JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

# Relationship between Red Wine Grade and Phenolics. 2. Tannin Composition and Size

Stella Kassara and James A. Kennedy*

The Australian Wine Research Institute, P.O. Box 197, Glen Osmond, SA 5064, Australia

**ABSTRACT:** Commercial red wines (*Vitis vinifera* L. cv. Shiraz) produced during the 2009 vintage underwent winemaker assessment for allocation grade soon after production. The wines were then subjected to phenolic analysis to measure wine color (total anthocyanin, $SO_2$ nonbleachable pigment, and wine color density) and tannins (concentration, composition, and average degree of polymerization). A positive relationship was found between wine phenolic concentration and projected bottle price. Tannin compositional analysis suggested that there was specifically a relationship between wine grade and skin-derived tannins. These results suggest that maximization of skin tannin concentration and/or proportion is related to an increase in projected wine bottle price.

**KEYWORDS:** red wine, grading, quality, price, tannin, composition, size, color, phloroglucinolysis

## INTRODUCTION

Wine is a beverage that is widely appreciated for its stylistic variation and overall complexity. During wine production operations, winery personnel invest significant time evaluating the sensory attributes of their wines to blend and assemble the various product lines prior to bottling. For red wines, a major attribute that is evaluated is the mouthfeel quality. It is generally recognized that tannins make up the core of red wine mouthfeel, contributing bitterness and astringency.[1] In addition to tannins, various other wine components influence our perception of mouthfeel.[2,3]

In large part, tannins found in red wine are derived from the skin and seed tissue of the grape berry.[4,5] The tannins in these tissues are flavonoid-based proanthocyanidins, with procyanidins found in both skin and seed tissue and prodelphinidins restricted to skin tissue.[6,7] Skin tannins are generally higher in molecular mass than seed tannins.[8] During winemaking, skin and seed tannins are extracted, and their relative contribution to the overall wine tannin pool can vary with such things as fruit maturity, berry crushing, soak time, and enzyme use.[9,10]

Considerable research has been directed to the understanding of the qualitative variation in red wine mouthfeel, and among winemakers, it is generally thought that tannin variation has an impact on overall mouthfeel quality. Published research on the relationship between red wine mouthfeel quality and tannin concentration and composition, however, is lacking. It has been shown that tannin perception can vary with tannin concentration and composition in model systems,[11−13] and tannin concentration and composition vary substantially in red wines.[9,10,13] Research that focuses on tannin concentration and structure variation in commercial wines relative to winemaker perception of mouthfeel and overall wine grade is lacking; however, this information can potentially provide direction with regard to understanding the underlying chemistry of mouthfeel quality.

In the first paper in this series,[14] a direct relationship was found between wine grade (highest = 1, lowest = 9) and tannin concentration. The utility of this finding from a wine production standpoint could vary depending on the underlying explanation for the observed wine grade−tannin concentration relationship. It may be that an increase in tannin concentration per se determines wine grade; therefore, an improvement in wine grade would result by simply increasing the tannin concentration of a given wine. Alternatively, an observed increase in tannin concentration may be a consequence of having the appropriate tannin composition and size; and therefore, to increase wine grade, a specific type of tannin would need to be selected. Finally, an observed increase in tannin concentration may be a consequence of having a wine matrix that can support more tannin; therefore, if a higher grade wine is desired then the wine matrix composition needs to be modified to support a higher tannin concentration (e.g., residual sugar, ethanol, polysaccharides, anthocyanins, flavor). Combinations of these explanations could be rationalized, and implicit in these explanations is that the perception of tannins in wine is in balance with the wine as a whole.

The purpose of this investigation was to explore the second hypothesis, that tannin composition and/or molecular mass changes could provide an explanation for the increase in tannin concentration with an increase in wine grade observed in the first paper.[14] Although a relationship between composition and concentration would not exclude the possibility that additional factors played a role, it could provide direction for future research. To conduct this investigation, a reasonably large sample set of winemaker-evaluated commercial wines was acquired from a large commercial winery located in South Australia.

## MATERIALS AND METHODS

**Chemicals.** All chromatographic solvents were of high-performance liquid chromatography (HPLC) grade, and all chemicals were of analytical reagent grade. Acetonitrile, absolute ethanol, methanol (Merck), formic acid (98−100%, Sigma), and glacial acetic acid (98%) and hydrochloric

Received: March 15, 2011
Revised: June 21, 2011
Accepted: June 25, 2011
Published: June 25, 2011

Table 1. Wine Phenolic Chemistry with Respect to Wine Allocation Grade and Projected Bottle Price (±SEM)

| | | | tannin | | | color | | |
|---|---|---|---|---|---|---|---|---|
| wine grade | no. of samples | projected bottle price (Australian dollars) | concentration (mg/L) | mDP | prodelphinidin (mol fraction)[a] | color density | anthocyanin (mg/L) | nonbleachable pigments |
| 1 | 2 | 105−110 | 1649 ± 51 | 10.71 ± 0.69 | 0.35 ± 0.02 | 24.08 ± 1.34 | 839 ± 30 | 5.14 ± 0.88 |
| 2 | 4 | 80−85 | 1664 ± 213 | 11.83 ± 1.10 | 0.36 ± 0.02 | 23.26 ± 1.05 | 882 ± 57 | 4.14 ± 0.05 |
| 3 | 4 | 50−55 | 1706 ± 202 | 12.34 ± 0.83 | 0.37 ± 0.00 | 22.26 ± 1.75 | 839 ± 44 | 4.30 ± 0.53 |
| 4 | 8 | 25−30 | 1608 ± 113 | 10.69 ± 0.80 | 0.35 ± 0.01 | 20.54 ± 0.88 | 782 ± 36 | 3.81 ± 0.26 |
| 5 | 15 | 15−18 | 1384 ± 136 | 10.50 ± 0.51 | 0.35 ± 0.01 | 20.06 ± 0.64 | 782 ± 26 | 3.82 ± 0.26 |
| 6 | 11 | 10−12 | 1137 ± 99 | 7.93 ± 0.41 | 0.29 ± 0.02 | 14.78 ± 1.32 | 498 ± 59 | 3.33 ± 0.33 |
| 7 | 10 | 7−10 | 968 ± 75 | 6.57 ± 0.40 | 0.26 ± 0.01 | 11.21 ± 0.39 | 344 ± 10 | 2.80 ± 0.22 |
| 8 | 11 | 5−7 | 1005 ± 79 | 7.13 ± 0.27 | 0.26 ± 0.01 | 11.18 ± 0.28 | 347 ± 12 | 2.67 ± 0.11 |
| 9 | 8 | <5 | 1117 ± 73 | 6.44 ± 0.18 | 0.26 ± 0.00 | 11.47 ± 0.43 | 316 ± 8 | 3.00 ± 0.21 |

[a] Faction (in moles) of prodelphinidin relative to known proanthocyanidin phloroglucinol products.

acid (HCl, 32%, Ajax fine chemicals) were obtained from Rowe Scientific (Lonsdale, SA, Australia). Acetaldehyde (99.5%) was sourced from Chem Supply (Gillman, SA, Australia), methyl cellulose (M-0387, viscosity of 2% aqueous solution at 20 °C = 1500 cP), ammonium sulfate crystals (A4915), and DL-tartaric acid (99% T400) were sourced from Sigma-Aldrich (St. Louis, MO). Sodium metabisulfite (Univar, A1184) was obtained from Ajax Finechem (Sydney, Australia).

**Wine Samples.** Seventy-three commercial Shiraz wines (2009 vintage) were sourced from multiple regions in South Australia (Constellation Wines, Australia). The wines were evaluated by winemaking staff and allocated a grade from 1 (high quality) to 9 (low quality) as described previously.[14] In addition, for the current study, targeted bottle price was assigned by the production staff at the winery. Wines were evaluated as new wines following fermentation/maceration and malolactic fermentation. Samples of new wines were collected, bottled in 750 mL wine bottles, and maintained at cellar temperature until analyzed (within 4 months).

Wines were centrifuged at 1500*g* for 5 min (Hettich 32R Universal centrifuge) prior to extraction and analysis, with no precipitate observed after centrifugation. Wine color was determined spectrophotometrically using Somers modified color measurements. Wine tannin concentration was determined using the methyl cellulose precipitable tannin assay (MCP) and tannin composition using acid catalysis in the presence of excess phloroglucinol (phloroglucinolysis). Collected data were averaged within each allocation grade and compared with winemaker assessment.

**Tannin Concentration and Pigmented Polymer Content.** MCP tannin assay and modified Somers color measurements were performed as reported by Mercurio et al.[15] Both assays were conducted using high-throughput methods in 96-well microplates. MCP tannin assays gave the tannin concentration of each wine in epicatechin equivalents (mg/L). The modified Somers method provided wine color density (WCD), $SO_2$ nonbleachable pigment, and total anthocyanin.

**Isolation of Total Polymeric Phenols.** Solid phase extraction (SPE) was used to isolate total polymeric phenols from each wine sample as previously described.[16] Briefly, Oasis HLB cartridges (3 mL, 650 mg, 30 μm) were conditioned with methanol (2 mL) followed by water (2 mL). Wine (1 mL) was applied to the cartridge under gravity. Once wine phenolics were adsorbed, the cartridge was dried with a gentle stream of nitrogen. Each cartridge was washed with 40 mL of 95% acetonitrile/5% 0.01 M HCl (v/v) under vacuum. This step removed phenolic acids, nonpolymeric flavanols, flavonols, anthocyanins, and other pigmented monomers. Polymeric phenolics were eluted with 300 μL of neat formic acid followed by 3 mL of 95% v/v methanol. The fraction was concentrated under nitrogen at 28 °C and dissolved in methanol (10 g/L, based on the tannin concentration obtained by the MCP tannin assay as described above).

**Validation of Total Polymeric Phenolics Recovery from SPE.** The MCP tannin assay (tannin concentration) and HPLC analysis (total peak areas) were used to validate the recovery of total polymeric phenolics with SPE. The eluted total polymeric phenolics fraction of several randomly selected wines were dried and redissolved in 1 mL of 10% v/v ethanol/0.1% v/v formic acid in water. The concentration of polymeric phenolics isolated by SPE was compared to the concentration of polymeric phenolics in the original wine. Using the MCP tannin assay, >90% recovery was obtained after SPE when polymeric phenolics were eluted with 300 μL of formic acid and 3 mL of 95% v/v methanol, indicating that this was efficient in recovering polymeric phenolic material from the SPE cartridge (data not shown). Recovery of total polymeric phenolics from SPE was also determined using RP-HPLC.[15] All material eluted from the SPE cartridge was analyzed at 280 and 520 nm, and peak areas were compared with those of the original wine sample. The recoveries using this method were also >90% (data not shown).

**Composition of Tannins Isolated from Wine.** Tannins obtained from wines were characterized by acid catalysis in the presence of phloroglucinol (phloroglucinolysis) and subsequent HPLC analysis to determine the mean degree of polymerization (mDP) and subunit composition.[6,17] Tannins isolated from wine using SPE were dissolved in methanol to 10 g/L. An aliquot of this solution (25 μL) was reacted with 25 μL phloroglucinol reagent (0.2 N HCl, 100 g/L phloroglucinol, and 20 g/L ascorbic acid in methanol) in a 0.2 mL PCR tube. The reaction was heated at 50 °C for 25 min, cooled on ice for 1 min, and then quenched with 70 mM (150 μL) sodium acetate. Controls were prepared by replacing phloroglucinol reagent with methanol, (−)-epicatechin was the HPLC standard, and calculations were as described earlier.[6] HPLC analysis was performed using a model 1100 HPLC (Agilent Technologies Australia Pty Ltd., Melbourne, Australia) equipped with Chemstation software. The concentration of isolated polymeric phenolics by phloroglucinolysis was found to be correlated ($r^2$ = 0.84) to the concentration of isolated polymeric phenolics when assessed using the MCPT assay.

## RESULTS AND DISCUSSION

**Wine Tannin Concentration.** The concentration of wine tannins was determined to confirm the relationship between tannin concentration and wine allocation grade observed previously.[14] Consistent with previous observation, the concentration of tannin and total phenolics generally increased with wine grade (Tables 1 and 2 (phenolics: $r^2$ = 0.833 (all grades), $r^2$ = 0.877

Table 2. Correlation between Chemical Information and Allocation Grading for Commercial Shiraz Wines

| | | correlation ($r^2$) | |
|---|---|---|---|
| chemistry | information | all grades | grades 3−7 |
| tannin | concentration | 0.818 | 0.984 |
| | degree of polymerization | 0.798 | 0.953 |
| | prodelphinidin in tannin | 0.790 | 0.904 |
| color | wine color density | 0.918 | 0.913 |
| | total anthocyanins | 0.867 | 0.866 |
| | nonbleachable pigments | 0.878 | 0.937 |

(grades 3−7)). The projected wine bottle prices for each of the wine grades have also been provided.

**Wine Color.** Various wine color attributes were measured, given that previous studies have found a relationship between overall red wine color and the perception of quality.[18,19] In the present study, an increase in wine color density, $SO_2$ nonbleachable pigment content, and total anthocyanin concentration was observed with an increase in projected bottle price (Table 1). These results indicated that wine color attributes generally increased with total tannin concentration, and given previous observations, this could explain winemaker assessment of wine grade, as found by others.[18−22]

**Wine Tannin Composition and Size Distribution.** Phloroglucinolysis was used to provide information on proanthocyanidin composition and apparent mDP. Phloroglucinolysis provided compositional information on isolated tannin, however by mass, only a fraction of the tannin is converted to known flavan-3-ol products.[23] Nevertheless, it was decided that phloroglucinolysis could yield important compositional information. In addition, the concentration of tannin in wine as measured by phloroglucinolysis was shown to be correlated with that determined using the MCP assay. From the results, an increase in mole proportion of prodelphinidins contained in extension subunits, as well as an increase in proanthocyanidin mDP, was associated with an overall increase in tannin concentration. Given that red wine proanthocyanidins are derived principally from seed and skin proanthocyanidins and that seed proanthocyanidins lack prodelphinidins, the observed increase in prodelphinidin proportion with tannin concentration suggests an increase in the proportion of skin tannins and/or an increase in the proportion of prodelphinidins within the skin tannin.

**Implications.** From this set of wines, a positive relationship between projected wine bottle price and tannin concentration, proportion of prodelphinidin, tannin mDP, and color chemistry was observed. Furthermore, the observed tannin chemistry differences suggest a relationship between skin phenolic extraction and projected bottle price. These results add to the previously observed relationship between tannin concentration and wine allocation grade.[14] Although not excluding that wine matrix may have an effect on allocation grade, the results here suggest the importance of skin tannin concentration on wine allocation grade. It should be noted that for this set of wines, the strongest relationship between skin-derived phenolics and allocation was found in the midrange of wine grade, suggesting that on either end of the projected bottle price, other factors were more important in determining the winemaker perception of value.

Assuming that skin tannin is related to red wine grade, managing skin tannin concentration in red wine could be achieved both in the vineyard and in the winery. In the winery, an increase in the proportion of skin tannin has been associated with increased berry crushing prior to fermentation, combined with reduced soak time.[9] Managing these variables in conjunction with temperature could result in an increase in the overall concentration and proportion of skin tannin during maceration.[24] Enzymes may also provide an avenue for increasing the proportion of skin phenolics including color.[25,26]

In the vineyard, positive relationships between skin tannin concentration and reduced vine vigor and vine water status have been observed.[27−29] In a viticulture study on Pinot noir fruit and wine, Cortell et al. observed an increase in total skin tannin, proportion of prodelphinidin, tannin molecular mass, and pigmented polymer with a reduction in vine vigor.[27] Moreover, these observations were associated with the winery's bottle price. In addition, an increase in fruit exposure has been associated with an increase in skin tannins and the proportion of prodelphinidins contained in skin tannins.[30−32] Fruit maturity may also be an important factor in the partition properties of skin phenolics into wine.[33,34] Finally, variations in skin tannin chemistry similar to those found in this study have been found to be associated with varietal differences in astringency perception.[35] Anecdotally, these vineyard-derived factors have all been associated with a general increase in wine quality.

Should further research confirm a consistent relationship between skin tannin proportion, wine color, and wine allocation grading, it may not necessarily be true that skin phenolic concentration provides a causal explanation for an observed increase in wine grade. For example, an increase in sun exposure may lead to an increase in the production of desirable flavor compounds, a reduction in titratable acidity, or the production of skin-derived phenolic compounds associated with velvety astringency descriptors.[36] Nevertheless, the results of this investigation provide information on correlative factors related to projected wine bottle price.

## AUTHOR INFORMATION

### Corresponding Author

*Present address: Department of Viticulture and Enology, California State University, 2360 East Barstow Avenue, MS VR89, Fresno, CA 93740-8003, United States. Phone: (559) 278-2089. Fax: (559) 278-7179. E-mail: jakennedy@csufresno.edu.

### Funding Sources

The Australian Wine Research Institute, a member of the Wine Innovation Cluster in Adelaide, is supported by Australian grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian government.

## ACKNOWLEDGMENT

We thank Constellation Wines, Australia, and Chris Bevin for the donation of wine samples for research and for providing winemaker assessment of wine grade and projected bottle price.

## REFERENCES

(1) Gawel, R. Red wine astringency: a review. *Aust. J. Grape Wine Res.* **1998**, *4*, 74–95.

(2) Vidal, S.; Courcoux, P.; Francis, L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual. Pref.* **2004**, *15*, 209–217.

(3) Lesschaeve, I.; Noble, A. C. Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. *Am. J. Clin. Nutr.* **2005**, *81*, 330s–335s.

(4) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric proanthocyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781–784.

(5) Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509–512.

(6) Kennedy, J. A.; Jones, G. P. Analysis of PA cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746.

(7) Kennedy, J. A.; Hayasaka, Y.; Vidal, S.; Waters, E. J.; Jones, G. P. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* **2001**, *49*, 5348–5355.

(8) Kennedy, J. A.; Taylor, A. W. Analysis of PAs by high-performance gel permeation chromatography. *J. Chromatogr., A* **2003**, *995*, 99–107.

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

(10) Sacchi, K. L.; Bison, L. F.; Adams, D. O. A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Vitic.* **2005**, *56*, 197–206.

(11) Cheynier, V.; Fulcrand, H.; Brossaud, F.; Asselin, C.; Moutounet, M. Phenolic composition as related to red wine flavor. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; Oxford University Publishing: New York, 1998; pp 125−141.

(12) Vidal, S.; Francis, L.; Guyot, S.; Marnet, N.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. J. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J. Sci Food Agric.* **2003**, *83*, 564–573.

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

(14) Mercurio, M. D.; Dambergs, R. G.; Cozzolino, D.; Herderich, M. J.; Smith, P. A. Relationship between red wine grade and phenolics. 1. Tannin and total phenolics concentration. *J. Agric. Food Chem.* **2010**, *58*, 12313−12319.

(15) Mercurio, M. D.; Dambergs, R. G.; Herderich, M. J.; Smith, P. A. High throughput analysis of red wine and grape phenolics − adaptation and validation of methyl cellulose precipitable tannin assay and modified Somers color assay to a rapid 96 well plate format. *J. Agric. Food Chem.* **2007**, *55*, 4651–4657.

(16) Jeffery, D. W.; Mercurio, M. D.; Herderich, M. J.; Hayasaka, Y.; Smith, P. A. Rapid isolation of red wine polymeric polyphenols by solid-phase extraction. *J. Agric. Food Chem.* **2008**, *56*, 2571−2580.

(17) Bindon, K. A.; Smith, P. A.; Kennedy, J. A. Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem.* **2010**, *58*, 2520–2528.

(18) Somers, T. C.; Evans, M. E. Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines. *J. Sci. Food Agric.* **1974**, *25*, 1369–1379.

(19) Jackson, M. G.; Timberlake, C. F.; Bridle, P.; Vallis, L. Red wine quality: correlations between colour, aroma, and flavour and pigment and other parameters of young Beaujolais. *J. Sci. Food. Agric.* **1978**, *29*, 715–727.

(20) Saenz-Navajas, M.-P.; Tao, Y.-S.; Dizy, M.; Ferreira, V.; Fernandez-Zurbano, P. Relationship between nonvolatile composition and sensory properties of premium Spanish red wines and their correlation to quality perception. *J. Agric. Food Chem.* **2010**, *58*, 12407–12416.

(21) Parpinello, G. P.; Versari, A.; Chinnici, F.; Galassi, S. Relationship among sensory descriptors, consumer preference and color parameters of Italian Novello red wines. *Food Res. Int.* **2009**, *42*, 1389–1395.

(22) Gishen, M.; Dambergs, R. G.; Cozzolino, D. Grape and wine analysis − enhancing the power of spectroscopy with chemometrics. A review of some applications in the Australian wine industry. *Aust. J. Grape Wine Res.* **2005**, *11*, 296–305.

(23) McRae, J. M.; Falconer, R. J.; Kennedy, J. A. Thermodynamics of grape and wine tannin interaction with polyproline: implications for red wine astringency. *J. Agric. Food Chem.* **2010**, *58*, 12510–12518.

(24) Cheynier, V.; Duenas-Paton, M.; Salas, E.; Maury, C.; Souquet, J. M.; Sarni-Manchado, P.; Fulcrand, H. Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.* **2006**, *57*, 298–305.

(25) Wightman, J. D.; Price, S. F.; Watson, B. T.; Wrolstad, R. E. Effect of processing enzymes on anthocyanins and phenolics in Pinot noir and Cabernet Sauvignon wines. *Am. J. Enol. Vitic.* **1997**, *48*, 39–48.

(26) Ducasse, M. A.; Canal-Llauberes, R. M.; de Lumley, M.; Williams, P.; Souquet, J. M.; Fulcrand, H.; Doco, T.; Cheynier, V. Effect of macerating enzyme treatment on the polyphenols and polysaccharide composition of red wines. *Food Chem.* **2010**, *118*, 369–376.

(27) Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T.; Kennedy, J. A. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. *J. Agric. Food Chem.* **2005**, *53*, 5798–5808.

(28) Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002**, *53*, 261–267.

(29) Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002**, *53*, 268–274.

(30) Downey, M. O.; Harvey, J. S.; Robinson, S. P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004**, *10*, 55–73.

(31) Cortell, J. M.; Kennedy, J. A. Effect of shading on accumulation of flavonoid compounds in *Vitis vinifera* L. Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* **2006**, *54*, 8510–8520.

(32) Ristic, R.; Downey, M. O.; Iland, P. G.; Bindon, K.; Francis, I. L.; Herderich, M.; Robinson, S. P. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin, and sensory properties. *Aust. J. Grape Wine Res.* **2007**, *13*, 53–65.

(33) Pérez-Magarino, S.; González-San José, M. L. Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *J. Agric. Food Chem.* **2004**, *52*, 1181–1189.

(34) Canals, R.; Llaudy, M. C.; Vallis, J.; Canals, J. M.; Zamora, F. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skins and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* **2005**, *53*, 4019–4025.

(35) Fernandez, K.; Kennedy, J. A.; Agosin, E. Characterization of *Vitis vinifera* L. cv. Carménère grape and wine proanthocyanidins. *J. Agric. Food Chem.* **2007**, *55*, 3675–3680.

(36) Hufnagel, J. C.; Hofman, T. Quantitative reconstruction of the nonvolatile sensometabolome of a red wine. *J. Agric. Food Chem.* **2008**, *56*, 9190–9199.