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Comparative Study of the Phenolic Composition of Seeds and Skins from Carménère and Cabernet Sauvignon Grape Varieties (*Vitis vinifera* L.) during Ripening

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The phenolic composition of skins and seeds from *Vitis vinifera* L. cv. Carménère and Cabernet Sauvignon grapes during ripening was evaluated by high-performance liquid chromatography−diode array detection and spectrophotometric analysis. As compared to Cabernet Sauvignon grape skins, Carménère grape skins presented higher contents of total anthocyanins, monomeric flavan-3-ols, and total flavonoids, a higher mean degree of polymerization, a higher percentage of galloylation, a higher average molecular weight of the flavanol fraction, and a higher color intensity. As compared to Cabernet Sauvignon grape seeds, Carménère grape seeds presented a lower content of monomeric flavan-3-ols, a higher mean degree of polymerization, a higher percentage of galloylation, a higher average molecular weight of the flavanol fraction, a lower content of (+)-catechin, and higher contents of (−)-epicatechin, epicatechin-3-*O*-gallate, gallic acid, and dimeric procyanidins esterified with gallic acid. Altogether, we conclude that Carménère grapes present a differential composition and evolution of phenolic compounds when compared to Cabernet Sauvignon grapes.

KEYWORDS: *Vitis vinifera* L.; proanthocyanidins; phloroglucinolysis; tannin; anthocyanins; ripeness; HPLC-DAD

INTRODUCTION

Phenolic compounds play an important role in the quality of grapes and wines. These compounds can be divided into two groups: nonflavonoids (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoids (anthocyanins, flavan-3-ols, and flavonols). Anthocyanins are responsible for the color in grapes and young wines, while flavan-3-ols (monomeric catechins and proanthocyanidins) are mostly responsible for the astringency, bitterness, and structure of wines (*1*), and flavonols (quercetin, myricetin, kaempferol, isorhamnetin, and their glycosides) seem to contribute to both bitterness and color (*1*, *2*). In grape berries, phenolic compounds are present mainly in skins and seeds. Flavonols and anthocyanins are the most abundant phenolic compounds in the skins of red grapes, whereas grape seeds are rich in flavan-3-ols (*2*, *3*). The content of phenolic compounds in grape berries depends on climatic and geographical factors, cultural practices, stage of ripeness, and vegetative vigor of the plant. The last two factors are closely related to genetic aspects such as vine cultivar, clone, and rootstock (*4*, *5*). Several authors working with different varieties have reported that phenolic compounds undergo an evolution during grape berry ripening (*6*−*12*). Carménère (*Vitis vinifera* L.), a late-maturing red grape variety, is cultivated significantly in Chile since its introduction from Bordeaux, France, in the middle of the 19th century, before the phylloxera devastation of European grapevines (*13*). This late-maturing variety is nowadays well-adapted to the Chilean ecosystems (soil diversity and dry climate during grape ripening). Nevertheless, until the mid-1990s, Carménère was confounded with Merlot and Cabernet Franc, two varieties with similar ampelographic characteristics. Chile and Southern Italy are the only two regions in the world that at the present have significant plantings of Carménère. In Chile, Carménère exists as single vineyards with a total production area of over 7000 ha (*14*). Currently, this variety is the subject of clonal selection programs that aim to promote it as the emblematic variety of Chile.

However, this variety has been less studied (*15*) with respect to either the phenolic composition of its grape berries or the eventual evolution of those compounds during ripening. The objective of the present study was to evaluate the phenolic composition of grape skins and seeds of the Carménère variety during ripening and to compare those data with the data obtained working in parallel with Cabernet Sauvignon, a widely distributed grape variety.

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MATERIALS AND METHODS

Materials. Standards of gallic acid (G-7384), vanillinic acid (V-2250), syringic acid (S-6881), protocatechuic acid (P-5630), caffeic acid (C-0625), quercetin (Q-0125), myricetin (M-6760), (+)-catechin (C-1251), (−)-epicatechin (E-1753), and (−)-epicatechin-3-*O*-gallate (E-3893), as well as phloroglucinol (P-3502) and 0.45 μm pore size membranes were acquired from Sigma Chemical Co. (St. Louis, MO). Vanillin 99% (code V-8510), trifluoroacetic acid, ethyl acetate, high-performance liquid chromatography (HPLC) grade acetonitrile, and pro-analysis solvents were purchased from Merck (Darmstadt, Germany). Gelatin (Gelarom) was purchased from Vinicas (Santiago, Chile). Sep-Pak Plus tC_{18} cartridges WAT 036810 and WAT 036800 were obtained from Waters (Milford, MA). Toyopearl TSK HW 40-F size exclusion resin (no. 807448) was obtained from Toso Haas (Stuttgart, Germany).

Instrumentation. The HPLC system (Agilent Technologies, Santa Clara, CA) consisted of a photodiode array detector model G1315B, a pump model Quat G1311A, and an autosampler model ALS G1329A. A reversed phase Nova Pack C_{18} column (4 μm, 3.9 mm i.d. × 300 mm; Waters Corp.) was used for HPLC-DAD analysis of individual phenolic compounds. A reversed phase LiChro Cart 100 RP-18 column (5 μm, 4 mm i.d. × 250 mm; Agilent Technologies) was used in phloroglucinolysis studies. Absorbances were measured using a Jasco UV−vis spectrophotometer model V-530 (JASCO International Co., Ltd., Tokyo, Japan).

Grape Samples. Self-rooted *V. vinifera* L. cv. Carménère and Cabernet Sauvignon vines, vintage 2008, planted in 2003 and grown in the Maule Valley at the VII Region of Chile, were used. Agronomic, photochemical, and technical variables were controlled. Three groups of 120 berries per variety were selected from four clusters per plant among a total of 30 plants. The samples were harvested monthly from February (veraison) to May.

Extracts of Phenolic Compounds. Phenolic compounds were extracted as described in previous reports (*16*−*21*). Briefly, skins and seeds were separated by hand from 120 berries, weighed, and ground. Forty milliliters of hydroalcoholic solution (1:9 v/v ethanol/water) containing 5 g/L of tartaric acid was added to the ground material (skins or grapes), and the weight of the resulting suspension was adjusted to 200 g with distilled water. After maceration for 2 h at 20 °C under mechanical stirring, the extract was filtered through a 0.45 μm pore size membrane.

Spectrophotometric Characterization. The total phenol content was determined by UV absorptiometry at 280 nm (*22*) using gallic acid as a standard. The total tannin content was measured by the method of Ribereau-Gayon and Stonestreet (*23*). The gelatin index of tannins was measured by the method of Glories (*22*) using 50 mL of the sample extract and 5 mL of a gelatin solution (70 g/L). Total anthocyanins were measured by diluting the extract with 2% hydrochloric acid in ethanol and by comparing spectrophotometric readings of single aliquots treated with either sodium metabisulfite or water (*24*). The color intensity was determined by visible absorptiometry at 420, 520, and 620 nm (*22*).

Fractionation of Proanthocyanidins into Monomers, Oligomers, and Polymers. Seed and skin extracts (see above) were fractionated by using Waters C_{18} Sep-Pak cartridges according to the method described by Sun et al. (*25*). Briefly, 5 mL of seed or skin extracts was concentrated to dryness in a rotary evaporator at < 30 °C. The residue was dissolved in 20 mL of 67 mM phosphate buffer, pH 7.0. The pH of the resulting solution was adjusted to 7.0 with NaOH or HCl. Two C_{18} Sep-Pak cartridges were assembled (WAT 36800 on top and WAT 36810 at the bottom) and conditioned sequentially with methanol (10 mL), distilled water (2 × 10 mL), and phosphate buffer, pH 7.0 (10 mL). Samples were passed through the cartridges at a flow rate not higher than 2 mL/min, and phenolic acids were then eliminated by elution with 10 mL of 67 mM phosphate buffer at pH 7.0. The cartridges were dried with nitrogen gas and eluted sequentially with 25 mL of ethyl acetate (fraction FI + FII containing monomeric and oligomeric flavan-3-ols) and with 15 mL of methanol (fraction FIII containing polymeric proanthocyanidins). The ethyl acetate eluate was taken to dryness under vacuum, redissolved in 3 mL of 67 mM phosphate buffer, pH 7.0, and reloaded onto the same series of cartridges that had been conditioned again as described above. The cartridges were dried with nitrogen and eluted sequentially with 25 mL of diethyl ether (fraction FI containing monomers) and 15 mL of methanol (fraction FII containing oligomers). Fractions F1, F2, and F3 were evaporated to dryness under vacuum and redissolved in 3 mL of methanol. The total content of flavan-3-ols in each fraction was determined by the vanillin assay (*26*).

Total Content of Flavan-3-ols. The vanillin assay was performed as described by Sun et al. (*26*). A 2.5 mL aliquot of 1:3 v/v sulfuric acid/methanol solution and a 2.5 mL aliquot of 1% (w/v) vanillin in methanol were mixed with 1 mL of the sample. The tubes were incubated at 30 °C for either 15 min (F1 fractions) or for a period of time long enough to allow maximal reaction (FII and FIII fractions). The absorbance was read at 500 nm. A blank was prepared by substituting the vanillin solution in the reaction mix with methanol.

Phloroglucinolysis. The procedure was performed as described by Kennedy and Jones (*27*) with some modifications. A proanthocyanidin-rich fraction from grape skins was first obtained by passing 15 mL of a grape skin extract through a Toyopearl TSK HW 40-F size exclusion column (100 mm × 10 mm). The column was equilibrated previously with 30 mL of ethanol/water (55/45) containing 0.5% v/v trifluoroacetic acid (solution A). After the extract was loaded, the column was rinsed with 50 mL of solution A to remove carbohydrates and monomeric flavan-3-ols. Proanthocyanidins were eluted with 30 mL of 60:40 v/v acetone/water (solution B), and the eluate was concentrated by removing acetone at 30 °C and reduced pressure, furtherly concentrated to dryness in a rotary evaporator at < 30 °C, and, finally, dissolved in 2.5 mL of methanol to proceed with phloroglucinolysis (*25*). For the phloroglucinolysis assay in seed samples, seed extracts were used directly. Thus, a 3 mL aliquot of a seed extract was concentrated under reduced pressure, further concentrated to dryness using a rotary evaporator at < 30 °C, and dissolved in 2.5 mL of methanol (*10*). One milliliter aliquots of each of the proanthocyanidin solutions in methanol (from skins or seeds) were allowed to react with 1 mL of solution C (0.25 g of ascorbic acid, 1.25 g of phloroglucinol, and 215 μL of concentrated hydrochloride acid in 25 mL of methanol) at 50 °C for 20 min (*22*). At the end of the incubation, the reaction was stopped with 1 mL of 200 mM sodium acetate. The chromatographic separation used a binary gradient with mobile phases of 1% v/v acetic acid (mobile phase A) and methanol (mobile phase B), and the elution was monitored at 280 nm. Elution conditions were as follows: flow rate, 1.0 mL/min; 100% A for 15 min, linear gradient from 95 to 80% A in 20 min, linear gradient from 80 to 60% A in 26 min, and 10% A for 10 min. The column was finally equilibrated with 10% A for other 6 min before the following chromatographic separation.

HPLC-DAD Analysis of Individual Phenolic Compounds. Seed and skin extracts of phenolic compounds were re-extracted with ethyl ether (3 × 20 mL) and ethyl acetate (3 × 20 mL). The resulting extracts were evaporated to dryness at 30 °C, redissolved in 2 mL of 50% (v/v) methanol/water, and membrane-filtered (0.45 μm pore size) (*17*−*21*). Fifty microliter aliquots of the final solution were subjected to reversed-phase chromatographic separation at 20 °C using a Nova Pack C_{18} column. A photodiode array detector was set at 280 nm. Two mobile phases were used as follows: A, water/acetic acid (98:2 v/v), and B, water/acetonitrile/acetic acid (78:20:2 v/v/v). A two-step gradient was carried out at a constant flow rate of 1.0 mL per min: 0−55 min, 100−20% A, and 55−70 min, 20−10% A. Equilibration times of 15 min were allowed between injections. Each major peak in the HPLC chromatograms of the extracts was characterized by both retention time and absorption spectrum (from 210 to 360 nm). Identification of specific compounds was achieved by comparison of UV spectra and retention times against those of pure standards. Glycosides of flavonols and myricetin, hydroxycinnamic acid esters, and procyanidins, for which standards were unavailable, were assigned by retention time and spectral parameters according to Peña-Neira et al. and Matus et al. (*17*, *18*, *20*). Quantitative determinations were made by using the external standard method and commercial standards. All of the qualitative and quantitative analyses of phenolic composition (including extraction) were performed in triplicate.

RESULTS AND DISCUSSION

General Analytical Parameters. Table 1 shows the content of total solids (°Brix), seed weight, and skin weight of Carménère and Cabernet Sauvignon grapes expressed on a per grape basis. The total solids of grapes from both cultivars increased from veraison to harvest time. By contrast, while the seed weight of Carménère grapes experienced a significant decrease over the

Table 1. General Analytical Parameters of Carménère and Cabernet Sauvignon Grapes during Ripening[a]

	date	Carménère	Cabernet Sauvignon
total solids (° Brix)	Feb 22[b]	17.3 ± 1.2 b	19.7 ± 1.2 a
	Mar 20	22.1 ± 1.0 a	23.3 ± 0.6 a
	Apr 20[c]	24.0 ± 1.7 a	25.7 ± 2.1 a
	May 22[d]	23.7 ± 0.6 b	25.3 ± 1.2 a
seed weight/grape (mg)	Feb 22[b]	36.1 ± 1.1 a	36.7 ± 2.9 a
	Mar 20	34.5 ± 2.0 a	36.7 ± 3.0 a
	Apr 20[c]	29.0 ± 3.1 b	36.7 ± 1.0 a
	May 22[d]	35.2 ± 2.1 a	35.7 ± 0.5 a
skin weight/grape (mg)	Feb 22[b]	221.7 ± 17.2 a	230.8 ± 7.1 a
	Mar 20	184.1 ± 15.1 a	207.5 ± 9.2 a
	Apr 20[c]	265.8 ± 7.3 b	233.3 ± 8.2 a
	May 22[d]	255.5 ± 15.1 a	210.2 ± 19.4 a

[a] Figures represent means ± standard deviations (triplicates). Values with different letters in the row are significantly different (Student's t test, $p < 0.05$). [b] Veraison. [c] Harvest time. [d] Over maturity.

same period, Cabernet Sauvignon seeds displayed a constant weight during all of the period under consideration. Although the skin weight did not show a clear tendency during maturation of both cultivars, Carménère skins presented a significantly higher weight at harvest, while Cabernet Sauvignon skin weight showed an apparent tendency to decrease between the first and the last sampling dates.

Phenolic Composition of Skins during Ripening. *Global Phenolic Composition.* **Table 2** shows the global extractable phenolic composition of skins from Carménère and Cabernet Sauvignon grapes during ripening. Because of climatic conditions in Chile (no rains between November and June), red varieties for reserve wines are harvested very late to reach proper phenolic maturity and reduced pyrazine concentrations (especially in Carménère grapes). Besides, as compared with other world regions, the period from veraison (the first week of February for both Carménère and Cabernet Sauvignon) to the traditional harvest time (between the last week of April and the last week of May) is longer. In Carménère skins, the extractable total phenols, total tannins, and total anthocyanins, as well as color intensity, decreased during the study period from 2.2 to 1.1 mg GAE/g, from 8.7 to 2.8 mg CE/g, from 2.2 to 0.9 mg ME/g, and from 3.4 to 1.5 A.U., respectively. In all of those parameters, the steepest decreases occurred between the second and the third samplings with practically no further changes observed at the fourth sampling date. In the skins of Cabernet Sauvignon grapes, the four parameters decreased by following the same tendency as the Carménère grape skins. In the particular case of anthocyanins, their decrease from the second sampling date onward is fully coincident with data from previous studies showing a similar decrease in Cabernet Sauvignon grapes during the ripening period in the Maipo Valley (Chile) (*20*). On the other side, the contents of extractable total anthocyanins and color intensity in Cabernet Sauvignon skins were markedly lower than those parameters in Carménère skins since the third sampling date onward. In contrast, the content of extractable total tannins in the skins of both varieties showed no differences during the ripening period. Altogether, values and trends observed in the phenolic analysis in this study are mostly coincident with those previously reported by different authors (*18*, *28*).

Distribution of Extractable Proanthocyanidins According to Polymerization Degree in Grape Skins during Ripening. As shown in **Figure 1**, the monomeric fraction was the more invariant and less abundant extractable fraction in the skins of the

Table 2. Global Extractable Phenolic Composition of Carménère and Cabernet Sauvignon Grape Skins and Seeds[a]

	date	Carménère	Cabernet Sauvignon
	skin		
total phenols (mg GAE/g)	Feb 20[b]	2.2 ± 0.2 b	1.8 ± 0.2 a
	Mar 20	2.9 ± 0.4 b	1.7 ± 0.2 a
	Apr 20[c]	1.1 ± 0.1 a	1.1 ± 0.1 a
	May 22[d]	1.1 ± 0.2 a	0.8 ± 0.3 a
total tannins (mg CE/g)	Feb 20[b]	8.7 ± 1.0 a	7.9 ± 0.5 a
	Mar 20	5.3 ± 0.9 a	4.9 ± 0.9 a
	Apr 20[c]	2.5 ± 0.4 a	2.9 ± 0.4 a
	May 22[d]	2.8 ± 0.4 a	3.0 ± 0.1 a
total anthocyanins (mg ME/g)	Feb 20[b]	2.2 ± 0.2 b	1.1 ± 0.2 a
	Mar 20	3.0 ± 0.6 b	1.3 ± 0.3 a
	Apr 20[c]	1.0 ± 0.2 a	0.5 ± 0.2 a
	May 22[d]	0.9 ± 0.2 b	0.5 ± 0.1 a
color intensity (A.U.)	Feb 20[b]	3.4 ± 0.2 b	2.0 ± 0.3 a
	Mar 20	4.2 ± 0.5 b	1.7 ± 0.4 a
	Apr 20[c]	1.6 ± 0.3 b	0.8 ± 0.1 a
	May 22[d]	1.5 ± 0.1 b	0.5 ± 0.2 a
	seed		
total phenols (mg GAE/g)	Feb 20[b]	21.8 ± 1.4 a	20.4 ± 0.6 a
	Mar 20	22.5 ± 1.7 b	17.9 ± 1.5 a
	Apr 20[c]	10.0 ± 1.3 a	8.5 ± 0.7 a
	May 22[d]	16.6 ± 2.8 a	17.5 ± 4.3 a
total tannins (mg CE/g)	Feb 20[b]	86.9 ± 4.8 a	90.3 ± 5.2 a
	Mar 20	50.2 ± 4.4 a	45.9 ± 6.7 a
	Apr 20[c]	42.9 ± 7.2 a	37.9 ± 2.9 a
	May 22[d]	32.9 ± 3.9 a	36.9 ± 9.1 a
gelatin index (%)	Feb 20[b]	64.2 ± 4.1 a	72.1 ± 4.3 a
	Mar 20	58.6 ± 1.6 a	54.4 ± 6.6 a
	Apr 20[c]	44.6 ± 12.2 a	49.2 ± 13.9 a
	May 22[d]	9.2 ± 6.0 a	15.3 ± 13.7 a

[a] Figures represent means ± standard deviations (triplicates). Values with different letters in single rows are significantly different (Student's t test, $p < 0.05$). GAE, equivalent gallic acid; CE, equivalent (+)-catechin; ME, equivalent malvidin-3-glucoside; and U.A., absorbance units. [b] Veraison. [c] Harvest time. [d] Over maturity.

Carménère and Cabernet Sauvignon grape varieties throughout the whole study period. In Carménère, extractable oligomers were also low-represented, except when a sharp 3-fold increase occurred at the second sampling date. By contrast, in the skin of Cabernet Sauvignon grapes, the levels of extractable oligomers were significant at the start of the study, and since then, they decreased continuously until the fourth sampling date. On the other hand, the extractable polymeric fraction was far predominant over the monomeric and oligomeric fractions in the skins of both grape varieties at the first sampling date. At this stage, the skins of Cabernet Sauvignon grapes showed a significantly higher content of extractable polymers than the skins of Carménère grapes. However, the skins of both grape varieties showed a marked and continuous decrease in the polymeric fraction from the first to the fourth sampling date (from 95.5 to 27.8 mg/K in Carménère as compared to a decrease from 165.4 to 27.9 mg/K in Cabernet Sauvignon) with an identical content in this fraction since the second sampling date onward.

This relative content of the various extractable fractions coincides fully with data reported in other studies (*29*). Nevertheless, the contents of extractable proanthocyanidins observed in this study are lower than those reported by Sun et al. in several

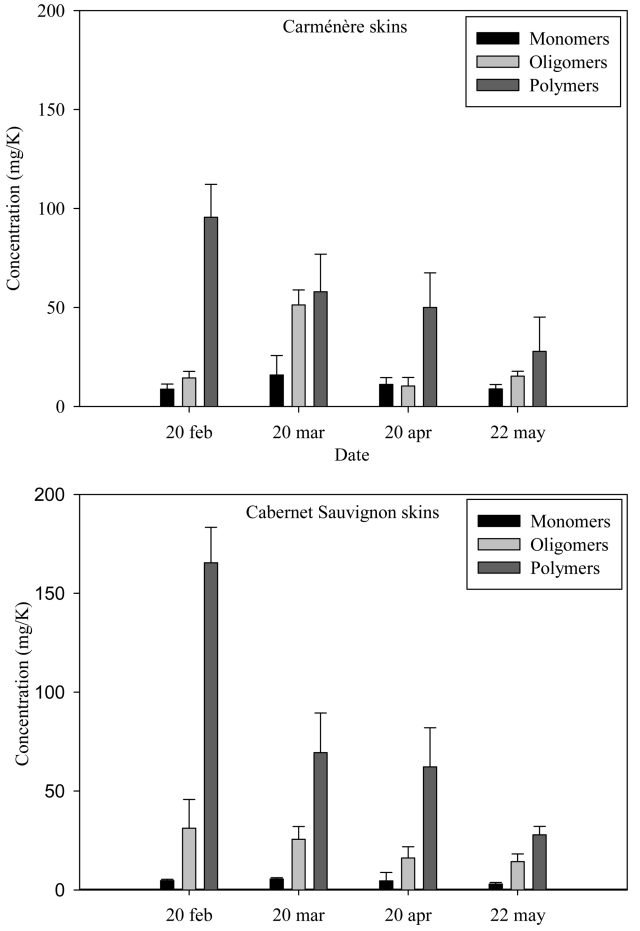

Figure 1. Flavan-3-ols content of monomeric, oligomeric, and polymeric fractions of Carménère and Cabernet Sauvignon skins during fruit ripening.

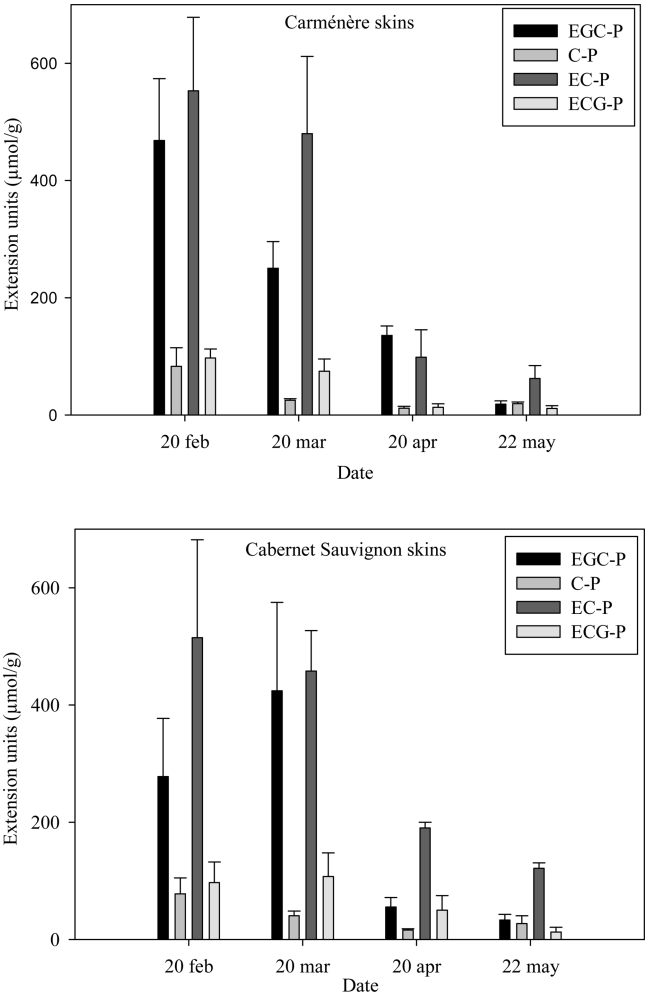

Figure 2. Change in procyanidin extension unit composition during fruit ripening of Carménère and Cabernet Sauvignon skins.

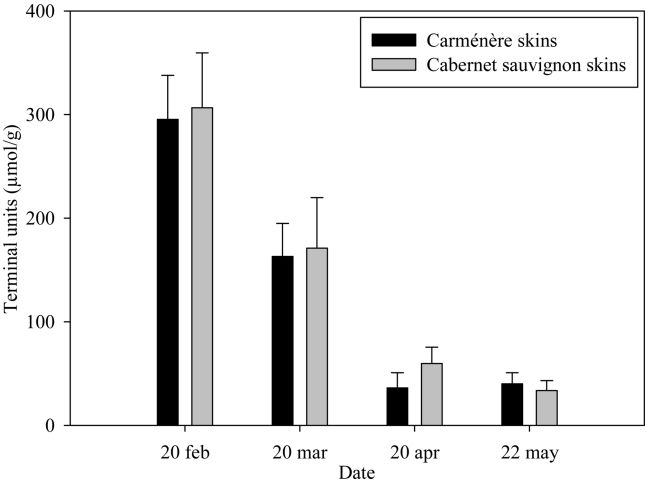

Figure 3. Change in procyanidin terminal unit composition during fruit ripening of Carménère and Cabernet Sauvignon skins.

grapevine varieties (*30*) and higher than the results obtained by Monagas et al. in Graciano, Tempranillo, and Cabernet Sauvignon grapes (*29*).

Phloroglucinolysis. **Figures 2** and **3** show the evolution of the extractable polymeric fraction isolated for pholoroglucinolysis from skins of Carménère and Cabernet Sauvignon grapes during ripening. Epigallocatechin-phloroglucinol (EGC-P), (+)-catechin-phloroglucinol (C-P), (−)-epicatechin-phloroglucinol (EC-P), and epicatechin-3-*O*-gallate-phloroglucinol (ECG-P) were identified as extension proanthocyanidin units, whereas only (+)-catechin (C) was identified as a terminal unit in the skins of both varieties, coinciding with other works (*7*, *8*, *25*, *29*−*31*). However, in contrast to previous reports (*7*, *32*), the terminal units (−)-epicatechin (EC) and (−)-epicatechin-3-*O*-gallate (ECG) were either insignificant or absent in all of the samples. By quantifying those compounds, we estimated both mean degree of polymerization (mDP), percentage of galloylation (% G), and average molecular weight (aMW) of flavanols. In the skins of Carménère and Cabernet Sauvignon grapes, the EC-P extension unit was the most abundant followed by the EGC-P extension unit, in agreement with previous reports (*8*, *9*). Also, in both varieties, all of the identified extension units displayed a similar progressive decrease since the first sampling date onward. Thus, in Carménère skins, the C-P, ECG-P, EGC-P, and EC-P contents of the extension units decreased between the first and the last samplings from 83.0 to 19.2, 97.3 to 11.2, 468.2 to 18.4, and 553.1 to 62.4 μmol/g, respectively. The C content of the terminal units underwent a continuous decrease from 295.3 to 40.0 μmol/g in the same period (**Figure 3**). Likewise, in the Cabernet Sauvignon skins, the C-P, ECG-P, EGC-P, and EC-P contents of the extension units decreased between the first and the last samplings from 77.8 to 27.1, 97.2 to 12.7, 277.8 to 33.1, and 514.8 to 121.4 μmol/g, respectively. In this variety, both the content and the continuous decrease of the C terminal units were parallel to the ones observed in Carménère skins (**Figure 3**). Altogether, these data confirm that extension and terminal units in skin

Table 3. Structural Characteristics and Compositions of Proanthocyanidins from Carménère and Cabernet Sauvignon Skins and Seeds[a]

		skin		seed	
	date	Carménère	Cabernet Sauvignon	Carménère	Cabernet Sauvignon
mDP	Feb 20[b]	4.9 ± 0.7 a	4.1 ± 0.6 a	3.8 ± 0.4 a	4.3 ± 1.2 a
	Mar 20	5.8 ± 2.5 a	7.1 ± 0.9 a	3.6 ± 0.3 b	2.8 ± 0.3 a
	Apr 20[c]	10.0 ± 3.7 a	6.4 ± 1.1 a	2.0 ± 0.2 a	1.8 ± 0.2 a
	May 22[d]	3.8 ± 0.3 b	7.1 ± 1.8 a	3.2 ± 0.0 a	2.7 ± 0.6 a
% G	Feb 20[b]	7.8 ± 3.2 a	7.5 ± 0.8 a	29.7 ± 0.8 a	26.8 ± 2.3 a
	Mar 20	9.9 ± 3.5 a	8.1 ± 3.4 a	31.8 ± 1.8 b	24.1 ± 1.2 a
	Apr 20[c]	12.2 ± 0.3 a	7.3 ± 2.9 a	20.6 ± 5.5 a	18.7 ± 5.5 a
	May 22[d]	12.5 ± 2.2 a	19.0 ± 8.6 a	27.5 ± 1.3 b	16.3 ± 3.0 a
aMW	Feb 20[b]	1480 ± 184 a	1248 ± 185 a	1260 ± 128 a	1422 ± 407 a
	Mar 20	1787 ± 802 a	2169 ± 309 a	1227 ± 98 b	894 ± 94 a
	Apr 20[c]	3162 ± 1244 a	1940 ± 365 a	655 ± 57 a	582 ± 51 a
	May 22[d]	1181 ± 93 a	2289 ± 636 a	1060 ± 6 a	850 ± 150 a

[a] Figures represent means ± standard deviations (triplicates). Values with different letters in single rows are significantly different (Student's *t* test, $p < 0.05$). [b] Veraison. [c] Harvest time. [d] Over maturity.

composition change during the growing season and decrease gradually during maturity in both varieties. Part of these observations is consistent with data from previous works (*7*, *8*).

As shown in **Table 3**, the mDP of extractable flavan-3-ols from Carménère skins increased continuously to a maximum of 10.0 at the third sampling date, and then, it decreased precipitously to 3.8 at harvest. By contrast, the mDP of extractable flavan-3-ols from Cabernet Sauvignon skins increased progressively from 4.1 to 7.1 between the first and the last sampling dates. On the other hand, the aMW of proanthocyanidins from the skins of both grape varieties showed significant increases up to the third (Carménère) or fourth (Cabernet Sauvignon) samplings. In spite of those variations, at technological maturity (third sampling date), the Carménère skins presented mDP and aMW values that are higher than those observed in Cabernet Sauvignon samples. Both trends, range of magnitude and maximum values of these parameters, coincided with technological maturity (24 °Brix) observed in previous works (*7*, *8*, *10*, *33*). On the other hand, the decrease in the mDP that we have observed in the last stages of berry development also coincided with studies from other laboratories (*7*, *9*). Finally, the % G in the extractable proanthocyanidins of the skins of Carménère grapes was slightly higher than in Cabernet Sauvignon grapes at the various sampling dates, except during the last one. However, both varieties showed a significant increase between the first and the last sampling dates, particularly in the case of the Cabernet Sauvignon skins.

Extractable Low Molecular Weight Phenolic Compounds in Grape Skins during Ripening. **Table 4** shows a group of 12 flavonoid and nonflavonoid compounds that were identified and quantified by HPLC-DAD analysis in the extracts of grape skins from the Carménère and Cabernet Sauvignon varieties during ripening. Among them, we identified four nonflavonoids (gallic acid, caftaric acid, vanillinic acid, and syringic acid) and eight flavonoids [procyanidin B3, (+)-catechin, myricetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, kaemferol-3-*O*-galactoside, kaemferol-3-*O*-glucoside, and isorhamnetin-3-*O*-glucoside], thus coinciding with data reported by other authors (*19*, *31*, *32*). In Carménère skins, the concentration of both groups of compounds experienced a significant (nonflavonoids) or drastic (flavonoids) decrease during ripening. In Cabernet Sauvignon skins, the flavonoid compounds displayed a drastic fall during ripening, while the nonflavonoids remained mostly unaffected. Vanillinic acid and gallic acid were the main nonflavonoid compounds identified in the skin extracts of both varieties. At the first two sampling dates, similar contents of non flavonoids were observed in the skins of both varieties, except for syringic acid, whose content in Carménère skins was over 2-fold the one in Cabernet Sauvignon skins. Contrarily, at the last two sampling dates, significantly higher contents of some nonflavonoids, such as gallic acid, caftaric acid, and vanillinic acid, were observed in Cabernet Sauvignon skins as compared with Carménère skins. A comparison of the total content of nonflavonoid compounds in the skins of both grape varieties showed the same relationship. On the other hand, quercetin-3-*O*-glucoside was found to be the more abundant flavonoid in the skins of both varieties. This observation coincides with those of other laboratories working with Cabernet Sauvignon grapes (*19*, *33*). We also observed a significantly higher concentration of this flavonoid in Carménère skins as compared with Cabernet Sauvignon skins, particularly in the first and last samplings. The rest of the extractable flavonols were more abundant in Carménère than Cabernet Sauvignon in three sampling dates. Finally, the total content of flavonoids was higher in Carménère skins than in Cabernet Sauvignon skins during the study period except for the third sampling date.

Phenolic Composition of Seeds during Ripening. *Global Phenolic Composition*. **Table 2** shows the global extractable phenolic composition of seeds from Carménère and Cabernet Sauvignon grapes during ripening. In Carménère seeds, total phenols and total tannins decreased from 21.8 to 16.6 mg GAE/g and from 86.9 to 32.9 mg CE/g, respectively, between the first and the last samplings. The gelatin index also decreased during ripening from 64.2 to 9.2%. In seeds of Cabernet Sauvignon, total phenols, total tannins, and the gelatin index decreased during the same period from 20.4 to 17.5 mg GAE/g, from 90.3 to 36.9 mg CE/g, and from 72.1 to 15.3%, respectively. Total phenols and total tannins were higher in Cabernet Sauvignon seeds than in Carménère seeds in the last two samplings. Nevertheless, the observed differences in these parameters did not reach statistical significance. The contents of total phenols and total tannins in our study, as well as the gelatin index, were concordant with those observed in previous studies with these and other varieties (*18*, *28*).

Distribution of Extractable Proanthocyanidins According to Polymerization Degree in Grape Seeds during Ripening. As compared with skins, seeds presented higher concentrations of monomers, oligomers, and polymers of flavanols. As shown in **Figure 4**, monomers were the less abundant fraction in seeds of the Carménère and Cabernet Sauvignon grape varieties, practically

Table 4. Extractable Low Molecular Weight Phenolic Compounds of Carménère and Cabernet Sauvignon Skins during Ripening[a]

compounds	date	Carménère	C. Sauvignon
gallic acid	Feb 20[b]	3.4 ± 0.2 a	3.3 ± 0.2 a
	Mar 20	2.1 ± 0.7 a	1.5 ± 0.1 a
	Apr 20[c]	2.5 ± 0.1 b	3.1 ± 0.2 a
	May 22[d]	2.8 ± 0.2 a	3.5 ± 0.5 a
caftaric acid	Feb 20[b]	0.8 ± 0.1 a	0.8 ± 0.1 a
	Mar 20	0.4 ± 0.2 a	0.3 ± 0.0 a
	Apr 20[c]	0.4 ± 0.0 b	0.5 ± 0.0 a
	May 22[d]	0.6 ± 0.1 a	0.7 ± 0.2 a
vanillinic acid	Feb 20[b]	7.5 ± 1.3 a	7.3 ± 0.3 a
	Mar 20	3.1 ± 0.7 a	2.9 ± 0.3 a
	Apr 20[c]	5.1 ± 0.1 b	6.3 ± 0.4 a
	May 22[d]	5.8 ± 0.4 a	7.1 ± 1.1 a
syringic acid	Feb 20[b]	3.1 ± 0.1 b	1.5 ± 0.2 a
	Mar 20	1.7 ± 0.5 a	0.7 ± 0.2 a
	Apr 20[c]	1.0 ± 0.1 a	1.1 ± 0.0 a
	May 22[d]	1.6 ± 0.2 a	1.8 ± 0.2 a
procyanidin B3	Feb 20[b]	3.5 ± 0.8 a	3.7 ± 0.5 a
	Mar 20	2.2 ± 0.4 a	1.1 ± 0.7 a
	Apr 20[c]	1.0 ± 0.4 a	0.9 ± 0.5 a
	May 22[d]	0.6 ± 0.2 a	0.7 ± 0.1 a
(+)-catechin	Feb 20[b]	3.1 ± 0.5 a	5.1 ± 3.0 a
	Mar 20	2.1 ± 0.4 b	1.0 ± 0.1 a
	Apr 20[c]	1.3 ± 0.2 b	0.8 ± 0.0 a
	May 22[d]	1.3 ± 0.4 a	0.5 ± 0.1 a
myricetin-3-*O*-glucoside	Feb 20[b]	15.1 ± 2.1 a	10.4 ± 1.8 a
	Mar 20	9.3 ± 1.6 a	6.8 ± 1.1 a
	Apr 20[c]	5.6 ± 1.2 a	3.7 ± 0.9 a
	May 22[d]	2.4 ± 0.3 a	3.1 ± 1.4 a
quercetin-3-*O*-galactoside	Feb 20[b]	18.1 ± 0.8 a	19.0 ± 6.9 a
	Mar 20	8.3 ± 2.0 a	9.0 ± 3.3 a
	Apr 20[c]	2.0 ± 0.4 a	2.6 ± 0.5 a
	May 22[d]	2.4 ± 0.3 a	2.2 ± 0.9 a
quercetin-3-*O*-glucoside	Feb 20[b]	181.2 ± 6.0 b	109.5 ± 10.8 a
	Mar 20	38.7 ± 11.4 a	31.8 ± 5.1 a
	Apr 20[c]	10.8 ± 2.3 a	13.4 ± 3.8 a
	May 22[d]	6.5 ± 1.6 b	0.9 ± 0.4 a
kaemperol-3-*O*-galactoside	Feb 20[b]	12.4 ± 0.7 b	8.3 ± 0.6 a
	Mar 20	2.6 ± 0.6 a	1.3 ± 0.3 a
	Apr 20[c]	0.8 ± 0.2 a	0.9 ± 0.5 a
	May 22[d]	0.5 ± 0.4 a	0.1 ± 0.2 a
kaemperol-3-*O*-glucoside	Feb 20[b]	39.9 ± 7.9 a	31.3 ± 3.4 a
	Mar 20	2.6 ± 0.6 a	1.3 ± 0.3 a
	Apr 20[c]	0.8 ± 0.2 a	0.9 ± 0.5 a
	May 22[d]	0.5 ± 0.4 a	0.1 ± 0.2 a
isorhamnetin-3-*O*-glucoside	Feb 20[b]	22.6 ± 0.6 b	12.5 ± 1.5 a
	Mar 20	25.6 ± 6.1 a	19.6 ± 1.5 a
	Apr 20[c]	2.5 ± 0.5 a	3.7 ± 0.9 a
	May 22[d]	1.9 ± 0.3 b	1.0 ± 0.2 a

[a] Figures are expressed in mg/kg and represent means ± standard deviations (triplicates). Values with different letters in the row are significantly different (Student's *t* test, $p < 0.05$). [b] Veraison. [c] Harvest time. [d] Over maturity.

at all of the sampling dates. In Carménère, monomers increased continuously up to a 10-fold peak at the third sampling date and returned to basal levels at the last sampling date. Oligomers

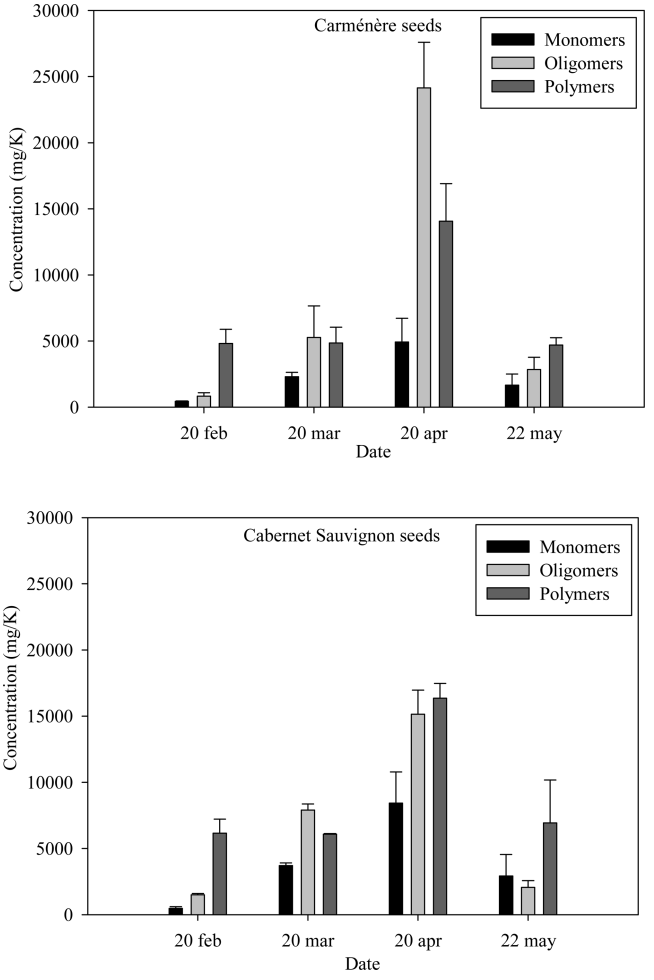

Figure 4. Flavan-3-ols content of the monomeric, oligomeric, and polymeric fractions of Carménère and Cabernet Sauvignon seeds during fruit ripening.

displayed a similar progression during the four-stage study protocol, thus becoming the main fraction of proanthocyanidins in the Carménère seeds with a 30-fold increase at the third sampling date. Similarly, polymers in Carménère seeds displayed a sharp 3-fold increase at the third sampling date. On the other hand, in seeds of the Cabernet Sauvignon grape variety, the contents of monomers, oligomers, and polymers displayed similar temporal progression profiles as those correspondingly observed in the Carménère variety. As compared with Carménère seeds, Cabernet Sauvignon seeds exhibited statistically significant higher concentrations of monomers at the second sampling date and higher concentrations of oligomers at three sampling dates. Likewise, the magnitude of the polymeric fraction in Cabernet Sauvignon seeds was higher than that in Carménère seeds, although the difference did not reach statistical significance. Altogether, relative contents of the various fractions fully coincide with those reported by other authors (*29*), although concentrations of flavanol fractions observed in our study are lower than those reported by Sun et al. (*30*) and higher than the ones reported by Monagas et al. (*29*).

Pholoroglucinolysis. As shown in **Figures 5** and **6**, both the terminal units and the extension units in the polymeric fraction of either Carménère or Cabernet Sauvignon grape seeds were found to be C, EC, and ECG. This observation is in agreement with those of various previous reports (*33*−*37*). On the basis of the quantification of those units, we determined both mDP, % G, and aMW of flavanols. In seeds of both grape varieties, the EC-P

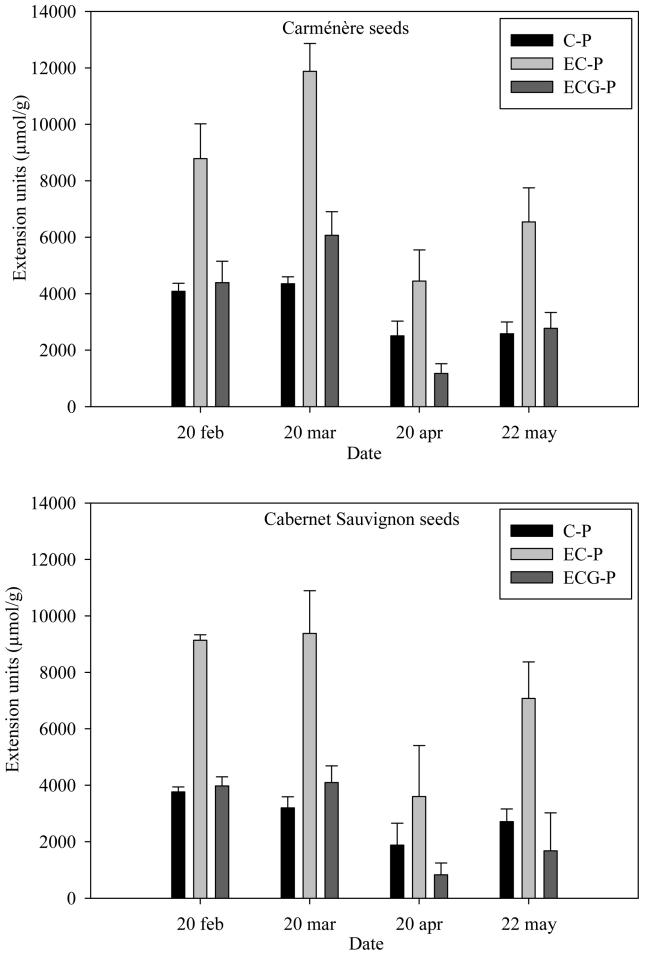

Figure 5. Change in procyanidin extension unit composition of Carménère and Cabernet Sauvignon seeds during fruit ripening.

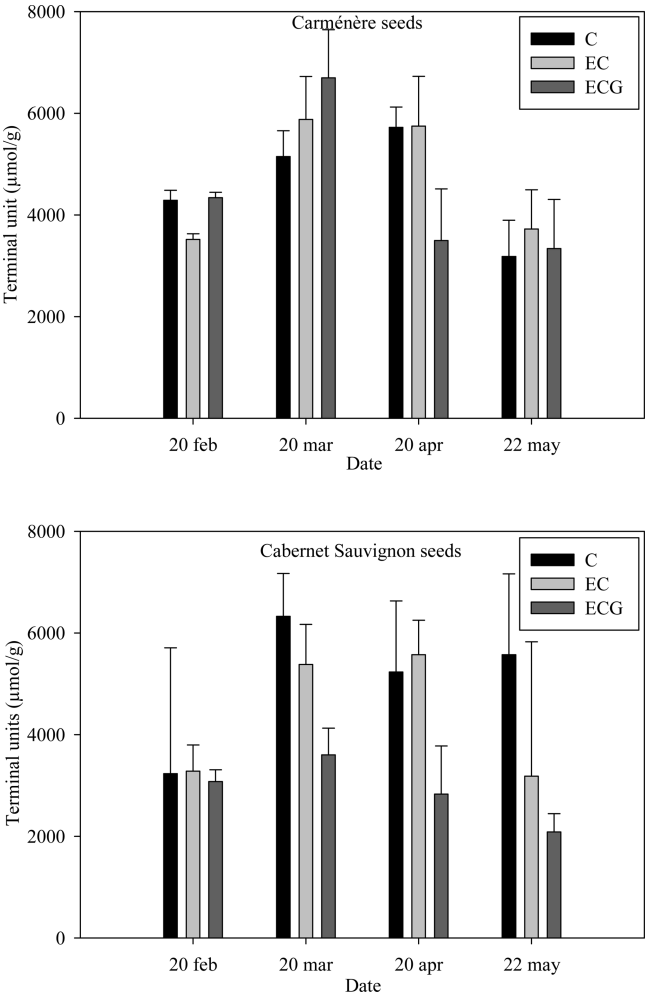

Figure 6. Change in procyanidin terminal unit composition of Carménère and Cabernet Sauvignon seeds during fruit ripening.

content of the extension units doubled those of C-P and ECG-P at each stage of the study. Such an observation coincides with results obtained by other authors (*8*−*12*, *26*, *34*, *35*). In Carménère seeds, contents of the three extension units decreased by half after the second sampling date. On the other hand, in seeds of this variety, the three terminal units, which were equally represented at the first sampling date (around 4000 μmol/g), experienced a significant increase at the second sampling date (around 6000 μmol/g) and a return to basal levels at the third (ECG) or fourth (C and EC) sampling dates. In Cabernet Sauvignon seeds, the contents of the three extension units remained invariable up to the second sampling date, experienced a 50% decrease at the third sampling date, and showed a partial recovery at the last sampling date. In this variety, the three terminal units, which were equally represented at the start of the study (around 3500 μmol/g), displayed differential changes during ripening. Thus, terminal units C and EC but not ECG experienced marked increases (to around 6000 μmol/g) at the second sampling date, which lasted up to the third (EC) or even the fourth (C) sampling dates (**Figure 6**). In a relative manner, the EC-P extension unit was somewhat more abundant in Carménère seeds than in Cabernet Sauvignon seeds, at both the second and the third sampling dates. Likewise, Carménère seeds presented higher contents of the C-P and ECG-P extension units than Cabernet Sauvignon seeds. On the other hand, despite the fact that C was the most abundant terminal unit in Cabernet Sauvignon seeds at three sampling dates, thus coinciding with other authors (*8*−*12*, *26*, *34*, *35*), in Carménère seeds, the most important terminal unit in two sampling dates was EC. Likewise, Carménère showed higher values of ECG terminal unit than Cabernet Sauvignon seeds. Also, when all of the extension and terminal units are taken together at each sampling date, Carménère seeds presented higher values than Cabernet Sauvignon seeds.

On the other hand, skin proanthocyanidins differed from seed proanthocyanidins in a lower content of galloylated derivatives and a higher mDP, as also observed by some other authors (*26*). On the other side, mDP, % G, and aMW of flavan-3-oles of Carménère seeds showed a significant decrease at the third sampling date followed by a marked recovery at the last sampling date (**Table 3**). Likewise, in Cabernet Sauvignon seeds, all of those parameters decreased progressively to minimal levels either at technological maturity or at the third sampling date (mDP and aMW) or fourth sampling date (% G). Similar trends and values of mDP and % G have been observed in seeds from berries of different varieties (*8*, *9*, *12*, *34*, *36*, *37*). Surprisingly enough, however, while the mDP values range from 1.8 to 4.3, which correspond in size to dimeric or trimeric procyanidins, the most important flavan-3-ol fraction was found to be the polymeric fraction, which has been associated to procyanidins larger than six flavan-3-ol units (*29*). This result might well suggest that the mDP determined by phloroglucinolysis does not necessarily represent the proportion of different flavan-3-ol fractions in the samples.

Finally, Carménère seeds presented higher mDP and aMW values than Cabernet Sauvignon in the last three sampling dates,

thus reaching a statistically significant difference at the second sampling date. Likewise, Carménère seeds presented statistically higher % G values than Cabernet Sauvignon at two sampling dates during ripening.

Extractable Low Molecular Weight Phenolic Compounds in Grape Seeds during Ripening. **Table 5** shows a group of 13 flavonoid and nonflavonoid compounds that were detected and quantified by HPLC-DAD analysis in the extracts of seeds from the Carménère and Cabernet Sauvignon grape varieties during ripening. Among them, we identified three monomers (C, EC, and ECG), four procyanidin dimers [catechin-(4α→8)-epicatechin (B4), epicatechin-(4β→8)-epicatechin (B2), catechin-(4α→8)-catechin (B3), and epicatechin-(4β→8)-catechin (B1)], three dimers esterified with gallic acid [catechin-(4α→8)-epicatechin-3-*O*-gallate (B4G), epicatechin-3-*O*-gallate-(4β→8)-catechin (BlG), and epicatechin-(4β→8)-epicatechin-3-*O*-gallate (B2G)], one procyanidin trimer [epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (C1)], one trimer esterified with gallic acid [epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-3-*O*-gallate (C1G)], and only one nonflavonoid compound (gallic acid). During ripening, the concentration of each of those compounds in the seeds of both grape varieties experienced a drastic and continuous fall. In accordance with the HPLC analyses, the concentrations of the monomers C and EC in the seeds were higher than those found in the skins. Coinciding with observations made by other authors working with other varieties (*38*−*40*), C, EC, and ECG were the most abundant flavonoid compounds. Also coinciding with other studies, we observed that in Cabernet Sauvignon seeds, the C monomer displayed the highest value during the essay (*8*, *11*, *38*). Likewise, although Cabernet Sauvignon seeds presented higher C values than Carménère while this last one presented higher EC and ECG values than Cabernet Sauvignon during the study, most of the times those differences did not reach statistical significance. Various authors have suggested that these are variety-dependent features (*39*−*41*). However, concentrations of all of these three flavanol monomers in the seeds of both varieties decreased markedly during the study. Relative proportions of these flavanol monomers also changed in both varieties during the same period. Thus, in Cabernet Sauvignon seeds, the C:EC:ECG ratio changed gradually from 55:32:13 on February 20th to 70:26:4 on May 22th, whereas Carménère seeds displayed a similar C:EC:ECG ratio on February 20th (50:36:13) that changed to 45:37:18 in the last sampling date. Flavan-3-ol monomer ratios that are similar to the ones that we have observed now in Carménère seeds have been reported in other grape varieties (*8*, *12*, *39*). According to Kennedy et al. (*12*), differences in the relative proportions of flavan-3-ol monomers are consistent with expected differences when C, EC, and ECG are exposed to radical-induced oxidation under aqueous conditions and a strong relation between the variety and the chemical evolution of monomeric composition in seeds during ripening. The rest of the compounds that were identified in the seed extracts in both grape varieties showed a significant decrease during the study period. Again, although Cabernet Sauvignon seeds presented higher procyanidin C1 values than Carménère samples while this last variety showed higher C1G values than Cabernet Sauvignon during the study, most of the times, those differences did not reach statistical significance either. Interestingly, contents of dimeric procyanidins esterified with gallic acid (B4G, B1G, and B2G) in Carménère seeds were higher as compared with Cabernet Sauvignon samples. With regard to the rest of the extractable flavonoid compounds, there was no differential trend between both varieties. Finally, Carménère seeds showed significantly higher contents of extractable gallic acid as compared with Cabernet Sauvignon seeds.

Table 5. Extractable Low Molecular Weight Phenolic Compounds of Carménère and Cabernet Sauvignon Seeds during Ripening[a]

compounds	date	Carménère	C. Sauvignon
gallic acid	Feb 20[b]	220.0 ± 13.3 a	113.2 ± 40.1 a
	Mar 20	144.0 ± 11.7 a	42.8 ± 14.0 a
	Apr 20[c]	48.6 ± 4.6 a	38.9 ± 2.8 a
	May 22[d]	37.7 ± 2.3 a	36.9 ± 7.7 a
procyanidin B3	Feb 20[b]	131.1 ± 35.6 a	134.8 ± 55.0 a
	Mar 20	54.3 ± 4.1 a	17.0 ± 5.3 a
	Apr 20[c]	43.3 ± 13.4 a	41.8 ± 5.6 a
	May 22[d]	36.8 ± 4.7 a	42.2 ± 9.1 a
procyanidin B1	Feb 20[b]	174.9 ± 30.5 a	186.0 ± 98.0 a
	Mar 20	40.0 ± 3.6 a	68.3 ± 29.1 a
	Apr 20[c]	35.3 ± 2.4 a	31.0 ± 2.3 a
	May 22[d]	37.6 ± 4.3 a	29.2 ± 5.4 a
C	Feb 20[b]	2952.0 ± 666.0 a	3045.0 ± 1007.0 a
	Mar 20	504.5 ± 69.1 a	1553.0 ± 583.0 a
	Apr 20[c]	790.0 ± 348.0 a	702.0 ± 271.0 a
	May 22[d]	467.3 ± 67.1 a	960.0 ± 324.0 a
procyanidin C1	Feb 20[b]	66.0 ± 32.0 a	92.0 ± 45.0 a
	Mar 20	25.1 ± 12.2 a	35.3 ± 16.5 a
	Apr 20[c]	15.5 ± 5.4 a	14.3 ± 1.7 a
	May 22[d]	16.1 ± 2.1 a	18.1 ± 4.3 a
procyanidin B4	Feb 20[b]	249.2 ± 33.8 a	237.0 ± 127.0 a
	Mar 20	49.7 ± 6.4 a	66.3 ± 27.0 a
	Apr 20[c]	41.6 ± 14.1 a	38.3 ± 6.1 a
	May 22[d]	37.1 ± 3.9 a	43.6 ± 9.4 a
procyanidin B2	Feb 20[b]	125.0 ± 12.7 a	147.3 ± 41.1 a
	Mar 20	137.9 ± 21.1 a	139.1 ± 45.6 a
	Apr 20[c]	84.0 ± 9.3 a	69.9 ± 5.3 a
	May 22[d]	84.3 ± 4.8 a	71.7 ± 12.7 a
EC	Feb 20[b]	2115.0 ± 251.0 a	1756.0 ± 897.0 a
	Mar 20	600.6 ± 23.6 a	678.0 ± 231.0 a
	Apr 20[c]	452.0 ± 111.0 a	371.2 ± 14.7 a
	May 22[d]	378.9 ± 29.3 a	360.4 ± 84.2 a
procyanidin B4G	Feb 20[b]	90.0 ± 18.8 a	66.1 ± 33.1 a
	Mar 20	16.1 ± 6.9 a	17.4 ± 5.0 a
	Apr 20[c]	30.8 ± 0.1 a	24.4 ± 9.3 a
	May 22[d]	29.7 ± 2.4 a	24.3 ± 4.4 a
procyanidin B1G	Feb 20[b]	345.0 ± 117.0 a	273.0 ± 132.0 a
	Mar 20	21.1 ± 1.6 a	17.1 ± 5.5 a
	Apr 20[c]	24.0 ± 1.4 b	19.6 ± 1.6 a
	May 22[d]	20.8 ± 1.6 a	20.0 ± 3.5 a
procyanidin B2G	Feb 20[b]	83.4 ± 33.1 a	52.0 ± 31.4 a
	Mar 20	55.3 ± 2.3 a	36.5 ± 14.4 a
	Apr 20[c]	51.2 ± 5.5 a	40.3 ± 13.5 a
	May 22[d]	57.5 ± 6.6 b	34.5 ± 6.5 a
ECG	Feb 20[b]	880.0 ± 374.0 a	734.0 ± 124.0 a
	Mar 20	214.0 ± 101.0 a	169.9 ± 48.7 a
	Apr 20[c]	175.8 ± 49.6 a	111.8 ± 81.1 a
	May 22[d]	182.1 ± 19.0 b	52.2 ± 11.3 a
procyanidin C1G	Feb 20[b]	198.5 ± 8.2 a	238.0 ± 130.0 a
	Mar 20	100.0 ± 51.2 a	88.7 ± 41.5 a
	Apr 20[c]	30.2 ± 1.6 a	25.0 ± 10.6 a
	May 22[d]	31.9 ± 4.3 b	15.1 ± 5.9 a

[a] Figures are expressed in mg/kg and represent means ± standard deviations (triplicates). Values with different letters in the row file are significantly different (Student's *t* test, $p < 0.05$). [b] Veraison. [c] Harvest time. [d] Over maturity.

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