Chemical and Sensory Effects of Saignée, Water Addition, and Extended Maceration on High Brix Must

James F. Harbertson,[1]* Maria S. Mireles,[1] Eric D. Harwood,[1]
Karen M. Weller,[2] and Carolyn F. Ross[2]

Abstract: Merlot grapes harvested from a single vineyard (11,209 kg/ha, Columbia River Basin) at ~28 Brix were vinified using five experimental winemaking techniques: (1) water addition to 24.3 Brix to compensate for high Brix must (control); (2) water addition to 26.8 Brix (high ethanol); (3) saignée (juice runoff) targeted to 16% of the total volume, paired with an equal volume water addition, yielding 24.1 Brix (low saignée); (4) low saignée treatment paired with a 20-day extended maceration (low saignée-EM); and (5) ~32% saignée with water addition to 24.3 Brix (high saignée). All five techniques were duplicated in 18,972 L fermentors at a commercial facility. Among the treatments there were differences in anthocyanin, polymeric pigments (small and large), tannin, and total iron reactive phenolics. The standard water addition (control) and low saignée treatments had comparable extraction of tannin, anthocyanin, and polymeric pigment formation. A significant enhancement in the amount of anthocyanin, tannin, and large polymeric pigments was observed when a greater volume of juice was removed (high saignée) and when extended maceration was used. Results indicated that the proportion of tannin extracted from the skins and seeds was altered by winemaking treatment. Low saignée-EM resulted in significantly greater seed extraction compared with the other treatments and a trained sensory panel found the resulting wines significantly less smooth and more drying than the other treatments. The high ethanol wines had significantly reduced fresh fruit flavors and increased "hot" mouthfeel.

Key words: red wine, tannin, anthocyanin, extended maceration, saignée, mouthfeel, polymeric pigments, water addition

Fruit that undergoes extended ripening may reach elevated Brix levels, which could possibly lead to a stuck fermentation. In the U.S. wine industry, water may be added to avoid stuck fermentation (Bisson 1999). However, in order to enhance the phenolic components in wine, winemakers often prefer to maintain the increased solids-to-juice ratio that results from extended fruit maturation. *Saignée*, a French word that means *bleed*, refers to the removal of juice before fermentation and has been shown to simulate a reduction in berry size (Singleton 1972). When saignée is paired with water addition, the net result is a lower Brix must that still possesses the desirable solids-to-juice ratio of the smaller, more mature berries achieved by extended ripening.

Phenolics are an important class of compounds in red wine, providing important sensory (aroma, astringency, bitterness, color) and chemical (antioxidant capacity) properties. Tannins are a subset of phenolics present in grape skins and seeds and are primarily responsible for wine astringency. Anthocyanins are pigments found in the skins of red grapes and in the pulp of tinteurier varieties. Anthocyanins and tannins are extracted from the grape skins and seeds during the alcoholic fermentation.

A variety of winemaking techniques have been used to enhance phenolic extraction in wine, although only a few have been successful. A review concluded that the most effective techniques are alteration of fermentation temperature, must freezing, saignée (juice runoff), pectolytic enzyme treatments, and extended maceration (Sacchi et al. 2005). Other techniques, such as sulfur dioxide (Bakker et al. 1998) and initial cold soak of the fruit (Gerbaux 1993), have largely shown no lasting gain or have led to a reduced final phenolic concentration.

During the winemaking process, anthocyanins form colored and colorless adducts with tannins, keto-acids, and aldehydes. These derived pigments were first identified by their ability to resist bisulfite bleaching and are referred to collectively as polymeric pigments (Somers 1966). These compounds are neither all polymeric and nor all completely resistant to bisulfite bleaching (Remy et al. 2000). Invariably, however, polymeric pigments are artifacts of the winemaking process and are the primary source of coloration of old red wines, whereas young red wines are colored by a combination of anthocyanins and polymeric pigments. There has been some speculation that tannin-anthocyanin adducts would have less astringent properties when compared to underived tannins (Singleton 1992). Work comparing purified

[1]School of Food Science, Washington State University, IAREC, 24106 N. Bunn Rd., Prosser, WA 99350, and [2]School of Food Science, Washington State University, FSHN, Pullman, WA 99164.

*Corresponding author (email: jfharbertson@wsu.edu; tel: 509 786-9296; fax: 509 786-9370)

Acknowledgments: The authors thank Washington Wine Grape Funds for support of the project.

The authors are grateful for the expert winemaking from Joshua Maloney and Robert Bertheau from Chateau Ste. Michelle and for John Thorngate's assistance with editing and verification of statistical data.

Manuscript submitted Mar 2009, revised Jul 2009, accepted Jul 2009. Publication costs of this article defrayed in part by page fees.

fractions of tannins and equally sized acetaldehyde-bridged tannin anthocyanin adducts indicated that the derived tannins were less astringent (Vidal et al. 2004), but there is not yet direct evidence of this in wine.

In recent work evaluating the tannin profiles from skin and seed extracts of different grape cultivars, tannin structure from both skins and seeds varied by cultivar and vintage (Chira et al. 2009, Cosme et al. 2009); however, there were no differences when the extracts were subjected to sensory evaluation (Chira et al. 2009). When the variation of tannin content in the skins and seeds of grape cultivars was examined, tannin per seed was fairly constant (Harbertson et al. 2002) and the amount of skin tannin varied ~two- to three-fold (Harbertson et al. 2002, Seddon and Downey 2008). A wide survey of wines (n = 1324) indicated a greater than 30-fold variation (Harbertson et al. 2008). The much broader range of tannin values in wine relative to the smaller differences in fruit suggests that winemaking plays the greater role in determining the final tannin content of finished wine. The duration of contact time between the fermenting juice with skins and seeds during the winemaking process heavily influences the extraction of anthocyanins and tannins into the wine. One factor thought to limit extraction is the differential solubility of anthocyanins and tannins in alcoholic solutions (Sacchi et al. 2005). Anthocyanins are considered more water soluble, whereas tannins are more ethanol soluble (Bate-Smith 1975).

Here we report on the effect of ethanol concentration, contact time, saignée, and water addition on the extraction of tannin, anthocyanin, and formation of polymeric pigments during the first 185 days of winemaking and on a sensory evaluation of the finished wine. Previous work indicates small-scale fermentations (3.5 kg) are reproducible but yield tannin concentrations that are much (36%) lower than commercial fermentations of the same fruit with similar skin contact times (Sampaio et al. 2007). To ensure commercial applicability, this experiment was carried on a commercial scale (7,892 kg per fermentation, each done in duplicate).

Materials and Methods

Reagents. Bovine serum albumin (BSA, Fraction V powder), sodium dodecyl sulfate (SDS; lauryl sulfate, sodium salt), triethanolamine (TEA), ferric chloride hexahydrate, and (+)-catechin were purchased from Sigma (St. Louis, MO). Malvidin-3-glucoside was purchased from Polyphenols Laboratories (Sandnes, Norway).

Fruit sampling and berry extraction. In 2007, fifteen 50-cluster (bunch) replicates were selected from 15 rows evenly distributed across a nine-year-old 8-ha own-rooted Merlot (Clone 3) vineyard located in the Columbia River Basin on the day of harvest. Samples were put in self-sealing bags, placed on ice, and transported to the research station where they were counted and weighed. For each replicate, the berries were separated from the cluster and placed onto a table where 30-berry replicates were selected at random. The remaining fruit was divided and stored for other analysis.

Winemaking. Merlot grapes were mechanically harvested and delivered immediately to the winery near the Columbia River in gondolas. The grapes were received into a hopper via a Delta E50 destemmer (Vaslin Bucher, Chalonnes sur Loire, France) with the crushers disengaged and then pumped through a must line with a progressive cavity pump to the fermentors. Each fermentor (18,972 L capacity) held between 7,892 and 8,165 kg must and was set to cool the juice to 21°C or to heat to 19°C if the fruit was cold. The jacketed fermentors were set to 29°C and were cooled with a stainless-steel jacket containing ethylene glycol. The yield was ~0.7 L juice per kg of fruit. After the fruit was destemmed, 30 mg/L of sulfur dioxide was added. Juice removal, diammonium phosphate (DAP), and water additions were performed the morning after receiving the grapes, and yeast was added the evening after receiving. *Saccaromyces cerevisiae bayanus* strain (Uvaferm, Fermentis, Oskaloosa, IA) (120 mg/L) was rehydrated according to the manufacturer's rehydration procedure and added prior to mixing the tank. The DAP was added to raise the yeast assimilable nitrogen to 225 mg/L. The amount of dechlorinated water added depended upon the treatment (Table 1).

The tanks were mixed at a rate of 1.10 min per metric ton of fruit. Fermentations were carried out in duplicate. On day one of maceration, the juice was mixed from the bottom tank valve over the top of the fermentor for 7 min through an irrigator. On day two the tank was only mixed during the morning additions, and on day three the pump-overs began. For each pump-over, wine was pumped from the racking valve over the top of the fermentor through an irrigator at ~1.10 min per metric ton, three times per day until the must was drained, at which time the pomace was pressed in a Willmes press (model TP15; Willmes, Lamperheim, Germany). Each treatment was given 7 days of contact time and pressed at 6.2 Brix ± 0.4 (standard error) with the exception of the treatment that included extended maceration, which received 20 days and was pressed at -1.3

Table 1 The volume changes and soluble solids at crush and inoculation for the different treatments (± standard error).

Treatment	Juice removal (%)	Water addition (%)	Volume change (%)	Initial Brix	Brix at inoculation
Control	0	18.7 ± 0.6	+18.7	28.3 ± 0.2	24.3 ± 0.9
High ethanol	0	4.5 ± 0.8	+4.5	28.0 ± 0.2	26.8 ± 0.6
Low saignée	18.1 ± 1.2	18.1 ± 1.2	0	28.1 ± 0.3	24.1 ± 0.4
Low saignée-EM	16.8 ± 0.4	16.8 ± 0.4	0	27.8 ± 0.1	24.2 ± 0.2
High saignée	32.7 ± 1.7	16.4 ± 0.8	-16.4	27.7 ± 0.2	24.2 ± 0.8

Brix ± 0.1 (standard error). The total press cycle was 46 min, beginning at 150 millibar and finishing at 350 millibar. Pressure was held for 6 min, the bladder deflated, and then rotated twice before reinflating. The free-run juice from pressing was pumped into a drain tank and combined with the press wine after the press cycle was finished. After the wines were dry (-1.3 Brix) they were sent to barrel and inoculated with lactic acid bacteria (*Leuconostoc oeanos* DSM 7008, Chris Hansen Laboratory, Denmark). Each replicate was monitored for Brix and temperature once a day after the morning pump-over. The temperature of the must sampled from the racking valve was measured with a hand-held digital thermometer. The same sample was analyzed with a hand-held densitometer calibrated for Brix (DMA 35N, Anton Paar, Austria).

Winemaking treatments. Four treatments plus a control were included in this study: (1) water addition to 24.3 Brix to compensate for high Brix must (control); (2) water addition to 26.8 Brix (high ethanol); (3) saignée (juice runoff) targeted to 16% of the total volume, paired with an equal volume water addition, yielding 24.1 Brix (low saignée); (4) low saignée treatment paired with a 20-day extended maceration (low saignée-EM); and (5) ~32% saignée with water addition to 24.3 Brix (high saignée). Initially, the fruit was ~28 Brix; after saignée the replicates were watered back to a consistent soluble solids concentration (see Table 1). The contact time was limited to 7 days for each of the treatments with the exception of the extended maceration (low saignée-EM) treatment, which had a 20-day contact period. The extended maceration treatment also had juice removal consistent with the low saignée treatment, but because the initial Brix was different the juice removal and water addition were slightly different to accommodate a final alcohol concentration that would be the same. After pressing, two 3.78 L self-sealing bags of pomace (skins and seeds) were collected from the press pan and stored at 2°C until analysis.

Fruit and pomace extraction. For tannin analysis 16 replicates (n = 16) containing 30 berries were dissected into skins and seeds separately and extracted with 70% acetone as described in previous work (Harbertson et al. 2002). Acetone was removed under reduced pressure at 38°C using a Büchi Syncore Polyvap (Flawil, Switzerland) equipped with a 24-well rack for 50-mL tubes. For pomace, the skins and seeds were separated and used to reconstruct ten 30-berry replicates. They were then subjected to the same extraction and solvent removal described earlier.

Fruit and wine analysis. Anthocyanins were measured according to Picciotto (2002). Samples were diluted into a pH 1.8 buffer containing 200 mM maleic acid with 170 mM NaCl and after a 10-min incubation were measured at 520 nm on a spectrophotometer (Beckman DU 640, Fullerton, CA). The absorbance at pH 1.8 was compared to the absorbance of a duplicate sample diluted in a pH 4.9 buffer comprised of 200 mM acetic acid and 170 mM NaCl. The difference between the two samples was then converted into malvidin-3-glucoside equivalents using a standard curve made by diluting pure malvidin-3-glucoside and subjecting it to the same pH shift conditions.

Tannins in the fruit, pomace, and wine were analyzed as previously described (Hagerman and Butler 1978) and adapted for wine (Harbertson et al. 2002) using protein precipitation and were standardized using catechin equivalents (CE). Dilution of samples was strictly evaluated and in accordance with previously reported best range of analysis (Jensen et al. 2008).

Phenolics in the wine were analyzed as previously described (Heredia et al. 2006). Briefly, a sample was diluted into an alkaline detergent buffer (5% SDS (w/v), 5% TEA, pH 9.4, buffer adjusted with HCl) to a finished volume of 875 µL and then incubated for 10 min. Absorbance at 510 nm was then recorded and then remeasured after adding 125 µL ferric chloride and incubated for 10 min. The difference was converted into catechin equivalents (CE), similar to the protein precipitable tannins.

Large polymeric pigments (LPP) and small polymeric pigments (SPP) were measured in the wine as previously described (Harbertson et al. 2003). SPP and LPP were summed to provide a measure of total polymeric pigments (TPP).

Experimental design. Microsoft Excel (Redmond, WA) was used for data entry and storage. Data analysis was performed with Statistica (StatSoft Tulsa, OK). Two-way completely randomized analysis of variance was performed on the winemaking trials and a one-way analysis of variance performed for the model extraction experiments. For each experiment, the variance from each treatment was examined and found to be similar to the other treatments. A 5% level for rejection of the null hypothesis was used for each experiment. Fisher's least significant difference (LSD) test was used as a post-hoc comparison of means. Similar to the ANOVA, 5% ($p < 0.05$) was considered a minimum for significance.

The winemaking trial was set up with a control and four treatments, each carried out in duplicate (two-way ANOVA, df = 4,4). The harvest fruit (n = 16) comparison to the pomace (n = 2) was carried out on skins and seeds and pomace separately (df = 5, 20).

Sensory evaluation. *Materials.* Tannic acid, carboxymethylcellulose, and alum were purchased (Sigma-Aldrich, St. Louis, MO) for preparing reference standards. All other materials for preparing standards were obtained through a local grocery store (Safeway, Pullman, WA). Unsalted-top saltine crackers (Safeway, Pleasanton, CA) and filtered deionized water were provided for rinsing the palate. The deionized water was filtered over a Milli-Q Reagent Water System (Millipore, Bedford, MA).

Difference testing. A duo-trio difference test with a constant reference (control) was used to explore differences between the winemaking treatments. Panels were conducted over two days, with 30 untrained panelists on each day (n = 30). On day 1, panelists compared the control wine to the high saignée and the high ethanol treatments. On day 2 untrained panelists compared the control wine to the low saignée and the low saignée-EM treatment.

On day 1, the panel was composed of 12 males and 18 females, and on day 2, the panel was composed of 15 males and 15 females. The age range was 21 to 65 years. The majority of panelists were light to moderate wine drinkers (consumed red wine from 5 to 20 times per month). For both panel days, panelists were recruited from the Washington State University community and were wine consumers. Minimal information on the nature of the study was provided in order to reduce potential bias. All participants signed an Informed Consent Form and the project was approved by the Washington State University Institutional Review Board.

Aliquots of wine (25 mL) bottled after one year of barrel aging were randomly presented in coded ISO/INAO wineglasses under white light. Each glass was covered to contain the wine aroma. Data were collected and analyzed using computer software (Compusense five, release 4.6; Compusense, Guelph, ON). Based on n = 30, duo-test test results ($p < 0.05$) required 20 correct responses (Roessler et al. 1978).

Trained panel evaluations. Panelists were trained over six sessions to recognize specific flavor and aroma attributes in the wines. The trained panel was composed of four males and eight females between the ages of 21 and 75. In the first training session, panelists were instructed on the proper use of the 15-cm unstructured line scale. Prior to participation in the panel, the panelists completed a blind evaluation of a bitter standard (13.3 mg quinine sulfate/L water) using a 15-cm unstructured line scale. Their evaluations were collected and examined to determine the intensity of bitterness assigned to this solution. From this initial screening, all panelists were found to be sensitive to bitterness and were retained on the panel. Panelists also evaluated the five experimental wines and generated appropriate terms to use for the description of wine aromas and flavors.

The second session included the training for mouthfeel and taste attributes. Reference standards for aroma and flavor and for taste were prepared in 100 mL base wine (Livingston Red Rosé; Gallo, Modesto, CA) (Table 2). Mouthfeel standards (with the exception of alcohol/hot) were dissolved in 750 mL base wine. The mouthfeel attributes smooth, dynamic, and drying were defined previously as grouping terms for mouthfeel characteristics (Gawel et al. 2000). *Smooth* was defined as the sensations of the mouth when the surfaces came in contact with each other; *dynamic* was an astringency term, specifically a sensation involving some sort of mouth movement; and *drying* was defined as the feeling of lack of lubrication in mouth. During the descriptive analysis discussion session, the panelists included an alcohol/hot mouthfeel term, which they defined as hot or burning. To avoid carryover effects panelists took about 8 min to assess each wine. There was a 2-min forced rest period between each wine and a 5-min rest midway through the evaluations. Crackers and water were used as palate cleansers before evaluations and between each sample.

In the four subsequent training sessions, panelists reviewed the standards, evaluated experimental wines, and discussed the intensity of the different attributes. Following each training session, data from each panelist were collected and evaluated to determine panelist and overall panel performance. Panelist performance was monitored and evaluated for reproducibility and agreement. For each attribute, a two-way analysis of variance (ANOVA) was performed, considering panelists, samples, and their interactions. Based on these results, additional training for specific panelists was suggested and performed.

During all of the evaluation and training sessions, a 15-cm line scale was used, labeled with terms low, moderate, and high of the desired attribute. Low and high were anchored at the 1 cm and 14 cm mark, respectively. The final training session was an orientation to the computer program (Compusense five, version 4.6) and the sensory laboratory used during data collection.

Clear ISO/INAO wineglasses were used for all evaluations. Each panelist was provided with deionized filtered water and unsalted crackers for palate cleansing. Twenty-five mL aliquots of room temperature wine were poured into the three-digit coded wineglasses and covered to trap volatiles. Wines were presented using a balanced design and panelists evaluated each wine in three evaluation sessions. Results were collected and analyzed by Compusense (version 4.8). The trained panel data were analyzed using a three-way fixed-effects analysis of variance (ANOVA) with replication (SAS, version 9.1; Cary, NC). Where appropriate, separation of the means was accomplished using Tukey's HSD. The significance value was established as $p \leq 0.05$.

Results

Acidity and ethanol. The finished wines were compared for basic wine attributes. There were no significant differences for pH or titratable acidity (Table 3). As intended, the high ethanol fermentation had a significantly

Table 2 Ingredients and preparation of sensory analysis standards. Mouthfeel standards (with the exception of alcohol/hot) were dissolved in 750 mL base wine; aroma and flavor and taste standards were dissolved in 100 mL base wine.

	Reference standard
Aroma, flavor	
Fresh fruit	5 mL raspberry jam, 10 mL cherry jam
Dried fruit	2 dried prunes, cut into pieces
Canned vegetal	5 mL juice from canned asparagus
Caramel	5 mL soy sauce
Woody	10 mL oak wood shavings
Earthy	10 mL potting soil
Spicy	6 clove buds, soak for 5 min, remove 4
Taste	
Bitter	20 mg quinine sulfate
Sour	3.5 g tartaric acid
Mouthfeel	
Drying	2 g tannic acid, 0.875 g alum
Weight	0.38 g carboxymethylcellulose
Alcohol/hot	6.4% ethanol (v/v)

different ethanol concentration ($p < 0.05$) yielding a wine with ~2% higher concentration. The high ethanol fermentations took 9 days to complete fermentation, compared with 7 days for the other treatments, but were otherwise not different (data not shown).

Anthocyanins. The evolution of anthocyanin through the first 185 days was marked by a general increase in all treatments until day 7, after which there was a decline (Figure 1). Anthocyanin concentrations for each of the treatments declined between press and day 185 (Figure 2, Table 4). At press, only the high saignée treatment reflected a significant difference in anthocyanin concentration. The other treatments had generally higher anthocyanin concentrations than the control, with the exception of the low saignée-EM, in which anthocyanins had peaked earlier. When anthocyanin content was reevaluated at 185 days, the high saignée treatment maintained an elevated anthocyanin content. Low saignée also resulted in elevated an anthocyanin value, but it was significantly lower than that of the high saignée. The low saignée-EM treatment had the lowest concentration of anthocyanins and was significantly different than the other treatments.

Polymeric pigments. Between 7 and 185 days, SPP increased by ~30% and LPP increased by 70%, providing an overall change in TPP of ~50% (Figure 2). For SPP, none of the treatments were different from each other at press (Table 5). Low saignée-EM was significantly greater in LPP concentration than each of the other treatments. For TPP, the low saignée-EM and high saignée treatments were significantly different than the other treatments; the order of the differences was low saignée-EM > high saignée > high ethanol ≥ low saignée ≥ control. At 185 days, the SPP content of the wines showed some significant differences, in the order of high saignée > high ethanol ≥ low saignée ≥ control = low saignée-EM. LPP concentrations also showed significant differences, in the order of low saignée-EM > high saignée ≥ high ethanol ≥ low saignée > control. TPP content of both low saignée-EM and high saignée were significantly greater than the other treatments.

Wine and grape tannins. For all winemaking treatments, the extraction of protein precipitable tannins increased during the contact period and thereafter remained fairly constant (Figure 3). When protein precipitable tannins were evaluated at press, the low saignée-EM was significantly more tannic than the other treatments (Table 4). On day 185, significant differences in tannin concentration were found among the treatments in the order of low saignée-EM > high saignée > high ethanol ≥ low saignée > control.

Tannin content of skins and seeds at harvest and the remaining skin and seed tannin content from the pomace of each treatment were determined (Table 6). For each treatment, the amount of tannin present in the pomace was significantly lower than the fruit from both tissues. The percentage of extracted skin tannin in each treatment was greater than the percentage of seed tannins extracted. None of the pomace treatments contained significantly different amounts of skin tannins; however, the low saignée-EM treatment showed a significantly lower amount of pomace seed tannins remaining compared with the other treatments.

The proportion of tannins extracted from skins and seeds and an estimated tannin concentration were determined (Table 7) based on the amount of tannins extracted from both skins and seeds during the fermentation (Table 6). The proportion of skin or seed tannins was determined

Table 3 pH, titratable acidity (TA), and ethanol (EtOH) of the finished red wines (± standard error).

Treatment	pH	TA (%)	EtOH% (v/v)
Control	3.81 ± 0.02 A[a]	0.50 ± 0.05 A	14.0 ± 0.31 A
High ethanol	3.89 ± 0.04 A	0.52 ± 0.03 A	15.9 ± 0.16 B
Low saignée	3.86 ± 0.03 A	0.52 ± 0.03 A	13.8 ± 0.23 A
Low saignée-EM	3.84 ± 0.04 A	0.51 ± 0.03 A	13.8 ± 0.13 A
High saignée	3.81 ± 0.04 A	0.50 ± 0.02 A	13.8 ± 0.13 A

[a]Letters within a column indicate significant differences at $p < 0.05$.

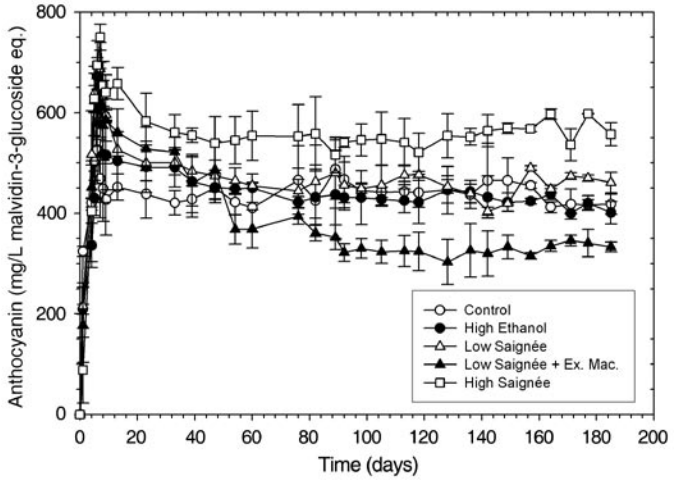

Figure 1 Extraction of anthocyanin during fermentation and aging (up to 185 days).

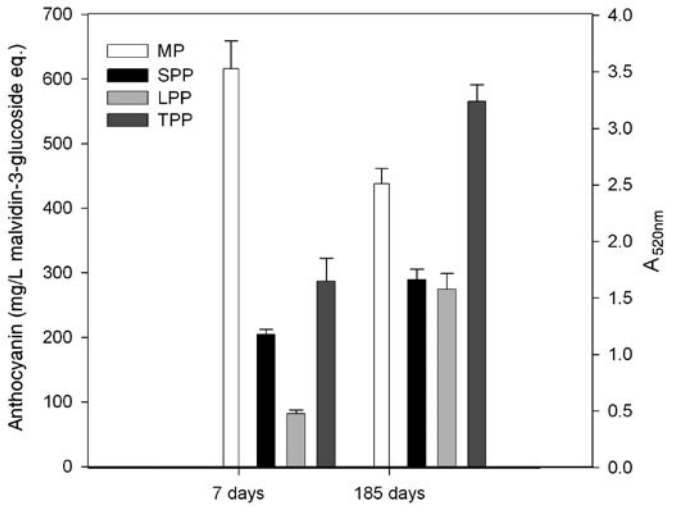

Figure 2 Polymeric pigment and anthocyanin content of treated wines at 7 days and 185 days (MP, anthocyanin; SPP, small polymeric pigment; LPP, large polymeric pigment; TPP, total polymeric pigment).

by the difference between what was found in either the skin or seed at harvest and the amount left in the pomace and then dividing by the total amount of tannin extracted. Pomace results were calculated based on a gram fresh weight basis by using the average berry weight at harvest (1.18 g, standard error 0.02, n = 16). Total wine tannins were estimated using the total amount extracted and compensating for the amount of liquid added or subtracted (Table 1). The control and low saignée treatments had similar proportions of skin and seed tannins extracted (nearly equal), while the high ethanol, high saignée, and low saignée-EM treatments had proportionally greater amounts of extracted seed tannins. However, in each case the amount of tannin found in the wine was less than what would be expected from the amount extracted (Table 4, Table 7).

Iron reactive phenolics. Concentrations of iron reactive phenolics (IRP) at press and at 185 days were significantly higher in the high saignée and low saignée-EM treatments than in the other treatments (Table 4). The high ethanol treatment contained more IRP than the control but was not significantly different from the low saignée treatment. After 185 days, the low saignée-EM continued to have significantly higher IRP concentrations than all the other treatments.

Replicate variability of standard analysis and phenolic components. A two-way ANOVA was performed for each of the standard wine analyses and the measured phenolic variables. There were no significant differences between replicates within a treatment for any of the variables, indicating that all the replicates of this large-scale experiment were well within acceptable limits.

Sensory evaluation. Difference tests revealed the wines to be significantly different (data not shown), therefore trained panel analysis was carried out. Descriptive analysis of the aroma and flavor of the wines showed fresh fruit flavor differences in the wines ($p < 0.05$) (Table 8). ANOVA results for taste and mouthfeel characteristics results revealed differences ($p < 0.05$) in several attributes, including sour, smooth, drying, dynamic, and alcohol/hot (Table 9).

Fresh fruit flavor was significantly lower in the high ethanol wine than in the control and low saignée wines (Table 10). Sourness was rated significantly lower in the control wine than in the high saignée and low saignée wines.

For mouthfeel, more differences were observed among the different winemaking treatments. The low saignée-EM and high ethanol wines were significantly less smooth than wines from the other treatments. Similarly, the low saignée-EM wine was significantly higher in the drying characteristic than all other wines. The high ethanol and high saignée wines were significantly more drying than the low saignée wine. The low saignée-EM wine was significantly more dynamic than the high saignée and control. In terms of alcohol/hot character, the high ethanol wine was rated

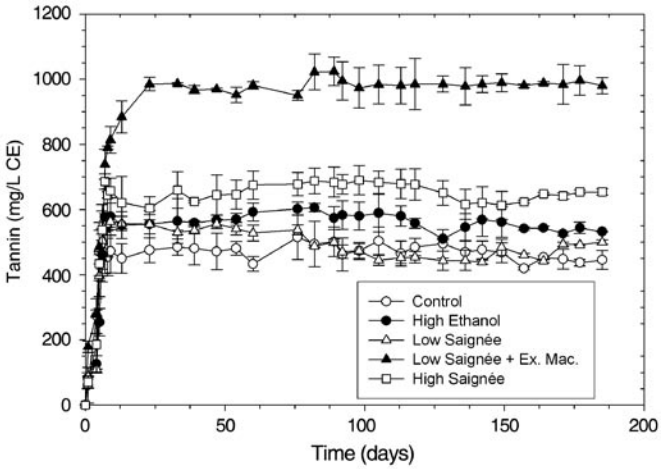

Figure 3 Protein precipitable tannins as measured in catechin equivalents (CE) during fermentation.

Table 4 Anthocyanin, tannin, and iron reactive phenolics concentrations at press and at 185 days (± standard error).

	Anthocyanin (mg/L M-3-glucoside equivalents)		Tannin (mg/L CE)		Iron reactive phenolics (mg/L CE)	
Treatment	**Press**	**185 days**	**Press**	**185 days**	**Press**	**185 days**
Control	468 ± 33.5 B[a]	418 ± 14.0 B	469 ± 85.3 A	399 ± 28.9 A	1145 ± 63.0 A	1338 ± 2.0 A
High ethanol	656 ± 44.5 B	401 ± 0.5 B	577 ± 10.2 A	512 ± 0.50 B	1338 ± 3.0 B	1578 ± 23 B
Low saignée	687 ± 52.0 B	461 ± 20.5 C	549 ± 16.2 A	500 ± 6.50 B	1300 ± 21 AB	1291 ± 10.0 A
Low saignée-EM	528 ± 101 A	352 ± 7.5 A	985 ± 45.1 B	980 ± 2.0 D	2026 ± 19.0 D	2353 ± 26.0 D
High saignée	750 ± 25.0 C	558 ± 1.5 D	686 ± 23.7 A	658 ± 10.0 C	1690 ± 80.0 C	1783 ± 30 C

[a]Letters within a column indicate significant differences at $p < 0.05$.

Table 5 Small polymeric pigment (SPP), large polymeric pigment (LPP), and total polymeric pigment (TPP) content at press (± standard error).

	SPP (AU)		LPP (AU)		TPP (AU)	
Treatment	**Press**	**185 days**	**Press**	**185 days**	**Press**	**185 days**
Control	1.04 ± 0.02	1.51 ± 0.06 AB[a]	0.41 ± 0.09 A	1.16 ± 0.11 A	1.46 ± 0.11 A	2.67 ± 0.09 A
High ethanol	1.13 ± 0.09	1.65 ± 0.09 B	0.52 ± 0.06 A	1.45 ± 0.02 AB	1.64 ± 0.14 A	3.10 ± 0.11 A
Low saignée	1.14 ± 0.01	1.60 ± 0.04 B	0.47 ± 0.05 A	1.36 ± 0.14 AB	1.61 ± 0.6 A	2.96 ± 0.18 A
Low saignée-EM	1.55 ± 0.20	1.37 ± 0.20 A	1.75 ± 0.04 B	2.35 ± 0.04 C	3.31 ± 0.23 C	3.72 ± 0.05 B
High saignée	1.28 ± 0.05	2.18 ± 0.05 C	0.55 ± 0.04 A	1.83 ± 0.09 B	1.83 ± 0.09 B	3.75 ± 0.13 B

[a]Letters within a column indicate significant differences at $p < 0.05$.

significantly higher than low saignée, but was not statistically different from the other wines.

Principal components analysis (PCA) was conducted to examine the interrelationships between the different variables and to allow the separation of the five wine treatments (Figure 4). PCA explained 86.2% of the variation in the data, with 68.3% and 17.9% explained by the first (F1) and second principal components (F2), respectively. F1 was defined primarily by the contrast between fresh fruit flavor and smoothness with dynamic, drying, and hot/alcohol attributes. F2 was primarily a function of perceived sourness. Separation was observed among the five wines. The low saignée, control, and high saignée wines were described more by their fresh fruit flavor and smoothness, with the high saignée wine higher in sourness than the other two wines. These wines were in contrast to the high ethanol wine, which was defined by its elevated perceived alcohol, and the low saignée-EM wine, which was defined by its drying and dynamic qualities.

Table 6 Tannin content in skins and seeds at harvest and in the pomace and percentage of extraction that occurred during winemaking from each tissue (± standard error).

Parameter	mg skin tannin per g FW	mg seed tannin per g FW	% skin extraction	% seed extraction
Harvest (n=16)	0.66 ± 0.01 A[a]	3.29 ± 0.05 A	NA	NA
Pomace control (n=2)	0.36 ± 0.02 B	2.77 ± 0.12 B	46%	16%
Pomace high ethanol (n=2)	0.40 ± 0.001 B	2.45 ± 0.02 B	40%	25%
Pomace low saignée (n=2)	0.36 ± 0.02 B	2.74 ± 0.26 B	45%	17%
Pomace low saignée-EM (n=2)	0.31 ± 0.02 B	1.83 ± 0.02 C	53%	44%
Pomace high saignée (n=2)	0.44 ± 0.04 B	2.54 ± 0.18 B	33%	22%

[a]Letters within a column indicate significant differences at $p < 0.05$.

Table 7 Estimated proportion of skin and seed tannins extracted into wine based on fruit and pomace comparison and estimated tannin concentration of the finished wine.

Treatment	Skin tannin	Seed tannin	Wine tannin (mg/L CE)
Control	42%	58%	620
High ethanol	26%	74%	977
Low saignée	40%	60%	782
Low saignée-EM	21%	79%	1749
High saignée	27%	73%	1031

Discussion

Five winemaking methods using very ripe Merlot grapes were examined, each method selected to provide insight into the factors influencing phenolic extraction. The extraction of anthocyanin followed the same trend as reported in other studies, reaching a maximum after 7 days of contact time and declining thereafter. The formation of polymeric pigments during the extraction and decline period of anthocyanins is consistent with previous studies (Nagel and Wulf 1979, Bakker et al. 1998, Ribéreau-Gayon et al. 1970).

At press, only the high saignée treatment showed a significantly increased concentration of anthocyanin relative to the other treatments. The high saignée wine was 55% higher in anthocyanin than the control, exceeding the dilution effect by ~20%, but the effect was transient and no longer detected at day 185. The low saignée-EM treatment yielded lower anthocyanin concentrations than the other fermentations because it was pressed at 20 days, well after the point (7 days) at which free anthocyanins were at their peak (Figure 1). The high ethanol fermentation was not lower in anthocyanin, providing evidence that anthocyanin extraction, at the concentration found in these wines, was not limited by ethanol solubility but rather by the conversion to other colored compounds and is consistent with previous findings (Gao et al. 1997).

On average, anthocyanin concentration declined by 20% between day 7 (peak extraction) and day 185 (the last date tested). A decline occurred in all treatments but did so to varying degrees, the control and low saignée-EM showed markedly different reductions. Anthocyanin declined ~10% in the control, whereas the low saignée-EM treatment showed a decline of 43% relative to its peak concentration. This discrepancy will be further explored in the analysis of polymeric pigments.

In this experiment, an analysis combining the traditional method of measuring polymeric pigments (by the addition of bisulfite) with protein precipitation (Harbertson et al.

Table 8 Degrees of freedom and F ratios from analysis of variance of trained panel evaluation of Merlot wine aroma and flavor attributes (n = 12).

		Aroma							Flavor						
Source of variation	df	Fresh fruit	Dried fruit	Canned vegetal	Caramel	Woody	Earthy	Spicy	Fresh fruit	Dried fruit	Canned vegetal	Caramel	Woody	Earthy	Spicy
Panelist (P)	11	7.26***[a]	5.27**	9.38***	17.32***	16.07***	31.53***	8.12***	19.79***	11.17***	12.16***	24.84***	18.51***	46.98***	8.67***
Session (S)	2	0.95	0.78	0.35	2.30	0.84	0.17	2.39	1.21	0.02	0.40	0.96	0.39	1.29	0.44
Wine (W)	4	0.77	2.05	1.17	1.63	0.09	0.97	1.46	2.84*	0.48	0.51	0.65	0.42	0.47	0.16
P*W	44	2.10**	1.15	1.27	1.31	1.03	0.97	1.45	1.24	0.70	0.91	1.02	0.54	1.0	0.81
P*S	22	0.74	1.01	1.03	2.19*	0.94	1.40	2.12*	0.77	1.70*	0.70	2.08*	2.09*	1.58	2.37*
S*W	8	1.98	0.76	0.73	0.31	0.65	1.96	0.49	1.06	0.98	0.54	0.65	0.68	0.56	1.07

[a]*, **, and *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

2003) was used to measure polymeric pigments. The analysis assumes that polymeric pigments that cannot precipitate protein are small (SPP), whereas those that are capable of protein precipitation are large (LPP). The sum of LPP and SPP was shown to be equivalent to the original method developed by Somers and Evans (1974) (Harbertson et al. 2003, Adams et al. 2004) and is provided here as total polymeric pigment (TPP). After 7 days, both SPP and LPP were formed in each of the wines. At press SPP formation did not differ significantly between the treatments. At press, there were no differences in the amount of LPP with the exception of low saignée-EM. The increase in LPP concentration found in low saignée-EM is likely due to the extra time provided for formation.

Although both SPP and LPP were formed during the period between 7 and 185 days (enough to double TPP), an obvious preference for LPP formation was observed, with a three-fold improvement. Similarly, previous work using the same method evaluated wines over a three-year period and found that LPP was preferentially formed, and although the time periods are unequal, the generalized feature is similar (Adams et al. 2004). SPP content was significantly greater in the high saignée than the control and at a rate that is consistent with dilution difference between the wines. There were no differences in SPP content at press, suggesting that the earlier high anthocyanin concentration led to greater SPP formation. The low saignée-EM treatment had significantly higher LPP content than the other treatments. It has been noted that the proportion of tannin and anthocyanin is an important parameter for the formation of polymeric pigments (Fulcrand et al. 2004). In the current study, the LPP fraction represented a pigmented polymer capable of precipitating protein. It is assumed that LPP are formed through reactions between anthocyanin and tannin. It is possible that the significantly higher concentrations of tannin in some treatments led to a corresponding increase in LPP in those treatments. The corresponding loss in anthocyanin for the low saignée-EM treatment also helps to explain this phenomenon, especially given that the decline was remarkably greater than in the other treatments. The control treatment showed the same trend but on a smaller scale, displaying a small decline in anthocyanin and had a corresponding small increase in SPP and LPP.

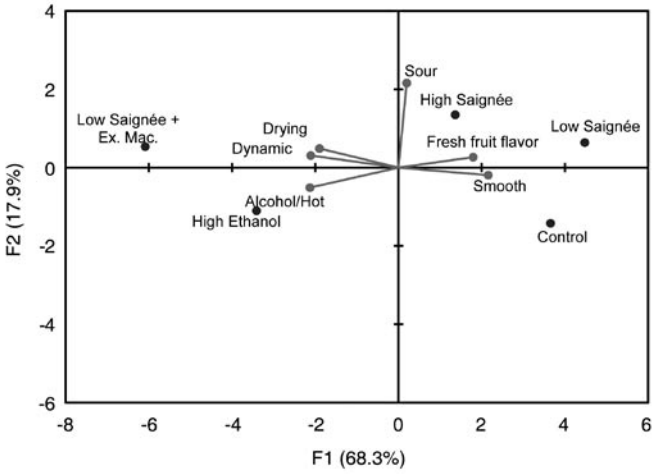

Figure 4 First two principal components (PC1 and PC2) and mean PC scores for sensory attributes of wines as evaluated by trained panel. PC1 illustrates the contrast between wine smoothness and fresh fruit flavor with dynamic/drying. PC2 is primarily a function of wine sourness. The plot illustrates the PC space for the five wines and six sensory attributes found to be significant using ANOVA.

Table 9 Degrees of freedom and F ratios from analysis of variance of trained panel evaluation of Merlot wine taste and mouthfeel (n = 12).

		Taste		Mouthfeel				
Source of variation	df	Bitter	Sour	Smooth	Drying	Dynamic	Weight	Alcohol/hot
Panelist (P)	11	11.65***[a]	9.49***	8.34***	11.61***	4.74***	18.77***	5.23***
Session (S)	2	2.69	1.85	0.48	1.92	0.02	2.04	0.57
Wine (W)	4	1.20	2.38*	2.34*	11.35***	3.00*	1.70	1.76*
P*W	44	1.18	1.08	1.00	1.08	1.19	1.84*	0.99
P*S	22	1.89*	0.73	1.11	0.57	2.61*	0.58	2.29*
S*W	8	1.31	0.34	2.08*	1.43	2.82*	0.80	2.74*

[a]*, **, and *** indicate significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 10 Means separation for sensory attributes of Merlot wines made using different winemaking techniques. Evaluations were made along a 15-cm line scale (n = 12).

Treatment	Fresh fruit flavor	Sour	Smooth	Drying	Dynamic	Alcohol/hot
Control	6.33 B[a]	5.50 A	6.91 B	6.64 AB	6.48 A	6.89 AB
High ethanol	5.33 A	5.80 AB	6.49 AB	6.88 B	7.21 AB	7.61 B
Low saignée	6.30 B	6.37 BC	7.04 B	6.00 A	6.76 AB	6.73 A
Low saignée-EM	5.78 AB	6.11 ABC	5.93 A	8.68 C	7.59 B	7.51 AB
High saignée	5.92 AB	6.66 C	6.76 B	7.10 B	6.72 A	6.99 AB

[a]Within a column, values followed by the same letter are not significantly different at $p \leq 0.05$.

The concentration of tannins in the wines ranged from 399 to 980 mg/L CE, which is consistent with data collected in a survey of 197 wines made from the same variety (Merlot) using the same analytical method (Harbertson et al. 2008). The saignée treatments showed increases in tannins proportional to the amount of juice removed. There is a 35% dilution effect for the high saignée and 35% increase in tannins and an 18% bleed-off effect for the low saignée and corresponding increase. Similarly, a significant increase in tannins was observed in Monastrell wines when a 20% saignée was used (Bautista-Ortín et al. 2004). The high ethanol treatment likewise had a similar increase due to a dilution effect that, once normalized, became the same as the control. The low saignée-EM treatment, not surprisingly, had significantly higher tannin concentration than the other treatments, and the effect is consistent with other research that found that extended maceration increases tannin concentration (Ribéreau-Gayon et al. 1970, Gómez-Plaza et al. 2001, Bautista-Ortín et al. 2004). The evaluation of skins and seeds from the fruit at harvest and the skins and seeds from the pomace after the winemaking trial provides information about the proportion of tannins extracted from the skins and seed during the winemaking trial. The evidence shows that extended maceration extracts significantly more seed tannins than the other treatments. The lengthy contact time has long been estimated to give greater seed tannin extraction (Singleton and Draper 1964, Oszmianski et al. 1986). Interestingly, the proportion extracted roughly resembles the proportions of total tannin in the berry (~20% skin tannin, 80% seed tannin). Although there were no significant differences among the remaining treatments, the data suggest that high saignée and high ethanol extract more seed tannins proportionally relative to skin tannins than the control and low saignée, which have closer to 50% skin and 50% seed extraction proportions. In the current study, the ethanol did appear to extract more seed tannins and leave behind more skin tannins, even though concentration of tannin in the wines was similar to the control. It is not clear why the high saignée treatment extracted less skin tannin relative to seed tannin than the control and low saignée. The results suggest that altering the ratio of solids to juice favors seed extraction. Recent work demonstrated that the extraction of skin tannins reaches a plateau readily whereas the seed tannins continue to increase as the contact time increases (Cerpa-Calderon and Kennedy 2008). The current study results are consistent with those results; however, we show a slight increase in the skin tannin extraction with time (Table 6).

Sensory evaluation of the wines revealed the winemaking techniques did not alter the aroma attributes. Since saignée is essentially a concentration of the berry, this finding suggests that the difference in concentration was not enough to alter the perception of the aroma classes found in these wines. However, some flavor attributes were altered. The high ethanol treatment yielded a wine rated significantly lower for fresh fruit flavor than the low saignée and control treatments. Although the pH and the titratable acidity of the wines were nearly identical, the trained panel deemed the low and high saignée wines more sour than the control. Sourness is known to impact astringency, but it is not known if the reverse effect is true. Curiously, this effect was not consistent with the most astringent wine being perceived as most sour, as the low saignée-EM treatment was not rated differently than the control.

The treatments examined resulted in wines with some significantly different mouthfeel characteristics. The high ethanol wine was rated significantly higher in alcohol/hot than the low saignée wine but was not significantly different than the other treatments in this attribute. The most tannic wine (low saignée-EM) was rated nearly the same for the alcohol/hot attribute as the high ethanol wine, even though the high ethanol wine contained nearly 2% higher ethanol concentration. The intensity of astringency is said to decline with greater quantities of ethanol (Lea and Arnold 1978). In this case, it appeared that the astringent properties of the low saignée-EM wine interfered with ethanol perception. The low saignée-EM wine was significantly less smooth and more drying than the other wines, a result consistent with greater tannin concentration. The high saignée and the high ethanol wines were significantly more drying than the low saignée but not the control. The wines with proportionally greater seed tannin extraction had both more drying mouthfeel descriptors as well as higher tannin concentrations. Seed tannins anecdotally have been considered problematic by winemakers, but it is not clear if the drying characteristic observed here is due to the seed tannins or the concentration of tannins. Similarly, an experiment with 20% juice runoff increased total phenolics and increased perceived astringency, although tannins were not measured (Gawel et al. 2001). The low saignée-EM wine was significantly more dynamic than the control and high saignée wines and slightly more dynamic than the other wines. Dynamic cannot be represented by a physical standard, as it involves multiple sensations of mouth movements (Gawel et al. 2000), so it is unclear why the low saignée-EM wine had the highest dynamic rating. The PCA illustrates an overall impression of the most important components and reemphasizes the sensory differences we observed in the wines (Figure 4).

The expected tannin concentration of the finished wines was estimated based on the tannin concentration that remained in pomace skins and seeds (Table 7). This estimate showed that between 20 and 46% of the tannins extracted were not present in the wine, with the low saignée-EM wine having the greatest discrepancy. Oxidation reactions and subsequent precipitation may have caused the loss of tannin. After exposing red wine to different amounts of catalytic metals, one study found up to 21% less tannin after an 80-day period (Cacho et al. 1995). Other researchers have also found tannins missing and have suggested that they are bound to cell wall components found in the grape skins and mesocarp present in the lees (Adams and Scholz 2008, Cerpa-Calderon and Kennedy 2008). It has also been suggested that the extraction of tannins from the seeds is

potentially influenced by the leakiness of the cells outside the true seed coat and the diffusion barrier represented by the cuticle on the outer surface of the seed (Adams and Scholz 2008). In the present study, the low saignée-EM treatment had the greatest amount of seed tannins extracted and the greatest amount of tannin unaccounted for in the wine. The results suggest that the amount of tannins extracted influences the fraction of tannins that are subsequently bound or oxidized.

The loss of tannins during the winemaking process and conversion of tannins into polymeric pigments make it difficult to assess the tannins present in the different wines. The pomace comparison provides evidence for the amount of tannins extracted from the various tissues during winemaking but does not provide evidence for what is actually left in the wine. Several methods are available for measuring the tannin polymer composition during fermentation. The methods are based on acidified depolymerization of the tannins and capture of the reactive carbocation subunits with a nucleophile that can be separated using chromatography. However, the methods are incapable of breaking down tannin polymers that do not have a regular C4-C8 configuration and anthocyanins found in the terminal position of the polymer (Fulcrand et al. 2004). Depolymerization in wine yields of only 50 to 60% have been reported (Pastor del Rio and Kennedy 2006), which, according to the authors of the method, make it difficult to assess composition (Peyrot des Gachons and Kennedy 2003). However, it has been reported that the conversion yield of tannins increases as the length of the maceration increases (Cerpa-Calderon and Kennedy 2008). Other methods are capable of measuring different sizes of tannins using soluble protein tannins complexes and matrix-assisted laser desorption ionization–time of flight (MALDI–TOF) mass spectroscopy (Mane et al. 2007). However, the mass spectroscopy method is limited by equipment and expertise and has not been tested with wine tannins. Thus, the methods to evaluate tannin structures found in wine and their origin are restricted; coupled with multiple factors that influence tannin extraction (the amount of tannin present in the fruit, the binding capacity of the grape insoluble cell wall matrix, winemaking technique used) there remains a need to explore the nature of the extraction of tannins during winemaking.

Conclusion

In this commercial-scale experiment, saignée at the same rate as water addition (low saignée) did not yield higher concentrations of phenolics or greater aroma or flavor attributes than a standard water addition (control). Therefore, it seems that saignée at the same rate as water addition is without merit from the perspective of phenolic and aroma/flavor enhancement. The high saignée treatment yielded significantly higher concentrations of tannins, anthocyanins, and LPP than the control and low saignée treatments, consistent with the amount of saignée used in the treatment. If extended maceration was combined with low saignée, significantly more seed tannins were extracted, resulting in a wine that was higher in tannin concentration and sensorially more drying and less smooth than the other treatments including the control. When only a small amount of water was added back, a significantly higher ethanol wine was produced, yielding higher tannin and anthocyanin concentrations than the control, but diminished fruity flavors with a drying and hot mouthfeel.

Literature Cited

Adams, D.O., J.F. Harbertson, and E.A. Picciotto. 2004. Fractionation of red wine polymeric pigments by protein precipitation and bi-sulfite bleaching. *In* Red Wine Color: Exploring the Mysteries. A.L. Waterhouse and J.A. Kennedy (eds.), pp. 275-288. ACS Symp. Ser. 886. Am. Chemical Society, Washington, DC.

Adams, D.O., and R.C. Scholz. 2008. Tannins–the problem of extraction. *In* Proceedings of the 13th Australian Wine Industry Technical Conference. R.J. Blair et al. (eds.), pp. 160-164. AWITC, Urrbrae, South Australia.

Bakker, J., P. Bridle, S.J. Bellworthy, C. Garcia-Viguera, H.P. Reader, and S.J. Watkins. 1998. Effect of sulphur dioxide and must extraction on colour, phenolic composition and sensory quality of red wine. J. Sci. Food Agric. 78:297-307.

Bate-Smith, E.C. 1975. Phytochemistry of proanthocyanidins. Phytochemistry 2:1107-1113.

Bautista-Ortín, A.B, J.I. Fernández-Fernández, J.M. López-Roca, and E. Gómez-Plaza. 2004. Wine-making of high coloured wines: Extended pomace contact and run-off of juice prior to fermentation. Food Sci. Tech. Int. 10:287-295.

Bisson, L.F. 1999. Stuck and sluggish fermentations. Am. J. Enol. Vitic. 50:107-119.

Cacho, J., J.E. Castells, A. Esteban, B. Laguna, and N. Sagristá. 1995. Iron, copper, and manganese influence on wine oxidation. Am. J. Enol. Vitic. 46:380-384.

Cerpa-Calderon, F.K., and J.A. Kennedy. 2008. Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. J. Agric. Food Chem. 56:9006-9014.

Chira, K., G. Schmauch, C. Saucier, S. Fabre, and P.L. Teissedre. 2009. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). J. Agric. Food Chem. 57:545-553.

Cosme, F. J.M. Ricardo-Da-Silva, and O. Laureano. 2009. Tannin profiles of *Vitis vinifera* L. cv. red grapes growing in Lisbon and from their monovarietal wines. Food Chem. 112:197-204.

Fulcrand, H., V. Atanasova, E. Salas, and V. Cheynier. 2004. The fate of anthocyanins in wine: Are there determining factors? *In* Red Wine Color: Exploring the Mysteries. A.L. Waterhouse and J.A. Kennedy (eds.), pp. 68-88. ACS Symp. Ser. 886. Am. Chemical Society, Washington, DC.

Gao, L., B. Girard, G. Mazza, and A.G. Reynolds. 1997. Changes in anthocyanins and color characteristics of Pinot noir wines during vinification processes. J. Agric. Food Chem. 45:2003-2008.

Gawel, R., A. Oberholster, and I.L. Francis. 2000. A mouth-feel wheel: Terminology for communicating the mouth-feel characteristics of red wine. Aust. J. Grape Wine Res. 6:203-207.

Gawel, R., P.G. Iland, P.A. Leske, and C.G. Dunn. 2001. Compositional and sensory differences in Syrah wines following juice runoff prior to fermentation. J. Wine Res. 12:5-18.

Gerbaux, V. 1993. Etude de quelques conditions de cuvaison susceptibles d'augmenter la composition polyphénolique des vins de Pinot noir. Rev. Oenol. 69:15-18.

Gómez-Plaza, E., R. Gil-Muñoz, J.M. López-Roca, A. Martínez-Cutillas, and J.I. Fernández-Fernández. 2001. Phenolic compounds and color stability of red wines: Effect of skin maceration time. Am. J. Enol. Vitic. 52:266-270.

Hagerman, A.E., and L.G. Butler. 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26:809-812.

Harbertson, J.F., R.E. Hodgins, L.N. Thurston, L.J. Schaffer, M.S. Reid, J.L. Landon, C.F. Ross, and D.O. Adams. 2008. Variability of tannin concentration in red wines. Am. J. Enol. Vitic. 59:210-214.

Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. Am. J. Enol. Vitic. 53:54-59.

Harbertson, J.F., E.A. Picciotto, and D.O. Adams. 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54:301-306.

Heredia, T.M., D.O. Adams, K.C. Fields, P.G. Held, and J.F. Harbertson. 2006. Evaluation of a comprehensive red wine phenolics assay using a microplate reader. Am. J. Enol. Vitic. 57:497-502.

Jensen, J.S., H.H.M. Werge, M. Egebo, and A.S. Meyer. 2008. Effect of wine dilution on the reliability of tannin analysis by protein precipitation. Am. J. Enol. Vitic. 59:103-105.

Lea, A.G., and G.M. Arnold. 1978. The phenolics of ciders: Bitterness, astringency. J. Sci. Food Agric. 29:478-483.

Mane, C., N. Sommerer, T. Yalcin, V. Cheynier, R.B. Cole, and H. Fulcrand. 2007. Assessment of the molecular weight distribution of tannin fractions through MALDI-TOF MS analysis of protein-tannin complexes. Anal. Chem. 79:2239-2248.

Nagel, C.W., and L.W. Wulf. 1979. Changes in the anthocyanins, flavonoids and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. Am. J. Enol. Vitic. 30:111-116.

Oszmianski, J., F.M. Romeyer, J.C. Sapis, and J.J. Macheix. 1986. Grape seed phenolics: Extraction as affected by some conditions occurring during wine processing. Am. J. Enol. Vitic. 37:7-12.

Pastor del Rio, J.L., and J.A. Kennedy. 2006. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. Am. J. Enol. Vitic. 57:125-132.

Peyrot des Gachons, C., and J.A. Kennedy. 2003. Direct method for determining seed and skin proanthocyanidin extraction into red wine. J. Agric. Food Chem. 51:5877-5881.

Picciotto, E.A. 2002. An investigation of polymeric pigments in red wine by means of a biochemical assay and synthesis. M.S. thesis, University of California, Davis.

Ribéreau-Gayon, P., P. Sudraud, J.C. Milhe, and A. Canbas. 1970. Reserches technologiques sur les composés phénoliques des vins rouges. Conn. Vigne Vin 4:133-144.

Remy, S., H. Fulcrand, B. Labarbe, V. Cheynier, and M. Moutounet. 2000. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. J. Sci. Food Agric. 80:745-751.

Roessler, E.B., R.M. Pangborn, J.L. Sidel, and H. Stone. 1978. Expanded statistical tables for estimating significance in paired-preference, paired-difference, duo-trio and triangle tests. J. Food Sci. 43:940-947.

Sacchi, K.L, L.F. Bisson, and D.O. Adams. 2005. A review of the effect of winemaking techniques on the phenolic extraction in red wines. Am. J. Enol. Vitic. 56:197-206.

Sampaio, T.L., Kennedy, J.A., and M.C. Vasconcelos. 2007. Use of microscale fermentations in grape and wine research. Am. J. Enol. Vitic. 58:534-539.

Seddon, T.J., and M.O. Downey. 2008. Comparison of analytical methods for determination of condensed tannins in grape skins. Aust. J. Grape Wine Res. 14:54-61.

Singleton, V.L. 1972. Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. Am. J. Enol. Vitic. 23:106-113.

Singleton, V.L. 1992. Tannins and the qualities of wines. *In* Plant Polyphenols. R.W. Hemingway and P.E. Laks (eds.), pp. 859-880. Plenum Press, New York.

Singleton, V.L., and D.E. Draper. 1964. The transfer of polyphenolic compounds from grape seeds into wines. Am. J. Enol. Vitic. 15:34-40.

Somers, T.C. 1966. Wine tannins–Isolation of condensed flavonoid pigments by gel-filtration. Nature 209:368-370.

Somers, T.C, and M.E. Evans. 1974. Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wine. J. Sci. Food Agric. 25:1369-1379.

Vidal, S., L. Francis, A. Noble, M. Kwiatkowski, V. Cheynier, and E. Waters. 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal. Chim. Acta 513:57-65.