Effect of early seed removal during fermentation on proanthocyanidin extraction in red wine: A commercial production example

Jungmin Lee, James A. Kennedy, Chuck Devlin, Mark Redhead, Christopher Rennaker

United States Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Laboratory Worksite, 29603 U of I Lane, Parma, ID 83660, USA

Oregon State University, Department of Food Science and Technology, Corvallis, OR 97331, USA

Ste. Chapelle Winery, Caldwell, ID 83607, USA

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Abstract

Wines (Vitis vinifera L. cv. Merlot) were made by a commercial winery to examine the effects of seed removal at ~10 °Brix on the extraction of proanthocyanidins during fermentation. Seeds were removed at the point when they fell to the bottom of the fermenter, and were thus easily removed during regular pump-over operations. Proanthocyanidin extraction was compared to wine made from traditional winemaking regime in which no seed removal occurred. Proanthocyanidin differences observed in the wines were minor. The control wine contained a slightly higher percentage molar proportion of seed proanthocyanidins ((−)-epicatechin-3-O-gallate extension and terminal subunits), demonstrating higher seed tannin extraction, and the seed removed wine contained a higher percentage molar proportion of skin proanthocyanidin indicators ((−)-epigallocatechin extension subunits). Seed removed Merlot wines had higher concentrations of total anthocyanins. Minor differences in colour measurement values between the two wines were also observed. The control wine was slightly more orange (larger hue angle, \( h^* \)), lighter (larger \( L^* \) value), and more saturated (higher chroma value, \( C^* \)) in colour. This appears to be the first paper to report the effects of early seed removal in Merlot winemaking, and demonstrates how winery tannin management techniques contribute to proanthocyanidin composition.

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1. Introduction

Wine tannins and colour are important red wine quality factors that can be manipulated by grape growing and winemaking practices. Tannin quality in wines has been a challenge to define and has been a strong research interest. Only a few studies have examined the impact of skin and seed tannins in winemaking (Berg & Akiyoshi, 1956; Kovac, Alonso, Bourzeix, & Revilla, 1992; Kovac, Alonso, & Revilla, 1995; Meyer & Hernandez, 1970; Peyrot des Gachons & Kennedy, 2003). Meyer and Hernandez (1970) reported a 10% decrease in total phenolics (determined by the Folin–Ciocalteu method) in the final product by early seed removal. Additional seeds added to must yielded wines that were stronger in varietal characteristics of Garnacha and Tempranillo wines (Kovac et al., 1995). Conflicting reports in regard to benefits or limitations of skin versus seed contact time during winemaking have added to the confusion for determining the best winemaking strategies for making wines that contain soft smooth
tannins. Phenolic extraction during winemaking is influenced by numerous factors, including temperature, maceration time, solvent composition, proportions of skin and seed present, and fruit ripeness among others (Kovac et al., 1995; Peyrot des Gachons & Kennedy, 2003; Pastor del Rio & Kennedy, 2006).

This experiment was initiated and carried out by a professional winemaker to determine the changes or similarities in wine tannin composition in a control wine without seed removal compared with one after seed removal. The objective of this study, therefore, was to examine the proanthocyanidin constituents of wine made with altered seed contact time during its alcohol fermentation.

2. Materials and methods

2.1. Wine samples

Commercial wines (Vitis vinifera L.) were made at Ste. Chapelle winery (Caldwell, Idaho, USA) in 2004, with commercially ripe fruit (ca. 25°Brix at harvest) obtained from one vineyard block. Wines were pressed after completion of alcohol fermentation (12-day maceration period). Seeds were kept either in the fermenter throughout fermentation (control wine), or were removed when the fermenting juice reached 10°Brix (seed removed wine). All other fermentation conditions were identical (same harvest date, yeast used, fermentation temperature, pump-over frequency and duration and sulfur dioxide additions). Identical nine-metric-ton conical bottom fermenters were used, which were monitored twice daily. After completion of alcohol fermentation, samples underwent stabilization and clarification in two 19 L carboys per treatment, from which young wine samples were collected before blending, and kept at 15 °C prior to further analysis. Basic chemical analyses (pH, TA, specific gravity, and alcohol content) of these wines were provided by the winery’s laboratory.

2.2. Reagents and standards

All chemicals (phloroglucinol, (+)-catechin hydrate, sodium acetate, potassium chloride, acetic acid, acetonitrile, methanol, hydrochloric acid and water) used in this study were of analytical and high performance liquid chromatography (HPLC) grade and were obtained from Sigma Chemical Co. (St. Louis, MO).

2.3. Sample preparation and HPLC/DAD analysis of proanthocyanidins

Phloroglucinolysis (acid catalysis of proanthocyanidins in the presence of excess phloroglucinol) was conducted on wine samples as described by Peyrot des Gachons and Kennedy (2003). Phloroglucinolysis provided detailed proanthocyanidin subunit composition and mean degree of polymerization. One milliliter of each sample was dried with a N-Evap 112 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA) under a constant nitrogen gas stream, in a 40 °C water bath, before conducting phloroglucinolysis in triplicate.

An HP1100 system equipped with a diode array detector (HPLC/DAD, Agilent Technologies Inc., Palo Alto, CA) was used, to determine molar proportion of the proanthocyanidins, as described by Kennedy and Taylor (2003), with the following modifications: two serially connected Oynx Monolithic (100 × 4.6 mm) columns, fitted with 5.0 × 4.6 mm, i.d. guard column of the same column properties as the analytical columns (Phenomenex, Torrance, CA) were used. Absorbance spectra were collected for all peaks. Identification was based on spectra, peak retention times, and previously reported peak identification (Peyrot des Gachons & Kennedy, 2003). Total proanthocyanidins were determined by summing the identified extension and terminal subunits quantified with external standard (+)-catechin. Terminal subunit calculations were corrected for the flavanol monomers found in the samples. Percent molar proportions, mean degree of polymerization, and galloylation rates were calculated (Peyrot des Gachons & Kennedy, 2003; Pastor del Rio & Kennedy, 2006). Details of performing phloroglucinolysis and calculations to obtain the values for percentage molar proportion, mean degree of polymerization, and galloylation rates are well documented (Kennedy & Jones, 2001; Kennedy & Taylor, 2003; Peyrot des Gachons & Kennedy, 2003; Pastor del Rio & Kennedy, 2006). A chromatogram of the proanthocyanidin phloroglucinolysis products can be found in Kennedy and Jones (2001).

2.4. Total anthocyanin content (ACY) and colour measurements

Total anthocyanins were determined by the modified pH differential method (Lee, Durst, & Wrolstad, 2005) using a SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA) and pH 1.0 buffer absorbance at 520 nm. ACY was expressed as malvidin-3-glucoside (molar extinction coefficient of 28,000 l cm⁻¹ mol⁻¹ and molecular weight of 493.3 g mol⁻¹). ACY was determined in triplicate.

A HunterLab CT1100 ColourQuest colorimeter (Hunter Associate Laboratories, Inc., Reston, VA) was used for the colour measurements. The colorimeter mode was as follows: total transmittance mode, Illuminant D65, and 10° observer angle. An optical 2.0 mm pathlength colorimeter cell (Hellma, Borough Hall Station, NY) was used. Five colour parameters were recorded: Hunter CIE lightness (L'), a' value, b' value, chroma (saturation, C'), and hue angle (colour itself, h°). All measurements were conducted in triplicate.

2.5. Statistical analysis

Statistica for windows version 7.1 was used (StatSoft, Inc., Tulsa, OK) for t-test calculation and one-way analysis
of variance for the two groups (control and seed removed wine samples) at α = 0.05 level for values in Tables 2 and 3.

3. Results and discussion

Table 1 indicates the pH, TA, specific gravity, and alcohol content of the two wines. Professional winemakers of Constellation Wine USA, Inc. (Canandaigua, NY) conducted an informal tasting of the two wines, and on the whole, control wine was preferred over the seed removed wine. Early seed removal during fermentation did not necessarily negatively impact the taste and mouthfeel of the Merlot wine, but the control wine was preferred. From a blind tasting, the professional winemakers gave the control wine descriptors, such as smoother tannins, softer tannins, and fruitier when compared to the seed removed wine, which are associated with more positive tannin quality. Control wine had a slightly lower pH (pH 3.14), higher titratable acidity (TA, 8.6 g of tartaric acid/l), a somewhat higher specific gravity (SG, 0.9953), and lower alcohol content (13.4%) than seed removed wine (pH 3.18; TA, 8.2 g of tartaric acid/l; SG, 0.9947; % alcohol, 14.1%), also in Table 1. These variations in basic measurements of the two wines may have contributed to the disparity in perceived astringency and bitterness, but were only minor compositional differences.

Table 2 summarizes the proanthocyanidin content and composition of these wines. Total proanthocyanidin content of seed removed wine (953 mg of catechin/l) was slightly higher than that of control (940 mg of catechin/l), although the changes were not statistically different (p = 0.60). This observation could also have been due to random error or bias. Seed removed wine contained more proanthocyanidins, and this could have been due to skin proanthocyanidins being more readily extracted during fermentation when compared to seed proanthocyanidins’ extractability (Cheynier, Prieur, Guyot, Rigaud, & Mou- tounet, 1997). The mean degrees of polymerization (mDP) for proanthocyanidins from the seed removed wine were greater (mDP = 4.7) than those of the control wine (mDP = 4.3), which might have been due to the increased proportion of skin proanthocyanidin extraction (Peyrot des Gachons & Kennedy, 2003). The percentage molar proportions of proanthocyanidins for the two wines were statistically different for (−)−epigallocatechin (EGC) extension subunits (skin-derived), and (−)−epicatechin-3-O-gallate (ECG) terminal subunits (seed-derived), which were statistically different (t-test, p ≤ 0.05). Although not statistically different, percentage molar proportions of ECG extension subunits (primarily from seeds) were slightly higher in control wines. Galloylation rate (molar proportion of galloylated flavanol subunits to total flavanol subunits) of the control (9.7%) was higher than that of the seed removed wine (9.0%), which also indicates that increased concentrations of seed proanthocyanidins diffused into control wine.

Seed removed wine had 14% higher levels of ACY than had control wine (Table 3), which again might be due to skin phenolics being more readily extracted than seed phenolics during alcohol fermentation. Total proanthocyanidin to total anthocyanin ratio was higher for control wine (3.61), than seed removed wine (3.13). Based on colour measurement results (Table 3), control wines were slightly lighter (larger L* value), more intense in colour (larger C*), and more orange than seed removed wine, although the differences between the two wines were not distinguishable by visual observation. The control wine might also have been slightly more orange due to the new anthocyanins formed with seed extracted proanthocyanidins. Seed removed wine had a more intense red colour (smaller hue angle than control), which was most likely due to higher amounts of ACY present.

Taste preference, from the informal tasting, for control wine may be due to the structural diversity as a result of the slightly elevated seed proanthocyanidin proportion present, when compared to seed removed wine. Proanthocyanidin structures that were not analyzable with the

| Table 1 Basic chemical analysis for the two wines provided by the winery |
|------------------|------------------|------------------|------------------|
|                  | pH               | TA (g/l tartaric acid eq.) | Specific gravity | Alcohol (% v/v) |
| Control wine     | 3.14             | 8.6              | 0.9953           | 13.4            |
| Seed removed wine| 3.18             | 8.2              | 0.9947           | 14.1            |

TA, titratable acidity; alcohol was determined by an ebulliometer; eq., equivalent.

| Table 2 Total proanthocyanidin and proanthocyanidin composition of the two wines analyzed by phloroglucinolysis and HPLC |
|------------------|------------------|------------------|------------------|
|                  | Total proanthocyanidin (mg/l C eq.) | mDP | Extension subunits (% molar proportion) | Terminal subunits (% molar proportion) |
|                  |                                |     | EGC | C     | EC | ECG | C     | EC | ECG |
| Control wine     | 940a                          | 4.32 | 20.4a | 7.8a | 44.3a | 5.9a | 13.0a | 6.4a | 4.0a |
| Seed removed wine| 953a                          | 4.70 | 23.8b | 7.9a | 44.3a | 5.7a | 12.6a | 5.8a | 3.6b |

eq., equivalent; mDP, mean degree of polymerization; terminal subunits were corrected for flavanol monomers found in samples; values in each column sharing the same letter are not significantly different from each other (t-test, p > 0.05); C, (+)-catechin; EGC, (−)-epigallocatechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-O-gallate.
method used in this study could also be contributors to the taste and mouthfeel difference. The perception of astringency was altered not only by the quality and quantity of the wine proanthocyanidins, but also by the different levels and combinations of acids, alcohol, polysaccharides, monosaccharides and pH of the wines (Gaylor, Francis, & Waters, 2007; Lesschaeve & Noble, 2005), and the influences of these factors were not examined, but were highly likely contributing to the differences in astringency and mouthfeel of these wines. Vast diversity among different grape varieties has been reported in anthocyanins and other phenolic monomers composition. Although not explored in this study, it was expected that there would be a large difference in the quality of the proanthocyanidins present, which may contribute to these different findings.

A winemaker at an Oregon winery performed a seed removal trial with cv. Pinot noir and the proanthocyanidins in the wines were analyzed using the same analytical methods. These wines were made in one ton plastic fermenters and punched down for cap management, with seeds being removed half way through alcohol fermentation. Pinot noir seed removed wine had a 31% reduction in total proanthocyanidins (mg/l) compared to traditionally made wine, and slightly elevated proportions of EGC extension subunits (molar proportions), and lower proportions of ECG extension subunits (molar proportions) compared to the control wine. Nevertheless, a subtle difference in astringency quality was observed. This example also emphasizes that differences in proanthocyanidin extraction depend on numerous grape and winemaking factors.

4. Conclusion

To the best of our knowledge, this is the first time the influence of seed removal on the proanthocyanidin composition of Merlot wines made on a commercial scale has been reported. This paper gives additional insight into different winemaking strategies for winemakers who would like to manipulate the proanthocyanidin composition of their wines. Grape seed proanthocyanidins might play a role in binding with undesirable flavour contributors, e.g. bitter compounds. More research is needed to better understand the quality and quantity of proanthocyanidins that end up in the final product by altering proanthocyanidin management in the vineyard as well as in the winery. Based on this study, the winery decided to continue with their traditional winemaking method, rather than attempt early seed removal for their Merlot winemaking protocol.

Acknowledgements

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References


Table 3

<table>
<thead>
<tr>
<th>ACY (mg/l)</th>
<th>L'</th>
<th>a' value</th>
<th>b' value</th>
<th>Chroma (C')</th>
<th>Hue angle (h°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>260a</td>
<td>44.9a</td>
<td>58.2a</td>
<td>12.7a</td>
<td>59.5a</td>
</tr>
<tr>
<td>Seed removed wine</td>
<td>304b</td>
<td>43.3b</td>
<td>58.0b</td>
<td>12.6b</td>
<td>59.3b</td>
</tr>
</tbody>
</table>

Hunter colorimeter setting was D65 illuminant, 10° observer angle, 2.0 mm colorimeter cell, and total transmittance mode. ACY, total anthocyanin content expressed as malvidin-3-glucoside (extinction coefficient = 28,000 l cm⁻¹/C0) and molecular weight = 493.3 g mol⁻¹. Values in each column sharing the same letter are not significantly different from each other (t-test, p > 0.05).