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Phenolic composition of skins from four Carmenet grape varieties (*Vitis vinifera* L.) during ripening

Elías Obreque-Slier [a,*], Álvaro Peña-Neira [a], Remigio López-Solís [b], Alejandro Cáceres-Mella [a], Héctor Toledo-Araya [b], Américo López-Rivera [b]

[a] Department of Agro-Industry and Enology, Faculty of Agronomical Sciences, University of Chile, P.O. Box 1004, Santiago, Chile
[b] Program of Cellular and Molecular Biology, Faculty of Medicine-ICBM, University of Chile, Independencia 1027, Santiago, Chile

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ABSTRACT

Phenolic composition of skins from *Vitis vinifera* L. cv Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) grapes during ripening was evaluated by HPLC-DAD and spectrophotometric analysis. At the time of harvest maturity, CS skins showed the highest contents of monomeric, oligomeric and polymeric flavan-3-ols with respect to other varieties. Likewise, the L^*, C^* and a^* CIElab parameters showed no statistical differences between the four varieties but the H^* and b^* CIElab parameters exhibited clear differences between the M and CS skins. Alike, the sum of glucoside and cumarilglucoside anthocyanins in CA identified by HPLC-DAD was higher than in CF and CS in some sampling date. Additionally, significant differences in the content of low molecular weight phenolic compounds quantified by HPLC-DAD were observed. Comparatively, the CS skins displayed higher mDP, %EG and aMW values than the rest of the cultivars on the last sampling date. Overall, we conclude that CA, M, CF and CS grape skins present marked differences in phenolic composition during ripening.

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1. Introduction

Phenolic compounds constitute a complex and highly diverse family of secondary metabolites of plants that are present in the fruits of *Vitis vinifera* L. Flavonoids and non flavonoids are the two major classes of phenolic compounds. Non flavonoids comprise phenolic acids, cinnamic acids and stilbenes whereas flavonoids are mostly represented by flavonols, anthocyans and flavan-3-ols or proanthocyanidins (Cheynier & Rigaud, 1986; Escribano-Bailón, Gutiérrez-Fernández, Rivas-Gonzalo, & Santos-Buelga, 1992; Matus et al., 2008; Peña Neira, Dueñas, Duarte, Hernández, Estrella & Loyola, 2004; Peña-Neira, Cáceres, & Pastenes, 2007; Santos-Buelga, Francia-Aricha, & Escribano-Bailón, 1995). In the fruits of *Vitis vinifera* L., phenolic compounds are found in skins, seeds, stems and pulps (teinturier grape varieties). During winemaking these compounds are differentially extracted thus providing red wine with a diversity of sensory features, such as color, aroma, astringency and bitterness (Gil-Muñoz, Gómez-Plaza, Martínez, & López-Roca, 1997; Guerrero et al., 2009; Obreque-Slier, Peña-Neira, & López-Solís, 2012; Pérez-Magariño & González-San José, 2003). Unlike grape seeds and stems, grape skins contain a large array of phenolic compounds, which comprise all those mentioned above (Ricardo da Silva, Rigaud, Cheynier, Cheminat, & Moutounet, 1991; Souquet, Labarbe, Le Guernevé, Cheynier, & Moutounet, 2000). Both composition and concentration of phenol compounds in grapes is largely dependent on a number of factors, such as climate (Bergqvist, Dokoozlian, & Ebisuda, 2001), stage of grape maturity (Obreque-Slier et al., 2010; Obreque-Slier, López-Solís, Castro-Ulloa, Romero-Díaz, & Peña-Neira, 2012), plant growing techniques (Intrieri, Filippetti, Allegro, Centinari, & Poni, 2008) and plant cultivar (Guendez, Kallithraka, Makris, & Kefalas, 2005; Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Ricardo Da Silva, 2003;Obreque-Slier et al., 2010; Obreque-Slier, López-Solís, et al., 2012; Rodríguez-Montealegre, Romero-Peces, Chacón-Vozmediano, Martínez Gascueña, & García Romero, 2006; Sun Ricardo da Silva & Spranger, 1998a).

Currently, more than 5000 *Vitis vinifera* cultivars are used for the production of wine (Hidalgo, 2003). These have been grouped into families. Carmenet is one of the most important families of *Vitis vinifera* in the world and comprises the cultivars Merlot, Cabernet Franc, Cabernet Sauvignon, Fer Servadou, Petit Verdot and

* Corresponding author. Tel.: +56 2 9786477; fax: +56 2 9785796.
E-mail address: eobreque@uchile.cl (E. Obreque-Slier).

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Carménère (Reynier, 2002). Carménère is a late-maturing cultivar that was devastated from the European vineyards by the Phylloxera plague in the 19th century. This scarcely known variety was rediscovered in Chile in 1994, after being mistaken during a long time for Merlot and Cabernet Franc, two Carmenet cultivars displaying similar ampelographic features. In Chile, Carménère is grown in single vineyards on a total surface area close to 8000 ha (Servicio Agrícola y Ganadero, 2008). To date, the cultivar Carménère has not been included in studies assessing the varietal effect on the phenolic composition of *Vitis vinifera* grape skins (Guendez et al., 2005; Guerrero et al., 2009; Monagas et al., 2003; Rodríguez-Montealegre et al., 2006; Sun et al., 1998a). In addition, comparative information on the phenolic composition encompassing more than two members of the Carmenet family is scarce. The objective of the present study was to evaluate the phenolic composition of grape seeds of the Carmenet varieties Carménère, Merlot, Cabernet Franc and Cabernet Sauvignon during maturation. All of them have been grown under similar edaphoclimatic and cultural conditions.

2. Materials and methods

2.1. Materials

Standards of gallic acid (G-7384), vanillinic acid (V-2250), syringic acid (S-6881), quercetin (Q-0125), myricetin (M-6760), kaempherol (K-0133), malvidin-3-glucoside (79311), (+)-catechin (C-1251) and (−)-epicatechin-3-*O*-gallate (E-3893), phloroglucinol (P-3502) and 0.45-μm pore size membranes were acquired from Sigma Chemical Company, Saint Louis, Missouri, USA. Vanillin 99% (code V-8510), trifluoroacetic acid, ethyl acetate, acetonitrile HPLC grade and pro-analysis solvents were purchased from Merck, Darmstadt, Germany. Sep-Pak Plus tC_{18} cartridges WAT 036810 and WAT 036800 were obtained from Waters (Milford, MA, USA). Toyopearl TSK HW 40-F size exclusion resin (N° 807448) was obtained from Toso Haas, Stuttgart, Germany.

2.2. Instrumentation

Absorbances were measured using a Jasco UV–Vis spectrophotometer Model V-530 (JASCO International Co., Ltd., Tokyo, Japan). A pH meter Hanna Instruments pH 211 was used. The HPLC system (Agilent Technologies Santa Clara, CA., USA) 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 ID × 300 mm; Waters Corporation, Milford, MA., USA) was used for HPLC-DAD analysis of individual phenolic compounds. A reversed phase LiChro Cart 100 RP-18 column (5 μm, 4 mm ID × 250 mm; Agilent Technologies, Santa Clara, CA., USA) was used in phloroglucinolysis and anthocyanins studies.

2.3. Grape samples

Self-rooted *Vitis vinifera* L. cv. Carménère, Merlot, Cabernet Franc y Cabernet Sauvignon vines, vintage 2008, planted in 2000 and grown in the Maipo Valley (Latitude 33° 38′ 23.3″ S, Longitude 70° 41′ 30.6″ O) at the Metropolitan Region of Chile were used. The growing site was characterized by a Mediterranean semi-arid climatological condition, cold winter with moderate rainfall and hot dry summer, and deep colluvial/alluvial soils of frank texture. Agronomic, photochemical and technical variables were controlled. Three groups of 120 berries per variety were selected from 4 clusters per plant among a total of 30 plants. The samples were harvested monthly from February 20 (*veraison*) to May 20 and analyzed in triplicate for all the assays in the study excepting phloroglucinolysis and flavan-3-ol fractionation. For convenience, these latter assays were performed only on samples taken at *veraison* and technical maturity. The phenolic compounds were extracted from grape skins as described in previous reports (Obreque-Slier et al., 2010a; Obreque-Slier, López-Solís, et al., 2012).

2.4. Spectrophotometric characterization

Total phenol content was determined by UV-absorptiometry at 280 nm (Ribéreau-Gayon, 1970) using gallic acid as standard. Total tannin content was measured by the method of Ribéreau-Gayon and Stonestreet (1966). Total anthocyanins were measured by diluting the extract with acidified ethanol (2 mL hydrochloric acid in 100 mL ethanol), and by comparing spectrophotometric readings of single aliquots treated either with sodium metabisulfite or water (Ribéreau-Gayon & Stonestreet, 1965). The CIElab parameters were determined according to the method of Ayala, Echávarri, and Negueruela (1997).

2.5. HPLC-DAD analysis of anthocyanins compounds

One-hundred milliliters of each extract was filtered through a 0.45 μm pore size and then subjected to reversed-phase chromatographic separation at 20 °C. The photodiode array detector was set from 210 to 600 nm. Two mobile phases were used as follows: A, water/formic acid (90:10 mL:mL), and B, acetonitrile. A gradient was applied at a flow rate of 1.1 mL/min from 0 to 22 min and 1.5 mL/min from 22 to 35 min as follows: 96–85% A from 0 to 12 min, 85–15% A from 12 to 22 min, 85–70% A from 22 to 35 min. The quantification was carried out by peak area measurements at 520 nm. Anthocyanins were quantified and expressed as mg/L of malvidin-3-glucoside. The calibration curves at 520 nm were obtained by injection of different volumes of standard solutions under the same conditions as for the samples analyzed (Peña-Neira et al., 2007).

2.6. HPLC–DAD analysis of individual phenolic compounds

Extracts of skin 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, re-dissolved in 2 mL (50 mL methanol/100 mL water) and membrane-filtered (0.45 μm pore size) (Matus et al., 2008; Obreque-Slier et al., 2010; Obreque-Slier, López-Solís, et al., 2012; Peña-Neira et al., 2007, 2004). 5 μL 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: A, water/acetic acid (98:2 mL:mL) and B, water/acetonitrile/acetic acid (78:20:2 mL:mL:mL). 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 identified both by retention time and absorption spectrum (from 210 to 360 nm) against those of pure standards. Glycosides of flavonols for which standards were unavailable were assigned by retention time and spectral parameters as was described in previous reports (Matus et al., 2008; Obreque-Slier et al., 2010; Obreque-Slier, López-Solís, et al., 2012; Peña-Neira et al., 2007, 2004). Quantitative determinations were made by using the external standard method and commercial standards.

2.7. Fractionation of proanthocyanidins into monomers, oligomers and polymers

Skin extracts (see above) were fractionated using Waters C_{18} Sep-Pak cartridges according to the method described by Sun, Leandro, Ricardo da Silva, and Spranger (1998). Briefly, 8 mL of skin extracts were concentrated to dryness in a rotary evaporator at <30 °C. The residue was dissolved in 20 mL of 67 mmol/L 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 mmol/L 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 mmol/L phosphate buffer, pH 7.0, and re-loaded 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.

2.8. Total content of flavan-3-ols in the monomer, oligomer and polymer fractions

The vanillin assay was performed as described by Sun, Leandro, Ricardo da Silva, and Spranger (1998b). A 2.5-mL aliquot of 1:3 mL:mL sulfuric acid/methanol solution and a 2.5-mL aliquot of vanillin solution (1 g vanillin/100 mL of 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). Absorbance was read at 500 nm. A blank was prepared by substituting the vanillin solution in the reaction mix with methanol.

2.9. Phloroglucinolysis

The procedure was performed basically as described by Kennedy and Jones (2001). Briefly, 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/trifluoroacetic acid 55:45:0.5 mL:mL:mL (solution A). After loading the extract, 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 mL:mL 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 0.5 mL of methanol to proceed with phloroglucinolysis. Half mL aliquots of each of the proanthocyanidin solutions in methanol from skins were allowed to react with 0.5 mL of solution C (0.25 g of ascorbic acid, 1.25 g of phloroglucinol, 215 μL concentrated hydrochloride acid in 25 mL of methanol) at 50 °C for 20 min. At the end of the incubation the reaction was stopped with 0.5 mL of 200 mmol/L sodium acetate. The chromatographic separation used a binary gradient with mobile phases of 1 mL acetic acid/100 mL water (mobile phase A) and methanol (mobile phase B) and the elution was monitored at 280 nm. Elution conditions were: 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. Both mean degree of polymerization (mDP), galloylation percentage (%G), epigalloylation percentage (%EG) and average molecular weight (aMW) were calculated as described by Kennedy and Jones (2001). All the qualitative and quantitative analyzes of phenolic composition (including extraction) were performed in triplicate.

2.10. Statistical analysis

Minitab Release software version 13.32 and Tukey's t test were applied to contrast quantitative variables with a 95% confidence interval.

3. Results and discussion

3.1. General analytical parameters

Grapes of the four cultivars were sampled monthly: 15 days after veraison (February 20th), 45 days after veraison (March 20th), during harvest maturity (April 20th) and over maturity (May 20th). Table 1 shows skin weights and the global phenolic composition of Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) grapes during ripening.

As for the grape skin weights, none of the four cultivars showed a distinctive behavior during ripening although the CA and CS skins presented weight values significantly higher on the last sampling date. This trend has been also observed in previous studies (Obreque-Slier et al., 2010; Peña-Neira et al., 2007).

Total phenol and total anthocyanin contents in grape skins reached their highest concentrations on the second sampling date and decreased gradually toward the last sampling date in

Table 1
General analytical parameters of Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) grape skins during ripening.

		20-February	20-March	20-April	20-May
Skin weight (g/100 grapes)	CA	31.2 ± 1.6aAB	26.3 ± 0.8aA	24.1 ± 0.1aA	36.2 ± 6.6aB
	M	33.6 ± 1.1aA	25.0 ± 2.3aA	32.2 ± 5.9abA	26.1 ± 4.0aA
	CF	35.7 ± 3.4aA	23.4 ± 1.1aA	31.2 ± 7.4abA	31.0 ± 5.2aA
	CS	31.2 ± 4.0aAB	23.0 ± 1.0aA	39.2 ± 5.0bB	35.6 ± 3.2aB
Total phenols (mg GAE/g skin)	CA	3.2 ± 0.4aA	3.9 ± 0.6aA	3.2 ± 0.6aA	1.9 ± 0.2bB
	M	2.6 ± 0.0abA	4.4 ± 0.2aB	2.9 ± 0.3aA	2.7 ± 0.5aA
	CF	2.0 ± 0.1bA	3.7 ± 0.3aB	2.5 ± 1.0aAB	2.2 ± 0.6abAB
	CS	2.2 ± 0.3bA	3.8 ± 0.5aB	1.9 ± 0.1aA	2.3 ± 0.5abA
Total tannins (mg CE/g skin)	CA	6.6 ± 0.8aA	7.9 ± 1.8aA	7.3 ± 0.6aA	7.8 ± 2.2aA
	M	5.6 ± 0.3abA	8.4 ± 1.1aAB	9.2 ± 0.7aB	8.4 ± 2.0aAB
	CF	3.7 ± 1.5bA	7.1 ± 1.5aA	7.8 ± 2.2aA	6.1 ± 1.6aA
	CS	3.5 ± 0.3bA	9.3 ± 1.8aB	7.0 ± 0.4aB	7.1 ± 1.3aB
Total anthocyanins (mg ME/g skin)	CA	3.3 ± 0.4aA	4.0 ± 0.6aA	2.8 ± 0.5aA	1.3 ± 0.0aB
	M	2.7 ± 0.2abA	4.3 ± 0.3aB	2.3 ± 0.4aA	2.2 ± 0.4aA
	CF	2.0 ± 0.1bA	3.7 ± 0.2aB	1.9 ± 0.8aA	1.8 ± 0.4aA
	CS	2.1 ± 0.3bA	3.7 ± 0.5aB	1.5 ± 0.0aA	1.9 ± 0.4aA

Figures represent mean ± standard deviation (triplicates). Different small letters in single columns stand for statistically significant differences between cultivars and different capital letters in single rows stand for statistically significant differences between sampling dates (Tuckey test, $p < 0.05$). GAE, gallic acid equivalent; CE, (+)-catechin equivalent; ME, malvidin-3-glucoside equivalent.

coincidence with studies previous (Matus et al., 2008; Peña-Neira et al., 2004). Either CA or M skins presented the highest contents of total phenol and total anthocyanins compared with the other two cultivars in some ripening stages.

With regard to the content of total tannins in the skins of the various cultivars, we observed no significant differences during ripening of CA and CF but significant increases in the CS and M skins on the second and third sampling dates, respectively. Likewise, while M exhibited higher values of tannins toward the last sampling dates, the CA skins presented significantly higher contents of tannins at the early stage of maturation when compared with the CF and CS skins.

3.2. Identification of CIElab parameters

The CIElab system describes chromatic characteristics by using several colorimetric coordinates (Pérez-Magariño & González-San José, 2003). Table 2 shows the values of clarity (L^*), chroma (C^*), hue (H^*), a^* and b^* in extracts of grape skins of the four varieties at different stages of maturity. The L^* coordinate varies between 100 (perfect white) and cero (black) (Gil-Muñoz et al., 1997). In this study, the four cultivars showed values in the range between 56.9 and 79.1. While CA and M exhibited higher values toward the latter maturation dates, the CF and CS skins presented no significant variation during ripening (Table 2). The a^* parameter goes from red (+) to green colors (−) (Pérez-Magariño & González-San José, 2003). In this study, all the varieties exhibited only positive values. Besides, CA and M presented lower a^* values toward the final stages of the study, thus coinciding with lower contents of total phenols and total anthocyanins observed in this study. The b^* values vary from yellow (+) to blue colors (−) (Pérez-Magariño & González-San José, 2003). In this study, all the four cultivars presented only negative values. It is interesting to note that while the CA and CS skins presented the highest values on the last sampling date, the M and CF grape skins showed no variations during ripening. Also, the M skins presented the most positive and CS the most negative b^* values during the study.

The C^* parameter indicates the contribution of a^* (red color) and b^* (yellow color) to total color (Gil-Muñoz et al., 1997). In this study, the chroma of CA and M skins were found to be significantly decreased on the last sampling date. Significant differences between varieties were observed only on the first sampling date between CA and CS. Finally, the H parameter indicates the tone or hue and varies between violet ($H = 315°$) and rose ($H = 0°$) (Pérez-Magariño & González-San José, 2003). The M skins showed the highest values of hue, while the CS skins showed the lowest values of hue throughout the study. Those differences were statistically significant on the last three sampling dates.

In sum, the L^*, C^* and a^* parameters showed no statistical differences between the four varieties but the H^* and b^* parameters exhibited clear differences between the M and CS skins. In addition, the CF skins presented no variation in any the CIElab parameters during maturation.

Several reports have shown changes in CIElab color parameters during maturation in different cultivars. However, studies comparing the colorimetric composition of skins from different grape varieties are scarce and most of them deal only with the characterization of wines produced with grapes collected at different maturity stages (Pérez-Magariño & González-San José, 2004, 2005). Although in this study we observed some significant but particular differences in some CIElab color parameters among members of the Carmenet family, our results would suggest a relationship between the variety and the colorimetric composition of grape skins.

3.3. Extractable anthocyanins in grape skins during ripening

Table 3 shows the content of anthocyanins after HPLC-DAD analysis of grape skin extracts from the CA, CS, M and CF varieties during ripening. In this study we identified delfinidin-3-glucoside (DPgl), cyanidin-3-glucoside (CYgl), petunidin-3-glucoside (PTgl), peonidin-3-glucoside (POgl), malvidin-3-glucoside (MVgl), delfinidin-3-acetilglucoside (DPac), cyanidin-3-acetilglucoside (CYgl), petunidin-3-acetilglucoside (PTac), peonidin-3-acetilglucoside

Table 2
CIELAB color space values.

		20-February	20-March	20-April	20-May
L^* (Lightness)	CA	59.6 ± 4.8aA	57.7 ± 6.6aA	71.1 ± 6.2aAB	79.1 ± 4.9aB
	M	65.3 ± 2.4abA	56.9 ± 1.6aB	66.3 ± 2.5aAC	73.0 ± 3.9aC
	CF	67.7 ± 1.4bA	64.8 ± 2.4aA	74.2 ± 9.3aA	73.6 ± 2.3aA
	CS	71.7 ± 3.1abA	65.8 ± 2.6aA	74.0 ± 3.6aA	71.8 ± 5.6aA
C^* (saturation)	CA	51.8 ± 4.7aA	51.8 ± 5.5aA	37.3 ± 7.8aAB	27.1 ± 6.6aB
	M	46.1 ± 2.2abAB	56.9 ± 1.6aA	43.7 ± 3.5aAB	37.9 ± 6.8aB
	CF	35.8 ± 1.6abA	45.3 ± 5.5aA	32.3 ± 12.1aA	37.2 ± 4.0aA
	CS	38.4 ± 9.7bA	43.4 ± 2.1aA	33.0 ± 4.6aA	35.9 ± 6.3aA
H^* (hue)	CA	350.6 ± 2.4aAB	350.5 ± 1.8abAB	349.0 ± 0.4aA	353.4 ± 0.5abB
	M	354.3 ± 3.3aA	354.5 ± 0.8aA	354.0 ± 1.3bA	356.0 ± 1.8aA
	CF	344.9 ± 30.9aA	350.1 ± 3.1abA	351.3 ± 2.7abA	351.6 ± 2.4bA
	CS	330.9 ± 1.3aA	347.3 ± 0.6bB	348.3 ± 0.4aB	349.3 ± 1.1bB
a^*	CA	51.1 ± 4.3aA	51.1 ± 5.6aA	36.6 ± 7.7aAB	26.9 ± 6.6aB
	M	45.8 ± 2.0aAB	53.2 ± 1.4aA	44.0 ± 2.7aAB	37.8 ± 6.9aB
	CF	30.5 ± 1.4aA	44.6 ± 5.6aA	32.0 ± 12.1aA	36.8 ± 4.2aA
	CS	37.1 ± 17.3aA	42.3 ± 2.1aA	32.3 ± 4.6aA	35.3 ± 6.3aA
b^*	CA	−8.6 ± 2.9aA	−8.4 ± 0.9aA	−7.1 ± 1.3aAB	−3.1 ± 0.8aB
	M	−4.6 ± 2.8aA	−5.1 ± 0.6bA	−4.6 ± 0.9aA	−2.6 ± 0.9aA
	CF	−10.1 ± 8.4aA	−7.6 ± 5.6abA	−4.8 ± 2.0aA	−4.7 ± 1.6abA
	CS	−12.8 ± 1.1aA	−9.5 ± 0.7aA	−6.7 ± 0.8aB	−6.6 ± 1.1bB

Figures represent mean ± standard deviation (triplicates). Different small letters in single columns stand for statistically significant differences between cultivars and different capital letters in single rows stand for statistically significant differences between sampling dates (Tuckey test, $p < 0.05$).

Table 3
Extractable anthocyanins (mg/Kg) of Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) skins during ripening.

		20-February	20-March	20-April	20-May
DPgl	CA	150.0 ± 50.6aAB	172.8 ± 48.4aA	84.3 ± 64.8aAB	48.2 ± 9.0aA
	M	165.2 ± 67.0aA	345.9 ± 35.2bB	142.1 ± 21.2aA	142.0 ± 50.8bA
	CF	97.5 ± 41.6aA	104.6 ± 6.4cA	108.9 ± 33.3aA	81.6 ± 36.1abB
	CS	37.4 ± 31.4aA	92.1 ± 84.6acA	52.3 ± 10.8aA	39.3 ± 9.5aA
CYgl	CA	25.6 ± 7.3aA	23.7 ± 9.6aA	19.6 ± 4.3aAB	5.6 ± 4.0aB
	M	89.7 ± 35.0bA	167.3 ± 12.0bB	79.8 ± 10.0bA	83.9 ± 36.7bA
	CF	29.7 ± 28.6aA	24.5 ± 1.7aA	36.4 ± 36.0abA	28.4 ± 31.9abA
	CS	4.7 ± 3.4aA	11.2 ± 8.0aA	2.5 ± 0.8aA	3.4 ± 1.9aA
PTgl	CA	146.3 ± 54.4aA	159.7 ± 17.8abA	118.8 ± 28.4aAB	55.7 ± 7.7aB
	M	139.8 ± 55.8bA	248.3 ± 16.6aB	116.0 ± 19.1aA	124.8 ± 41.6bA
	CF	95.1 ± 14.0abA	64.5 ± 3.1abB	100.1 ± 25.4abA	81.8 ± 27.5abA
	CS	45.6 ± 37.5bA	77.0 ± 68.6bA	53.7 ± 10.8bA	51.7 ± 7.3aA
POgl	CA	71.2 ± 38.1aA	106.0 ± 32.1aA	89.7 ± 9.6aA	46.5 ± 5.5aA
	M	195.4 ± 77.1bA	350.5 ± 23.7bB	183.0 ± 36.1bA	220.4 ± 79.7bAB
	CF	78.4 ± 24.5abAB	64.9 ± 2.0aA	121.3 ± 47.9abB	101.7 ± 56.2abAB
	CS	37.9 ± 31.0aA	56.0 ± 9.7aA	37.6 ± 9.4acA	45.8 ± 12.5aA
MVgl	CA	809.2 ± 321.1aAB	1155.0 ± 124.5aA	912.6 ± 152.3aAB	507.6 ± 25.4aB
	M	579.0 ± 207.8aA	1082.2 ± 116.3aB	475.0 ± 60.4bA	612.5 ± 145.3aA
	CF	475.8 ± 23.8aA	417.8 ± 4.5bB	595.0 ± 222.4abA	546.8 ± 57.6aA
	CS	429.1 ± 351.1aA	777.5 ± 737.1abA	531.4 ± 98.1abA	684.8 ± 195.5aA
DPac	CA	20.5 ± 17.0aA	19.4 ± 5.8aA	9.0 ± 1.4abA	4.2 ± 2.4aA
	M	20.2 ± 7.8aA	38.9 ± 4.1bB	15.3 ± 5.8aA	15.5 ± 7.3bA
	CF	19.6 ± 5.5aA	10.9 ± 0.4cB	15.4 ± 6.1aA	11.7 ± 4.3abAB
	CS	5.3 ± 5.3aA	7.8 ± 6.9acA	4.1 ± 1.8bA	2.2 ± 0.4aA
CYac	CA	8.4 ± 4.8abA	5.4 ± 1.8aA	2.8 ± 0.1aA	2.2 ± 0.5aA
	M	13.8 ± 6.2aA	22.5 ± 1.2bA	43.7 ± 7.9bA	12.4 ± 10.7aA
	CF	6.3 ± 2.8abA	3.5 ± 0.2cA	5.5 ± 2.5aA	5.1 ± 4.5aA
	CS	1.4 ± 1.1bA	1.7 ± 1.9cA	2.0 ± 1.3aA	8.2 ± 3.6aB
PTac	CA	39.8 ± 13.8aA	45.8 ± 8.4aA	27.0 ± 1.0abAB	12.2 ± 1.7aB
	M	33.9 ± 13.6aA	60.9 ± 3.0aB	26.6 ± 6.1abA	29.8 ± 8.7bA
	CF	35.7 ± 4.0aA	21.2 ± 0.7bB	31.4 ± 8.2aA	27.1 ± 3.3abA
	CS	16.8 ± 14.4aA	27.0 ± 24.9abA	14.4 ± 1.9bA	14.6 ± 4.3aA
POac	CA	16.2 ± 9.9aA	20.5 ± 13.8aA	20.3 ± 1.2abA	9.7 ± 1.8aA
	M	31.0 ± 11.8aA	57.0 ± 3.1bB	28.7 ± 7.0abA	36.2 ± 11.9bAB
	CF	27.6 ± 3.5aA	20.8 ± 0.4aB	36.1 ± 13.4aA	31.2 ± 6.6bA
	CS	9.4 ± 8.0aA	14.7 ± 12.9aA	10.0 ± 3.0bA	11.3 ± 2.7aA
MVac	CA	273.2 ± 68.9aAB	422.0 ± 127.4aA	303.3 ± 21.3aAB	156.1 ± 25.0aB
	M	157.1 ± 53.4aA	308.3 ± 30.6aB	140.4 ± 18.1aA	183.1 ± 52.6aA
	CF	191.6 ± 29.7aAB	157.7 ± 0.5bA	276.1 ± 155.1aB	256.3 ± 42.7aB
	CS	224.0 ± 183.2aA	393.1 ± 369.4aA	241.4 ± 59.2aA	300.7 ± 71.8aA
DPcu	CA	1.0 ± 0.7aA	NDaA	NDaA	NDaA
	M	2.5 ± 2.2aA	3.0 ± 2.6aA	NDaA	NDaA
	CF	1.2 ± 1.1aA	NDaA	NDaA	NDaA
	CS	NDaA	NDaA	NDaA	NDaA
CYcu	CA	11.7 ± 2.3aA	11.5 ± 0.6aA	5.3 ± 1.3aB	2.3 ± 0.2aB
	M	5.2 ± 1.7bAB	6.2 ± 2.1abA	1.7 ± 0.2bB	2.2 ± 0.6aB
	CF	4.9 ± 0.9bA	1.8 ± 1.0bB	3.4 ± 2.3abAB	2.1 ± 1.0aAB
	CS	4.4 ± 3.8bA	8.1 ± 8.3abA	3.7 ± 1.1abAB	8.9 ± 5.2bA
PTcu	CA	5.6 ± 4.1aA	5.4 ± 2.8aA	3.6 ± 1.9aA	0.6 ± 0.5aA
	M	2.4 ± 1.0aA	6.3 ± 1.0aB	3.8 ± 1.3aAB	3.3 ± 2.0aAB
	CF	2.2 ± 0.6aA	2.0 ± 0.0bA	3.5 ± 1.8aA	2.5 ± 2.4aA
	CS	0.4 ± 0.5aA	1.4 ± 0.4bA	1.1 ± 0.3aA	0.0 ± 0.0aA
POcu	CA	5.6 ± 1.0aA	5.0 ± 0.8aA	3.0 ± 0.6abB	1.4 ± 0.8aB
	M	5.9 ± 2.3aA	13.2 ± 1.8bA	7.0 ± 3.3aA	7.5 ± 4.7aA
	CF	5.5 ± 0.2aA	4.0 ± 0.1cB	6.2 ± 1.9abA	6.0 ± 2.9aA
	CS	1.4 ± 1.2bA	1.8 ± 1.6cA	1.0 ± 1.0bA	0.7 ± 0.2bA

Table 3 (*continued*)

		20-February	20-March	20-April	20-May
MVcu	CA	65.8 ± 35.2aA	83.4 ± 31.0aA	58.3 ± 33.3aA	27.4 ± 11.4aA
	M	22.9 ± 6.0abA	52.0 ± 5.2abB	27.6 ± 5.3aA	30.8 ± 9.1aA
	CF	20.7 ± 1.6bAB	20.7 ± 0.3bA	38.2 ± 20.4aB	33.9 ± 7.5aB
	CS	14.0 ± 12.2bA	30.2 ± 29.8cA	21.3 ± 5.1aA	21.9 ± 7.9aA

Figures represent mean ± standard deviation (triplicates). Different small letters in single columns stand for statistically significant differences between cultivars and different capital letters in single rows stand for statistically significant differences between sampling dates (Tuckey test, $p < 0.05$). ND, not detected.

(POac), malvidin-3-acetilglucoside (MVac), delfinidin-3-*p*-cumarilglucoside (DOcu), cyanidin-3-*p*-cumarilglucoside (CYcu), petunidin-3-*p*-cumarilglucoside (PTcu), peonidin-3-*p*-cumarilglucoside (POcu) and malvidin-3-*p*-cumarilglucósido (MVcu). Interestingly, all these anthocyanins were present in all the four varieties. Quantitatively, we identified MVgl as the most abundant anthocyanin in all the four varieties. This observation was in full accordance with those of other authors (Boss, Davies, & Robinson, 1996; Burs et al., 2002). The CA, M and CS skins presented the highest contents of the most abundant anthocyanins on the second sampling date, thus coinciding with the behavior of total anthocyanins and with previous studies (Matus et al., 2008; Peña-Neira et al., 2004). In addition, eight of fifteen anthocyanins of the CA skins exhibited a decreasing tendency to the last sampling date while the other seven anthocyanins remained mostly unchanged. On the other hand, while eleven anthocyanins identified in the M skins presented their maxima concentrations on the second sampling date, nine anthocyanins were exhibiting their lowest concentrations in the CF skins. The concentrations of all the anthocyanins of the CS skins (with exception of CYac) showed no significant variations during the study. On the other side, the sum of glucoside and cumarilglucoside anthocyanins in CA was higher than in CF on the second sampling date. Likewise, concentrations of glucoside and cumarilglucoside anthocyanins in CA skins was higher than in CS skins on the first (cumarilglucoside) and third (glucoside) sampling dates (Fig. 1). Interestingly, the concentration of acetylglucoside anthocyanins was similar among all the four cultivars during ripening. Finally, the sum of the contents of all the anthocyanins identified in the grape skins of the cultivars showed that the CA skins have concentrations significantly higher than the CF skins, but only on the second sampling date (Fig. 1). It is worth mentioning that the anthocyanin concentrations found in our study are in full agreement with those observed in previous works (Peña-Neira et al., 2007) and that similar differences have been observed between CS and other grape varieties (Guerrero et al., 2009).

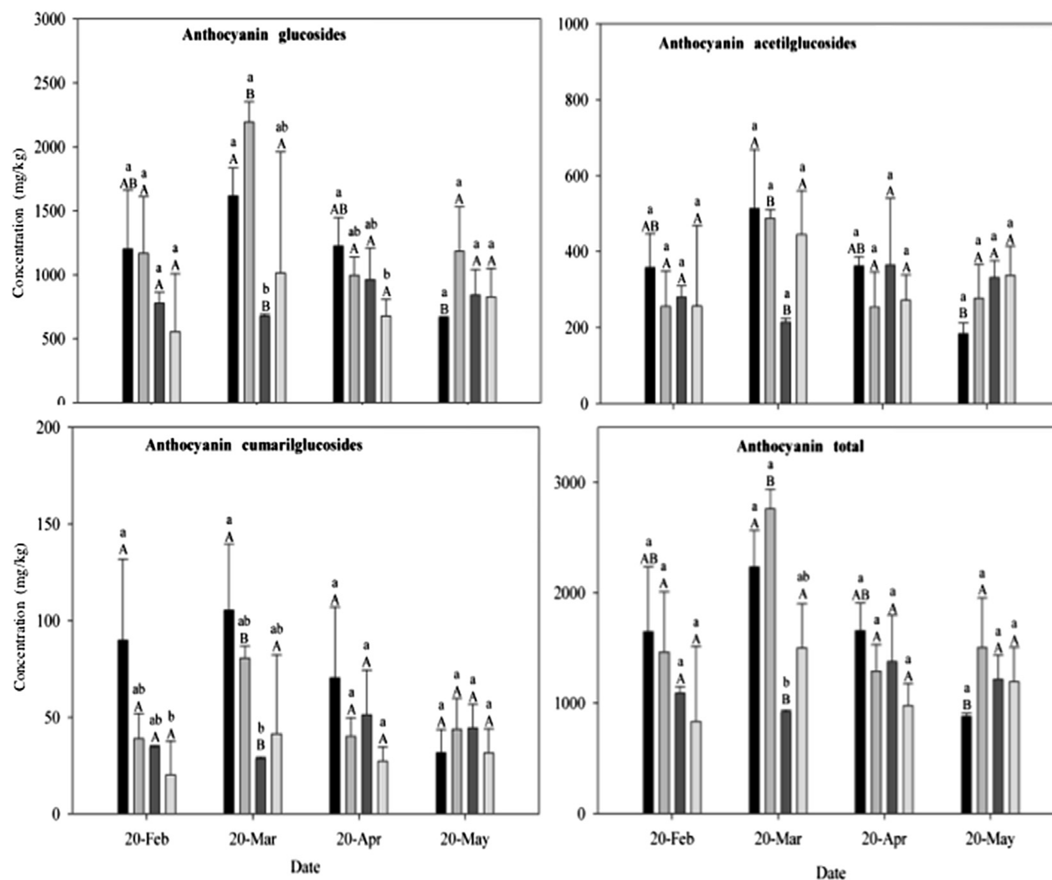

Fig. 1. Anthocyanin-glucosides, -acetylglucosides and -cumarilglucosides and total extractable anthocyanins of varietal grape skins during ripening. Different small letters on top of the bars stand for statistically significant differences between cultivars at a single date. Different capital letters on top of the bars stand for statistically significant differences between samples of single cultivars taken at different dates (Tuckey test, $p < 0.05$). Carménère, Merlot, Cabernet Franc, Cabernet Sauvignon.

3.4. Extractable low molecular weight phenolic compounds in grape skins during ripening

Table 4 shows contents of flavonoids and non–flavonoids compounds in skin extracts of the CA, CS, CF and M grape varieties during ripening. HPLC–DAD analysis showed three non-flavonoids [gallic acid (GA), vanillinic acid (VA) and syringic acid (SA)], two flavan-3-ols [(+)-catechin (C) and epicatechin-3-*O*-gallate (ECG)] and various flavonoids [myricetin-3-galactoside (Mga), myricetin -3-glucoside (Mgl), quercetin-3-galactoside (Qga), quercetin-3-glucuronide (Qgr), quercetin-3-glucoside (Qgl), kaempherol-3-galactoside (Kga), kaempherol-3-glucoside (Kgl), isorhamnetin-3-glucoside (Igl) and isorhamnetin-3-glucoronide (Igr)]. These compounds have been previously reported by different authors (Castillo-Muñoz et al., 2009; Matus et al., 2008; Peña-Neira et al., 2007; Rodríguez-Montealegre et al., 2006). In agreement with studies from other laboratories using different analytical approaches, Qgl was found to be the most abundant low molecular weight phenolic compound in the skins of all grape varieties (Castillo-Muñoz et al., 2009; Matus et al., 2008). On the other hand, GA presented the higher concentrations on the second (CF skins) or third (M skins) sampling dates. No variation was observed in the concentration of GA in the skins of CA and CS. Comparatively, it was possible to observe that the M skins presented higher concentrations in the third sampling date, while the CS skins exhibited a significantly higher content of GA on the second sampling date. Also, we observed a significant increase in the concentration of VA in the M skins, while the CS skins displayed a decreasing tendency during the study. It is interesting to note that no statistical difference between cultivars was observed at any maturation stage.

Table 4
Extractable low molecular weight phenolic compounds (mg/K) of Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) skins during ripening.

		20-February	20-March	20-April	20-May
GA	CA	2.6 ± 0.3aA	2.9 ± 0.0aA	3.0 ± 0.2aA	2.5 ± 0.3aA
	M	2.4 ± 0.1aA	4.1 ± 2.1aAB	6.3 ± 2.1bB	2.2 ± 0.2aA
	CF	2.4 ± 0.3aA	4.5 ± 0.9aB	2.1 ± 0.5aA	2.5 ± 1.1aA
	CS	2.9 ± 0.4aA	4.3 ± 0.6bA	2.0 ± 0.2aA	2.6 ± 0.4aA
C	CA	1.4 ± 0.6bA	3.2 ± 0.7abB	1.1 ± 0.2aA	4.0 ± 0.7abB
	M	1.1 ± 0.1bAB	2.4 ± 1.0abA	0.4 ± 0.7aB	4.3 ± 0.6abC
	CF	0.6 ± 0.6abA	3.0 ± 0.7aB	1.3 ± 0.7aAB	3.0 ± 0.8aB
	CS	1.8 ± 0.4aA	4.7 ± 0.7bB	1.2 ± 0.0aA	6.1 ± 0.6bC
VA	CA	2.0 ± 0.1aA	2.1 ± 0.2aA	2.2 ± 0.0aA	2.0 ± 0.5aA
	M	2.0 ± 0.2aA	2.8 ± 0.6aAB	1.8 ± 0.2aA	3.3 ± 0.7aB
	CF	2.1 ± 0.2aA	2.8 ± 0.5aA	2.0 ± 0.7aA	3.2 ± 1.0aA
	CS	2.4 ± 0.3aA	2.4 ± 0.2aA	1.4 ± 0.1aB	1.9 ± 0.6aAB
SA	CA	0.8 ± 0.3aA	2.3 ± 0.1aB	2.1 ± 0.1aB	1.9 ± 0.4aB
	M	0.5 ± 0.0aA	2.2 ± 1.2aB	2.0 ± 0.2aAB	1.8 ± 0.2aAB
	CF	1.0 ± 0.9aA	1.5 ± 0.4aA	1.4 ± 0.1aA	1.3 ± 0.6aA
	CS	3.1 ± 0.5bA	3.2 ± 0.7aA	2.6 ± 0.4aB	2.1 ± 0.6aB
ECG	CA	0.8 ± 0.1aA	0.9 ± 0.1aA	0.9 ± 0.1aA	3.3 ± 0.4aB
	M	0.8 ± 0.1aA	1.0 ± 0.2aA	1.1 ± 0.9aA	3.7 ± 1.5aB
	CF	0.7 ± 0.1aA	0.9 ± 0.3aA	1.0 ± 0.5aA	2.0 ± 1.1aA
	CS	0.6 ± 0.1aA	1.2 ± 0.4aA	1.4 ± 0.6aA	3.3 ± 0.4aB
MGa	CA	7.7 ± 1.3aA	8.5 ± 7.6aA	7.7 ± 3.4aA	2.4 ± 0.6aA
	M	8.8 ± 2.6aA	15.4 ± 2.2aB	8.9 ± 2.1aA	7.1 ± 1.2bA
	CF	4.4 ± 1.9aA	12.0 ± 5.0aA	19.6 ± 28.5aA	3.9 ± 1.5aA
	CS	4.2 ± 1.2aA	10.3 ± 2.6aB	2.2 ± 0.5aA	1.9 ± 0.4aA
MGl	CA	24.0 ± 4.9aAB	38.4 ± 12.6aA	28.0 ± 9.1bAB	9.8 ± 0.1aB
	M	13.1 ± 1.7bA	27.5 ± 2.9aB	20.3 ± 4.6abAB	13.8 ± 2.5aB
	CF	10.9 ± 2.0bA	32.7 ± 3.0aB	10.1 ± 8.5bA	13.0 ± 3.5aA
	CS	11.5 ± 3.7bA	34.9 ± 7.6aB	11.9 ± 3.0abA	10.7 ± 1.8aA
QGa	CA	22.4 ± 3.2aA	20.8 ± 5.8aA	14.6 ± 3.9aAB	8.0 ± 0.5aB
	M	17.1 ± 1.8aA	27.5 ± 4.2aB	13.8 ± 2.4aA	22.1 ± 5.6bAB
	CF	17.1 ± 1.8aA	28.5 ± 0.9aA	16.6 ± 8.5aA	19.7 ± 5.4abA
	CS	16.5 ± 3.6aBC	23.4 ± 4.9aB	7.6 ± 0.9aA	11.4 ± 1.9abAB
QGr	CA	49.9 ± 8.0aA	33.0 ± 8.1aB	19.9 ± 3.6aB	NDaC
	M	32.8 ± 6.0aAB	39.1 ± 11.2aA	17.0 ± 1.6aB	NDaC
	CF	41.9 ± 10.4aA	35.4 ± 1.6aAB	17.0 ± 10.7aB	NDaC
	CS	47.9 ± 11.9aA	37.1 ± 3.9aA	8.7 ± 1.8aB	NDaB
QGl	CA	118.2 ± 17.1aA	109.6 ± 29.3aA	29.8 ± 2.6aB	23.0 ± 0.6aB
	M	116.4 ± 10.0aA	169.8 ± 24.6aB	73.2 ± 4.5aC	59.9 ± 14.3bC
	CF	95.3 ± 6.6aA	158.8 ± 10.7aB	45.6 ± 9.0aA	50.0 ± 15.0bA
	CS	97.3 ± 22.1aA	136.9 ± 29.5aA	33.6 ± 0.1aB	36.3 ± 4.2abB
KGa	CA	4.7 ± 0.5abA	8.4 ± 1.8aA	65.4 ± 17.1aB	2.8 ± 0.4aC
	M	3.9 ± 0.2abA	7.5 ± 0.9aA	58.8 ± 38.2aB	3.6 ± 0.8abA
	CF	2.7 ± 0.5aA	10.0 ± 1.4abA	39.7 ± 38.6aB	4.6 ± 0.8bA
	CS	5.1 ± 1.0bA	12.8 ± 1.9bB	38.4 ± 5.1aC	4.6 ± 0.7bA
KGl	CA	13.2 ± 3.1bA	12.3 ± 4.4aA	7.8 ± 0.2aA	1.7 ± 0.3aB
	M	7.4 ± 0.7aA	11.7 ± 1.9aB	6.8 ± 0.4aB	2.9 ± 0.9aC
	CF	7.2 ± 0.7aA	10.6 ± 0.1aB	6.5 ± 0.6aA	2.0 ± 0.7aC
	CS	11.2 ± 2.3abA	12.0 ± 2.7aA	7.2 ± 0.8aB	1.9 ± 0.3aB
IGl	CA	4.6 ± 1.1bA	2.5 ± 0.2aA	11.5 ± 3.8aB	13.1 ± 0.5aB
	M	2.5 ± 0.6abA	0.3 ± 0.5aA	8.4 ± 0.7aB	23.1 ± 4.3bC
	F	2.1 ± 0.4aA	1.1 ± 1.8aA	8.2 ± 3.8aA	21.9 ± 5.6abB
	CS	4.4 ± 1.0bA	1.7 ± 1.7aA	5.2 ± 0.5aA	20.1 ± 3.0abB
IGr	CA	51.4 ± 10.7bA	36.3 ± 35.7aA	36.7 ± 12.6aA	3.5 ± 0.4aB
	M	31.9 ± 2.0aA	55.3 ± 7.8aB	28.4 ± 2.1aA	4.9 ± 0.4aC
	CF	30.0 ± 2.5aA	51.1 ± 6.2aB	28.6 ± 16.0aAC	8.9 ± 2.4bC
	CS	45.4 ± 9.2abA	57.2 ± 14.3aA	18.7 ± 1.2aB	9.4 ± 1.4bB

Figures represent mean ± standard deviation (triplicates). Different small letters in single columns stand for statistically significant differences between cultivars and different capital letters in single rows stand for statistically significant differences between sampling dates (Tuckey test, $p < 0.05$).

With respect to flavan-3-ols, ECG displayed its highest concentration in the skins of all the grape varieties on the last sampling date, with the exception of the CF skins that remained unaltered at the various stages of maturation. The C monomer also showed its highest concentrations on the last sampling date in all the varieties. In addition, as compared with the other varieties, the CS skins displayed the highest concentrations of C in most of the sampling dates. This marked increase of flavan-3-ols during maturation differs from previous data from our laboratory dealing with varieties grown under different edaphoclimatic conditions (Obreque-Slier et al., 2010). By contrast, the significantly higher content of C in CS compared to other grape cultivars fully coincides with data from previous and independents studies (Monagas et al., 2003; Obreque-Slier et al., 2010).

On the other hand, most of the flavonols, excepting Kga and Igl, displayed their highest concentrations on the first or second sampling dates. Comparatively, the CS skins showed the highest contents of Kga in three sampling dates, the M skins displayed the highest values of Mga and Qgl and the CA skins exhibited the highest values of Mgl, Qgr, Kgl and Igl, in at least three sampling dates. Interestingly, the M skins showed the highest concentrations of the sum of the flavonols on the last three sampling dates. In conclusion, differences in the content of low molecular weight phenolic compounds would be somewhat influenced by the varietal factor. Similar results have been reported previously by other authors by comparing M with CS grapes (Rodríguez-Montealegre et al., 2006) and CA with CS (Obreque-Slier et al., 2010).

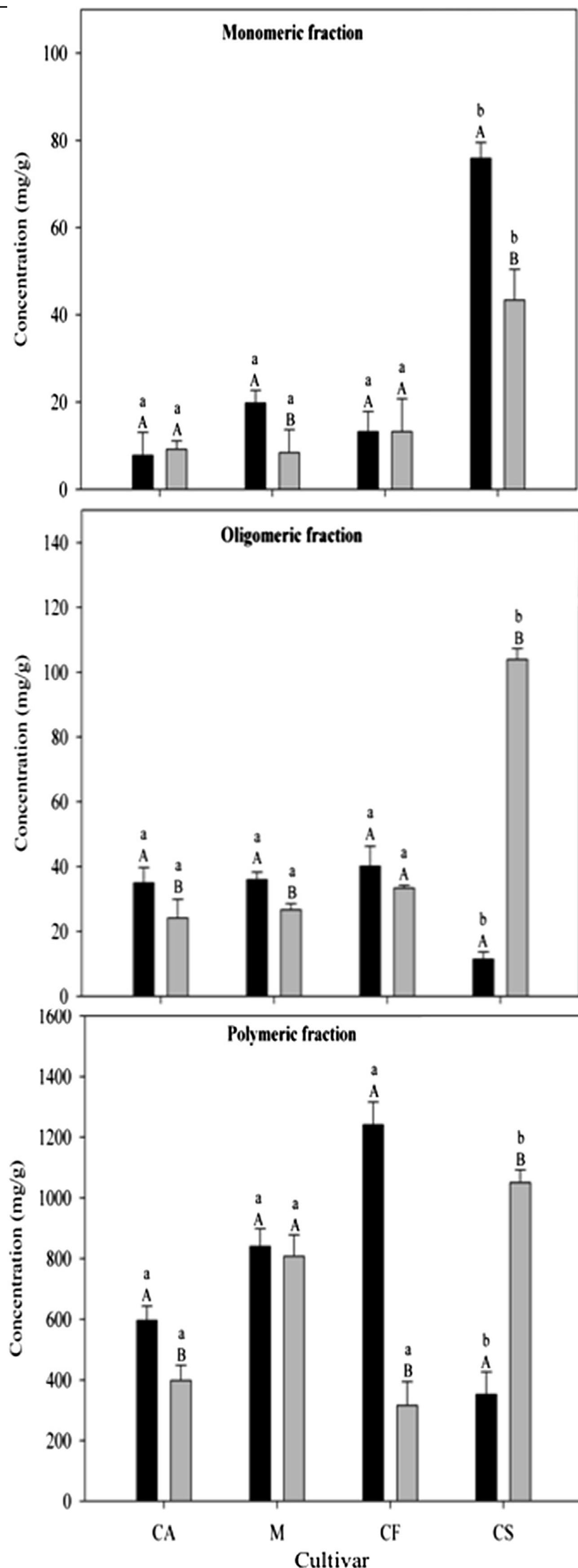

Fig. 2. Monomeric, oligomeric and polymeric fractions of flavan-3-ols from Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) skins at two stages of ripening. Different small letters on top of the bars stand for statistically significant differences between cultivars. Different capital letters on top of the bars stand for statistically significant differences between sampling dates (Tuckey test, $p < 0.05$). ▬ 20 February, ▭ 20 April.

3.5. *Distribution of extractable proanthocyanidins from grape skins according to polymerization degree during ripening*

Fig. 2 shows the mono-, oligo- and polymeric fractions of flavan-3-ols in grape skins at two sampling dates during maturation: veraison (February 20th) and technological maturity (April 20th). Monomeric flavan-3-ols represented the less abundant extractable fraction in skins of CA, CS, CF and M grapes throughout the whole study period. By contrast, polymeric flavan-3-ols represented the most abundant fraction. These observations are in full agreement with previous studies dealing with other *Vitis vinifera* L. varieties (Monagas et al., 2003; Obreque-Slier., 2010).

The content of the monomeric fraction of flavanols [(+)-catechin, (−)-epicatechin, (−)-epigallocatechin and (−)-gallocatechin] in CS was higher than in other cultivars. According to Sun et al. (1998), the oligomeric fraction is constituted by two to four monomer units, while the polymeric fraction includes flavanols with 5 or more units (Rodríguez-Montealegre et al., 2006). Although the CS skins presented the lowest concentrations of oligomeric and polymeric fractions on the first sampling date (February 20th), at the time of harvest maturity (April 20th) these skins showed the highest contents of these fractions with respect to other varieties. Overall, the oncentrations of flavanol fractions observed in this study are lower than those reported by Sun et al. (1998) and higher than those reported by Monagas et al. (2003). In our view, such differences may be associated to differences in the methods used for the extraction of phenolic compounds (Obreque–Slier et al., 2010a).

3.6. *Phloroglucinolysis*

Fig. 3 shows significant differences in mDP, % G, % EG and aMW values between *veraison* (February 20th) and technological maturity (April 20th) in all four cultivars, with the exception of % EG in the CS skins and mDP-aMW in the CA and M skins, which showed no significant differences between both sampling dates.

Comparatively, the CS skins displayed higher mDP and aMW values than the rest of the cultivars on the second sampling date (Fig. 3). This observation coincides with the high concentration of the polymeric fraction in skins of the CS cultivar (Fig. 2). Likewise, while the CA skins presented the lowest % G value on February 20th, the CS skins showed the highest % G value on this date. Nevertheless, all the four cultivars showed similar % G values (around 13.5%) on the second sampling date (on April 20th). On the other hand, statistically major differences between cultivars were observed in the % EG values, where the CS skins showed the highest value on April 20th (Fig. 3). Altogether, the mDP, aMW, % G and % EG values observed in grape skins of the four cultivars at two maturation stages (*veraison* and harvest) are highly coincident with those shown in reports from various laboratories working with other grape varieties grown in different parts of the world (Kennedy, Hayasaka, Vidal, Waters, & Graham, 2001; Kennedy & Jones, 2001). On the other hand, in this study, and in previous ones, no major differences in the mDP, aMW and % G of the flavan-3-ol fraction have been observed (Obreque-Slier et al., 2010). However, Monagas et al. (2003) working with grape skins of Cabernet Sauvignon, Graciano and Tempranillo have reported differences in those parameters.

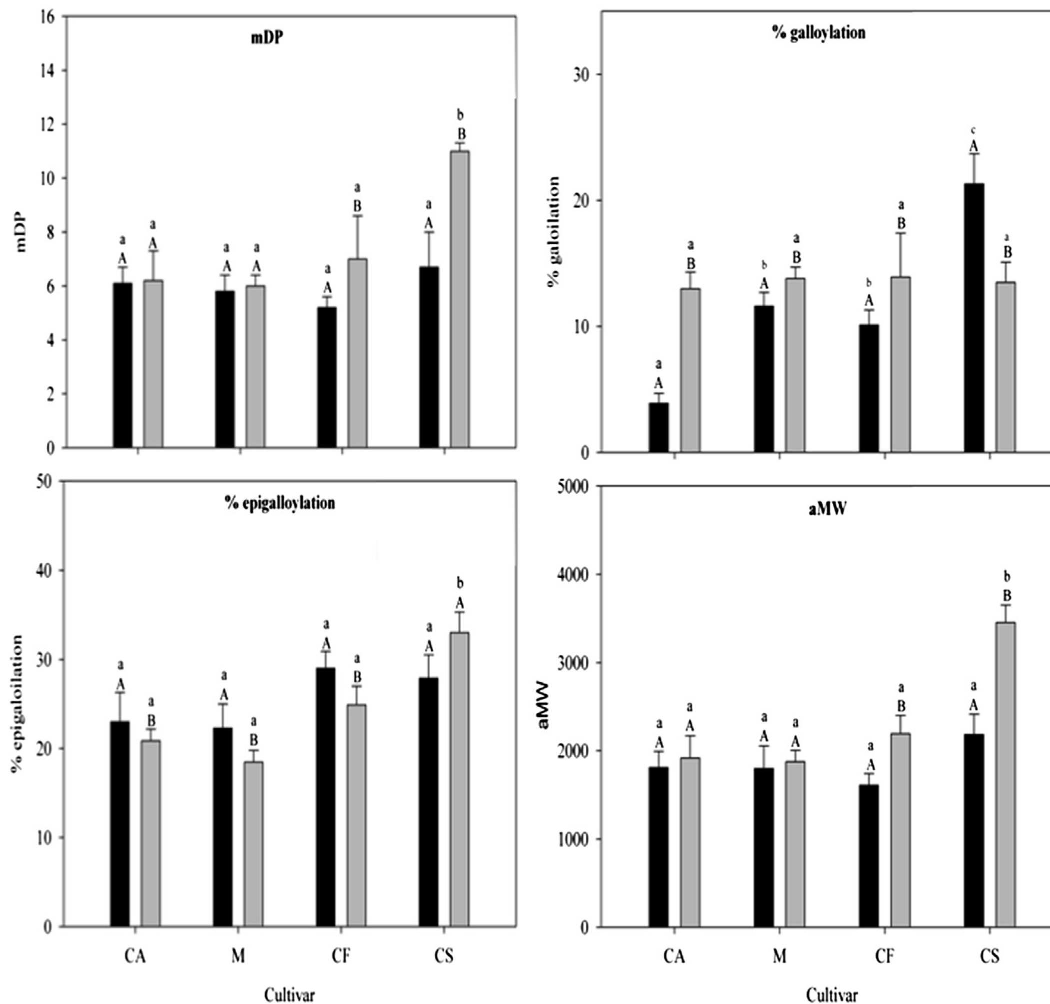

Fig. 3. Mean degree of polymerization (mDP), percentage of galloylation (% G) and epigalloylation (% EG) and average molecular weight (aMW) values of Carménère (CA), Merlot (M), Cabernet Franc (CF) and Cabernet Sauvignon (CS) skins at two stages of ripening. Different small letters in the single columns are statistically different between cultivars and different capital letters in the single rows are statistically different between sampling dates (Tuckey test, $p < 0.05$). ■ 20 February, ▭ 20 April.

4. Conclusion

Compositional differences between cultivars were observed only in some ripening stages. Nevertheless, Cabernet Sauvignon skins showed the highest contents of monomeric, oligomeric and polymeric flavan-3-ols with respect to other varieties at harvest maturity. Likewise, H^* and b^* CIElab parameters exhibited clear differences between the Merlot and Cabernet Sauvignon skins. Alike, the sum of glucoside and cumarilglucoside anthocyanins in Carménère was higher than in Cabernet Franc and Cabernet Sauvignon in some sampling date. Additionally, significant differences in the content of low molecular weight phenolic compounds were observed. Comparatively, the Cabernet Sauvignon skins displayed higher mean degree of polymerization, epigalloylation porcentage and average molecular weight values than the rest of the cultivars on the last sampling date. Altogether, Carménère, Merlot, Cabernet Franc and Cabernet Sauvignon grape skins may present significant differences in phenolic composition at some maturation stages.

Acknowledgments

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