and they emphasise the importance of better understanding yeast strain effects as a function of fermentation process, particularly in the case of a novel process like microwave maceration with early pressing. No inhibition was observed between the malolactic fermentation strain PN4 and the three yeast strains used in this study, and wines completed AF and MLF simultaneously which meant they were stable at 17 days post-harvesting. This study provided proof of concept for very rapid wine making of tannic styles of Pinot noir by co-inoculation for simultaneous AF and MLF. The rapid method of winemaking described in this paper warrants

further investigation for application at commercial scale, and in a range of varieties beyond Pinot noir.

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CONFLICT OF INTEREST

No conflict of interest declared.

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TABLES

Table 1. L-malic acid concentration in early pressing microwave macerated Pinot noir must early (day 1) and late (day 13) in fermentation with three yeast treatments (±SE). Different lowercase letters denote significant difference between treatments within and between time periods (Tukey's P≤0.05).

	L-malic acid concentration (g/L)	
	day 1	**day 13**
EC1118	2.74±0.03 **a**	0.13±0.001 **b**
RC212	2.70±0.02 **a**	0.09±0.008 **c**
AWRI1176	2.71±0.03 **a**	0.14±0.004 **b**

Table 2. Yeast treatment and bottle age main effects and interactions for phenolic and colour parameters in Pinot noir wine.

	ANOVA Significance (P)		
	yeast	**age**	**yeast x age**
Total phenolics (AU)	0.776	< 0.001	0.765
Tannin (g/L)	0.546	< 0.001	0.866
Anthocyanin (mg/L)	< 0.001	< 0.001	0.289
Non-bleachable pigment (AU)	< 0.001	< 0.001	< 0.001
Colour density (AU)	< 0.001	< 0.001	< 0.001
Hue	< 0.001	< 0.001	0.004
Hue SO_2	< 0.001	< 0.001	< 0.001

FIGURES

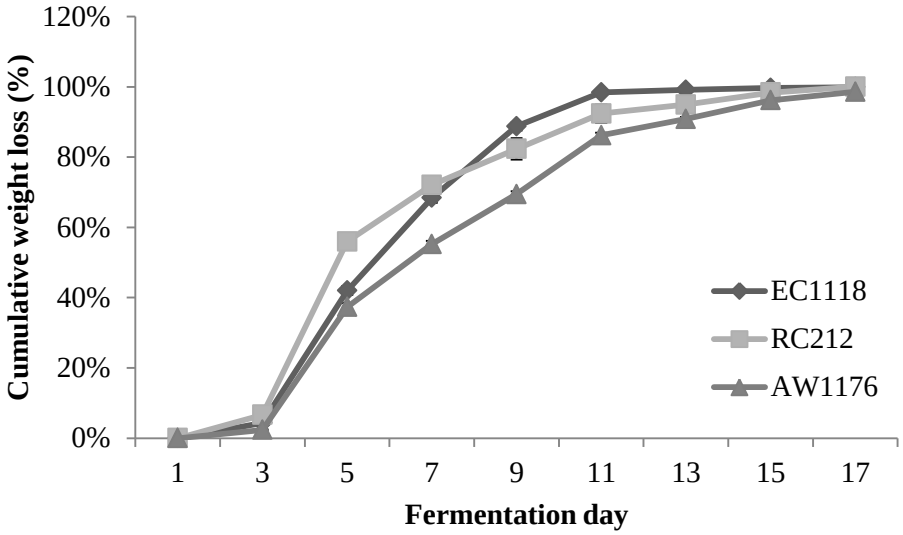
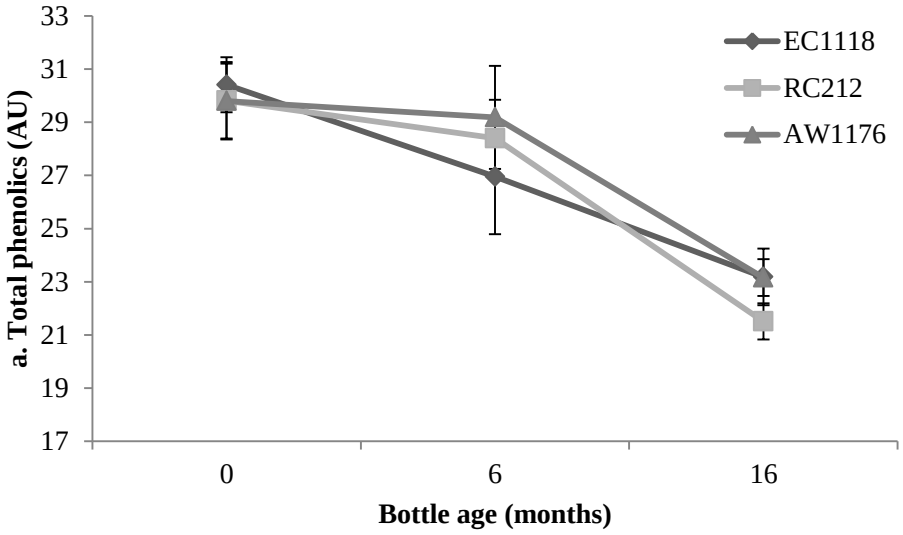
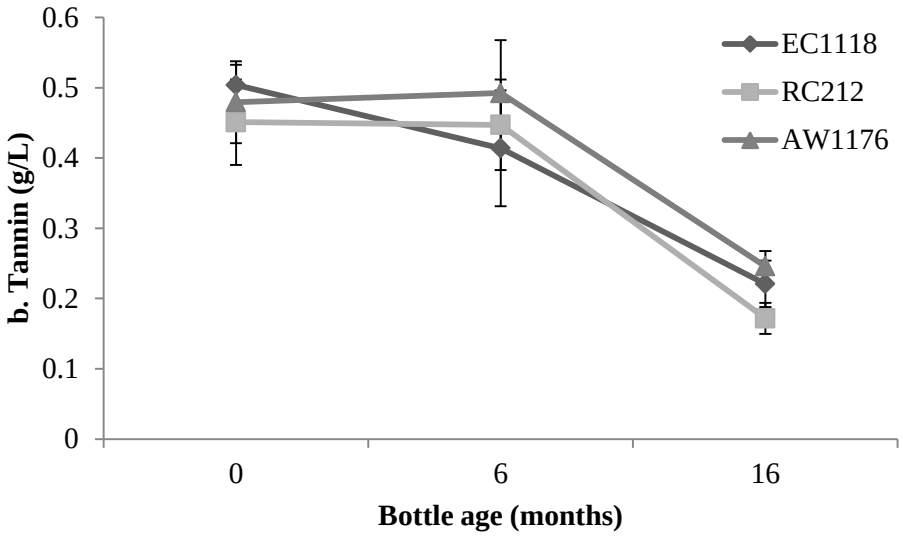
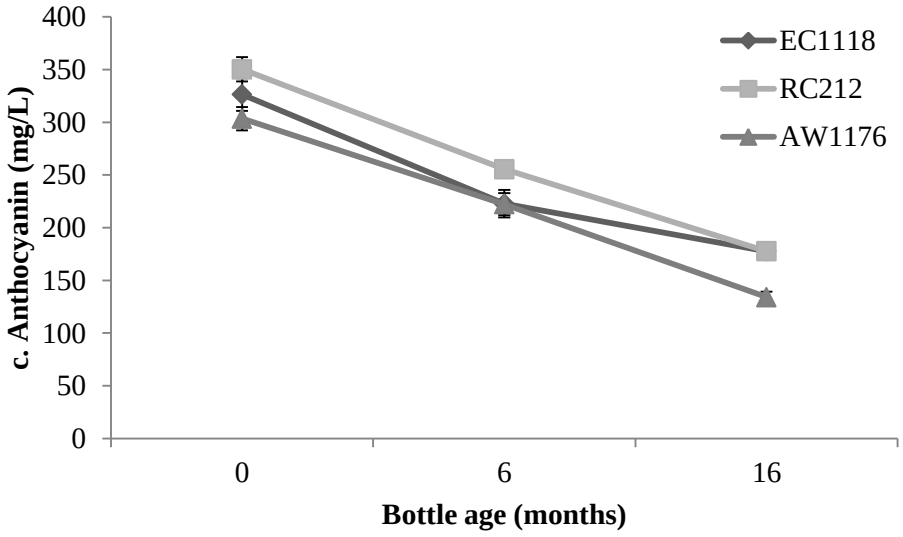
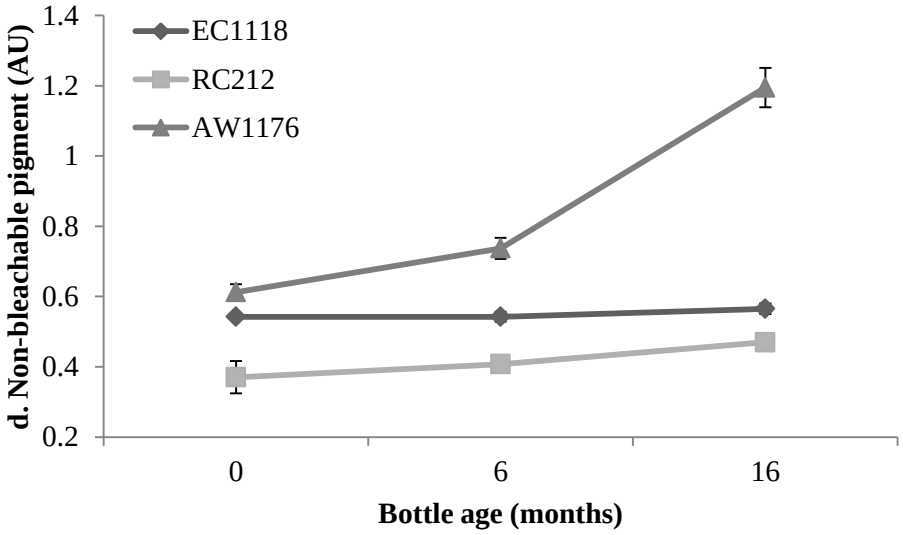


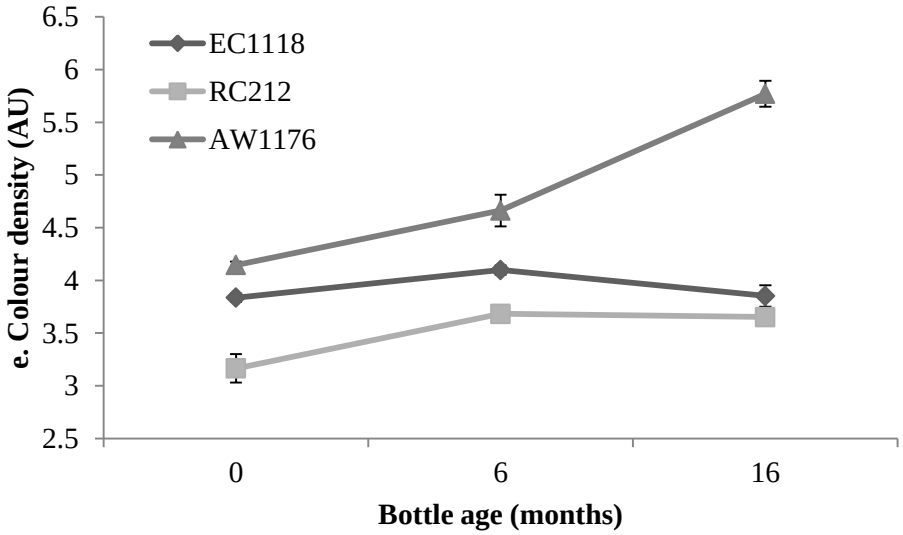
Figure 1. Fermentation kinetics for early press-off microwave macerated Pinot noir must fermented at 19(±2)°C under three yeast treatments (±SD).




0.6
0.5
0.4
0.3
0.2
0.1
0
b. Tannin (g/L)
0
6
16
Bottle age (months)
EC1118
RC212
AW1176


400
350
300
250
200
150
100
50
0
c. Anthocyanin (mg/L)
0
6
16
Bottle age (months)
EC1118
RC212
AW1176


d. Non-bleachable pigment (AU)
1.4
1.2
1
0.8
0.6
0.4
0.2
EC1118
RC212
AW1176
0
6
16
Bottle age (months)


e. Colour density (AU)
6.5
6
5.5
5
4.5
4
3.5
3
2.5
EC1118
RC212
AW1176
0
6
16
Bottle age (months)

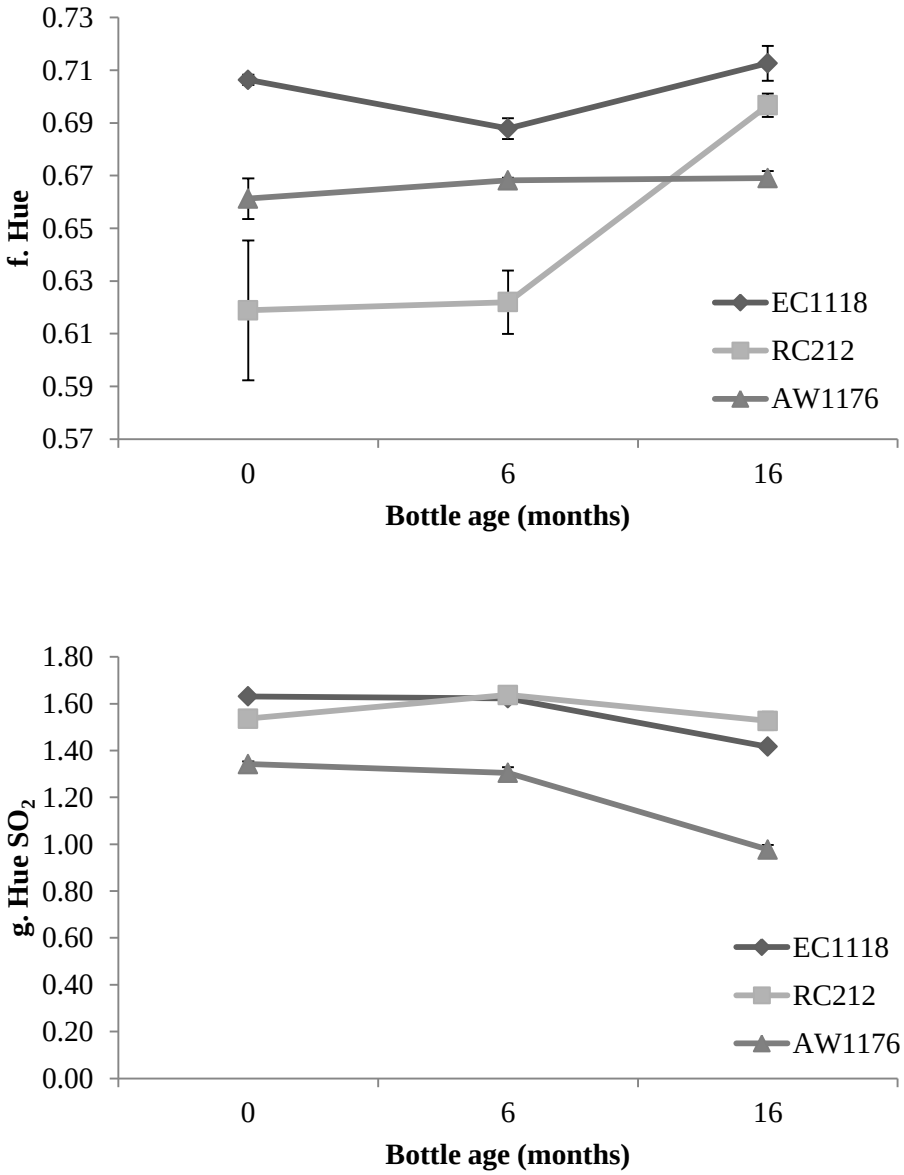


Figure 2. Phenolic concentration in early press-off microwave macerated Pinot noir wine fermented at 20°C under three yeast treatments (±SD). a. Total Phenolics; b. Tannin; c. Anthocyanin; d. Non-bleachable pigment; e. Colour Density; f. Hue; g. Hue SO_2

Chapter 9. SUMMARY & IMPLICATIONS

A novel process for Pinot noir wine making

This thesis has detailed the development of a novel red wine maceration process based on microwave, and reported exploratory research into the interaction between microwave maceration and several important winemaking parameters and decision points (eg. yeast strain, must nutrient status, wine aroma, viticultural interventions). The main conclusions from this body of research are that microwave maceration with managed hold time may provide wine makers with greater control over phenolic outcomes in Pinot noir wines, and that microwave maceration with early press off may offer substantial production efficiencies in red wine making.

Microwave maceration followed by fermentation on skins

Phenolics

Three independently replicated trials were conducted which compared control fermentation on skins with fermentation on skins of must that had been microwave macerated. The initial trial applied a ten minute hold time to must after microwave maceration, and the subsequent two trials applied a one hour hold time. Wine phenolic concentration was examined at storage ages ranging from 'at bottling' to 30 months, providing insight into the comparative impact of microwave maceration on young wine (eg. bottling to 6 months) and at a time period that approximated release and consumption of wine (eg. 18 to 30 months age).

Regardless of hold time, young microwave macerated wines were consistently higher in mean total phenolics, total pigment, total tannin, anthocyanin and colour density, than control wines.

For 10 minute hold time wines, difference in concentration of non-bleachable pigment was only apparent in the wines after 30 months bottle aging, although non-bleachable pigment concentration appeared to be trending towards a difference at 18 months bottle age. With a one hour hold time, young microwave wines were higher than control wines for mean concentration of non-bleachable pigment. This may indicate precocious formation of non-bleachable pigment during hold time, in part due to greater concentration (availability) of liberated anthocyanin and tannin in the longer hold time musts. An investigation of extended hold time which is reported in the appendix to this thesis suggested that longer hold time resulted in a comparatively stronger non-bleachable pigment concentration in wines, and this effect became more pronounced as wines aged.

As tannin concentration can be difficult to manage in Pinot noir wine making, and tannin is important for wine quality (Harbertson et al., 2008), our findings suggest that microwave maceration may assist wine makers to more often produce wines with tannin concentration similar to those observed in some Old World Pinot noir wines (around 2.0 g/L; Dambergs *personal communication*). Further investigation of the impact on tannin concentration of hold time after microwave maceration may offer winemakers an alternative approach to using extended maceration for building tannin in Pinot noir wines. Extended maceration can over-extract seed tannin which has been associated with bitterness in red wines.

Fermentation kinetics

Alcoholic fermentation (AF) was faster to initiate in microwave macerated musts than control musts. Rapid initiation of AF is important in industry as it reduces the risk of must oxidation and of colonisation by undesirable microbes, and may also contribute to production efficiency in the

winery. The reasons for consistent rapid initiation of fermentation in microwaved must remain speculative: sanitation of must by microwave may have reduced microbial competition or inhibition; inhibition of *S.cerevisiae* by SO_2 may have been alleviated by the presence of excess phenolics in microwaved must and/or; substantial physical disruption at the grape cellular level by microwave may have released cellular contents that enabled AF (eg. grape sugars, nutrients – note YAN in must was significantly higher when under microwave maceration, see subsequent section). Research into the underlying mechanisms or causes of early initiation of fermentation in this novel system would provide a basis for reliable fast starts, and could provide insights into the mechanisms of inhibition in early fermentation that slow initiation in standard red wine ferments.

Sanitation effect

Pinot noir must was plated for total yeast count directly after microwave maceration. This showed that microwave maceration was substantially more effective than SO_2 treatment for suppressing grape-associated yeast flora in must. In winemaking, the use of SO_2 to achieve around a two log reduction in grape-associated yeast flora to ~10^4 cfu/mL was sufficient sanitation to allow inoculated yeast to dominate a wine ferment (Cavazza et al., 2011). From that perspective, the yeast count in microwaved must demonstrated very effective must sanitation and was a novel outcome for winemaking. Due to its role as an antioxidant, SO_2 addition is unlikely to be replaced by microwave maceration, however, microwaving may offer winemakers the option of reducing their use of SO_2 during crushing. Reduced SO_2 addition at crushing could alleviate the lag phase following inoculation that is commonly seen in commercial ferments. An effectively sanitised microwaved must could improve winemakers' success in inoculation and earlier initiation of AF with a preferred fermentation strain.

YAN and enzyme effects

Microwave maceration had a significant impact on the concentration of yeast assimilable nitrogen (YAN) in must. Microwave macerated must was 40 mg/L higher in YAN than must prior to microwaving (control), and the difference in YAN was almost entirely attributable to a significant increase in primary amino acid nitrogen (PAAN) which was 38 mg/L higher in microwave must compared with control must. The early liberation of PAAN likely has significance for the successful initiation of fermentation, may alleviate the need for diammonium phosphate (DAP) addition in some musts, and may influence the metabolism of yeast (with flow on effects for wine aroma). These effects could be examined through further research.

Additionally microwave maceration can inactivate heat labile enzymes in grape must. Microwaving has proven more effective than thermal processing for enzyme inactivation in other food products (Keying et al., 2009; Matsui et al., 2008; Salazar-González et al., 2012), and the rapidity of heating by microwave may alleviate the problem of enzyme stimulation during thermovinification when a must transitions through 45-50°C (Rankine, 2004). During this project, microwave maceration was trialled on Shiraz fruit with Botrytis infection levels ranging from 1% to 40% by visual inspection. Oxidative risk fell from 8.2 μL/mL to 0.9 μL/mL (Carew et al., 2013a) which is evidence the process can impact heat labile enzymes. This application in Botrytis affected fruit suggests that the process has direct and immediate potential to reduce the impact of disease in the grape and wine industry.

Microwave maceration with early press-off and fermentation off-skins

Phenolics

The efficient and effective early extraction of phenolics from grape solids into juice that was observed from microwave maceration suggested an opportunity for an additional novel wine making process – microwave maceration with early press off, followed by fermentation off skins. By investigating and adjusting post-microwave hold time, we achieved sufficient phenolic extraction to justify investigating this early press off approach.

Three independently replicated trials were conducted to compare phenolic outcomes in wines from microwave maceration with early press off ('off skins') and control fermentation for seven days on skins. The hold time applied across the three trials was the same (one hour) and demonstrated that extraction of phenolics under the microwave maceration and hold time regime resulted in equivalent, or greater, phenolic extraction depending on the fruit parcel. In two trials, the six month old Pinot noir wine from microwave maceration treatment was largely equivalent to control wine for six of the phenolic measures examined. The third trial resulted in microwave macerated wines with greater concentration of total phenolics, total pigment, tannin and colour density than control wines. Across all three trials, there was substantial variation in wine tannin concentration between replicates which may have overshadowed a tannin effect - microwave wine tannin was numerically higher for all three trials, but the difference was non-significant for two trials.

Based on our trials, it is clear that microwave maceration with early press off after a one hour hold time can produce Pinot noir wine which is similar in phenolic concentration to, or has

greater phenolic concentration than, wine fermented on pomace for seven days. Similar results were recorded in a small-scale comparison of control and microwave macerated Shiraz must, however that variety required a three hour hold time to produce microwave wine equivalent in phenolic profile to the control treatment (Carew et al. 2013a). These findings suggest that microwave treatment with early press off may offer an efficient way to make Pinot noir wine, and potentially other red wines, with phenolic concentration equivalent or greater than submerged cap fermentation.

Fermentation kinetics

Early press off microwave macerated must fermented off skins was consistently faster to initiate and finish fermentation than control must fermented on skins. In the three independently replicated trials conducted, off skins microwave wines completed fermentation early (~day five) compared with control treatment (day seven). Fermentation kinetics graphs showed that microwave off skins treatments delivered an abrupt end to fermentation whereas control wine fermentation slowed around day five. This suggests the novel off skins fermentation process may aid winery efficiency in two ways. Removal of pomace would reduce the volume of must (tank space requirement) by approximately 40% and additional winery capacity would be created by short fermentation time (~30% faster than standard fermentation). There would also be potential cost savings due to the absence of cap to manage (plunge, pump over, chemical additions). The fermentation kinetics results reported in this thesis suggest that the novel microwave maceration with off skins fermentation process may also reduce risks associated with sluggish or stuck fermentation, although this assertion would need further investigation.

Aroma effects

Physical and chemical conditions in must influence yeast metabolism, and hence aroma compound generation. Few researchers have related the chemical and physical impact of thermal maceration processes to red wine aroma outcomes, but one study concluded that thermovinified red wines were significantly higher in ester compounds than control wines, and displayed 'fruity' character (Fischer et al., 2000). Given the important role of esters in Pinot noir wine aroma (Fang and Qian, 2005), investigating the impact of microwave maceration on Pinot noir aroma compounds like esters was of importance to understanding the overall viability of the proposed process.

The findings presented in this thesis show that the concentration of aroma active compounds differed substantially between control and microwave maceration with early press off treatment Pinot noir wines, and that microwave maceration wines were instrumentally higher in many important aroma active compounds. Microwave macerated wines were slightly higher than control wines for higher alcohols, these compounds tend to be perceived as fruity at low concentrations and fusel or spirituous at higher concentration. Differences between treatments were comparatively high for the three acetate compounds examined, with response ratios four to six times higher in microwave wines than control wines. Most of the ethyl esters examined were also higher in microwave wines than control wines. This is significant as ethyl octanoate and ethyl decanoate have been identified as key odorants in the varietal aroma of Pinot noir wine (Fang and Qian, 2005) and these compounds were two-fold higher in the microwave wines than the control wines. Wine aroma results from complex interactions between compound concentration and human perception so formal sensory appraisal of these wines would be required to establish if the differences revealed by GCMS would translate into consumers of microwave macerated wines having different aroma experiences to control wines.

We proposed seven factors that may contribute to the differences observed between thermovinified and standard wines: liberation of grape aromas and aroma precursors; fermentation temperature differences; differences in CO_2 evolution rate; volatilisation of aroma compounds during cap management; heat inactivation of aroma degrading enzymes and transferases; the presence or absence of pomace; and the use of semi-open versus semi-closed fermentation systems.

Simultaneous alcoholic and malo-lactic fermentation

Due to the sanitising effect of microwave maceration, SO_2 application was not required in microwave musts at crushing (Carew et al., 2013b). Additionally, rapid phenolic extraction from microwave obviated the need for high temperature alcoholic fermentation. This meant it was possible to inoculate early press off juice for simultaneous alcoholic and malo-lactic fermentation. An replicated experimental trial of this approach to winemaking showed it was effective and resulted in wines that had finished AF and MLF day 17 of the fermentation period. Early, rapid extraction of phenolics via the microwave method used in this study allowed alcoholic fermentation to proceed at a temperature suited to the malolactic culture (20-25°C) (Peynaud, 1984). This option for lower temperature fermentation may offer wine makers a means to preserve volatile aroma compounds. In this study, low fermentation temperature extended the AF and MLF period by approximately 10 days compared with previous experimental ferments (Carew et al., 2013b), however conventional MLF after AF, can add 40 or more days to the duration of red wine vinification (Abrahamse and Bartowsky, 2012). While this approach represents the most radical departure from standard wine making practice that we have proposed, the potential benefits in terms of cooler fermentation options (aroma), expansion of

effective winery capacity and faster stabilisation of wine (simultaneous AF and MLF) justify serious consideration of the approach as an industry scale process for red wine making.

Microwave maceration as a novel, efficient winemaking process

In Chapter 5, microwave maceration was compared with heat maceration and shown to cause the greater intracellular damage to grape skins. Whilst the resulting bottle aged wines in that trial did not show significant difference in phenolic profile between heat and microwave maceration treatments, the results inferred more substantial and extensive intracellular disruption associated with microwave. As described in the introductory chapter, microwave heating occurs at a distributed, molecular scale and this differs from conventional heat transfer mechanisms operating in heat macerated must; heat macerated must relies on conductive heating from the sleeved element and either physical stirring, pumping or convection to distribute point source heat throughout the must. An open question remains as to whether part of the effective liberation of phenolic compounds with microwave maceration was due to non-thermal effects. These effects, in theory, would involve disruption to electrical processes within biological cells (eg. maintenance of semi-permeability) and smaller scale chemical binding (eg. Van der Waals forces). The body of work presented here did not examine non-thermal effects empirically and so further research would be required to examine the role of these, as well as the role of a potential synergy in mass and heat transfer processes (eg. cellular and molecular origin, same direction of transfer).

Unique features and possible mechanisms of microwave assisted extraction

<u>Cell-level effects</u>

As indicated in Chapter 1, microwave heating differs from conductive heating in that it is a penetrating form of heat generation which acts at a molecular level, and is distributed throughout the material being heated. Conductive heating relies on molecule-to-molecule transmission of heat from the point source. The research reported in Chapter 5 established there were histological and juice phenolic differences between musts heated in these two ways. The size of effect reported in Chapter 5 between the two heating methods may not have been sufficient to pass the 'industry significance' test, in that the gains from microwave would be unlikely to justify industry investing in a novel technology (and its associated risks). Scientifically, however, the mechanisms behind the observed difference merit consideration. The main mechanism driving the observed differences between conventional heating and microwave heating of musts is likely to have been differential physical damage to cell structures. Gross differences between heat and microwave treatment impacts on vacuolar integrity were suggested by the depletion of vacuolar contents apparent in microwave treated grape skin (Chapter 5, Figure 1, plate c: pg 144) and evidence of nitrogen release (Chapter 3: significant difference in YAN), however, the specific forms of degradation were not examined. These differences could be examined and quantified by aser scanning confocal fluorescence microscopy (LSCM), Raman spectroscopy, atomic force microscopy (AFM), electron microscopy (SEM, TEM) or nuclear magnetic resonance (NMR) (Chundawat et al., 2011). The possible forms of cellular degradation from microwave include: distortion of existing intra- and inter-cellular pores which mediate semi-permeability (Gekas et al., 2002; Chundawat et al., 2011), or heat-mediated breaching of cell walls and membranes (eg. cellulose gelatinisation). Alternately, microwave heating may have changed the conformation of, or degraded, specific cell components (eg. cell wall proteins). Each of these speculated mechanisms could be termed a 'heat-mediated effect', and if these mechanisms were associated

with observed extraction outcomes, they would suggest that the distributed nature of heating in the microwave process was important in achieving greater extraction than conventional conductive heating.

Some authors have suggested that non-thermal effects may aid in phytoextraction by microwave. While there has been no published evidence to support this thesis to-date, the possible mechanisms of non-thermal effects include; changed dielectric properties in the heated material, disruption of hydrogen-bonding or Van Der Waals forces (Dincov et al., 2004) which mediate cell structure and possibly phenolic compound attachment to cell membranes on a fine scale. A better understanding of the polarity of the specific phenolic compounds targeted would be needed to underpin investigation of non-thermal effects, as polarity likely affects detachment of phenolics from the grape solids matrix (Wijngaard et al., 2012).

Mass transfer effects

The differences in phenolic extraction observed between microwave-macerated musts held at peak temperature for 10 minutes (Chapters 3 and 4), those held for one hour (Chapters 5, 6, 7 and 8) and unpublished data from hold time trials (Appendix C) demonstrated that extraction in this system was in-part, due to the effect of time on diffusion of phenolics from grape solids into juice. Microwave heating of foods has been modelled using Maxwell's equations which are based on the properties of the microwave system, the interface between the microwave cavity space and material to be heated, and the dielectric properties of the material to be heated (Campañone et al., 2012; Routray and Orsat, 2012). Mass transfer of compounds in phytoextraction has been modelled using Fick's second law (Gekas et al., 2002; Ziaedini et al., 2010), which assumes time and temperature-driven diffusion as the main mechanism of mass

transfer. This emphasises that variation in hold time and peak temperature are likely important factors in controlling the rate and extent of phenolic extraction in the microwave-mediated extraction process described in this thesis. Routray and Orsat (2012), however, identified three additional variables which may influence mass transfer in microwave extraction; dielectric properties of the solvent, microwave power level, and solvent-plant material contact surface area.

The change in grape juice viscosity with heating was likely an important mechanism influencing diffusion of phenolics into grape juice. It is unlikely that fluid viscosity changes differed between heating by microwave and conductive heating, except in relation to the distribution and efficiency of heating. However, the impact of temperature on fluid viscosity may have contributed in two ways: the change to fluid viscosity may have reduced juice resistance to the transmission of molecules thereby decreasing the time to equilibrium (more efficient extraction), and higher temperature and associated increase in molecular excitation may have enhanced the rate at which molecules 'escaped' the cellular wreckage (ie. breached, damaged, porated, distorted, perforated grape solids).

Phenolic extraction and polymerisation under microwave

The body of research presented in this thesis demonstrates that microwave is highly effective in extraction of primary grape phenolic compounds (eg. anthocyanins, tannins). This is the simplest and most direct benefit of the application of the CPR process in red winemaking. Chapter 5 however, showed CPR wines with early press-off to be differentiated from control and microwave wines fermented on skins on the basis of colour stabilisation; these wines were significantly higher in non-bleachable pigment which is an important outcome in terms of rapid stabilisation and finishing of wines. The two mechanisms that have been proposed for pigmented

polymer formation are direct condensation and bridging. Bridging has been identified as the faster and more prevalent process of co-pigment formation in normal red wine systems (Hayasaka, Birse, Eglinton, & Herderich, 2007; Timberlake & Bridle, 1976). The hold time data presented in Appendix C, however, may contradict this contention. Bridging is mediated by yeast secondary metabolites (eg. acetaldehyde, pyruvate) and the creation of non-bleachable pigment prior to yeast inoculation strongly supports the thesis that the mechanism of polymerisation in this system is direct chemical condensation. This is a novel concept and system, and further research into this phenomenon would be of value including: characterisation of the polymers in terms of identifying the catechin species contributing (ie. whether the skin-associated epigallocatechin played a greater role than seed-associated epicatechin-3-gallate) and understanding their long term stability (ie. beyond the 30 months examined in this body of research). A further interesting question to answer would be whether the observed high concentration of pre-AF non-bleachable pigment in CPR with early pressing wines was attributable to the heating regime speeding or aiding chemical condensation, or whether this effect was more strongly associated with the synchronisation of extracted anthocyanin and tannin. These questions might best be answered initially in model system research, and further explored by selective removal or addition of phenolics to CPR juice by reverse osmosis, for example.

Future research

Engineering process optimisation

The rapid and effective phenolic extraction from grape must from using microwave maceration in this study could be investigated via biophysical effects of microwave on grape material. As described above, there are several potential mechanisms at work in microwave phytoextraction. Investigation at the mechanistic scale would assist in developing the process for larger scale

industrial application and optimising extraction outcomes. Engineering optimisation relies on modelling systems as a precursor to process design. As discussed above, modelling the relationship between microwave heating and the extraction of phenolic compounds from grape solids into grape juice to support engineering process optimisation would require integration of such factors with Maxwell's and Fick's models.

Additionally a 'black box' research approach - varying microwave parameters which may affect the rate and effectiveness of extraction - could assist with immediate process development for industry application. Parameters which could be varied include: hold time at 70°C after microwave maceration (extended hold time was shown to increase extraction, with asymptotes varying by target phenolic compound), peak temperature (early trials suggested an extraction threshold at 65°C; some of the research literature indicated some classes of phenolic compound may be heat labile at ~95°C); and the effect of agitation or stirring on mass transfer (the power law suggests mass transfer from grape solids to juice may be aided by stirring or agitation).

Business case and practicality

Based on extensive microvinification trials, the innovation proposed in this thesis of rapid, efficient red wine making by microwave maceration and early press off appears to be technically feasible. Technical feasibility or 'proof of concept' is a necessary first step in the development and introduction of new processes for industry. It is not, however, the final step. Additional research would be required to understand the costs, benefits and risks (perceived and real) of this innovative approach. The development of a business case for use of microwave maceration for red wine making in industry would need to consider the capital and operating costs of this approach in light of the efficiency and quality gains it offers. For example, the capital cost of

microwave equipment may be comparable with investment in a thermovinification or Flash Détente unit, however the running cost for these technologies would likely differ as the energy conversion rate for microwave is substantially higher than convective heating which would suggest lower running costs and lower energy footprint for the microwave option. The capital and operating cost of microwave maceration with early press off needs to be analysed against the offset capital expenditure on winery capacity (or resulting increase in effective winery capacity), compared with standard fermentation of Pinot noir must on skins.

Further research is also required to understand the practicality of microwave in an industry setting. The microvinification research reported in this thesis included two batch processes (batch heating in microwave, batch hold time). Upstream unit operations in the wine industry are mostly inline (eg crushing, destemming) and downstream operations are mostly batch (eg alcoholic fermentation, cooperage). Industry scale microwave units tend to be of the tunnel variety which would offer the opportunity to incorporate microwave maceration as an inline process, however further research would be required to understand how wine phenolic profile is affected by pumping through a tunnel microwave unit, and possibly through lagged heating tubes (inline hold time) and through a counter current exchange cooler (chilling after hold time). Pinot noir wine makers tend to avoid pumping and agitation of must due to perceived damage to the integrity of grape solids and resulting impacts on wine quality, so the impact of microwave as an inline process on wine quality would need investigation.

Conclusion

Microwave maceration was associated with effective and rapid extraction of phenolics in Pinot noir winemaking, and offered a range of exciting opportunities for better controlling phenolic outcomes in red wine making, and substantial potential efficiencies in the red wine making process. The next step for this research is to engage winemakers in large scale trials of microwave maceration for red wine making. The novel process described herein may be a valuable new tool which will complement the wine maker's existing options for red wine maceration to create beautiful red wines.

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APPENDIX A – Poster presented at the 8th International Cool Climate Symposium, Hobart, Tasmania, February 2012

Pinot noir phenolics: outcomes from inoculated & wild fermentation

ICCS February 2012, Hobart

Anna L Carew (TIA), Robert Dambergs (AWRI/TIA) & Chris Curtin (AWRI)

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Background

- Extraction and stabilisation of phenolics is a challenge for Pinot noir winemaking
- Yeast important for phenolics in red wine? Research results are 'variable'
- Limited yeast strains and strategies favoured by Pinot noir winemakers (eg. RC212, wild, EC1118)

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Yeast trials

Q1: Are there yeast strain effects on phenolics in Pinot noir?

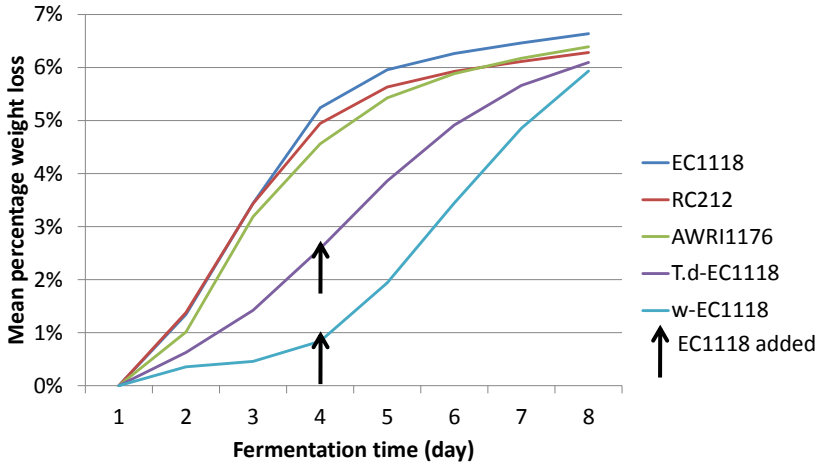
Strains:
- *Saccharomyces cerevisiae* EC1118, RC212
- *Saccharomyces bayanus* AWRI1176
- non-*Saccharomyces* sequential inoc (*Torulaspora delbruekii* + EC1118)
- 'wild' sequential inoculation (EC1118)

Q2: Are phenolics from 'non-*Sc*' ferments more like 'wild' or inoculated ferments?

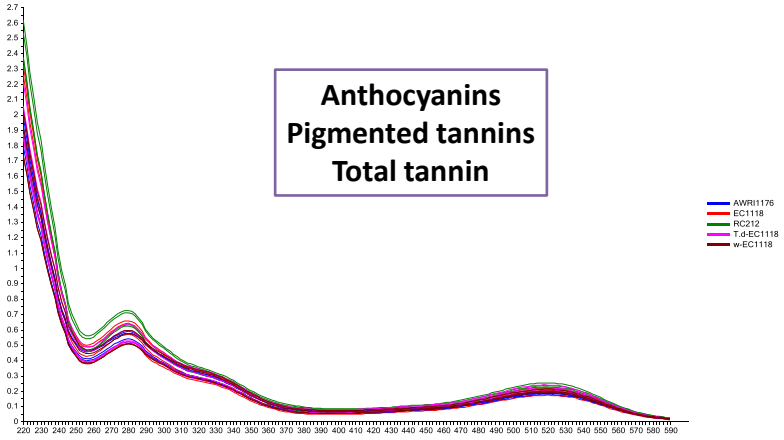
Microvinification

1. **Crush & destem**
2. Add SO_2
3. **Inoculate**
4. **Ferment**
5. **Cold settle**
6. Rack & bottle

Fermentation kinetics (D)



Raw UV/Visible Spectra



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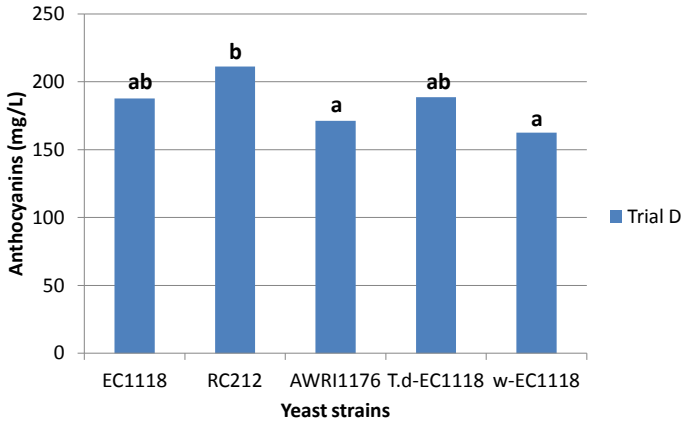
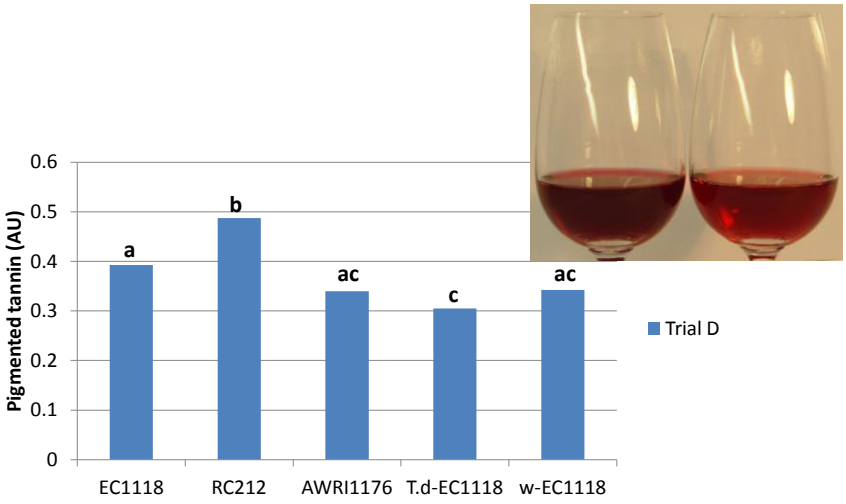
Anthocyanins - 6 mths



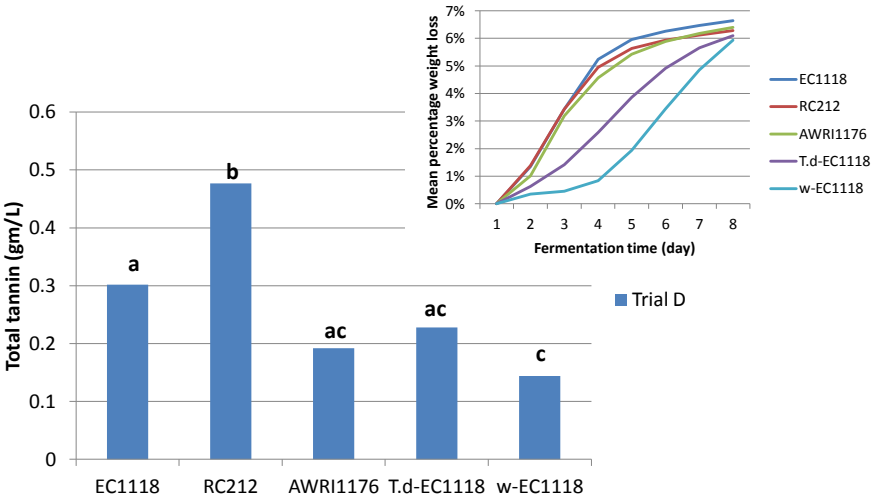
Different letter denotes significant difference between strains within trial (P<0.05)

Pigmented tannins (6 mths)



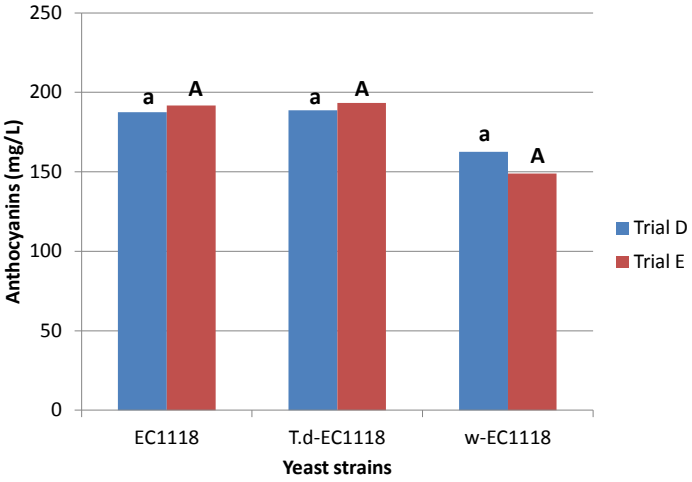
Different letter denotes significant difference between strains within trial (P<0.05)

Total tannin (6 mths)



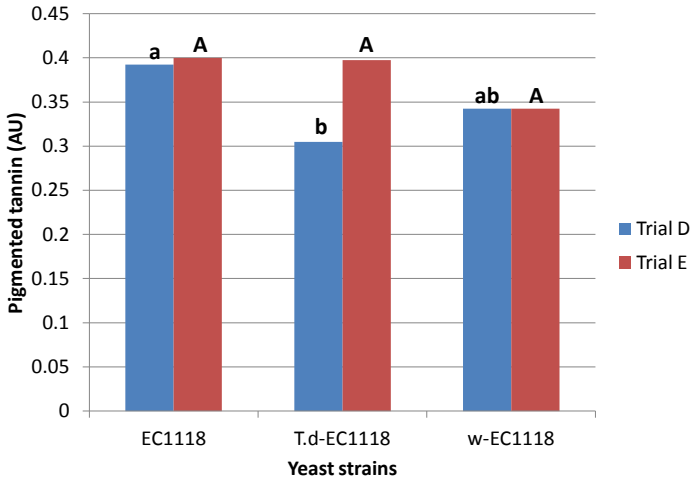
Different letter denotes significant difference between strains within trial (P<0.05)

Anthocyanins - 6 mths



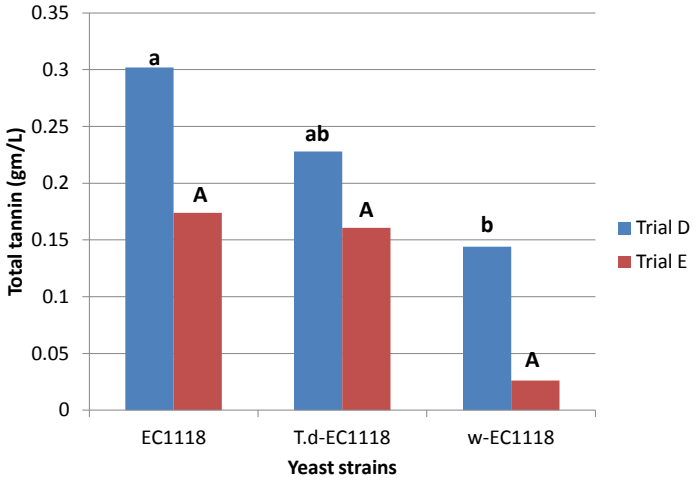
Different letter denotes significant difference between strains within trial (P<0.05)

Pigmented tannins - 6 mths



Different letter denotes significant difference between strains within trial (P<0.05)

Total tannin - 6 mths



Different letter denotes significant difference between strains within trial (P<0.05)

Conclusions from 2011

- Yeast treatment had significant impact on phenolics
  - stable colour effect
  - effect on tannin greater magnitude
- Yeast strategy may assist Pinot noir makers to improve phenolic outcomes & colour stabilisation
- But…

…questions remain

AROMA? 

Does strain affect extraction…

…or is result due to differential tendency to bind/complex with phenolics?

Acknowledgements

• Dr Dugald Close (TIA)
• Lallemand Australia
• Mauri Yeast Australia
• Tamar Ridge Estates

• Carew is supported by an Australian Postgraduate Award, the Tasmanian Institute of Agriculture, University of Tasmania and the Australian Wine Research Institute

Thank you! Questions?

APPENDIX B – Poster presentation from the 8th International Cool Climate Symposium, Hobart, Tasmania, February 2012

Maceration Effects and Pinot Noir Phenolics

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[2] Australian Wine Research Institute, Tasmanian Institute of Agriculture, Hobart, Tasmania

Introduction

Extraction of phenolic compounds for adequate colour, colour stability and balanced mouthfeel can be a significant challenge for Pinot noir winemakers. A range of maceration techniques is used by red wine makers to optimise extraction, with varying effect (Sacchi et al 2005). During 2011 vintage, phenolic extraction outcomes from seven maceration techniques for Pinot noir were evaluated (n=28) using 1kg microvinification in Bodum™ pots (Fig. 1). All maceration treatments were applied to grapes that had been crushed, destemmed, and dosed with 50gm/L SO_2. The maceration treatments were:

1. Control
2. High sulphite (+100gm/L SO_2)
3. Enzyme addition (name enzyme)
4. Extended maceration for 4 days
5. Cold soak at 4°C for 4 days
6. Freeze at -20°C for 4 days, & thaw
7. Dry ice soak for 4 days

Fig. 1: Microvinification of Pinot noir in 'submerged cap fermenters'

Fermentation kinetics

Treatments 1-4 were inoculated with yeast at day 1 and treatments 5-7 at day 5 (Fig. 2). All pots were fermented at 27°C (±3°C) and weighed periodically to track CO_2 loss as an indicator of fermentation kinetics. Figure 2 shows that all treatments conformed to typical fermentation profiles with initial lag phase, rapid fermentation, and coming dry around 7 days after inoculation. The high sulfite treatment was notably slow to enter lag-phase fermentation and the freeze & thaw treatment finished relatively rapidly.

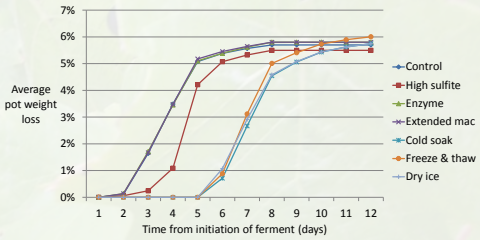

Fig. 2: Fermentation kinetics for seven Pinot noir maceration treatments

Phenolic effects

Phenolics were quantified using UV-Visible Spectrophotometry when wine was at 6 months bottle age. Asterisks in Figures 3, 4 and 5 denote significant differences between treatment and control for three phenolic measures that are important indicators of: colour potential (free anthocyanins), stable colour (pigmented tannins) and astringent mouthfeel (total tannin). The extended maceration treatment achieved high stable colour by 6 months (Figs 3 and 4) as did dry ice maceration (Fig. 4). Significantly high tannin in the extended maceration treatment (Fig. 5) may signal an over-extracted, astringent wine. Significantly higher free anthocyanin and tannin in freeze & thaw (Figs 3 and 4) calls into question the use of frozen Pinot noir grapes for winemaking research. Interestingly, there were no significant differences in the three phenolic measures for commonly used maceration techniques (eg. high SO_2, enzyme, cold soak) at this stage of the experimental wine's maturation.

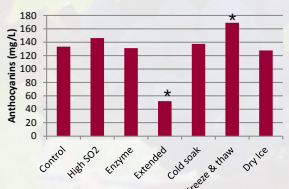

Fig. 3: Free anthocyanin at 6 months bottle age

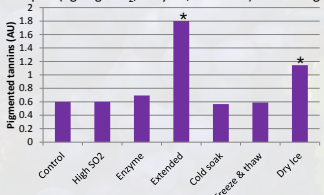

Fig. 4: Pigmented tannin at 6 months bottle age

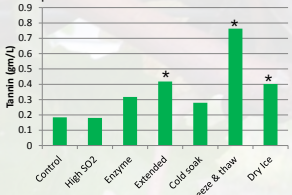

Fig. 5: Total tannin at 6 months bottle age

Fig. 6: Pinot noir wine at 6 months

Conclusion

Effective extraction of phenolic compounds is important to achieve Pinot noir wine that is visually appealing and has a balanced mouthfeel. Of the experimental maceration treatments applied, only extended contact time treatments delivered significant differences in the phenolic parameters measured. This suggests that to produce strongly coloured (Fig. 6), tannic Pinot noir wine may require extended time on skins using means other than cold soaking. Trade-offs to consider would be reduction in winery throughput, risks of oxidation or over-extraction, and additional inputs (eg. additives, energy). The results also signal caution for Pinot noir winemaking research based on frozen grapes, given significant differences observed from the freeze & thaw treatment.

Acknowledgements

Carew and Sparrow - Australian Postgraduate Award, the Tasmanian Institute of Agriculture, University of Tasmania and the Australian Wine Research Institute. Additional support - Lallemand Australia, Tamar Ridge Estates and the Australian Grape and Wine Research and Development Corporation.

TIA is home to the AWRI's Tasmanian Node which is jointly funded by TIA, UTAS, AWRI and the GWRDC.

PDF available at http://winetasmania.com.au/technical/research/8th_iccs/posters

APPENDIX C – draft of Materials and Methods, and Results and Discussion sections for a manuscript on effects of extended hold time after microwave maceration on phenolics diffusion in must, and wine

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Author contributions to date: Carew 88%, Close 10%, Dambergs 2%

JOURNAL

Journal of Food Engineering

TITLE

Diffusion of phenolic compounds from Pinot noir grape solids into juice during high temperature hold time, and phenolic characteristics of wine.

AUTHORS

Carew, Close, Dambergs

ABSTRACT

KEYWORDS

INTRODUCTION

MATERIALS & METHODS

Fruit and phenolics analysis

Pinot noir grapes were harvested on 25th March, 2013 from a vineyard in Northern Tasmania, Australia. Fruit was analysed for ripeness by assessment of pH (3.28) and titratable acidity (8.34) (Crison, pH-Matic 23, Italy), and total soluble solids (22.4) measured by digital hand-held pocket refractometer Pocket PAL-87S (Atago, Japan).

Grape juice and wine phenolics were quantified by UV-visible spectrophotometry as previously described (Mercurio, Dambergs et al. 2007; Dambergs, Mercurio et al. 2012). MORE here – measures and why used those.

Impact of hold time on juice and wine

The impact of hold time after microwave maceration on the concentration of phenolics in Pinot noir juice was examined. Fruit bunches were randomly allocated to 1 kg lots (n=4) and each lot was crushed in a custom bench top crusher, then destemmed by hand. Resulting must was subjected to microwave maceration as previously described (Carew, Sparrow et al. 2013) to a peak temperature of 70°C followed by hold time in a 70°C waterbath. A 1.5 mL juice sample was taken from each replicate hourly over the six hours hold time period, and samples were frozen for later analysis.

The impact of hold time on wine phenolics was examined using a 3x2 factorial experiment. There were three wine making treatments - control fermentation on skins (ctl); microwave maceration of must with three hours hold time followed by pressing-off prior to fermentation (3hr); microwave maceration with six hours hold time and press-off prior to fermentation (6hr), and two bottle age treatments: phenolics were analysed at bottling, which was approximately 40 days post-harvest (bott); and at six months bottle age, which was approximately 220 days post-harvest (6mo).

Must for the wine making experiment was prepared from the same fruit used for the hold time and juice experiment. As above, fruit bunches were randomly allocated to 1 kg lots and crushed and destemmed, with four replicates for each winemaking treatment (n=12). Control (ctl) wines were made using the French Press method (Dambergs and Sparrow 2011; Carew, Sparrow et al. 2013). Briefly, musts were treated with 50 mg/L sulfur dioxide in the form of a 20% aqueous solution of potassium metabisulfite and inoculated with cultured yeast strain *Saccharomyces cerevisiae* EC1118 (Lallemand Australia). Submerged cap fermentation at 28°C for 7 days in Bodum™ coffee plungers followed.

For microwave treatment wines, 8 replicates were microwave macerated as previously described (Carew, Sparrow et al. 2013) to a peak temperature of 70°C and then held at peak temperature in a 70°C waterbath. For this experiment, hold time was extended to three hours for the '3hr' winemaking treatment and six hours for the '6hr' winemaking treatment, rather than the 10 minute hold time described in our previous work (Carew, Sparrow et al. 2013). For the 3hr and 6hr treatments, must was pressed off after the allocated hold time using a custom-made basket press, juice was transferred to 500 mL Schott™ laboratory bottles, and cooled to 28°C. Replicates were inoculated for alcoholic fermentation with *S.cerevisiae* EC1118 (Lallemand Australia) then transferred to a 28°C constant temperature room. Schott™ bottles were loosely capped for the first five days of the alcoholic ferment which allowed CO^2 to release when pressure build up was sufficient to lift the Schott™ bottle lid and exhaust a portion of CO^2. Schott™ bottles where agitated (swirled) on days five and six to re-suspend settled yeast and ensure completion of the ferment.

At day two of the alcoholic ferment, XX mg/L of diammonium phosphate (20% aqueous solution) was added to each winemaking replicate (ctl, 3h, 6hr). Fermentation kinetics were monitored by weighing Bodum™ pots and Schott™ bottles each day. Weights stabilized at day 7 after inoculation and wines were assessed for residual sugar using Clinitest™ tablets. All wines were found to have completed

alcoholic fermentation by day 7 (≤2.5 g/L residual sugar). Control wines were pressed-off by firm application of the Bodum™ screen for 10 seconds, and pouring to 375 mL green glass bottles which were capped with a crown seals and cold settled at 4°C. Microwave wines were transferred to 375 mL green glass bottles and capped with crown seals for cold settling at 4°C. Wines were not subjected to malo-lactic fermentation or filtration, all wines were stabilised at racking with 80 mg/L SO_2 in the form of a 20% aqueous solution of potassium metabisulfite, and bottled under CO_2 cover to 50 mL amber glassware with wadded polypropylene capping. Bottled wines were stored under controlled conditions and a new bottle of wine from each replicate was opened for phenolics analysis at each bottle age time period.

Statistical Analysis

Absorbance data from UV-visible spectrophotometry of juice and wine samples was exported into a calculator developed by the Australian Wine Research Institute (REF). As reported previously (REF), the calculator used absorbance data from a modified Somers method to calculate phenolic concentration; A520 nm in a metabisulfite solution for non-bleachable pigment concentration, A420 and A520 nm in an acetaldehyde solution for colour density and hue, A520 nm in metabisulfite solution and 1MHCl for anthocyanin concentration, A420 nm in 1MHCl for total phenolics and A250, A270, A280, A290 and A314 nm in 1MHCl to quantify tannin.

The impact of hold time on Pinot noir juice phenolics was analysed using GenStat 14th edition. Means and standard errors were calculated, and data was analysed by one-way ANOVA with post-hoc analysis by Tukey's test ($P \leq 0.05$) to identify significant differences in the concentration of each phenolic parameter at each sampling time. Non-linear regression analysis (right-hand exponential fitted curve) in Genstat 14th edition was used to identify those parameters where concentration was correlated with hold time. A correlation cut-off of $R^2 \geq 0.59$ was set, and the fitted curve equation identifying asymptote and rate of change over time was reported for those parameters which met this correlation cut-off.

R version 2.15.1 was used for two-way ANOVA and post-hoc analysis by Tukey's ($P \leq 0.05$) of phenolic concentration in wines from the three wine making treatments at two bottle age time periods – bottling and 6 months.

RESULTS & DISCUSSION

Hold time juice phenolics

Significant changes during the six hour 70°C hold period were found for five of the six parameters examined, non-bleachable pigment levels during the hold period did not significantly differ (Table 1). Mean juice total phenolics increased from ~33 AU at one hour hold time to ~51 AU at four hours hold time (Figure 1) and anthocyanin from ~320 to ~430 mg/L over the same period (Figure 2). Mean juice tannin concentration increased from 0.81 g/L at one hour hold time to 1.71 g/L at four hours hold time (Figure 3) and mean juice colour density increased from 3.5 AU at 1 hour hold time to 4.5 AU at 4 hours hold time (Figure 4). Between one and three hours hold time, juice hue declined (0.59 AU to 0.55 AU) (Figure 6). A lower hue value is indicative of a shift towards blue-purple colouration which has been attributed to co-pigmentation between anthocyanins (Boulton 2001). The hue result appeared to align with the relatively rapid extraction of anthocyanin apparent in Figure 2.

Non-linear regression analysis suggested there was a correlation between hold time and juice concentration for total phenolics (Figure 1), anthocyanin (Figure 2) and tannin (Figure 3). These phenolic parameters returned R^2 values ≥0.59 when data was fitted to a standard right-hand exponential curve (Table 1). As indicated in Table 1, the asymptote for anthocyanin concentration was reached at approximately 4 hours hold time (436 mg/L) suggesting greater than four hours hold time offered limited value for additional anthocyanin extraction. The asymptotes identified for total phenolics (59.2 AU) and tannin (2.35 g/L) (Table 1) were extrapolations beyond the concentrations realised in this study and so need to be interpreted with caution. Solving the fitted curve equations suggested maximum extraction for total phenolics and tannin would be expected at 20 and 27 hours, respectively, however experimental work would need to be undertake to validate these estimates. Figures 2 and 3 show that anthocyanin and tannin extraction processes proceeded differently; maximum anthocyanin extraction was achieved relatively quickly, whereas tannin extraction proceeded more gradually but the change in tannin

concentration was substantial (ie. tannin concentration more than doubled between one and six hours hold time).

DRAFT paragraph: An integral part of red wine making is the management of mass transfer of phenolic compounds out of grape skins and seeds, and into grape juice. In this study, high temperature hold time after microwave maceration of Pinot noir must was associated with a rapid increase in juice phenolic concentration. This demonstrated that hold time was important for extraction of phenolics. Previous studies have shown similar extraction outcomes resulting from pomace contact at normal temperature (ie. 30°C), but those similar extraction outcomes have taken days, rather than hours, in normal temperature systems.

DRAFT paragraph: For three of the phenolic parameters that were examined – total phenolics, anthocyanin, tannin –concentration in juice was correlated with hold time, and extraction followed a decaying curve. This suggests the system under examination may conform to Michaelis-Menton/Fick's 2nd Law which describes extraction as a time/temperature dependent phenomenon. These results are highly significant to industry because they suggest the option of 'dial-up' phenolic concentration. This may be a compelling option in the case of Pinot noir, where grape anthocyanin levels are generally low compared with other red varieties, and where tannin extraction can be challenging.

DRAFT Segue: Important to examine whether the increases in grape juice phenolic concentration that were observed from extended hold time translated into stable wine phenolic outcomes.

Table 1. One-way ANOVA and non-linear regression analysis for phenolic concentration in Pinot noir grape juice sampled hourly over a six hour hold time at 70°C following microwave maceration.

	ANOVA Significance (P)	Regression (R^2)	Fitted curve*
Total Phenolics	≤0.001	0.87	$=59.2+(-37.5*0.70^{hrs})$
Anthocyanin	0.002	0.59	$=436+(-268*0.43^{hrs})$
Tannin	≤0.001	0.90	$=2.35+(-2.04*0.76^{hrs})$
Non-Bleachable Pigment	0.277	0.18	NA
Colour Density	0.003	0.57	NA
Hue	0.025	0.36	NA

* Fitted curve is only reported for phenolic parameters with R^2 value ≥0.59. General form for the fitted regression curve is phenolic concentration = $A+(BxR^{hrs})$; where A is the asymptote, and BxR^{hrs} describes the change in extraction rate over time.

NA = not applicable due to low correlation (R^2).

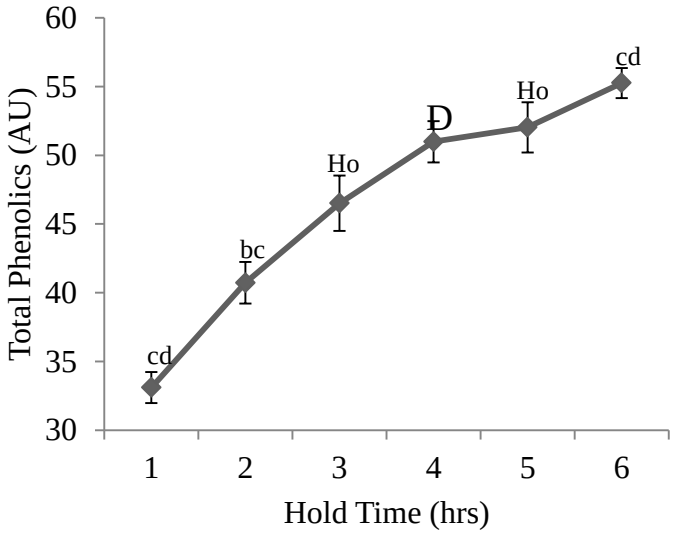

Figure 1. Total phenolic concentration in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Different letters indicate a significant difference in concentration between sampling periods (Tukeys Test: $P \leq 0.05$).

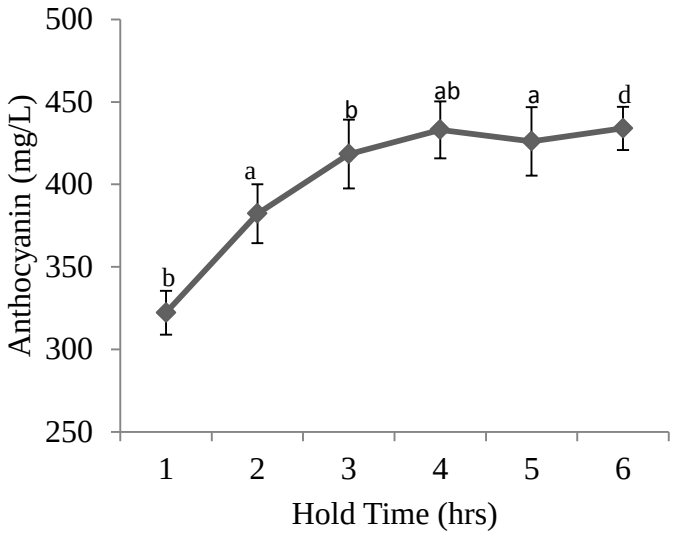

Figure 2. Anthocyanin concentration in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Different letters indicate a significant difference in concentration between sampling periods (Tukeys Test: $P \leq 0.05$).

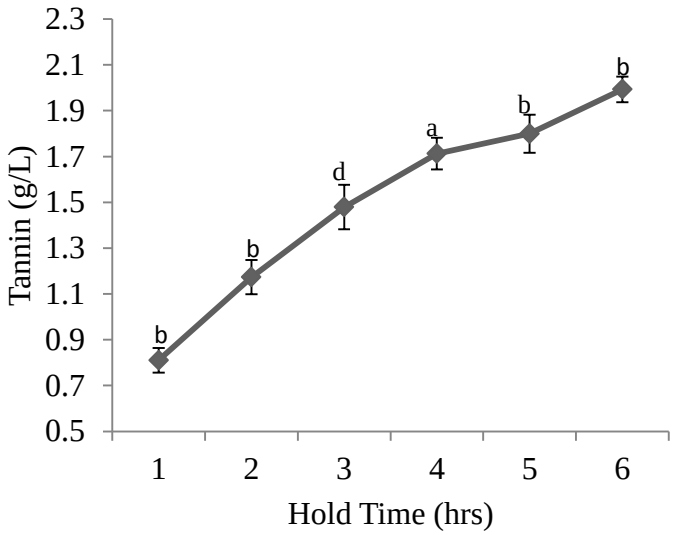

Figure 3. Tannin concentration in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Different letters indicate a significant difference in concentration between sampling periods (Tukeys Test: $P \leq 0.05$).

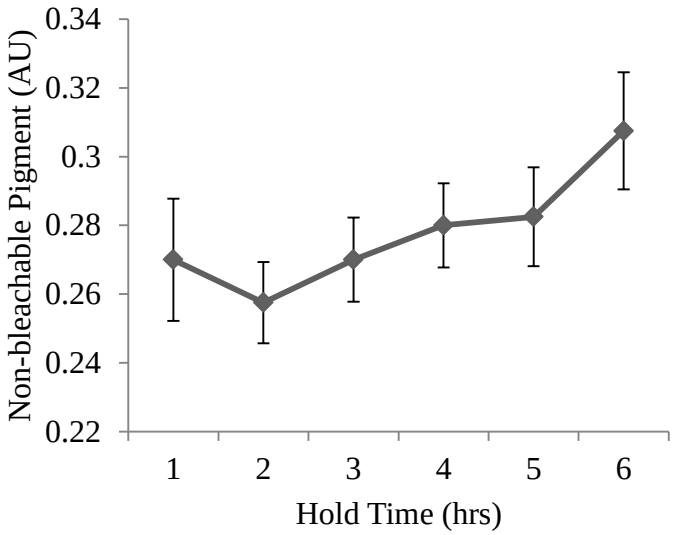

Figure 5. Non-bleachable pigment concentration in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Differences between sampling periods were non-significant (Tukeys Test: $P \leq 0.05$).

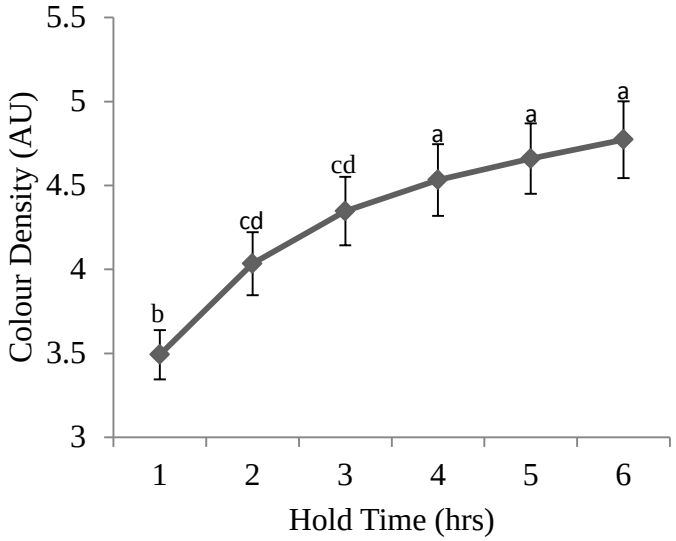

Figure 4. Colour density in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Different letters indicate a significant difference in concentration between sampling periods (Tukeys Test: $P \leq 0.05$).

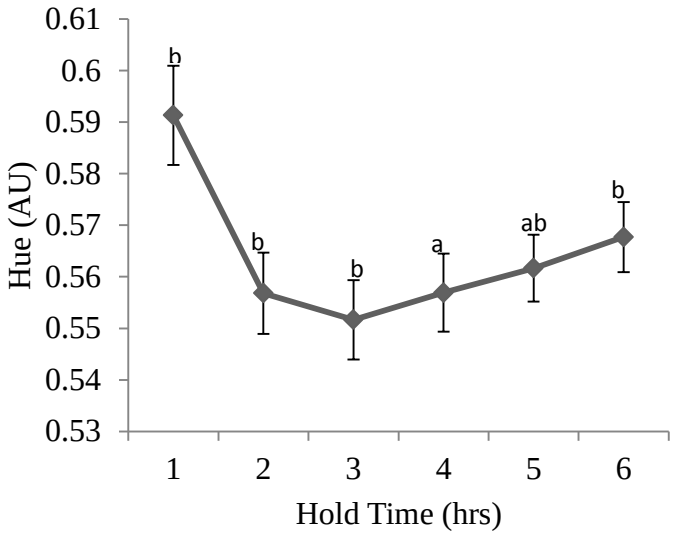

Figure 6. Hue in Pinot noir grape juice over a six hour hold time at 70°C following microwave maceration. Different letters indicate a significant difference in concentration between sampling periods (Tukeys Test: $P \leq 0.05$).

Wine phenolics

There were interactions between hold time and bottle age for wine total phenolics, anthocyanin and hue (Table 2). At six months bottle age, the difference in total phenolics concentration between 3 hour and 6 hour hold time wines that had been observed at bottling was no longer apparent. Significant differences between treatments for anthocyanin and hue at bottling, were no longer apparent by six months bottle age, which was likely due to anthocyanin binding into polymeric pigment (REF) which would have impacted both the anthocyanin concentration and the hue readings.

Main effects of bottle age for total phenolics, anthocyanin, colour density and hue, and of hold time for all six phenolic parameters were found (Table 2). Total phenolics, anthocyanin and colour density declined in concentration with bottle age (Table 3) which is usual in red wine due to changes in wine pH, oxidation, and the formation of polyphenols and their sedimentation out of the liquid phase (REFS). Hue values

increased with bottle age as the wines transitioned from younger, more purple hues, towards older, more garnet hues. These hue changes are likely attributable to the transition from colour dominated by the transient monomeric and co-pigmented forms of anthocyanin, to colour from polyphenols which form from complexing between anthocyanin and tannin (REF). Co-pigmented anthocyanins lend a purple hue to wine, and polyphenols are associated with a garnet hue.

Table 3 shows wine making treatment main effects, with control wines significantly lower in most phenolic parameters than the two microwave treatments. For example, tannin concentration in control wines was less than half the concentration of 3 hour treatment wines, and less than one third that of 6 hour treatment wines. Non-bleachable pigment is an indicator of stable colour in red wine (REF) and there was twice the non-bleachable pigment concentration in 6 hour treatment wines, compared with control wines. For a variety like Pinot noir where long term colour stability can be problematic (REF), a non-bleachable pigment concentration approaching 1 AU is promising as … (REF – or Bob's AWRI Roadshow).

For some phenolic parameters, concentration levels in 6 hour hold time wines significantly exceeded 3 hour hold time wines (Table 3). These differences did not endure with bottle age for total phenolics and tannin concentration, however the high tannin and anthocyanin concentrations at bottling in 6 hour wine may have driven the significantly higher non-bleachable pigment concentration observed in these wines at 6 months. This suggests that the rate limiting factor for non-bleachable pigment formation in this system may have been tannin.

Table 2. Two-way ANOVA for phenolic concentration in Pinot noir wine with three hold time treatments (control, 3 hours, 6 hours) at two bottle ages (bottling, 6 months).

	ANOVA Significance (P)		
	hold time	bottle age	hold time x bottle age
Total Phenolics	≤0.001	0.01	0.03
Anthocyanin	0.01	≤0.001	0.04
Tannin	≤0.001	0.17	0.09
Non-Bleachable Pigment	≤0.001	0.79	0.18
Colour Density	≤0.001	0.01	0.49
Hue	0.04	≤0.001	≤0.001

Table 3. Mean phenolic concentration in Pinot noir wine with three hold time treatments (control, 3 hours, 6 hours) at two bottle ages (bottling, 6 months). Different letters within rows indicate a significant difference between treatments at that bottle age (Tukeys Test: $P \leq 0.05$). NS indicates differences in mean concentration at that bottle age were non-significant.

		Mean Concentration		
		control	3 hours	6 hours
Total Phenolics (AU)	bottling	24 a	37 b	46 c
	6 months	23 a	35 b	37 b
Anthocyanin (mg/L)	bottling	158 a	209 ab	232 b
	6 months	124	161	131 NS
Tannin (g/L)	bottling	0.40 a	1.05 b	1.65 c
	6 months	0.42 a	1.05 b	1.33 b
Non-Bleachable Pigment (AU)	bottling	0.41 a	0.71 b	0.84 b
	6 months	0.45 a	0.61 a	0.93 b
Colour Density (AU)	bottling	3.2 a	4.5 b	5.4 b
	6 months	2.9 a	3.6 a	4.8 b
Hue (AU)	bottling	0.62 a	0.70 b	0.68 b
	6 months	0.77	0.75	0.74 NS

CONCLUSIONS

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Yeast strain and maceration effects on Pinot noir phenolics

TASMANIAN INSTITUTE OF AGRICULTURE

AWRI 30th May, 2012

Anna L Carew (TIA), Robert Dambergs (AWRI/TIA), Chris Curtin (AWRI), Dugald Close (TIA)

UTAS TIA is a joint venture of the University of Tasmania and the Tasmanian Government Tasmania Explore true possibilities

Background

tia TASMANIAN INSTITUTE OF AGRICULTURE

- Extraction and stabilisation of phenolics is a challenge for Pinot noir winemaking
- **Yeast** choice important for phenolics in Pinot noir?

→ Research contradictory or inconclusive

- **Maceration** methods to optimise phenolics in Pinot?

→ Yes, but disadvantages

Tasmanian Institute of Agriculture – research • development • extension • education • training

Methods

- random bunch allocation, ~1kg per pot
- four replicates per treatment
- modified Somers, AWRI tannin calculator, PCA

Yeast

Q1: Is yeast choice important for phenolics in Pinot noir?

Diverse strains and strategies:
- *Saccharomyces cerevisiae* EC1118, RC212
- 'wild' sequential inoculation (EC1118)
- *Saccharomyces bayanus* AWRI1176
- non-*Saccharomyces* sequential inoc (*Torulaspora delbruekii* + EC1118)

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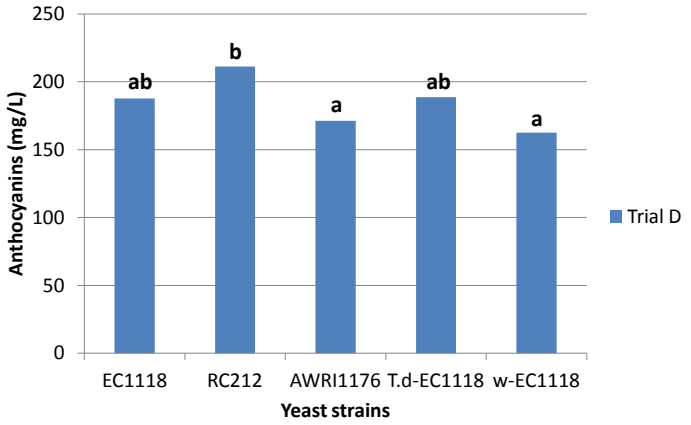
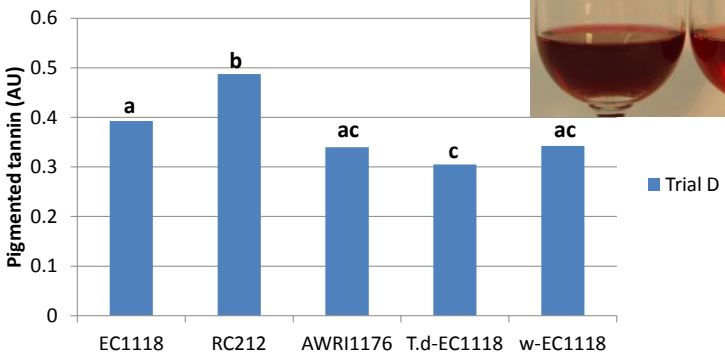
Anthocyanins - 6 mths



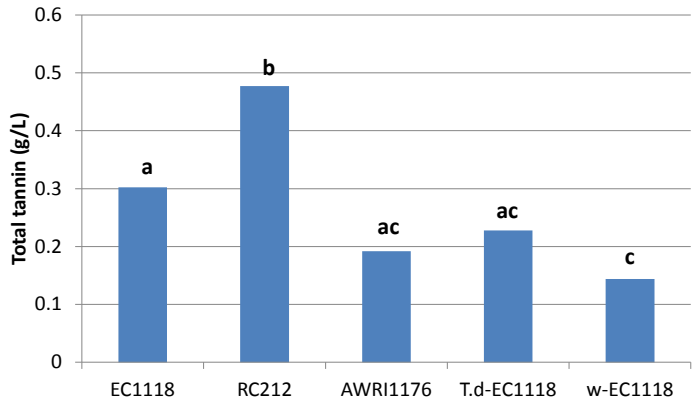
Different letter denotes significant difference between strains within trial (P<0.05)

Pigmented tannins - 6 mths



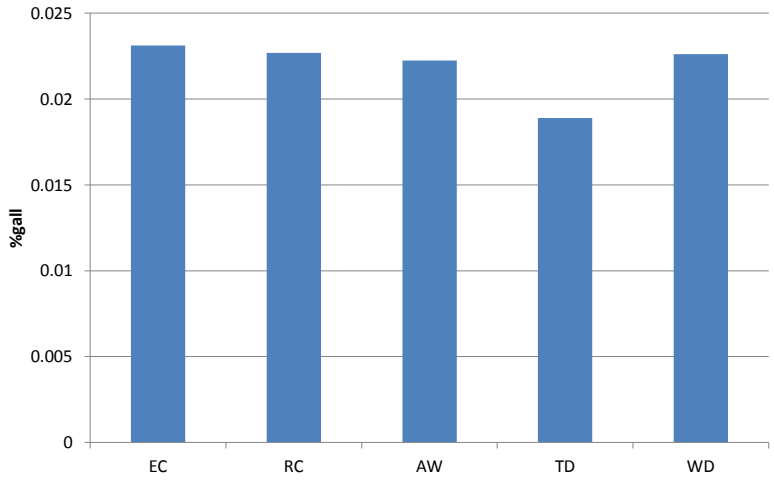
Different letter denotes significant difference between strains within trial (P<0.05)

Total tannin - 6 mths



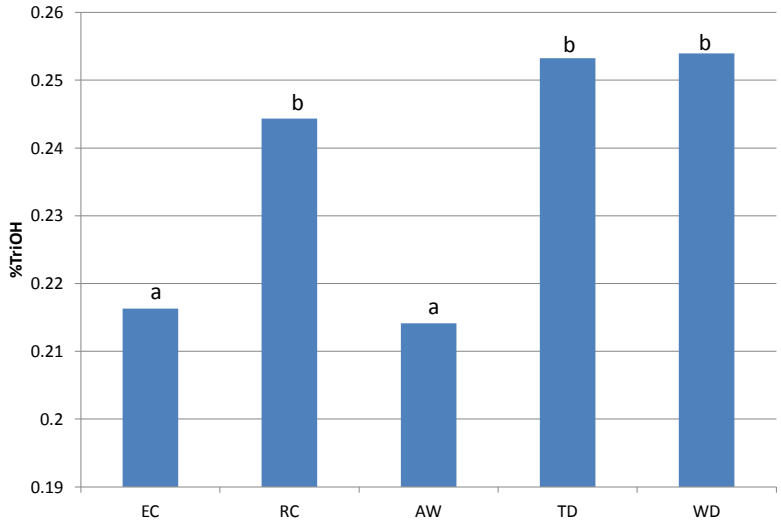
Different letter denotes significant difference between strains within trial (P<0.05)

% galloylation



Acknowledging Paul Smith, Stella Kassara AWRI.
Different letter denotes significant difference amongst treatments (P<0.05)

% Trihydroxylation



Acknowledging Paul Smith, Stella Kassara AWRI.
Different letter denotes significant difference amongst treatments (P<0.05)

Yeast effects

- Yeast treatment had significant impact on phenolics
  - magnitude of effect - tannin **concentration**
  - substantial differences - tannin **composition**
- Yeast strategy important for Pinot noir makers
- Return to strains in 2013

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Maceration

Q2: Maceration methods to optimise phenolics in Pinot noir wine?

- cold soak
- extended maceration
- high late fermentation temperature
- thermal treatment

- 2011 trials (with Angela Sparrow)
- thermal treatment looked promising

Microwave maceration method

1. Intermittent microwave & stirring

2. Monitor for peak temperature (70°C)

3. Hold in 70°C waterbath

4. Icebath to ~24°C, inoculate with ADY for AF

Why microwave?

- novel application
- energy efficient
- heat transfer mechanism

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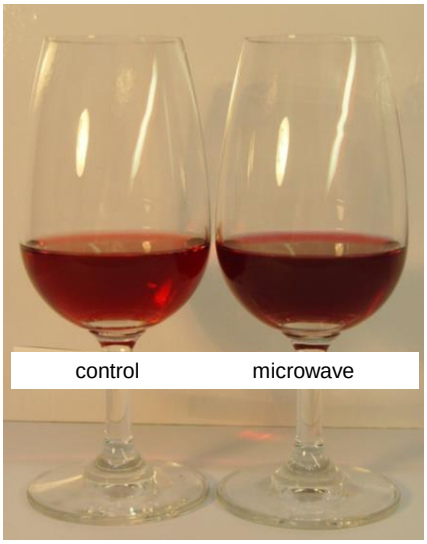
Control vs. microwave wine



Informal tasting
(Godden, Wilkes, Dambergs)

Microwave:
AROMA – more (red & dark) fruit, pinot-like
MOUTH – more palate weight, nice length, soft and mouth-coating, not hard tannin

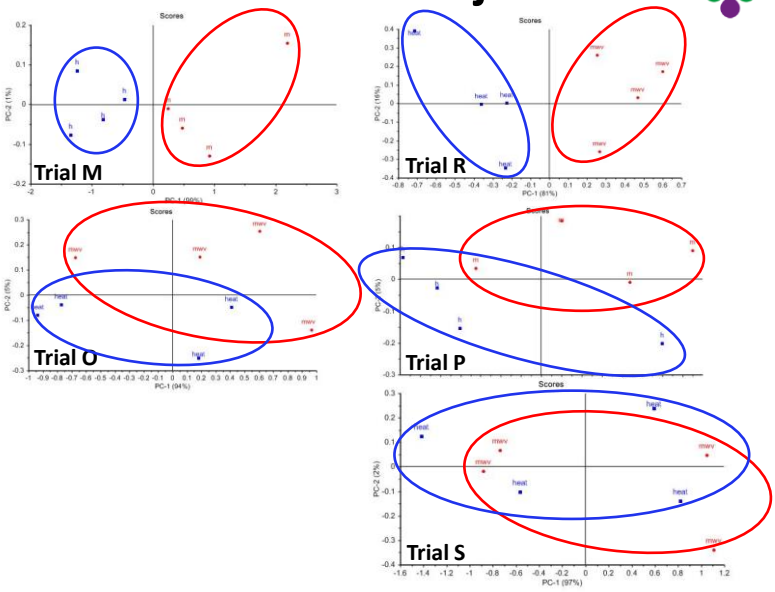
Control:
AROMA and MOUTH – no faults but pissy

Maceration 2012

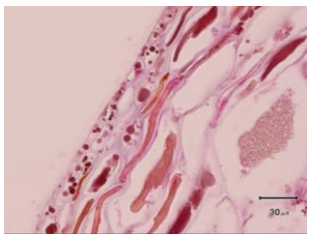
- refine and investigate microwave method
- compare phenolic outcomes from microwave, with heat

Heat Exchanger Mk4

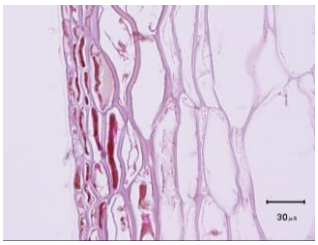
Heat vs. Microwave – juice PCA 230-590



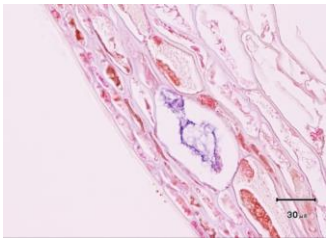
Pinot noir grape skin sections



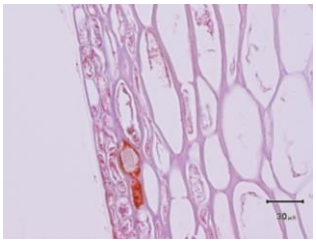
A. Fresh grape skin



B. Post-fermentation (8 days)



C. Post-heating (70°C)



D. Post-microwave (70°C)

Acknowledging Dane Hayes, DPIPWE.

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Ctl, heat, mwv wine - on-skins

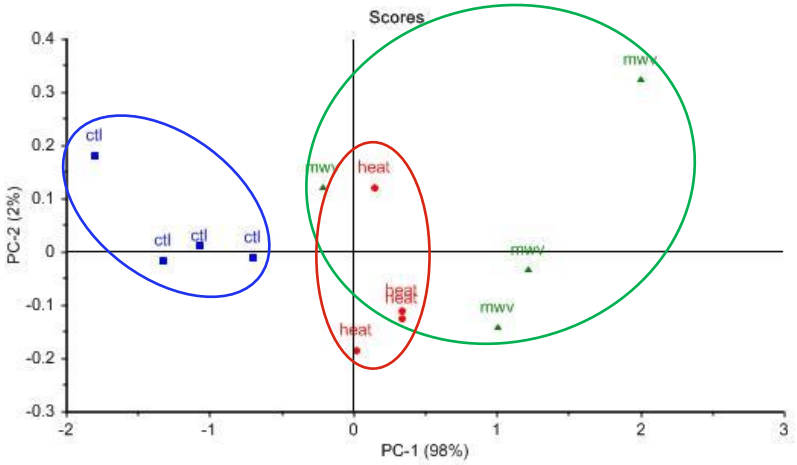
Photo: Bottled Pinot noir wine, 80mg/L SO_2
O1 = control
O2 = heat macerated
O3 = microwave macerated

	CTL	HEAT	MWV
Anthocyanins (mg/L)	230 a	332 b	350 b
Pigmented tannin (AU)	0.43 a	0.58 b	0.65 b
Total tannin (g/L)	0.25 a	0.70 ab	0.94 b

Different letter denotes significant among treatments within trial (P<0.05)

Control, heat, microwave wine

PCA 230-590nm



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Control, Heat & Microwave

	CTL	HEAT	MWV
Yeast (cfu/mL)	$1x10^4$	<20	<20
AF lag phase	long	short	short
YAN (mg/L)	202 a	215 a	242 b
Pre-AF juice (ml/840gm)	~340	~380	~400

+ potential to heat denature laccase (*Botrytis*)

On skins vs. early press-off

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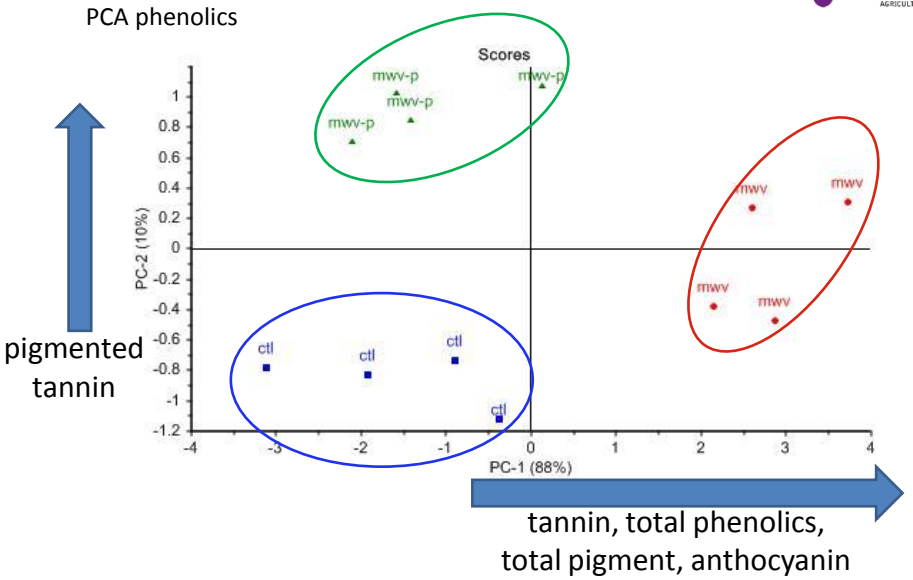
On skins vs. early press off wine

PCA phenolics



pigmented tannin

tannin, total phenolics, total pigment, anthocyanin

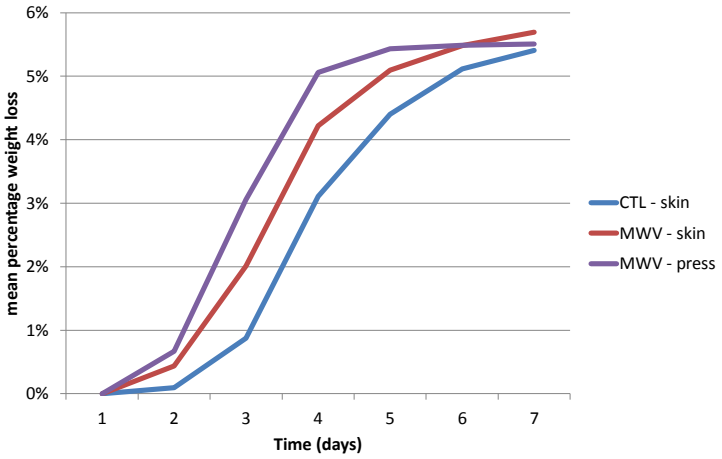
Wine phenolics

On skins | Early press off

	CTL	MWV	MWV-p
Anthocyanins (mg/L)	292 a	412 b	272 a
Pigmented tannin (AU)	0.31 a	0.50 b	0.45 b
Total tannin (g/L)	0.25 a	0.72 b	0.27 a

Different letter denotes significant among treatments within trial (P<0.05)

Fermentation kinetics (on skins vs early press off)



Conclusions

YEAST

- Yeast treatment had significant impact on concentration of phenolics
  - substantial effect on tannin **concentration**
  - changed tannin **composition**

MACERATION

- Heat and microwave both delivered wine with higher phenolic extraction than control
- Microwave resulted in higher phenolic concentration in juice and wine, compared with heat
- Microwave maceration may enable better quality and more efficient Pinot noir production

...YEAST + MACERATION?

Acknowledgements

• TIA, AWRI & DPIPWE colleagues
• Lallemand Australia
• Mauri Yeast Australia
• Tasmanian Estates

• Carew is supported by an Australian Postgraduate Award, the Tasmanian Institute of Agriculture, University of Tasmania and The Australian Wine Research Institute.

Thank you! Questions?

APPENDIX E – poster presented at the 15th Wine Industry Technical Conference, July 2013, Sydney, Australia

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Microwave Pinot noir: phenolics and aroma

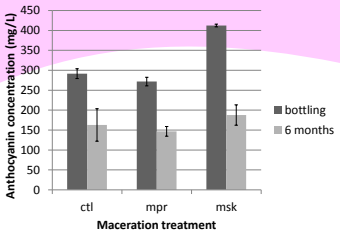
Anna Carew[1]; Natoiya Lloyd[2]; Dugald Close[1]; Bob Dambergs[1,3]
[1]Perennial Horticulture Centre, Tasmanian Institute of Agriculture, Hobart & Launceston, Tasmania
[2]Metabolomics Australia, Urrbrae, South Australia
[3]The Australian Wine Research Institute; Tasmanian Institute of Agriculture, Hobart, Tasmania

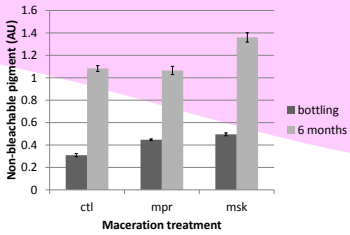
Wines from microwave with early press-off were phenolically equivalent to control wines

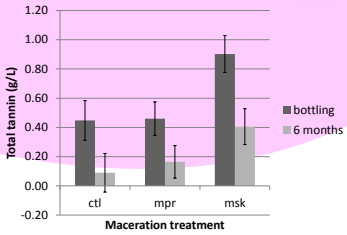
Rapid extraction of phenolics by microwave maceration offers winemakers alternatives for alcoholic fermentation of red wine. This trial examined three options for Pinot noir winemaking:

- Control fermentation on skins for 7 days (**ctl**)
- Microwave maceration & fermentation on skins for 7 days (**msk**)
- Microwave, press-off at 3 hours & fermentation as juice (**mpr**)

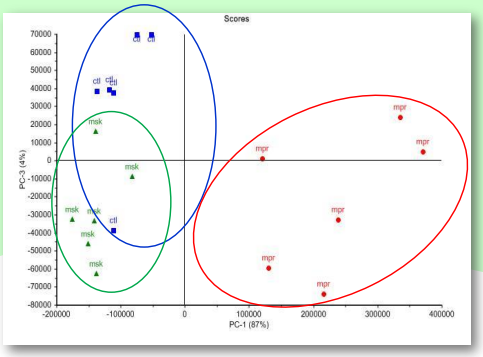
Control (ctl) and microwave with press-off (mpr) wines were mostly equivalent for phenolics concentration, but microwave fermented on skins (msk) produced wines with greater phenolics concentration (graphs below).







Wines from microwave with early press-off were aromatically distinct from control wines



Principal component analysis of volatile aroma compounds examined by GCMS. Response ratios suggested microwave with press-off (mpr) was aromatically distinct.

Loadings analysis showed PC1 separation was driven by three compounds: 2&3-methylbutanol (nail polish aroma), 2-methylpropanol (fusel aroma) and ethyl octanoate (red cherry, raspberry aromas).

Wine aroma is complex. GCMS analysis showed treatment effects but formal sensory analysis would be required to establish the true sensory impact of these alternate wine making processes.

Microwave maceration offers potential control over phenolic outcomes in Pinot noir wine, and an opportunity for aromatic differentiation

Contact Dr Anna Carew
anna.carew@utas.edu.au

TIA is home to the AWRI's Tasmanian Node which is jointly funded by TIA, UTAS, AWRI and the GWRDC

APPENDIX F – Oral presentation from the 15th Wine Industry Technical Conference, July 2013, Sydney, Australia

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Microwave Maceration of Pinot noir: phenolic similarities, aromatic differences

Anna Carew (TIA/AWRI)
Natoiya Lloyd (Metabolomics Australia)
Bob Dambergs (TIA/AWRI)
Dugald Close (TIA)

TIA is a joint venture of the University of Tasmania and the Tasmanian Government

Controlled Phenolic Release (CPR)

Microwave maceration (70°C) + Hold time (10 min – 6 hours)

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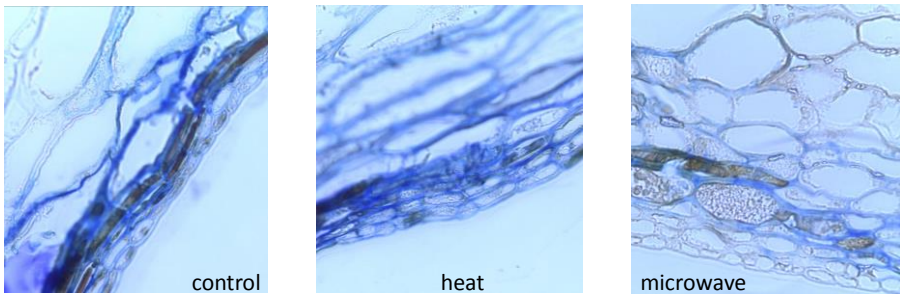
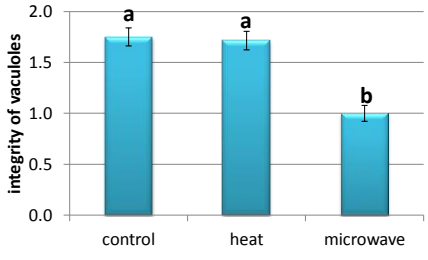
Commercial Microwave

Semi-commercial scale - 15 kW pentagonal microwave unit, ~200kg/hr
(with Dr Kai Knoerzer, CSIRO Animal, Food and Health Sciences, VIC)

Phenolic Release by Microwave





Histology: Dr Warwick Gill, TIA

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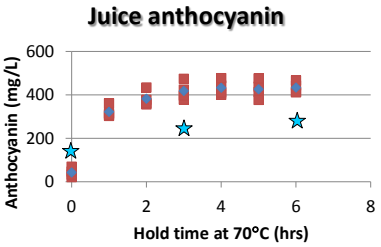
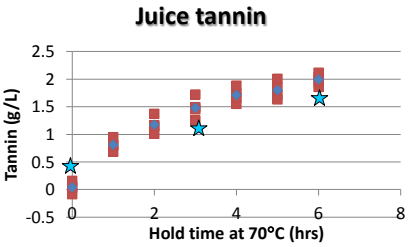
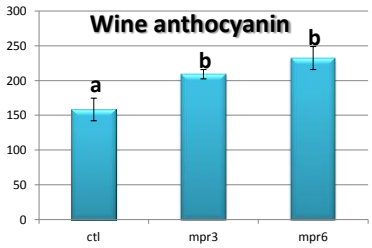
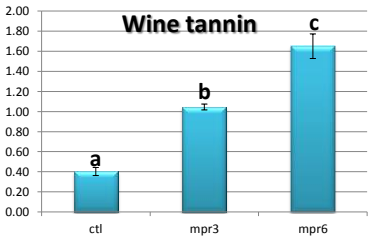
Controlled Diffusion by Hold Time

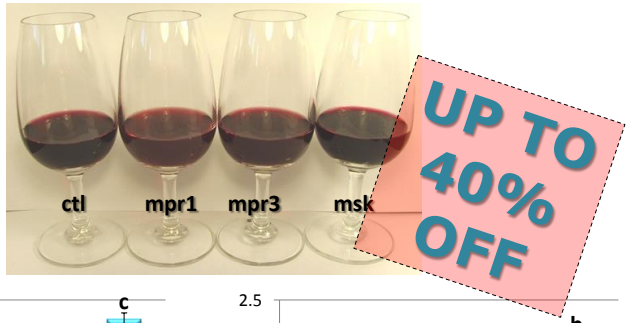


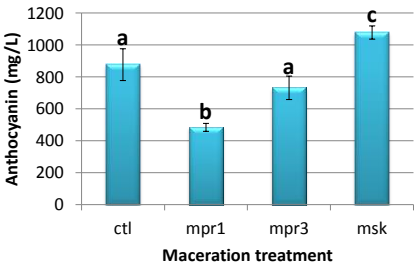


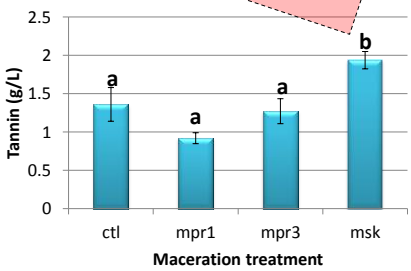




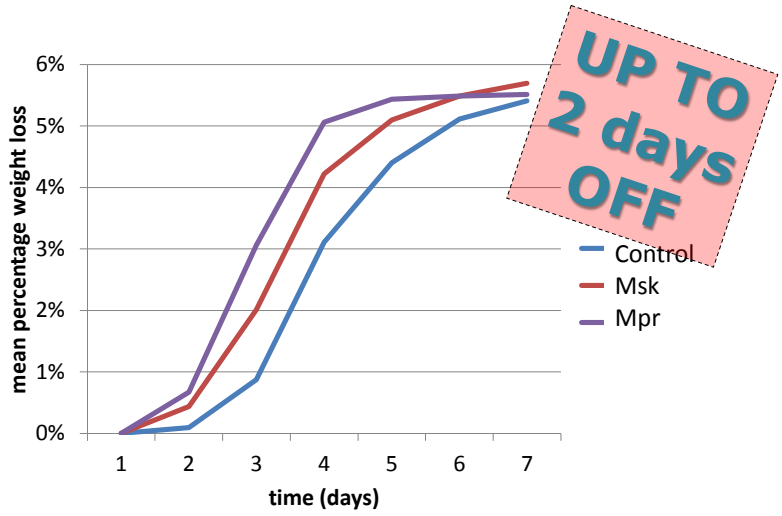
CPR Options - Shiraz





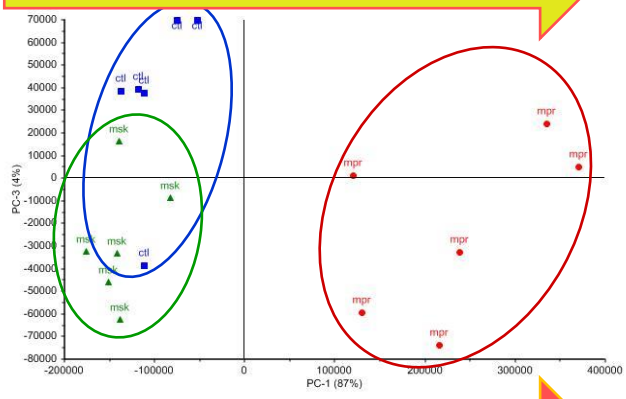


CPR Fermentation Kinetics



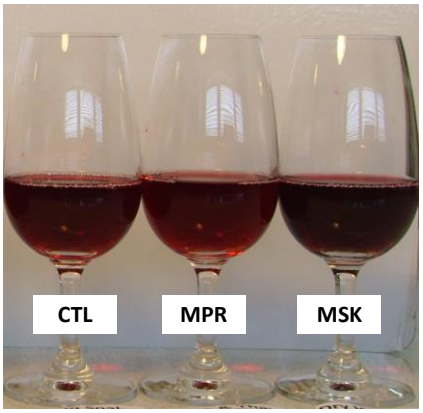
Aroma Differences by GCMS

Ethyl acetate ctl & msk ~1.4 mpr 2.3



Ethyl octanoate ctl & msk ~1.7 mpr 3.7

How does it taste?



Informal tasting
- 1x experienced wine judge
- 2x WSET trained tasters
- 1x experienced wine scientist ('Bob')

Control:
AROMA and MOUTH – no faults but pissy
MSK:
AROMA – more (red & dark) fruit, pinot-like
MOUTH – more palate weight, soft and mouth-coating, not hard tannins
MPR:
AROMA – fruit, confectionary, pretty
MOUTH – soft, round tannin

CPR Conclusions

- Control over phenolic outcomes in red winemaking
- Aroma differences – new product/differentiation
- Process efficiency – shorter/less tank space, fast fermentation
- Seeking industry partners for semi-commercial trials - VIC, NSW

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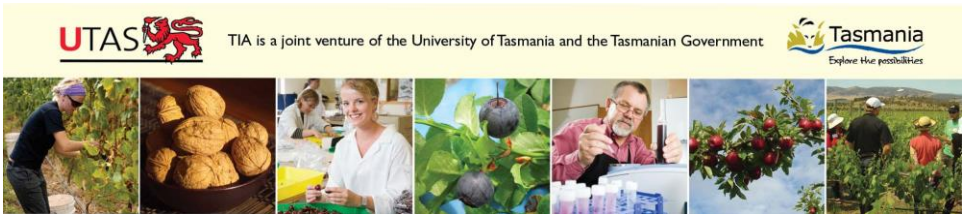
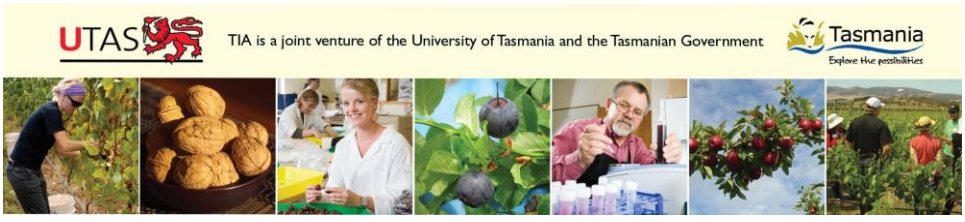
Acknowledgements



Thank you!

Questions?

contact: anna.carew@utas.edu.au



APPENDIX G – poster presented at the 15th Wine Industry Technical Conference, July 2013, Sydney, Australia

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Microwave Pinot noir: finished in 37 days

Anna Carew[1]; Dugald Close[1], Bob Dambergs[2]
[1]Perennial Horticulture Centre, Tasmanian Institute of Agriculture, Hobart & Launceston, Tasmania
[2]The Australian Wine Research Institute; Tasmanian Institute of Agriculture, Hobart, Tasmania

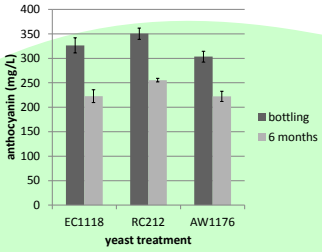
Microwaved must was pressed-off prior to inoculation

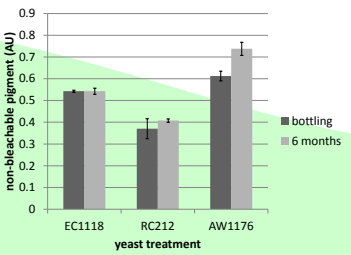
This trial generated proof of concept for rapid red winemaking from microwave maceration.

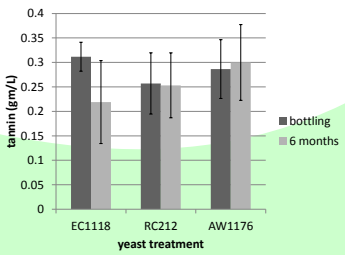
- Microwave macerated musts were pressed-off after 3 hrs skin contact time
- Musts were co-inoculated for alcoholic and malolactic fermentation
- All replicates completed AF and MLF by 17 days post-inoculation

Wines from this trial were settled, stabilised and bottled at 37 days post-harvest. This process has the potential to significantly increase winery efficiency.

Yeast strain affected wine phenolics concentration







Three yeast treatments were co-inoculated with *Oenococcus oenii* PN4 into microwave macerated, pressed off Pinot noir juice:

- V1 *Saccharomyces cerevisiae* EC1118
- V2 *S. cerevisiae* RC212
- V3 *S. bayanus* AWRI1176

The graphs above show phenolics at bottling and six months post-bottling. Strain effects on non-bleachable pigment were apparent at six months bottle age and can be seen in the photo to the right.

Research into yeast strain effects is important for understanding and managing the impact of novel maceration processes like microwave maceration for red wine making.

Microwave maceration allowed simultaneous malolactic & alcoholic fermentation, & yeast strain influenced wine phenolics

Contact Dr Anna Carew
anna.carew@utas.edu.au

TIA is home to the AWRI's Tasmanian Node which is jointly funded by TIA, UTAS, AWRI and the GWRDC