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Yeast Effects on Pinot noir Wine Phenolics, Color, and Tannin Composition

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ABSTRACT: Extraction and stabilization of wine phenolics can be challenging for wine makers. This study examined how yeast choice affected phenolic outcomes in Pinot noir wine. Five yeast treatments were applied in replicated microvinification, and wines were analyzed by UV−visible spectrophotometry. At bottling, yeast treatment *Saccharomyces cerevisiae* RC212 wine had significantly higher concentrations of total pigment, free anthocyanin, nonbleachable pigment, and total tannin and showed high color density. Some phenolic effects were retained at 6 months' bottle age, and RC212 and *S. cerevisae* EC1118 wines showed increased mean nonbleachable pigment concentrations. Wine tannin composition analysis showed three treatments were associated with a higher percentage of trihydroxylated subunits (skin tannin indicator). A high degree of tannin polymerization was observed in wines made with RC212 and *Torulaspora delbruekii*, whereas tannin size by gel permeation chromatography was higher only in the RC212 wines. The results emphasize the importance of yeast strain choice for optimizing Pinot noir wine phenolics.

KEYWORDS: *wild fermentation, sequential inoculation, anthocyanin, pigmented tannin*

INTRODUCTION

Phenolic compounds are important to the aesthetic, flavor, and mouthfeel qualities of red wine,[1] but the extraction and stabilization of phenolics can be a particular challenge for Pinot noir winemakers.[2] Red wine color depends on anthocyanin extraction from grape skin and its stabilization 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 nonacylated form.[3,4] Stabilization 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 optimize 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 byproducts 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 comparison 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 focused 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

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dryness", which appeared to undermine the finding of strain-attributable difference due to potential effects of nonuniform pomace contact time;[18] however, Wädenswil 27 was reported to have "fermented at a slower rate" (p 4016 of ref 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. Little attention has been paid to the impact of yeast strain on tannin concentration and tannin composition. Insight into tannin concentration and composition is important for Pinot noir as this is a variety for which a well-balanced wine tannin profile can be difficult to achieve, and long-term color stabilization 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

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 randomized to 1.1 kg 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 benchtop crusher and destemmed by hand before the resulting must was decanted to a 1.5 L Bodum "Kenya" plunger coffee pot. The coffee pots acted as pilot-scale submerged cap fermenters. Free sulfur dioxide (SO_2; 20 mg/L) in the form of potassium metabisulfite solution was applied to each pot; 20 mg/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 2 h, were inoculated according to Table 1. All yeast strains were commercially available active dried yeast cultures that were rehydrated according to the manufacturers' instructions. Hemocytometer counts showed inoculation resulted in between 1.9×10^6 and 6.0×10^6 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 level 2 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 *Saccharomyces bayanus* strain AWRI1176 (AW) and *Torulaspora 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 7 days on skins. Pots were weighed daily, and weight loss through evolution of carbon dioxide was used as an indicator of fermentation kinetics. Yeast assimilable nitrogen (60 mg/L) in a 20% diammonium phosphate solution was applied at day 3 of the ferment. Pots were all pressed off at day 7 by hand-pressing down the Bodum filter screen with 10 s 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 stabilized by application of 0.5 mL of a 20% potassium metabisulfite 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 1 month's further settling prior to bottling in amber glassware with polypropylene screw-cap closures. Bottled wines were stored at 14 °C. Wines were analyzed for phenolics at bottling and 6 months after bottling. Wines were analyzed for tannin composition 8 months 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 analyzed using UV–visible spectrophotometry in HCl, acetaldehyde, and metabisulfite buffers to quantify total phenolics, total pigment, anthocyanins, total tannin, nonbleachable 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 noncolored tannins and anthocyanins plus low molecular weight nonpigmented 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 nonpigmented tannins. Nonbleachable 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 (e.g., 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.

Tannin Composition. For each wine at 8 months bottle age, a 4 mL sample was loaded onto a solid phase extraction (SPE) cartridge, and total tannins were isolated following the method of Kassara and Kennedy.[38] For treatment RC, 4 mL of sample overloaded the SPE cartridge so findings for this treatment were confirmed via analysis of 2 mL samples. Isolated tannins were subjected to acid-catalyzed depolymerization in the presence of phloroglucinol.[39] Four tannin composition measures were calculated from total tannin isolated from wine samples: percent trihydroxylated subunits (an indicator of the proportion of skin tannin in wine[38]); percent galloylated subunits (an indicator of the proportion of seed tannin in wine[38]); mean degree of polymerization (mdp), percent 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,

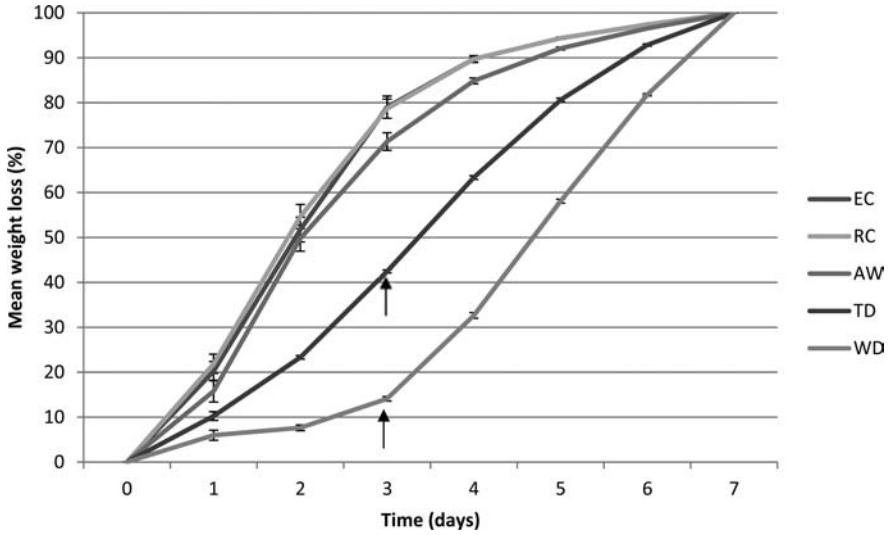

Figure 1. Fermentation kinetics for Pinot noir wine made with five yeast treatments (SE) (arrows indicate sequential inoculation with EC1118).

Table 2. Concentrations of Phenolic and Color Indicators in Pinot noir Wine at Bottling and Six Months Bottle Age (SE)

		yeast treatment[a]				
phenolic/color measure	bottle age	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 ± 0.08 cd	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

[a]Lower case letters denote significant difference among treatments at specified bottle age (Tukey's test $P < 0.05$).

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 depolymerizable. Tannin with a high percent conversion yield indicates a higher proportion of unmodified, grape-like tannins. The measures mdp, percent trihydroxylation, and percent galloylation can only be applied to converted tannins and hence are interpreted relative to percent 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 to 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 7 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 3 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 5 or 6. At day 3, WD and TD pots were inoculated with a log phase culture of EC1118, which did not appear to affect the fermentation rate for TD treatment but coincided with a fermentation rate increase in WD treatment pots. Figure

Table 3. Tannin Size Indicators and Subunit Composition for Pinot noir Wine at 8 Months Bottle Age (SE)

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

1 shows that all experimental ferments finished alcoholic fermentation prior to pressing off at day 7, and this provided equivalent pomace contact time for all treatments.

Phenolic and Color Measures. Significant differences were observed among yeast treatments for all measures related to color intensity (Table 2). Treatment effects were discernible 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 6 months in bottle, with RC wines significantly higher in mean total pigment and anthocyanin at 6 months bottle age than AW and WD. By 6 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 affected 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 toward more garnet and 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 affected formation of stable color as shown by significant differences in nonbleachable pigment concentration between the experimental wines (Table 2). Analysis of wines at 6 months bottle age showed RC and EC yeast treatments were associated, respectively, with 37 and 21% higher nonbleachable pigment than the TD treatment. Treatments RC and EC also showed significant increase in mean concentration of nonbleachable pigment between bottling and 6 months bottle age ($P < 0.001$), whereas the change in mean nonbleachable pigment concentration for the three remaining yeast treatments was nonsignificant ($P > 0.05$).

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 6 months had a mean total tannin concentration 70% lower than RC treatment wine.

Tannin Composition. In this study, yeast treatment affected 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. By comparison of those treatments with similar conversion ratios, mdp results showed RC treatment wine tannin had a depolymerizable portion 1.7 subunits longer than that of the AW treatment and 0.9 subunit longer than in the WD treatment. Similarly, depolymerizable TD wine tannin was 0.9 subunit 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: percent galloylation (epicatechin gallate) and percent trihydroxylation (epigallocatechin). Wines with similar percent conversion yield showed differences in percent 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 percent trihydroxylated subunits, compared with AW wine, and TD wine was higher in trihydroxylated subunits than EC wine.

DISCUSSION

A limited range of yeast strains have tended to be employed by New World Pinot noir winemakers,[2] and more research into yeast strain effects on Pinot noir phenolics is needed to assist winemakers to optimize 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 a high concentration of wine phenolics. For example, RC wines were significantly higher in anthocyanin concentration at 6 months than AW and WD wines. Differential fining of phenolics via adsorption to yeast cell walls has been demonstrated elsewhere.[9,19,55] 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%.[8] Although 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 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 6 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 of practical importance 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 demonstrate that choice of yeast strain may greatly assist winemaker's efforts to enhance tannin in Pinot noir wine.

Whereas 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 courseness.[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 polymerization (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 (nonbleachable 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 nonbleachable pigment over that period. Two pathways have been described for the 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 nonbleachable 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 stabilization. The nonbleachable pigment results demonstrated that yeast treatment had the capacity to affect 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 trihydroxylated 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 affecting phenolic outcomes in red wine. Previous research on a closely related strain concluded that *S. bayanus* strains might offer positive outcomes for color stabilization in red wines; compared with a control strain, the *S. bayanus* strain AWRI1375 was found to optimize pigmented polymer formation in Cabernet Sauvignon wine.[15] Our results contrast with this finding and emphasize the importance of a better understanding of yeast strain effects by grape variety.

Fermentation Kinetics and Phenolics. Novel strategies such as use of non-*cerevisiae* and non-*Saccharomyces* strains, and co-incoulation 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 colonization 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 sulfite application (20 mg/L) to reduce inhibition of the yeast inoculum. This may limit application of the TD strain trialed 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 shows that EC, RC, and AW had largely completed alcoholic fermentation by day 5, which meant pomace in those treatments was in contact with a relatively high ethanol environment for 3 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 concentrations 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 that of 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 being influenced by the physical breakdown of grape solids,[53] differential yeast fining of tannin from the liquid phase of wine,[9,19,55] or differential expression by yeast of extracellular enzymes contributing to the release of tannins from the grape matrix (e.g., β-glucosidase, pectinase, proteolytic enzymes).[56−58] The mechanisms by which yeast mediates 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 affected 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 polymerization in wine at 8 months bottle age. Although the importance of seed-to-skin tannin ratio, and of tannin polymerization, 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.

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Notes

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ABBREVIATIONS USED

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 polymerization; 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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