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Characterization of *Vitis vinifera* L. Cv. Carménère Grape and Wine Proanthocyanidins

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A formal compositional study of the proanthocyanidins of *Vitis vinifera* L. cv. Carménère was conducted in this work. We first characterized the polymeric proanthocyanidins of Carménère skins, seeds, and wines. In addition, the wine astringency was analyzed and compared with Cabernet Sauvignon. Although Carménère wines had a higher proanthocyanidin concentration and mean degree of polymerization than Cabernet Sauvignon wines, the former wines were perceived as less astringent. The low seed/skin proportion in Carménère wines as compared to other varieties, as evidenced by the reduced number of seeds per berry and the higher amount of epigallocatechin subunits of Carménère wine proanthocyanidins, could explain this apparent paradox.

KEYWORDS: ***Vitis vinifera*; Carménère; red wine; grapes; proanthocyanidins; phloroglucinolysis; Chile**

INTRODUCTION

Vitis vinifera L. cv. Carménère is a red grape variety recently rediscovered in Chile. Carménère vines were imported from Bordeaux, France, to Central Chile in the middle of the 19th century, before the phylloxera devastation of European grapevines (*1*). This late-maturing variety adapted well to the Chilean soil and to its dry climate. Nevertheless, until the mid-1990s, it was confused with Merlot and Cabernet Franc, which have similar ampelographic characteristics. Chile and southern Italy are the only two regions in the world that currently have significant plantings of Carménère (*2*). Today, Carménère exists as single vineyards, and the variety is the subject of clonal selection projects to promote Carménère as the emblematic variety of Chile.

Carménère wines are defined as deeply colored, with well-structured tannins (*3*, *4*). These organoleptic properties are related to the wine phenolic compounds, particularly to the proanthocyanidins. Proanthocyanidins are polymeric flavonoid compounds composed of flavan-3-ol subunits, and they play a role in long-term red wine color stability (*5*) and influence the sensory properties of astringency (*6*).

Proanthocyanidins are localized in the solid parts of the grape berry. Seed proanthocyanidins consist of (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin-3-*O*-gallate (ECG) subunits linked by C_4-C_8 and/or C_4-C_6 bonds (*7*). Grape skin proanthocyanidins also contain (−)-epigallocatechin (EGC) and small amounts of gallocatechin (*8*). The proanthocyanidin amount, composition, and mean degree of polymerization (mDP) differ between berry skins and seeds. Skin proanthocyanidins have a higher mDP and a lower proportion of galloylated subunits than those from seeds (*7*−*9*). Experimental evidence has shown that the mDP and galloylation of wine proanthocyanidins are important structural variables affecting wine astringency perception (*9*).

The aim of this study was to characterize Carménère proanthocyanidins and the astringency of wines produced from Carménère grapes. The characterization was carried out by acid-catalyzed depolymerization in the presence of phloroglucinol (phloroglucinolysis), which provided information on the concentration, mDP, and conversion yield. The investigation was also extended to quantify proanthocyanidins in several varietal Carménère wines from different Chilean regions. Finally, the astringency of Carménère and Cabernet Sauvignon wines produced under similar fermentation conditions was compared.

MATERIALS AND METHODS

Materials. All solvents were of high-performance liquid chromatography (HPLC) grade. Acetone, acetonitrile, methanol, and glacial acetic acid were purchased from J. T. Baker (Phillipsburg, NJ). Phloroglucinol and C were purchased from Sigma (St. Louis, MO). Ammonium phosphate monobasic and orthophosphoric acid were purchased from Fisher Scientific (Santa Clara, CA). Hydrochloric acid and anhydrous sodium acetate were purchased from E. M. Science (Gibbstown, NJ) and Mallinckrodt (Phillipsburg, NJ). Alum (aluminum potassium sulfate dodecahydrate) was purchased from Merck (Germany).

Instrumentation. A Hewlett-Packard model 1100 HPLC (Palo Alto, CA) consisting of a vacuum degasser, autosampler, quaternary pump, diode array detector, and column heater was used. A computer workstation with Chemstation software was used for chromatographic analysis.

Clone Studies. *Plant Material*. Three, 4 year old Carménère clones growing at the Research Station of Copeval S.A. in San Fernando

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(Colchagua Valley, VI region, Chile) were used in this study. The plant identity of clonal vines was checked by ampelography and polymerase chain reaction-based molecular markers, including microsatellite DNA markers (simple sequence repeats), which were compared with DNA profiles of previously authenticated vines: Cabernet franc and Merlot from the Foundation Plant Materials Service, University of California (Davis, CA), and Carménère from the Institut National de la Recherche Agronomique (INRA) plant collection (Montpellier, France) (*10*).

Fruit Sampling and Extraction. Whole grape bunches of the three selected clones (vintage 2004) were harvested at technological maturity (24° Brix). Two groups of 100 berries were randomly selected from the collected samples of each clone and weighed. Grape seeds and skins were manually separated, and the respective proanthocyanidins were extracted as described in Kennedy and Jones (*11*). The resulting skin and seed extracts were frozen at 20 °C until further fractionation (see below).

Winemaking. Triplicate wines were produced from each of the three selected Carménère clones at the Wine Research Station of the Faculty of Agricultural Sciences of the Universidad Católica de Chile. One hundred kg of fruit was destemmed, crushed, and collected into 120 L stainless steel wine vats. The musts were inoculated with *Saccharomyces cerevisiae bayanus* EC1118 yeast strain (20 g/hL) according to the manufacturer's guidelines. The fermentations were carried out at a temperature up to 28 °C. The cap was punched down daily. The malolactic fermentation was conducted spontaneously. At the end of the fermentation, the wines were racked and stabilized for 3 weeks at 0−10 °C. Before bottling, the free SO_2 was adjusted to 30 mg/L. The phenolic profile of the resulting wines was determined after 6 months of storage at room temperature.

Fractionation and Chemical Characterization of Proanthocyanidins. Four milliliters of the skin and seed extracts obtained above, as well as the clonal wines, were concentrated using a Centrivap concentrator (Labconco Corp., Kansas City, MO); after reconstitution in 2 mL of phosphate buffer, pH 7.0 (67 mM), the samples were applied to two columns (0.5 g) connected in series: Discovery C_{18} Lt (top) and C_{18} (bottom) cartridges from Supelco/Sigma-Aldrich (St Louis, MO), following the procedure of Monagas et al. (*12*). The proanthocyanidin fraction was chemically characterized using double-strength phloroglucinolysis, as described elsewhere (*13*).

Carménère Varietal Wine Studies. Seventeen Carménère varietal wines vintage 2004 were analyzed. The wines were produced and kindly donated by different wineries situated in the Cachapoal (valley 1), Colchagua (valley 2), and Curicó (valley 3) valleys in Central Chile. Special care was taken that the wines contained proanthocyanidins exclusively derived from the original grapes (no barrel or any other wood addition).

The wines (400 mL) were dealcoholized under reduced pressure, filtered through a nylon membrane (0.45 μm, Whatman, United States), and applied to a column (4.8 cm × 23.5 cm) containing Toyopearl HW 50-F resin (Sigma-Aldrich). The wines were fractionated according to Kennedy and Jones (*11*). The proanthocyanidin fraction was collected and freeze-dried. Its composition was determined by phloroglucinolysis, followed by reversed-phase HPLC, as described (*11, 14*).

Quantitative recovery of proanthocyanidins from the column was determined as follows: A known amount of proanthocyanidins, previously purified and characterized, was first loaded onto the column. After they were rinsed, the proanthocyanidins were eluted and dried, and the resulting phenolic extract was quantified and compared with the initial quantity. The extract recovery yield approached 94% by weight, indicating that this fractionation procedure was suitable for proanthocyanidin purification. Furthermore, the proanthocyanidin subunit composition and conversion yield were similar before and after column fractionation.

Sensory Studies. *Wine Samples.* Five Carménère and five Cabernet Sauvignon, vintage 2006, collected in pairs from five wineries were used to compare the wine astringency of these two varieties. The wines were produced under similar conditions for both grape varieties, i.e., without prefermentative stage, inoculated with commercial yeasts, and fermented in stainless steel wine vats under tight temperature control (25−28 °C) until dryness. It is worth mentioning that the wines contained only proanthocyanidins derived from the original grapes. The wines were fractionated according to Vidal et al. (*9*). Sugars, anthocyanins, phenolic acids, organic acids, flavan-3-ol monomers, and oligomers eluted first and were discarded. The proanthocyanidins were collected and freeze-dried, and their compositions were determined by phloroglucinolysis.

Panel Selection and Training. A similar salivary flow rate among the panelists was used as selection criterion to limit the effect of individual differences in astringency perception due to subject's saliva characteristics (*15*). Salivary flow evaluation was carried out according to ref *16*. Each panelist ingested 15 mL of an aqueous solution of citric acid (4 g/L), which was expectorated after 10 s. The panelists then spat the stimulated saliva into a weighed container for 1 min. The saliva was collected in triplicate and weighed on an analytical balance. Ten panelists—from a total of 16—were selected from those having the closer flow rates. Characteristic values of a percentile distribution (first and third quartiles) were used in order to define the selected group; the latter had a mean salivary flow rate of 2.2 ± 0.5 g/min.

Selected panelists were trained over four sessions to evaluate the astringency by tasting model standard solutions. The standard solutions were prepared by dissolving alum (0.55 and 1.6 g/L), skin (0.3, 0.6, and 1.2 g/L), and seed (0.3, 0.6, and 1.2 g/L) proanthocyanidins in 1% v/v ethanol in distilled water (*17*).

Astringency Comparison between Carménère and Cabernet Sauvignon Wines. During the formal sessions, Carménère and Cabernet Sauvignon wines, vintage 2006, were evaluated. The panelists tasted 15 mL of the wines at room temperature and in individual booths, illuminated with red light. The subjects were asked to hold each sample in their mouth for 8 s, spit it out, and rate the astringency intensity using a 0−10-point scale. Between samples, the panelists were asked to rinse their mouth with distilled water for 45 s, to eat some plain crackers for 30 s, and finally to rinse again with distilled water for a further 45 s. The wine evaluation consisted of three repetitions for each sample, with a total of six sessions. In each session, six samples were presented. There was a 30 min interval between the first three samples and the subsequent ones. The order of sample presentation was balanced for first-order and carry-over effects.

Comparison of Astringency Intensity between the Proanthocyanidin Fraction and the Corresponding Wine. The astringency elicited by extracted proanthocyanidins was contrasted with the astringency of the corresponding wine. For this purpose, the proanthocyanidin extracts of a Carménère and a Cabernet Sauvignon wine were sensory evaluated by the trained panel after extract reconstitution in 400 mL of 1% v/v ethanol (same as original wine volume). The panelists received 15 mL of the proanthocyanidin extract and were asked to evaluate its intensity with respect to the original wine at room temperature, as described. The evaluation was carried out in duplicate for each sample in two sessions. The order of sample presentation was balanced for first-order and carry-over effects.

Statistical Analyses. Statgraphics Plus for Windows 4.0 (Herndon, VA) was used for statistical analyses. The analysis of variance (ANOVA) and the least significant difference (LSD) test were used to determine statistically different values at a significance level of $p \leq 0.05$ for the chemical parameters of seed, skin, wine clones, and sensorial analysis with an equal number of samples. The Scheffe's F test was applied to the varietal wine data ($p \leq 0.05$). This multiple comparison procedure was chosen based on the unequal sample sizes and the efficiency of the method, that is, the one with the smallest type I error rate.

RESULTS AND DISCUSSION

Proanthocyanidins from Carménère Clones. Skin and seed proanthocyanidin concentrations and compositions from grape berries of three clones of *V. vinifera* L. cv. Carménère were first evaluated.

On a per berry basis, the Carménère seed proanthocyanidin concentration was higher than in skins (**Table 1**). However, the seed/skin proanthocyanidin ratio in terms of content was

Table 1. Structural Composition (% in Mol) and Characteristics of Proanthocyanidins from Carménère[a] Seeds, Skins, and Wine Clones

			extension (%)				terminal (%)				
clone	concentration (mg/berry)	seeds/ berry	C	EC	ECG	EGC	C	EC	ECG	mDP	%G
					seed						
1	3.36 ± 0.23	0.98	10.7	78.1	11.2	0	44.6	32.9	22.5	6.1	13.1
2	3.51 ± 0.24	0.93	13.8	77.4	8.9	0	43.4	31.4	25.3	5.4	11.1
3	3.52 ± 0.14	1.10	13.0	75.5	11.5	0	49.1	30.1	28.5	7.4	13.8
					skin						
1	1.81 ± 0.15		3.4	67.3	2.1	27.2	100	0	0	12.1	1.9
2	1.52 ± 0.24		5.4	68.1	1.8	24.6	100	0	0	8.7	1.6
3	1.65 ± 0.02		4.2	65.3	2.5	28.0	100	0	0	11.0	2.3
					wine						
1	246 ± 15		4.5	68.2	3.4	23.9	64.8	35.2	0	5.9	2.8
2	239 ± 18		3.9	70.1	3.6	20.4	73.5	26.5	0	4.7	3.6
3	286 ± 44		6.4	68.8	2.7	22.1	51.0	48.9	0	4.9	2.1

[a] Abbreviations: %G, percentage of galloylation.

approximately 2, much lower than most grape varieties, where this proportion has been found to be at least 3, even reaching 10 for some varieties (*12*, *18*, *19*). This lower ratio of Carménère proanthocyanidin results from both the lower concentration of Carménère seed proanthocyanidins and the higher content of skin proanthocyanidins, as compared with other varieties (*12*, *18*−*20*).

The higher relative amount of skin vs seed proanthocyanidins in Carménère could be the consequence of the lower amount of average seed number per berry in this variety (**Table 1**), in comparison with other grape varieties (*19*−*21*). The latter may have resulted from the inflorescence characteristics of Carménère, where a certain percentage of anomalous flowers with spiraled stamen normally occurs (*22*).

In seeds, C, EC, and ECG were identified as terminal, as well as extension proanthocyanidin subunits (**Table 1**). The extension subunits were characterized by a predominance of EC. A similar extension subunit profile has been observed in seeds of Syrah (*20*), Pinot noir (*19*), and Cabernet Sauvignon (*12*), with small variations among varieties. For terminal subunits, when compared with the varieties previously mentioned, a major variability in subunits ratio was found.

In skins, in addition to the subunits reported in seeds, EGC was identified as a specific extension subunit, and C was the only terminal subunit determined. Proportionally, EC was the most abundant subunit, followed by EGC (**Table 1**). Furthermore, skin proanthocyanidins differed from seed proanthocyanidins by their lower amounts of galloylated derivatives and higher mDP.

Finally, the proanthocyanidin composition of clonal wines was closer to the skin phenolic profile than to the seed one, particularly with regard to the %EGC in wine. The proanthocyanidin concentration among the wines produced from the three clones was not significantly different (LSD, 5%), the same as for proanthocyanidin concentration of seeds and skins (LSD, 5%).

Proanthocyanidins from Carménère Varietal Wines. The proanthocyanidin concentrations and compositions of several varietal Carménère wines from three valleys of Central Chile were assessed. Proanthocyanidin concentrations of Carménère wines—determined from the corresponding dried phenolic extracts—varied between 643 and 1857 mg/L (average, 1151 ± 451 mg/L) and depended on their geographical origin (**Figure 1**, $p < 0.024$, Scheffe 5%). The highest proanthocyanidin concentrations were found in wines from the Curicó valley (valley 3), where the highest number of growing degree days and diurnal temperature fluctuations (till 20 °C) were reached. This agrees with previous studies (*23*, *24*), where higher sun exposure positively influenced proanthocyanidin concentration.

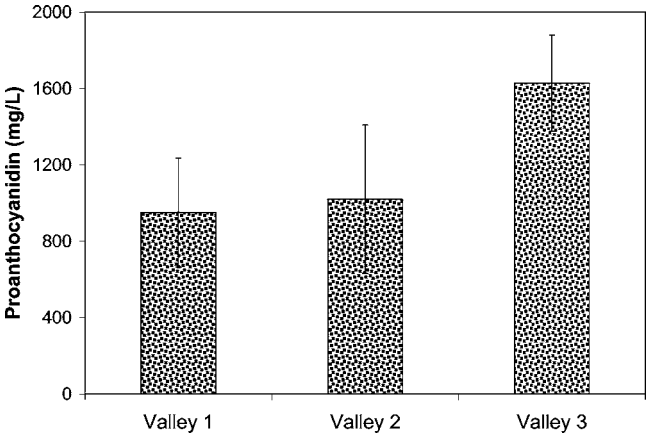

Figure 1. Concentration (mg/L) of proanthocyanidins from varietal Carménère wines from three Chilean valleys. Vertical bars represent the standard deviation ($n = 5$, 6, and 6).

The conversion yield of proanthocyanidins into their constitutive units was calculated as the ratio between total released units (flavan-3-ols and adducts) and the phenolic polymer content of the initial wine extract (gravimetrically determined). For vintage 2004 wines (**Table 2**), we found an average conversion yield of 23.1% (w/w), with significant differences among valleys ($p < 0.026$, Scheffe 5%); that is, the warmer the region, the higher the conversion yield. A similar mDP was found in all regions; furthermore, the percentage of galloylation of wine proanthocyanidins was similar to previously reported values (*12*, *25*).

Wine conversion yields were much lower than those determined for seed or skin extracts (*26*). In grapes, tannins reach their maximum conversion yield close to veraison (above 85% w/w). During fruit ripening, the conversion yields decrease (*27*, *28*). The changes in proanthocyanidins during fruit ripening are consistent with oxidation, as suggested by Kennedy et al. (*26*), and the conversion yield declines during this time. It is also likely that during winemaking the proanthocyanidins become modified and that these modifications lead to a reduced conversion yield. For example, during aging, anthocyanins and proanthocyanidins react with each other to form secondary pigments (*26*, *29*), leading to a reduction in conversion yield.

Table 2. Structural Characteristics and Composition (% in Mol) of Wine Proanthocyanidins from Carménère[a] 2004

					extension (%)				terminal (%)	
valley	*n*	yield (% w/w)	mDP	G (%)	C	EC	ECG	EGC	C	EC
1	5	17.8 a	6.9 a	2.4 a	3.3 a	56.4 ab	2.9 a	37.4 a	65.3 b	34.7 b
2	6	20.5 a	7.1 a	2.7 a	4.7 a	58.2 b	3.2 a	33.9 a	60.9 a	39.0 a
3	6	33.3 b	8.5 a	3.0 a	4.0 a	54.4 a	3.4 a	38.2 a	64.8 b	35.2 b
average		23.1	7.4	2.7	4.3	56.9	3.2	35.5	62.6	37.4

[a] ANOVA to compare data: Values with different letters within each column are significantly different ($p < 0.05$, Scheffe). Abbreviations: *n*, number of wines; %G, percentage of galloylation.

Table 3. Structural Characteristics and Composition (% in Mol) of Proanthocyanidins from Carménère 2006 and Cabernet Sauvignon 2006 Wines[a]

						extension (%)				terminal (%)	
wine	*n*	concentration (mg/L)	yield (% w/w)	mDP	G (%)	C	EC	ECG	EGC	C	EC
Carménère	5	3182 b	57.8 b	13.6 b	3.7 a	3.5 a	55.7 a	4.1 a	36.7 b	68.6 b	31.4 a
Cabernet Sauvignon	5	2497 a	46.1 a	8.7 a	3.6 a	5.3 b	59.1 b	4.2 a	31.4 a	63.7 a	36.3 b

[a] ANOVA to compare data: Values with different letters within each column are significantly different ($p < 0.09$, LSD). Abbreviations: *n*, number of wines; %G, percentage of galloylation.

Structurally, the proanthocyanidins in the Carménère wines were composed of C, EC, EGC, and ECG as extension subunits; only the first two were present as terminal subunits (**Table 2**). As expected, EC was the major extension subunit. Extension subunits also contained a high proportion of EGC, suggesting a higher proanthocyanidin contribution from the grape skins in Carménère. For terminal subunits, C was the most abundant component. Thus, wine proanthocyanidins contained both procyanidins and prodelphinidins.

Comparative Sensory and Chemical Analysis of Carménère and Cabernet Sauvignon Wines. The proanthocyanidins from Carménère and Cabernet Sauvignon wines (same wineries and vintage 2006) were compared. Major differences were found in concentration, conversion yield, and mDP (**Table 3**). For Carménère wines, these parameters were higher than for Cabernet Sauvignon wines. The proportion of EGC was higher in Carménère, significantly different than Cabernet Sauvignon (LSD, 9%). In addition, when wines were segregated by winery, the proportion of EGC was in all cases higher for Carménère than for Cabernet Sauvignon wines (data not shown).

The intensity of astringency of these Carménère vs Cabernet Sauvignon wines was then evaluated. The panelists rated the Cabernet Sauvignon wines higher than the Carménère wines in astringency (**Figure 2**). On a scale of intensity of 0−10 points, the average scores for astringency were 6.0 and 5.3, respectively ($p < 0.03$, LSD 5%).

These results contradict previous studies (*9*, *30*, *31*), where increasing chain length (mDP) resulted in a higher overall astringency. However, the presence of EGC units in the proanthocyanidins has been shown to lower the "coarse" perception (*9*) through the increase of the degree of B ring trihydroxylation. Moreover, Poncet-Legrand et al. (*32*) recently highlighted the strong influence of structural features of flavan-3-ol monomers on the interactions with proline-rich proteins (PRP). These authors demonstrated that EGC did not form aggregates with poly (L-proline), a model protein reminiscent of structural characteristics of salivary PRPs. Astringency may therefore be modulated by accessibility of interaction sites and molecular conformation (*33*).

Wine-extracted proanthocyanidins for either a Cabernet Sauvignon or a Carménère wine elicited similar astringency to

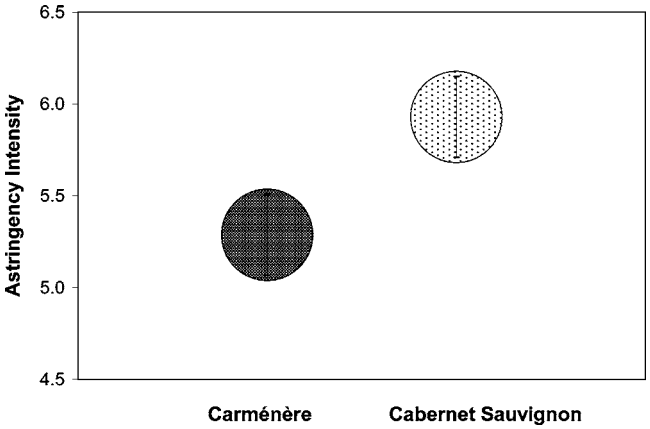

Figure 2. Astringency perceived in Carménère and Cabernet Sauvignon wines.

Table 4. Comparison of Astringency Perceived in Proanthocyanidins Fractions and Wines[a]

	astringency intensity	
sample	Carménère	Cabernet Sauvignon
proanthocyanidin extract	4.8 a	5.9 ab
wine	5.8 ab	6.5 b

[a] ANOVA to compare data: Values with different letters within each column are significantly different ($p < 0.05$, LSD).

the whole wine (**Table 4**, LSD 5%), strongly suggesting a close relationship between the proanthocyanidins and the resulting wine astringency. Nevertheless, several wine components—other than phenolics—are known to influence the perception of astringency, including ethanol, acidity, pH, and polysaccharides, among others (*33*); however, at least the former three did not show significant differences among the studied samples (**Table 5**), strengthening, therefore, the importance of the proanthocyanidin compounds in wine astringency.

Overall, the results of this research showed that although Carménère wines had a higher proanthocyanidin concentration and mDP than Cabernet Sauvignon wines, the former wines were perceived as less astringent than the latter. The higher amount of EGC in Carménère, as compared with Cabernet

Table 5. Total Acidity, Ethanol, and pH from Sensory Analysis Wines[a]

wine variety	*n*	total acidity (g/L) sulfuric acid	ethanol % (v/v)	pH
Carménère	5	3.08 a	13.54 a	3.71 a
Cabernet Sauvignon	5	2.96 a	13.33 a	3.65 a

[a] ANOVA to compare data: Values with different letters within each column are significantly different ($p < 0.05$, LSD); *n*, number of wines.

Sauvignon wines, could at least partly explain this apparent paradox. EGC subunits are only found in skin proanthocyanidins, which also have higher mDPs and elicit less astringency intensity than seeds (*7*, *8*, *34*). Furthermore, the seed/skin ratio determined here for Carménère is the lowest reported so far (*12*, *18*, *19*). Therefore, the increased proportion of EGC and the higher mDP in Carménère wines suggest that these wines might contain a higher proportion of skin tannins than Cabernet Sauvignon (this study; *12*), Tempranillo, and Graciano (*12*) or Pinot noir (*19*). Therefore, while the higher concentration and mDP would suggest that Carménère wines should have more astringency, they do not. The apparent increase in the proportion of skin proanthocyanidins as predicted by EGC in addition to the overall low conversion yields observed in this study suggests that there are aspects of proanthocyanidin structure that influence perceived astringency and are not yet understood.

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