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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)

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Grape variety [Cabernet Sauvignon (CS) and Merlot (M)] effect on the proanthocyanidin composition and sensory perception of wine grapes from Bordeaux vineyards for two successive vintages (2006 and 2007) is reported. The flavan-3-ol monomers [(+)-catechin = C, (−)-epicatechin = EC, (−)-epicatechin-*O*-gallatte = ECG] and the proanthocyanidin oligomers [dimers B1, B2, B3, and B4 and trimer Cat-Cat-Epi (T)] in grape seed and skin tannin extracts were identified and quantified at harvest. Proanthocyanidin subunit compositions, percentage of galloylation (%G), and percentage of prodelphinidins (%P) as well as mean degree of polymerization (mDP) of the proanthocyanidin fraction were determined. Sensory analysis concerning the astringency and bitterness intensity of the proanthocyanidins of skin and seed tannin extracts was also performed. The results showed that proanthocyanidin composition can be greatly affected by grape variety. For both vintages between CS and M, significant differences were found on mDP ($p < 0.05$) in seed tannin extracts, whereas in skin tannin extracts, significant differences were observed for %G and %P ($p < 0.05$). Sensory analysis showed that grape variety influenced neither astringency nor bitterness intensity perception for both skin and seed tannin extracts for the two successive vintages studied. A positive correlation was found between astringency intensity, mDP, and B3 content in skin tannin extracts.

KEYWORDS: Cabernet Sauvignon; Merlot; grapes; proanthocyanidins; astringency; bitterness; mean degree of polymerization; percentage of prodelphinidins; percentage of galloylation

INTRODUCTION

Cabernet Sauvignon (CS) and Merlot (M) are the world's most widely recognized red wine grape varieties. CS became internationally recognized first through its prominence in Bordeaux wines. From France, the grape spread across Europe and to the New World, where it found new homes in habitats such as California's Napa Valley, Australia's Coonawarra region, Chile's Maipo Valley, Argentina, South Africa (*1*), and southern Brazil (*2*). Despite its importance in the world of wine, the grape is a relatively new variety, being the product of a chance crossing between Cabernet Franc and Sauvignon Blanc sometime during the 17th century in southwestern France (*3*). On the other hand, M is produced primarily in France (where it is the third most planted red grape), Italy (where it is the country's fifth most planted grape), California, Romania, and to a lesser extent in Australia, Argentina, Canada's Niagara peninsula, Chile, New Zealand, South Africa, Switzerland, Croatia, Hungary, Montenegro, Slovenia, and other parts of the United States such as Washington state and Long Island (*4*).

The tannic nature of CS and M is of paramount interest, particularly in the Bordeaux wine-growing region, which is mostly planted with these two varieties. Proanthocyanidins, or condensed tannins, are grape-derived flavonoid compounds of great importance to red wine quality due to their astringent, bitter properties (*5, 6*) and their role in the long-term color stability (*7*). Astringency is a tactile sensation, whereas bitterness is a taste. Molecular size of proanthocyanidins affects their relative bitterness and astringency (*6*). Monomers are more bitter than astringent, whereas the reverse is true for large molecular weight derivatives. Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG),

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and (−)-epigallocatechin (EGC) (*8*−*11*). Skin proanthocyanidins differ from those found in seeds in that skin tannins include prodelphinidins (EGC) and have a higher degree of polymerization and a lower proportion of galloylated subunits (*5, 7, 12*).

Relatively little is known about the variety effect on grape proanthocyanidin composition and on sensory perception. Previous studies (*13*−*15*) have examined the phenolic content of wines often in relation to a single variety, but they tended to focus on rather few compounds of interest. In the literature, data concerning variety effect on yield and quality of wine grapes (*16*) and variety differences in response to environmental parameters are also reported (*17*−*19*). Interestingly, the comparison of polyphenol content from different varieties has received little attention, in particular when defining features that may be specific to the grape, juice, and wine (*20*−*23*). The aim of this work was to investigate the variety effect on proanthocyanidin composition and sensory perception of the Bordeaux wine grape varieties CS and M in order to determine if the proanthocyanidin composition and sensory perception of skin and seed extract may discriminate the two varieties CS and M.

MATERIALS AND METHODS

Reagents. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, MA). Acetonitrile (HPLC grade), ethyl alcohol (HPLC grade), acetic acid, orthophosphoric acid, L-ascorbic acid, L-tartaric acid, hydrochloric acid, ammonia, and sodium acetate were obtained from Prolabo-VWR (Fontenays/Bois, France). (+)-Catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-*O*-gallate (ECG), B1 [(−)-epicatechin-(4β-8)-(+)-catechin], B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin], and ammonium phosphate were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). B3 [(+)-catechin-(4α-8)-(+)-catechin], B4 [(+)-catechin-(4α-8)-(−)-epicatechin], and trimer (T) [(+)-catechin-(4β−-8)-(+)-catechin-(4β-8)-(−)-epicatechin] were synthesized by the Laboratory of Organic Chemistry and Organometallic, Universiteì Bordeaux 1 (*24*).

Selection of Experimental Area and Samples. The study was carried out with samples from five vineyards located in the Bordeaux vine-growing region in southwestern France. They are situated in Pauillac (V1), Margaux (V2), Saint Emillion (V5), Saint Emillion (V6), and Côtes de Bourg (V7). The vineyards are all planted with *Vitis vinifera* L. cv. Cabernet Sauvignon (CS) and Merlot (M). One grape sample of each vineyard and of each variety was collected at maturity in September 2006 and 2007.

Preparation of Extracts. Seeds and skins were removed by hand from grapes, washed with distilled water, lyophilized for 2 days, and stored at −20 °C. The frozen seeds and skins were finally ground in a ball grinder. A 5 g portion of the obtained powder was extracted using 45 mL of acetone/water (80:20, v/v) followed by 45 mL of methanol/water (60:40, v/v). The centrifugal supernatants were evaporated under reduced pressure at 30 °C to remove organic solvents, and then the residue was dissolved in water and freeze-dried to obtain a crude tannin extract.

Fractionation of Seed Proanthocyanidins. The crude seed tannin extract was first dissolved at a concentration of 80 g/L in distilled water containing 5% ethanol to help solubilization. This solution was extracted three times with chloroform to remove lipophilic material, and then the aqueous phase was finally extracted three times with ethyl acetate to obtain low molecular weight procyanidins (oligomeric tannins) in the organic phase. The oligomeric proanthocyanidin extract was concentrated and freeze-dried to yield the dry powder. Then it was analyzed by reverse phase HPLC-UV.

Fractionation of Skin Proanthocyanidins. Crude skin tannin extract (2.4 g) was dissolved in 10 mL and then fractionated on Toyopearl TSK HW-50 (F) gel from Tosoh Corp. After loading, the column was washed with 200 mL of distilled water. Anthocyanins were eluted with 900 mL of ethanol/water/trifluoroacetic acid (20:79:1, v/v/v). Then the residual monomers and oligomers were retrieved with methanol (100%). After the evaporation of the organic solvent, skin tannin extract was freeze dried and analyzed by reverse phase HPLC-UV.

Table 1. Ternary Mobile Phase Gradient of the HPLC Method

		% of mobile[a]		
time (min)	flow rate (mL/min)	phase A	phase B	phase C
initial	0.5	97	3	0
5.00	0.5	97	3	0
15.00	0.5	92	8	0
18.00	0.5	0	8	92
30.00	0.5	0	13	87
55.00	0.5	0	20	80
60.00	0.5	0	25	75
70.00	0.5	0	30	70
75.00	0.5	0	80	20
80.00	0.5	0	97	3
82.00	0.5	97	3	0
84.00	0.5	97	3	0

[a] A, 50 mM ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid; B, 20% A with 80% acetonitrile; C, 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5.

HPLC Analysis of Monomeric and Oligomeric Flavan-3-ols. The equipment used for the HPLC analysis consisted of a Finnigan UV−vis detector (UV−vis 200), a Finigan autosampler, and a Finnigan ternary pump coupled to an Xcalibur data treatment system. Separation was performed on reversed-phase Agilent Nucleosil C18 (250 mm × 4 mm, 5 μm). The mobile phases were (mobile phase A) 50 mM dihydrogen ammonium phosphate adjusted to pH 2.6 with orthophosphoric acid, (mobile phase B) 20% A with 80% acetonitrile, and (mobile phase C) 0.2 M orthophosphoric acid adjusted with ammonia to pH 1.5. The solvent gradient is described in **Table 1**. Eluting peaks were monitored at 280 nm. Calibration curves were established at 280 nm using external standards, either commercial (C, EC, ECG, B1, B2) or synthesized (B3, B4, T). Each sample was injected three times. Unknown concentrations were determined from the regression equations, and the results were converted into grams of dried weight.

LC-MS and HPLC Apparatus and Absorbance Measurements. LC-MS analyses were performed on a Micromass Platform II simple quadruple mass spectrometer (Micromass-Beckman, Roissy Charlesde-Gaulle, France) equipped with an electrospray ion source. The mass spectrometer was operated in negative-ion mode. The source's temperature was 120 °C, the capillary voltage was set at 3.5 kV and the cone voltage was −30 eV. HPLC separations were performed on a Hewlett-Packard 1100 series (Agilent, Massy, France) including a pump module and a UV detector. Both systems were operated using Masslynx 3.4 software. The absorbance was recorded at 280 nm and mass spectra were recorded in the range of 50 to 1500 amu. Separation was performed on a reversed-phase Waters XTerra RR C18 (100 mm × 4.6 mm, 3.5 μm) column at room temperature. The method uses a binary gradient with mobile phases containing 1% (v/v) aqueous acetic acid (mobile phase A) and MeOH (mobile phase B). The solvent gradient described below for oligomeric proanthocyanidins was applied at a flow rate of 1 mL/min. The elution conditions were: 5% B for 1 min, a linear gradient from 5 to 16% B in 1 min, a linear gradient from 16 to 22% B in 6 min a linear gradient from 22 to 35% B in 1 min, a linear gradient from 35 to 42% B in 7 min, a linear gradient from 42 to 100% B in 1 min. The column was then washed with 100% B for 3 min and re-equilibrated with 5% B for 4 min before the next injection.

Mean Degree of Polymerization (mDP). The proanthocyanidin mDP concentrations were quantified by phloroglucinolysis (*25*).

Sensory Analysis. *Samples.* Model wine solutions had the following composition: deionized water, ethanol (12%), tartaric acid (5 g/L) with pH adjusted at 3.2 with NaOH. To evaluate bitterness and astringency of model wine solutions, the tannin concentration of 1 g/L was chosen as a minimum tannin concentration typically found in red wines.

Judges. Twelve judges, six women and six men, from the Oenology Department at the University of Bordeaux took part in the experiment.

They were all selected on the basis of interest and availability. They were trained to evaluate the astringency and bitterness of tannin extracts by tasting two model solutions, which were assigned the medium score on the intensity scale (3.5 points). The standard solutions were 0.15 g/L quinine sulfate and 1.0 g/L aluminum sulfate for bitterness and astringency, respectively. The judges tasted 15 mL of the model wine solution at room temperature in individual booths, illuminated with red light. Each judge was asked to hold each sample in his/her mouth, spit it out, and rate the astringency and bitterness intensity using a 0−7 point scale. Between samples, the panelists were asked to rinse their mouths with distilled water, to eat some plain crackers for 30 s, and finally to rinse again with distilled water for another 45 s. The evaluation consisted of two repetitions for each sample, with a total of 16 sessions.

Data Analysis. Statistical data analysis was performed using the analysis of variance (ANOVA) of Statistica V.7 software (Statsoft Inc., Tulsa, OK). Tukey's HSD and Duncan's tests were used as comparison tests when samples were significantly different after ANOVA ($p < 0.05$) for chemical and sensory analysis, respectively. Principal component analysis (PCA) was used to examine any possible grouping of samples according to variety and vintage. PCA was performed on the correlation matrix using the attributes that differed significantly by ANOVA. Pearson's correlation analysis was used to investigate relationships between proanthocyanidin composition and sensory perception.

RESULTS AND DISCUSSION

Proanthocyanidin Composition. The catechin monomers (C, EC, and ECG) and the proanthocyanidin oligomers (dimers B1, B2, B3, and B4 and trimer T) in grape skin and seed tannin extracts were identified and quantified at harvest in September 2006 (**Table 2**) and 2007 (**Table 3**). As shown in **Tables 2** and **3**, concentrations of flavan-3-ols in skin tannin extracts were lower than in seed tannins extracts; this coincides with the findings mentioned previously (*26*). Dimer B4 was absent in grape skin as has already been reported by other researchers for red and white grape varieties (*27*−*29*).

The percentage of galloylation (%G) and the percentage of prodelphinidins (%P) as well as the mean degree of polymerization (mDP) of the proanthocyanidin fraction of seed and skin tannin extracts for the two vintages are presented in **Tables 4** and **5**. Skin and seed proanthocyanidin profiles differed by their low amounts of galloylated derivatives and higher mDP. These results are consistent with data concerning mDP values of polymeric proanthocyanidins in some publications, where values for grape seeds extracts ranged from 2.7 to 18.6 (*29*−*31*) for other *V. vinifera* varieties. However, literature data concerning mDP values of skin polymeric proanthocyanidins largely vary, from 11 to 83 approximately depending on the fractionation technique employed and the grape variety and vintage (*9, 30*).

Comparative Proanthocyanidin Composition between CS and M Grapes in 2006. *Seed Extracts.* The variables shown in **Table 2** (C, EC, ECG, B1, B2, B3, B4, and T) and in **Table 4** (mDP, %G) were analyzed by ANOVA to determine those variables (C, B1, B3, T, mDP, and %G) that significantly differentiated between the varieties at 95% of confidence. PCA was carried out on the correlation matrix of the variables that differed significantly by ANOVA ($p < 0.05$); the six variables across the 10 samples resulted in a three-factor solution explaining 86.26% of the total variance. The first two principal components explained 75.21% of the total variance, as shown in **Figure 1**. This figure shows the scores of the samples according to these first two components and overlays the loadings (the location in the PC space of the original variables). The first principal component was heavily negatively correlated with C, B1, B3, T, and mDP. The second principal component

Table 2. Levels of Monomeric and Oligomeric Flavan-3-ols in Seed and Skin Tannin Extract in 2006[a]

	CS[a] from vineyard					M[a] from vineyard					CS[b]	M[b]
	V1	V2	V5	V6	V7	V1	V2	V5	V6	V7	mean value, $n = 5$	mean value, $n = 5$
Seed Extract												
C	8.546 ± 1.330	13.342 ± 0.560	6.248 ± 1.890	4.745 ± 0.980	9.460 ± 0.780	3.388 ± 1.110	10.572 ± 2.340	4.379 ± 1.560	2.602 ± 1.970	4.313 ± 0.890	8.468a	5.051b
EC	2.722 ± 0.750	6.768 ± 0.990	1.447 ± 0.080	6.799 ± 0.050	8.094 ± 0.450	2.411 ± 0.430	3.556 ± 0.980	10.101 ± 1.120	9.563 ± 2.100	3.798 ± 1.870	5.166a	5.885a
ECG	0.202 ± 0.050	0.581 ± 0.077	0.463 ± 0.011	0.428 ± 0.013	0.676 ± 0.011	0.277 ± 0.004	0.155 ± 0.003	2.429 ± 0.008	0.702 ± 0.022	0.176 ± 0.001	0.470a	0.748a
B1	3.698 ± 0.003	4.677 ± 0.077	3.128 ± 0.102	4.767 ± 0.022	5.660 ± 0.036	3.356 ± 0.049	2.897 ± 0.410	4.952 ± 0.068	1.608 ± 0.020	3.035 ± 0.030	4.386a	3.170b
B2	0.768 ± 0.384	1.781 ± 0.891	0.665 ± 0.007	1.441 ± 0.720	0.747 ± 0.374	0.846 ± 0.423	0.700 ± 0.550	1.922 ± 0.963	1.708 ± 0.854	0.847 ± 0.424	1.080a	1.205a
B3	1.092 ± 0.492	1.944 ± 0.660	1.623 ± 0.165	1.070 ± 0.135	1.766 ± 0.133	0.545 ± 0.072	1.342 ± 0.269	0.767 ± 0.834	0.165 ± 0.383	0.478 ± 0.239	1.499a	0.659b
B4	0.451 ± 0.070	0.637 ± 0.004	0.275 ± 0.065	0.171 ± 0.005	0.256 ± 0.002	0.280 ± 0.002	0.278 ± 0.005	0.891 ± 0.014	1.648 ± 0.034	0.266 ± 0.008	0.358a	0.673a
T	1.001 ± 0.240	0.329 ± 0.080	4.263 ± 0.049	2.472 ± 0.030	3.423 ± 0.139	0.598 ± 0.025	1.597 ± 0.016	0.685 ± 0.068	0.595 ± 0.073	0.428 ± 0.040	2.298a	0.781b
Skin Extract												
C	0.020 ± 0.010	0.129 ± 0.050	0.103 ± 0.020	0.212 ± 0.150	0.170 ± 0.130	0.231 ± 0.010	0.040 ± 0.002	0.350 ± 0.000	0.330 ± 0.030	0.610 ± 0.120	0.127a	0.312a
EC	0.016 ± 0.001	0.111 ± 0.001	0.115 ± 0.002	0.119 ± 0.011	0.128 ± 0.012	0.021 ± 0.001	0.032 ± 0.001	0.006 ± 0.001	0.025 ± 0.002	0.072 ± 0.003	0.098a	0.031b
ECG	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B1	0.001 ± 0.0001	0.103 ± 0.001	0.034 ± 0.003	0.005 ± 0.001	0.023 ± 0.001	0.024 ± 0.001	0.002 ± 0.001	0.021 ± 0.002	0.013 ± 0.003	0.015 ± 0.001	0.033a	0.020a
B2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B3	0.120 ± 0.011	0.060 ± 0.001	0.190 ± 0.002	0.040 ± 0.001	0.010 ± 0.002	0.010 ± 0.000	0.030 ± 0.001	nd	nd	0.019 ± 0.001	0.084a	0.015b
B4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
T	nd	0.010 ± 0.001	nd	tr	tr	0.035 ± 0.001	nd	tr	nd	nd	0.010a	0.035b

[a] Data are means of triplicate determination. In units of mg/g dw for seed and skin tannin extract, ± standard deviation over three replications in one grape sample. CS, Cabernet Sauvignon; M, Merlot; nd, not detected; tr, traces (<0.001). [b] ANOVA to compare data; values with different letters within each row are significantly different (Tukey's test, $p < 0.05$); *n*, number of grape samples.

Table 3. Levels of Monomeric and Oligomeric Flavan-3-ols in Seed and Skin Tannin Extract in 2007[a]

	CS[a] from vineyard					M[a] from vineyard					CS[b]	M[b]
	V1	V2	V5	V6	V7	V1	V2	V5	V6	V7	mean value, $n = 5$	mean value, $n = 5$
						Seed Extract						
C	6.678 ± 0.040	2.876 ± 0.010	15.332 ± 1.550	20.951 ± 0.980	41.240 ± 4.511	9.044 ± 0.099	8.484 ± 0.054	10.426 ± 0.080	14.604 ± 0.960	14.362 ± 0.140	17.415a	11.384a
EC	4.448 ± 0.023	1.871 ± 0.028	7.144 ± 0.760	6.003 ± 0.010	23.247 ± 2.650	11.077 ± 0.030	9.723 ± 0.070	10.420 ± 1.150	11.019 ± 0.825	11.156 ± 1.240	8.542a	10.679a
ECG	1.258 ± 0.030	2.771 ± 0.04	2.364 ± 0.060	2.327 ± 0.070	2.681 ± 0.050	1.075 ± 0.014	0.575 ± 0.010	8.107 ± 0.020	2.656 ± 0.090	0.697 ± 0.010	2.280a	2.622a
B1	0.362 ± 0.016	0.200 ± 0.012	0.416 ± 0.029	2.033 ± 0.150	0.551 ± 0.015	0.518 ± 0.038	0.576 ± 0.041	0.624 ± 0.050	0.624 ± 0.050	0.849 ± 0.042	0.712a	0.638a
B2	2.592 ± 0.024	1.882 ± 1.724	0.801 ± 0.188	10.711 ± 0.161	17.648 ± 0.200	4.670 ± 0.040	3.818 ± 0.091	4.209 ± 0.121	1.002 ± 0.082	5.190 ± 0.138	6.727a	3.778a
B3	0.415 ± 0.210	0.211 ± 0.110	0.753 ± 0.430	1.999 ± 0.960	4.998 ± 2.540	0.949 ± 0.480	0.926 ± 0.480	1.044 ± 0.560	1.872 ± 0.950	1.108 ± 0.030	1.675a	1.180a
B4	1.436 ± 0.030	0.797 ± 0.014	2.410 ± 0.350	5.882 ± 0.146	8.183 ± 0.123	2.924 ± 0.150	2.642 ± 0.015	2.120 ± 0.095	2.410 ± 0.100	3.648 ± 0.145	3.741a	2.750a
T	nd	nd	nd	0.265 ± 0.020	0.191 ± 0.010	9.046 ± 0.058	0.080 ± 0.006	0.026 ± 0.008	8.093 ± 0.028	0.137 ± 0.010	0.228a	3.476b
						Skin Extract						
C	0.292 ± 0.001	1.383 ± 0.046	0.592 ± 0.037	0.476 ± 0.037	1.067 ± 0.008	0.149 ± 0.020	0.691 ± 0.010	0.065 ± 0.001	0.059 ± 0.003	0.252 ± 0.012	0.762a	0.243a
EC	2.026 ± 0.014	0.684 ± 0.012	0.248 ± 0.002	0.060 ± 0.003	0.105 ± 0.004	0.084 ± 0.000	0.154 ± 0.004	0.721 ± 0.024	0.717 ± 0.030	0.727 ± 0.017	0.625a	0.481a
ECG	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B1	0.037 ± 0.001	0.131 ± 0.018	nd	nd	nd	nd	nd	0.048 ± 0.002	nd	0.361 ± 0.007	0.084a	0.205a
B2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B3	0.183 ± 0.001	6.799 ± 0.032	0.202 ± 0.008	0.094 ± 0.002	0.232 ± 0.002	0.056 ± 0.003	1.690 ± 0.110	nd	0.231 ± 0.002	0.445 ± 0.011	1.502a	0.606a
B4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
T	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

[a] Data are means of triplicate determination. In units of mg/g dw for seed and skin tannin extract, ± standard deviation over three replications in one grape sample. CS, Cabernet Sauvignon; M, Merlot; nd, not detected. [b] ANOVA to compare data; values with different letters within each row are significantly different (Tukey's test, $p < 0.05$); n, number of grape samples.

was strongly negatively correlated with %G. Projection of the cases on the first two components showed that two groups of samples could readily be differentiated. The first group includes the CS samples, which generally showed a strong correlation with B1, B3, C, T, mDP, and %G; the majority of them are found on the left side of the first factorial plane. The second group was formed by the five M samples, placed on the right side of the first factorial plane, which shows that these individuals are opposed to the variables B1, B3, C, T, mDP, and %G. This fact allows us to assume that there was a discrimination in tannin composition of CS and M seed tannin extracts.

Skin Extracts. Among the eight studied variables (C, EC, B1, B3, T, mDP, %G, %P), five were significantly different (EC, B3, T, %P, %G, $p < 0.05$) (**Table 6**). Three significant components, which accounted for 94.42% of the total variance, were computed. A biplot constructed from the first two principal components, explaining 81.39% of the variance, is displayed in **Figure 2**. As can be seen in this figure, the first principal component is strongly negatively correlated with EC, %P, and %G, whereas the second principal component is negatively correlated with B3 and heavily positively correlated with T. Likewise, the data seems to form a pattern on this plot. For instance, the CS variety is placed in the left hand of the first factorial plane, because this variety shows a strong correlation with EC, B3, T, %G, and %P. In contrast, the M variety is placed in the right-hand corner of the first factorial plane, which shows that this variety has a weak negative correlation with EC, B3, T, %G, and %P. Thus, M and CS skin tannins can be clearly differentiated by their composition.

Comparative Proanthocyanidin Composition between CS and M Grapes in 2007. *Seed Extracts.* Identically as in the 2006 vintage, the same variables were examined for the vintage 2007 (**Tables 3** and **5**). Tannin CS and M profiles differed significantly on mDP and T concentration ($p < 0.05$). In vintage 2007 the M presented higher concentration of T, whereas the opposite was observed in vintage 2006. These contradictory findings could be attributed to the plot variation. As shown in **Table 3**, important variability was observed within the vineyards for the same variety. For example, in the case of CS seed extracts, the T was not detected in three vineyards. Because the data concerning the climatic conditions of each plot were unavailable, no further exploitation of this finding could be made. There were no significant differences for the other studied variables.

Skin Extracts. The same tannin aspects of vintage 2006 were studied, and important differences between CS and M were found for %G and %P ($p < 0.05$, **Table 5**). **Figure 3** indicates that for these variables CS showed a richer profile compared to M.

It is interesting to note that for both vintages grape variety influenced the tannin composition of both skin and seed tannin extracts, but it has showed different behaviors according to the vintage. The only consistent differences between CS and M in both vintages were the mDP in seed extracts and the %G and %P in skin extracts. A correlation study was performed on the data generated by chemical analysis of grapes from vintages 2006 and 2007. The data sets corresponding to each vintage were combined and submitted to a two-way ANOVA analysis with factor 1 variety (CS and M) and factor 2 vintage (2006 and 2007). In the case of seed extracts, grape variety effect was observed for mDP (**Table 6**), whereas a vintage effect was found for C, ECG, B1, B2, and B4 and for %G (**Figure 4**). **Figure 4**

Table 4. Structural Characteristics and Composition (Percent in Moles) of Seed and Skin Tannin Extract in 2006*

		CS[a] from vineyard					M[a] from vineyard					CS [b]	M[b]
		V1	V2	V5	V6	V7	V1	V2	V5	V6	V7	mean value, $n = 5$	mean value, $n = 5$
Seed Extracts													
terminal units	C	65	67.7	61.2	70	72.5	43.9	45.9	49.1	76.1	59.1	67.3	54.8
	EC	32.7	33	34.5	29.4	26.6	55.9	53.5	49.6	37.2	40.3	31.2	47.3
	ECG	2.3	2.7	1.9	0.6	1.0	0.2	0.6	1.5	2.1	0.6	1.7	1.0
extension units	C	46.2	21.5	31.1	23.5	40.3	24.0	24.9	44.2	22.7	20.2	32.5	27.2
	EC	34.4	54.6	33.5	58.9	32.8	67.6	68.1	41.9	39.1	45.3	42.8	52.4
	ECG	19.3	23.9	35.5	17.6	26.9	8.4	6.9	13.8	38.2	34.5	24.6	20.4
	EGC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
mDP		4.4	2.7	3.8	2.5	4.7	2.5	2.2	2.6	2.2	2.6	3.6 a	2.4 b
%G		17.9	15.9	26.7	11.6	21.4	5.0	4.1	9.1	10.9	21.3	18.7 a	10.1 b
%P													
Skin Extracts													
terminal units	C	63.9	71.1	41.7	35.6	40.3	34.7	43.3	52.6	40.6	78.8	50.5	50.0
	EC	36.1	28.9	61.9	60.7	59.7	65.3	56.7	47.4	59.4	21.2	49.5	50.0
	ECG	0	0	0	0	0	0	0	0	0	0	0	0
extension units	C	38.1	40.5	34.4	53.8	11.9	20.3	59.8	49.6	54.8	24.7	35.7	41.8
	EC	56.3	40.5	46.4	38.7	73.3	76.1	33.5	49.2	41.6	70.3	51.0	54.1
	ECG	1.5	39.5	2.9	2.1	2.9	1.1	2.6	0.4	0.8	2.4	9. 8	1.5
	EGC	4.1	15.1	16.3	6.0	12.0	2.5	4.1	0.7	2.8	2.6	10.7	2.5
mDP		15.7	25.1	13.8	27.4	27.7	16.3	15.7	27.1	26.3	35.4	21.9 a	24.2 a
%G		1.4	4.6	2.7	2.0	2.8	1.0	2.4	0.4	0.8	2.4	2.7 a	1.4 b
%P		3.9	14.5	15.1	5.8	11.6	2.4	3.8	0.7	2.7	2.5	10.2 a	2.4 b

[a] CS, Cabernet Sauvignon; M, Merlot; %P, percentage of prodelphinidins; %G, percentage of galloylation; mDP, mean degree of polymerization; nd, not detected. [b] ANOVA to compare data; values with different letters within each row are significantly different (Tukey's test, $p < 0.05$); *n*, number of grape samples.

Table 5. Structural Characteristics and Composition (Percent in Moles) of Seed and Skin Tannin Extract in 2007[a]

		CS[a] from vineyard					M[a] from vineyard					CS[b]	M[b]
		V1	V2	V5	V6	V7	V1	V2	V5	V6	V7	mean value, $n = 5$	mean value, $n = 5$
Seed Extract													
terminal units	C	51.1	47.4	52.1	35.9	10.6	46	29	45	50.5	18.5	39.4	42.6
	EC	35.1	41.5	30.3	57.9	87	45.2	60.9	40.2	46.5	76.6	50.3	48.2
	ECG	13.9	11.1	17.6	6.2	2.4	15.4	10.1	14.8	3.0	4.9	10.2	10.8
extension units	C	6.8	6.8	18.7	80.3	28.6	7.6	8.3	8.6	52.5	18.5	28.3	19.2
	EC	30.2	34.5	24.5	7.5	50.9	30.6	29.5	31.2	17.0	76.6	29.5	27.1
	ECG	63.0	58.7	56.8	12.2	20.5	61.8	62.2	60.1	30.5	4.9	42.2	53.7
	EGC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
mDP		3.8	3.9	7.2	8.8	4.1	3.5	2.8	3.3	2.3	2.0	5.5 a	3.0 b
%G		50.1	46.5	51.3	11.6	16.1	48.7	34.2	46.5	17.0	20.3	35.1 a	36.6 a
%P													
Skin Extract													
terminal units	C	87.2	99.8	64.0	40.9	82.0	99.6	98.9	99.0	92.0	99.8	74.8	97.4
	EC	4.3	0	5.2	48.3	0	0	0	0	6.7	0	11.5	1.7
	ECG	4.2	0.2	12.1	10.8	8.0	0.4	1.1	1.0	1.2	0.5	7.1	0.9
extension units	C	76.5	12.7	84.9	56.1	53.3	31.3	82.2	92.8	92.4	19.9	56.7	74.7
	EC	2.6	65.3	1.4	27	40.6	61.9	7.8	2.4	2.3	79.2	27.4	18.6
	ECG	2.1	1.9	9.3	3.3	1.8	1.5	1.5	1.8	2.4	0.2	3.7	1.8
	EGC	2.1	19.3	4.4	13.6	2.7	5.2	8.5	3	2.9	0.7	8.4	4.9
mDP		29.4	15.7	48.8	7.8	31.9	4.3	13.1	22.4	24.2	11.7	26.7 a	16.0 a
%G		2.2	2.5	9.4	4.2	3.5	1.3	1.4	1.8	2.3	0.2	4.4 a	1.7 b
%P		18.2	19.4	4.3	11.9	2.5	4.0	7.8	2.9	2.7	0.6	11.3 a	4.4 b

[a] CS, Cabernet Sauvignon; M, Merlot; %P, percentage of prodelphinidins; %G, percentage of galloylation; mDP, mean degree of polymerization; nd, not detected. [b] ANOVA to compare data; values with different letters within each row are significantly different (Tukey's test, $p < 0.05$); *n*, number of grape samples.

shows the PCA score plot for the first two PCs, which explain 82.28% of the total variance. The first component is positively represented by the variables C, ECG, B2, and B4. B1 was positively and negatively represented by the first and the second component, respectively. The second component is strongly positively represented by %G. The projection of the grape seed samples in the first two components showed that the seed samples were well separated by vintage. The 10 grape seed samples of 2006 (two replications of each vineyard) are concentrated in the bottom left quadrant of the first factorial plan and showed rather small variability within this vintage. The 2007 grape seed samples (also two replications of each vineyard) are localized entirely on the second factorial plan, showing a great variability within this vintage. In the case of skin extracts the grape variety has an influence on %G and %P and the vintage on EC concentration (**Table 6**). The magnitude of the influence that the variety had on the seed tannin extracts was less than that of the vintage year, whereas the opposite was observed in skin tannin extracts. These results suggest that tannins are dependent on variety, on vintage (*32*), and, according to our findings, on tannin source (skins or seeds). Preys et al. (*33*)

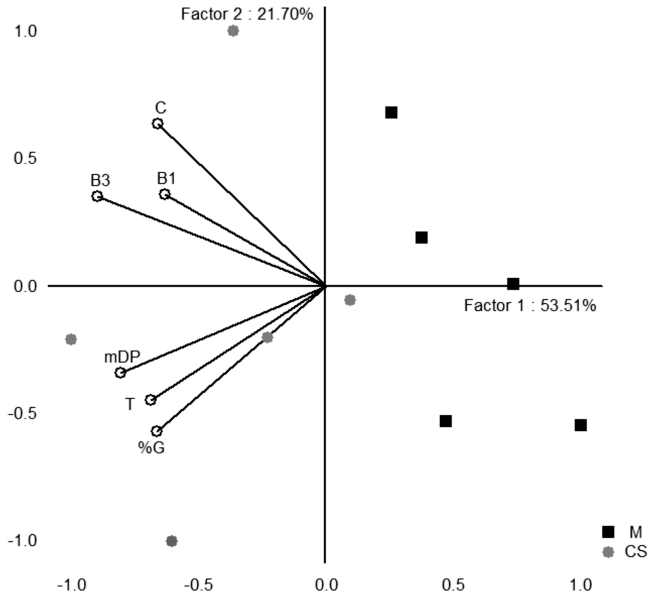

Figure 1. Principal component analysis of Seed Tannin Extract composition for 2006 (factor 1 vs factor 2).

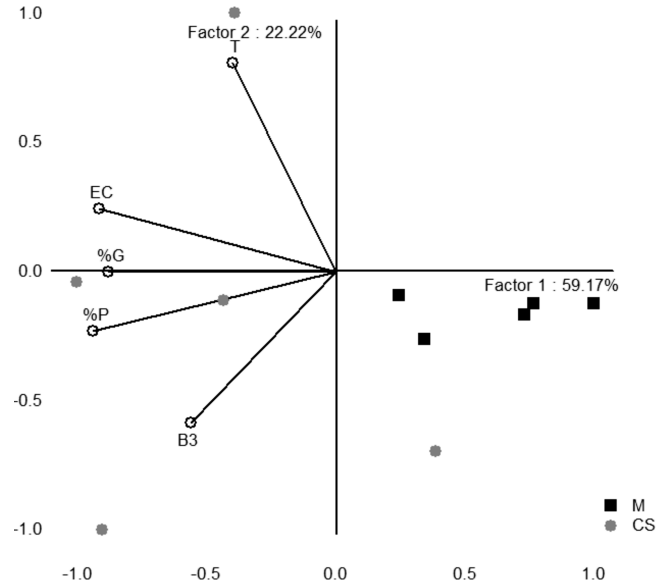

Figure 2. Principal component analysis of Skin Tannin Extract composition for 2006 (factor 1 vs factor 2).

Table 6. Comparison of Grape Variety and Vintage Effect of the Studied Variables on Skin and Seed Tannin Extract[a]

	variety[b]		vintage[b]	
	CS[a] mean value (mg/g), $n = 10$	M[a] mean value (mg/g), $n = 10$	2006 mean value (mg/g), $n = 10$	2007 mean value (mg/g), $n = 10$
		Seed Extract		
C	12.94 ± 3.58 a	8.19 ± 1.38 a	6.76 ± 1.12 a	14.37 ± 3.39 b
EC	6.85 ± 1.96 a	8.28 ± 1.12 a	5.53 ± 0.98 a	9.61 ± 1.82 a
ECG	1.38 ± 0.33 a	1.68 ± 0.76 a	**0.60 ± 0.21 a**	**2.45 ± 0.68 b**
B1	2.55 ± 0.66 a	1.90 ± 0.49 a	**3.77 ± 0.38 a**	**0.67 ± 0.16 b**
B2	3.90 ± 1.79 a	2.80 ± 0.55 a	**1.14 ± 0.16 a**	**5.56 ± 1.58 b**
B3	1.59 ± 0.43 a	0.92 ± 0.15 a	1.08 ± 0.19 a	1.43 ± 0.43 a
B4	2.05 ± 0.88 a	1.71 ± 0.39 a	**0.52 ± 0.14 a**	**3.25 ± 0.70 b**
T	1.18 ± 0.5 a	2.13 ± 1.09 a	1.54 ± 0.44 a	1.77 ± 1.14 a
mDP	**4.59 ± 0.61 a**	**2.60 ± 0.16 b**	3.02 ± 0.30 a	4.18 ± 0.68 a
%G	26.91 ± 5.10 a	21.71 ± 5.15 a	**14.4 ± 2.37 a**	**34.24 ± 5.15 b**
		Skin Extract		
C	0.45 ± 0.14 a	0.28 ± 0.07 a	0.22 ± 0.05 a	0.50 ± 0.14 a
EC	0.36 ± 0.20 a	0.26 ± 0.09 a	**0.07 ± 0.02 a**	**0.55 ± 0.18 b**
ECG	nd	nd	nd	nd
B1	0.02 ± 0.01 a	0.05 ± 0.035 a	0.03 ± 0.01 a	0.05 ± 0.04 a
B2	nd	nd	nd	nd
B3	0.79 ± 0.67 a	0.25 ± 0.16 a	0.05 ± 0.02 a	0.99 ± 0.66 a
B4	nd	nd	nd	nd
T	nd	nd	nd	nd
mDP[a]	24.30 ± 3.78 a	19.60 ± 2.90 a	23.10 ± 2.27 a	20.94 ± 4.20 a
%G[a]	**3.53 ± 0.73 a**	**1.40 ± 0.70 b**	2.06 ± 0.40 a	2.87 ± 0.80 a
%P[a]	**10.54 ± 1.98 a**	**2.97 ± 1.6 b**	6.30 ± 1.70 a	7.21 ± 1.21 a

[a] In units of mg/g dw for seed and skin tannin extract. CS, Cabernet Sauvignon; M, Merlot, %P, percentage of prodelphinidins; %G, percentage of galloylation; mDP, mean degree of polymerization. [b] ANOVA was made separately to compare vintage and variety effect; values with different letters within each row are significantly different (Tukey's test, $p < 0.05$); ±, standard error over three replications in one grape sample; n, number of grape samples; nd, not detected.

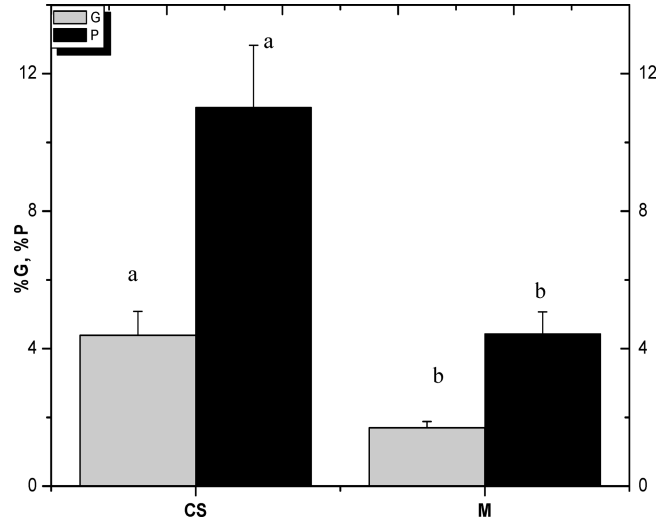

Figure 3. %P and %G (percentage of prodelphinidins and galloylation, respectively) in Skin Tannin Extract for the two grape varieties studied in 2007. Values with different letters are significantly different (Tukey's test $p < 0.05$). Bars represent standard deviation ($n = 5$ and 5).

have clearly observed this for Dornfelder and for Gamay wines, for two vintages, for which differences associated with tannin content, galloylation percentage, and mDP were reported.

Besides the mentioned significant differences in tannin profile between CS and M, a closer look at **Table 6** could lead to further remarks. For both vintages, in seed extracts EC content in M was higher than in CS, whereas CS presented a higher percentage of G than M. These findings are in accordance with Soleas et al. (*23*), who found that M wine exhibited a higher content of epicatechin than CS.

Sensory Analysis. Although several researchers have studied the impact of some media characteristics on bitterness and astringency, they often involved simple molecules such as flavan-3-ols (*34*−*36*), alums, or acids. The influence of grape variety on tannin extract perception has been investigated to a lesser extent (*22, 37*). The next step after the chemical analyses was to check if the factor variety could influence the tannin bitterness and astringency intensity.

Comparative Sensory Analysis between CS and M in 2006. **Table 7** shows the notes that were attributed by the judges. The panelist effect was not significant ($p > 0.05$), suggesting that the evaluation of astringency and bitterness of tannin extracts was made by homogeneous judges. On a scale of intensity of 0−7 points, the average scores for astringency were 5.2 and 4.2 for seed tannins extracts and 5.5 and 4.9 for skin tannin extracts in the cases of CS and M, respectively. As far

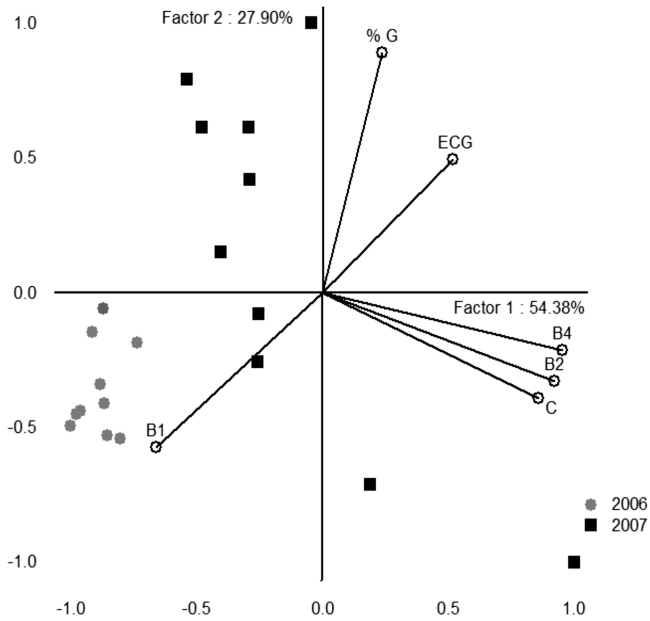

Figure 4. Principal component analysis of Seed Tannin Extract composition for 2006 and 2007 (factor 1 vs factor 2).

Table 7. Comparison of Astringency and Bitterness Intensity between Varieties in 2006[a]

	astringency intensity		bitterness intensity	
tannin extract	CS[a]	M[a]	CS[a]	M[a]
seeds	5.2 ± 2.1 a	4.2 ± 1.1 a	4.7 ± 1.3 a	3.9 ± 1.3 a
skins	5.5 ± 2.3 a	4.9 ± 1.4 a	5.2 ± 1.2 a	4.8 ± 1.3 a

[a] CS, Cabernet Sauvignon; M, Merlot. Mean astringency and bitterness over two repetitions; ±, standard deviation over the two repetitions; values with different letters in a row are significantly different (Duncan's test $p < 0.05$).

as bitterness intensity is concerned in seed tannin extracts the average scores were 4.7 and 3.9 and in skin tannin extracts the average scores were 5.2 and 4.8 for CS and M, respectively. Even if the panel is trained, standard deviation may still be important due to personal perception. No significant differences were found on astringency or on bitterness intensity ($p > 0.05$) for both skin and seed tannin extracts between CS and M. CS and M varieties did not have a significant difference in sensory perception of tannin extracts despite the chemical differences in tannin composition between them. With the exception of variations in proanthocyanidin composition (such as polymer size, extent of galloylation, and formation of derivatives) the other factors that influence these sensations (including sensory methods and interactions in mixtures as well as physiologic factors) could explain our findings. The intensity of astringency and bitterness builds up when several samples are tasted. Increasing the ethanol concentration of wine from 8 to 14% (by vol) approximately doubled the bitterness intensity but had no effect on astringency (*38, 39*). Ethanol enhancement of bitterness was also observed with oligomeric tannins in model wine solutions (*40*). Saliva may also influence astringency; it has been speculated that when subjects were partitioned into groups on the basis of their salivary flow rates and the data were analyzed separately, low-flow subjects perceived the maximal intensity of astringency later and rated it more intensely and for a longer time than did high-flow subjects, for both red (*41*) and white wines (*38*).

Comparative Sensory Analysis between CS and M in 2007. In vintage 2007, the average values for astringency were 3.9 and 4.2 in seed tannin extracts and 4.0 and 4.2 in skin tannin extracts for CS and M, respectively (**Table 8**). The average scores attributed by the judges for bitterness intensity were 3.8 and 4.2 in seed tannin extracts and 3.1 and 3.6 in skin tannin extracts for CS and M, respectively (**Table 8**). Statistical analyses showed that the grape varieties did not affect the astringency and bitterness intensity in either skin or seed tannin extracts ($p > 0.05$). Again, these observations could be attributed to the above-mentioned factors (sensory methods and interactions in mixtures as well as physiologic factors).

Table 8. Comparison of Astringency and Bitterness Intensity between Varieties in 2007

	astringency intensity		bitterness intensity	
tannin extract	CS[a]	M[a]	CS[a]	M[a]
seeds	3.9 ± 1.2 a	4.2 ± 0.9 a	4.0 ± 1.1 a	4.2 ± 1.4 a
skins	3.8 ± 1.1 a	4.2 ± 1.1 a	3.1 ± 0.8 a	3.6 ± 1.1 a

[a] CS, Cabernet Sauvignon; M, Merlot Mean astringency and bitterness over two repetitions; ±, standard deviation over the two repetitions; values with different letters in a row are significantly different (Duncan's test $p < 0.05$).

For both vintages, the grape variety showed the same behavior pattern. It influenced neither the bitterness nor the astringency intensity. A closer look at the data reveals similar assessment of bitterness and astringency intensity, but in general, lower intensity values were attributed to the orally perceived attributes in 2007.

Some studies have examined the mouthfeel properties of grape proanthocyanidins (*42*−*45*) so as to improve our understanding of the relationship between polyphenol sensory properties and their structure.

To investigate the extent to which our sensory measures could be explained by the proanthocyanidin composition, Pearson's correlation was performed. Because the grape variety did not influence the astringency or bitterness intensity, we have grouped the chemical and sensory data for seed tannin extracts in 2006 and 2007 vintage and also for skin tannin extracts in 2006 and 2007 vintages. In skin tannin extracts, a positive relationship was found between B3 content ($r = 0.74$, $p < 0.04$, vintage 2006), mDP ($r = 0.647$, $p < 0.04$, vintage 2007), and astringency intensity. No correlation was pointed out between both astringency and bitterness intensity and chemical data for vintages 2006 and 2007 in seed tannin extracts. The correlation between astringency and mDP has been also confirmed by another study (*31*). Vidal et al. (*31*) have previously evaluated the mouthfeel properties of different proanthocyanidin fractions in wine-like solutions and showed that the higher the mDP, the higher the overall astringency. In another study, Fernandez et al. (*22*) demonstrated that Carmenère wines were perceived as less astringent than CS wines, despite the fact that they presented a higher proanthocyanidin concentration and a higher mDP than CS wines. The apparent paradox was explained by the higher amount of epigallocatechin subunits of Carmenère wine proanthocyanidins. The positive correlation of B3 concentration with astringency intensity in skin tannin extracts is also interesting. Peleg et al. (*6*) demonstrated that the maximum intensity of the oral astringency of monomers was significantly lower than that of the dimers or trimers, which did not differ significantly. There are a number of reasons that all of the previous research findings (*42*−*46*) correlating both astringency and bitterness intensity with chemical data have not been confirmed. In this study, the trained judges defined astringency as drying or puckering in the mouth without attention to subqualities. They did not evaluate model wine samples using the mouthfeel wheel and specific mouthfeel descriptors with astringency subqualities.

Thus, the judges may have not been able to specifically express the mouthfeel quality perceived. Instead, they classified their perceptions as astringent and bitter. The relationship between tannin extracts and astringency should be further explored with a panel trained to define more specific subqualities of astringency, such as fine, grainy, dry, and chalky. Moreover, judges, even if they are trained, do not always describe bitter and astringent perceptions with the expected descriptors. Recently, Lesschaeve (unpublished observations, 2003) studied the relationship between the consumer language used to express likes and dislikes and sensory descriptors of red wines. She found that when consumers tended to like the wines, they did not use bitter as a descriptor; bitter was used to express dislike and tended to be associated with acid and astringent sensory characteristics, not bitterness. In the same study, consumers who liked astringent wines described them as having "a lot of character" or "a long aftertaste".

In addition, for the bitter taste, no general rule has ever been reported to relate the structure of the molecules and bitterness (*47*). Robichaud and Noble investigated the bitterness of some phenolic compounds (*42*); they found that (+)-catechin and gallic acid were more bitter than astringent. Boselli et al. (*37*) studied the sensory aspects and the related chemical components of five minor Italian Denominazione di Origine Controllata (DOC) red wines; they created a predictive model for astringency but not for bitterness. Landon et al. (*48*) indicated that perceived astringency as well as bitterness was significantly correlated with tannin levels in Washington wines. Schlosser et al. (*49*), who studied Chardonnay wines from three regions of the Niagara peninsula, have demonstrated no differences for aroma taste (perceived acidity, bitterness) and mouthfeel terms (astringency, body). Little is currently known about the relationship between bitterness and molecular structure; the number and diversity of bitter tastants indicate that several transduction mechanisms may be involved (*50*).

In our work we investigated the influence of grape variety on proanthocyanidin composition and sensory perception of grape skin and seed tannin extracts from Bordeaux wine grapes. Until now, grape variety effect on proanthocyanidin composition and sensory perception has never been studied in Bordeaux predominant wine grape varieties. We have demonstrated that there are aspects of proanthocyanidin (%P and %G in skin tannin extracts, mDP in seed tannin extracts) that might differentiate tannin composition of skin and seed tannin extracts according to variety. The astringency and bitterness intensity for both skin and seed extracts did not differentiate between CS and M. Although difficulties in creating a predictive model for sensory perception of skin and seed tannin extracts occurred, relationships between astringency and proanthocyanidin composition (particularly with mDP and B3 content) were observed in tannin skin extracts. This work will be continued focusing on the grape variety effect on mouth textural characteristics of tannin extracts. Despite recent findings, grape variety discrimination according to grape proanthocyanidin composition and sensory perception remains a major challenge of wine research.

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LITERATURE CITED

(1) Robinson, J. *The Oxford Companion to Wine*; Oxford University Press: Oxford, U.K., 2006; pp 119−121.
(2) Falcao, L. D.; de Revel, G.; Perello, M. C.; Moutsiou, A.; Zanus, M. C.; Bordignon-Luiz, M. T. A Survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C13-norisoprenoids, and the sensory profile of brazilian Cabernet Sauvignon wines. *J. Agric. Food Chem.* **2007**, *55*, 3605–3612.
(3) Bowers, I. E.; Meredith, C. P. The parentage of a classic vinegrape Cabernet Sauvignon. *Nat. Genet.* **1997**, *16*, 8–87.
(4) Clarke, O. *Oz Clarke Encyclopedia of Grapes*; Harcourt: New York, 2001; pp 132−133.
(5) Gawel, R. Red wine astringency: a review. *Aust. J. Grape Wine Res.* **1998**, *4*, 73–95.
(6) Peleg, H.; Gacon, K.; Schlich, P.; Noble, A. C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric.* **1999**, *79*, 1123–1128.
(7) Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175–2186.
(8) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781–784.
(9) Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509–512.
(10) Romeyer, F. M.; Macheix, J.-J.; Sapis, J.-C. Changes and importance of oligomeric procyanidins during maturation of grape seeds. *Phytochemistry* **1985**, *25*, 219–221.
(11) Czochanska, Z.; Foo, L. Y.; Porter, L. J. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **1979**, *18*, 1819–1822.
(12) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds—nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* **2000**, *80*, 1094–1117.
(13) Oszmianski, J.; Ramos, T.; Bourzeix, M. Fractionation of phenolic compounds in red wine. *Am. J. Enol. Vitic.* **1988**, *39*, 259–262.
(14) Baranowski, J. D.; Nagel, C. W. Isolation and identification of the hydroxycinnamic acid derivatives in white Riesling wine. *Am. J. Enol. Vitic.* **1981**, *32*, 5–13.
(15) Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890–894.
(16) Striegler, R. K.; Morris, J. R. Yield and quality of wine grape cultivars in Arkansas. *Am. J. Enol. Vitic.* **1984**, *35*, 216–219.
(17) Muniz, J. E.; Wample, R. L.; Loescher, W. H. Cultivar differences in response to low temperatures in *Vitis vinifera* callus in vitro. *Am. J. Enol. Vitic.* **1991**, *42*, 341–346.
(18) Perez, J. R.; Kliewer, W. M. Influence of light regime and nitrate fertilization on nitrate reductase activity and concentrations of nitrate and arginine in tissues of three cultivars of grapevines. *Am. J. Enol. Vitic.* **1982**, *33*, 86–93.
(19) Kobriger, J. M.; Kliewer, W. M.; Lagier, S. T. Effects of wind on water relations of several grapevine cultivars. *Am. J. Enol. Vitic.* **1984**, *35*, 164–169.
(20) Threlfall, R. T.; Morris, J. R.; Meullenet, J. F.; Striegler, R. K. Sensory characteristics, composition, and nutraceutical content of juice from *Vitis rotundifolia* (Muscadine) Cultivars. *Am. J. Enol. Vitic.* **2007**, *58*, 268–273.
(21) Gonzalez-Neves, G.; Charamelo, D.; Balado, J.; Barreiro, L.; Bochicchio, R.; Gatto, G.; Gil, G.; Tessore, A.; Carbonneau, A.; Moutounet, M. Phenolic potential of Tannat, Cabernet-Sauvignon and Merlot grapes and their correspondence with wine composition. *Chim. Anal.* **2004**, *513*, 191–196.
(22) 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.
(23) Soleas, G. J.; Dam, J.; Carey, M.; Goldberg, D. M. Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic

constituents in Ontario wines. *J. Agric. Food Chem.* **1997**, *45*, 3871–3880.
(24) Tarascou, I.; Barathieu, K.; Andre, Y.; Pianet, I.; Dufourc, E. J.; Fouquet, E. An improved synthesis of procyanidin dimers: regio- and stereocontrol of the interflavan bond. *Eur. J. Org. Chem.* **2006**, *536*, 7–5377.
(25) Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P. L.; Saucier, C. Ethylidene-bridged flavan-3-ols in red wine and correlation with wine age. *J. Agric. Food Chem.* **2007**, *55*, 6292–6299.
(26) Cheynier, V.; Moutonet, M.; Sarni-Machado, P. Les composés phénoliques In *Œnologie: Fondements Scientifiques et Technologiques*; Flanzy, C., Ed.; Lavoisier Tec & Doc: Paris, France, 1998; pp124−164.
(27) Mateus, N.; Marques, S.; Goncalves, A. C.; Machado, J. M.; De Freitas, V. Proanthocyanidin composition of red Vitis vinifera varieties from the Douro valley during ripening: Influence of cultivation altitude. *Am. J. Enol. Vitic.* **2001**, *52*, 115–121.
(28) De Freitas, V. A. P.; Glories, Y. Concentration and compositional changes of procyanidins in grape seeds and skin of white *Vitis vinifera* varieties. *J. Sci. Food Agric.* **1999**, *79*, 1601–1606.
(29) Monagas, M.; Gomez-Cordoves, C.; Bartolome, B.; Laureano, O.; Ricardo Da Silva, J. M. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003**, *51*, 6475–6481.
(30) Sun, B. S.; Pinto, T.; Leandro, M. C.; Ricardo-Da-Silva, J. M.; Spranger, M. I. Transfer of catechins and proanthocyanidins from solid parts of the grape cluster into wine. *Am. J. Enol. Vitic.* **1999**, *50*, 179–184.
(31) 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.
(32) Brossaud, F.; Cheynier, V.; Asselin, C.; Moutounet, M. Flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *Am. J. Enol. Vitic.* **1999**, *50*, 277–284.
(33) Preys, S.; Mazerolles, G.; Courcoux, P.; Samson, A.; Fischer, U.; Hanafi, M.; Bertrand, D.; Cheynier, V. Relationship between polyphenolic composition and some sensory properties in red wines using multiway analyses. *Anal. Chim. Acta* **2006**, *563*, 126–136.
(34) Gacon, K.; Peleg, H.; Noble, A. C. II-29. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Food Qual. Pref.* **1996**, *7*, 343–344.
(35) Kallithraka, S.; Clifford, M. N.; Bakker, J. II-2. Evaluation of astringency, mouth drying, mouth roughing and bitterness elicited by monomeric phenolic compounds in red wine and in model wine solution. *Food Qual. Pref.* **1996**, *7*, 334–65.
(36) Vidal, S.; Francis, L.; Noble, A.; Kwiatkowski, M.; Cheynier, V.; Waters, E. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Chim. Anal.* **2004**, *513*, 57–65.
(37) Boselli, E.; Boulton, R. B.; Thorngate, J. H.; Frega, N. G. Chemical and sensory characterization of DOC red wines from Marche (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **2004**, *52*, 3843–3854.
(38) Fischer, U.; Boulton, R. B.; Noble, A. C. Physiological factors contributing to the variability of sensory assessments: relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Qual. Pref.* **1994**, *5*, 55–64.
(39) Fischer, U. The influence of ethanol, pH, and phenolic composition on the temporal perception of bitterness and astringency, and parotid salivation M.Sc. Thesis, University of California, Davis, CA, 1990.
(40) Fontoin, H.; Saucier, C.; Teissedre, P. L.; Glories, Y. Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Qual. Pref.* **2008**, *19*, 286–291.
(41) Ishikawa, T.; Noble, A. C. Temporal perception of astringency and sweetness in red wine. *Food Qual. Pref.* **1995**, *6*, 27–33.
(42) Robichaud, J. L.; Noble, A. C. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **1990**, *53*, 343–353.
(43) Brossaud, F.; Cheynier, V.; Noble, A. C. Bitterness and astringency of grape and wine polyphenols. *Aust. J. Grape Wine Res.* **2001**, *7*, 33–39.
(44) Arnold, R. A.; Noble, A. C. Bitterness and astringency of grape seed phenolics in a model wine solution. *Am. J. Enol. Vitic.* **1978**, *29*, 150–152.
(45) Ricardo Da Silva, J. M.; Cheynier, V.; Souquet, J. M.; Moutonet, M.; Cabanis, J. C.; Bourzeix, M. Interaction of grape seed procyanidins with various proteins in relation to wine fining. *J. Sci. Food Agric.* **1991**, *57*, 111–125.
(46) Kallithraka, S.; Bakker, J.; Clifford, M. N. Evaluation of bitterness and astringency of (+)-catechin and (−)-epicatechin in red wine and in model solution. *J. Sensory Stud.* **1997**, *12*, 25–37.
(47) Maga, J. A. In *Bitterness in Foods and Beverages*; Rousseff, R. L., Ed.; Elsevier: Amsterdam, The Netherlands, 1990; Vol. 3, p 35.
(48) Landon, J. L.; Weller, K.; Harbertson, J. F.; Ross, C. F. Chemical and sensory evaluation of astringency in Washington State red wines. *Am. J. Enol. Vitic.* **2008**, *59*, 153–158.
(49) Schlosser, J.; Reynolds, A. G.; King, M.; Cliff, M. Canadian terroir: sensory characterization of Chardonnay in the Niagara peninsula. *Food Res. Int.* **2005**, *38*, 11–18.
(50) Andres-Barquin, P. J.; Conte, C. Molecular basis of bitter taste: the T2R family of G protein-coupled receptors. *Cell Biochem. Biophys.* **2004**, *1*, 99–112.

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