Research Article

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Phenolic composition and antioxidant capacity of pomaces from four grape varieties (*Vitis vinifera* L.)

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Abstract

BACKGROUND: Phenolic compounds are widely distributed secondary metabolites in plants usually conferring them with unique taste, flavour and health-promoting properties. In fruits of *Vitis vinifera* L., phenolic composition is highly dependent on grape variety. Differential extraction of these compounds from grapes during winemaking is critically associated with wine quality. By-products of winemaking, such as grape pomace, can contain significant amounts of polyphenols. However, information concerning the varietal effect on wine grape pomace is scarce. In this study, pomaces from Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape varieties were characterized spectroscopically and by HPLC-DAD analysis.

RESULTS: White grape pomaces (SB and CH) presented higher antioxidant capacities and higher contents of total phenols and total proanthocyanidins compared with red grape pomaces (CS and CA), whereas the latter showed much higher anthocyanin levels and colour intensities. Concentrations of monomeric proanthocyanidins and low-molecular-weight phenols in the four grape pomace varieties were significantly different.

CONCLUSION: Grape pomaces from four varieties showed high but diverse contents of polyphenols and antioxidant capacities. Thus grape pomaces represent an important potential source of polyphenols, which could be useful for nutritional and/or pharmacological purposes.
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Keywords: by-products; pomace; winemaking; polyphenols; proanthocyanidins

INTRODUCTION

Polyphenols are secondary metabolites found in plant leaves, fruits, floral tissues, stems, bark and roots.[1] These compounds have been associated with the prevention of degenerative diseases, cardiovascular diseases and several types of cancer.[2] In addition, polyphenols play an important role in the sensory characteristics of food, including colour, flavour, astringency and bitterness.[3−6] In *Vitis vinifera* L. grapes, the most abundant polyphenols can be categorized according to their chemical structures into flavonoids and non-flavonoids. Flavonoid compounds are predominantly found in the skins, seeds and stems,[3,5−8] whereas non-flavonoid compounds are most abundant in the berry pulp.[9]

A number of studies have demonstrated that the phenolic composition of grapes is strongly dependent on edaphic, geographical and weather-related factors as well as on grape variety.[3,6−8] With respect to the varietal effect, it has been observed that berry skins of Carménère contain higher levels of anthocyanins (1 mg g^{-1}), monomeric proanthocyanidins (15 mg kg^{-1}) and total flavonoids (16.5 mg g^{-1}) compared with berry skins of Cabernet Sauvignon (0.5 mg g^{-1}, 5 mg kg^{-1} and 9.1 mg g^{-1} respectively).[7] In addition, significant differences in the polyphenol content of seeds from Cabernet Franc, Cabernet Sauvignon, Merlot and Carménère cultivars have been observed; among the four varieties, Carménère seeds presented the highest concentration (16.4 mg g^{-1}) and Cabernet Franc seeds the lowest concentration (7.9 mg g^{-1}) of total proanthocyanidins.[8]

Phenolic compounds are extracted from grapes during the winemaking process.[10,11] However, the by-products of winemaking (e.g. grape pomace and stems) can contain significant amounts of phenolic compounds owing to their incomplete extraction during the wine production process.[12,13] It has been shown that approximately 60−65% of phenolic compounds remain in the grape pomace after red wine production.[14] Furthermore, it has been shown that pomace skins from Morio Muscat, Muller Thurgau, Pinot Noir, Cabernet Sauvignon and Merlot varieties have higher levels of total polyphenols, flavonols and proanthocyanidins compared with other residues generated during agricultural

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1521

fruit processing.[15] It should also be noted that grape pomace accounts for approximately 20% of harvesting by-products.[16] In total, approximately 13 million tons of pomace residues are generated worldwide each year,[17] of which only a small part is recycled or processed.[18]

Thus grape pomace is produced in large quantities and represents an important source of polyphenols. However, information concerning the varietal effect on wine grape pomace is scarce. Moreover, differences in the processes for red and white wine production may affect the availability of polyphenols in the resulting pomace. In this study we characterized the phenolic composition and evaluated the antioxidant properties of pomace derived from four *V. vinifera* L. grape varieties, including the less-known Carménère.

MATERIALS AND METHODS

Materials
Standards of gallic acid (G-7384), vanillic acid (V-2250), protocatechuic acid (P-5630), quercetin (Q-0125), myricetin (M-6760), kaempferol (K-0133), (+)-catechin (C-1251) and (−)-epicatechin-3-*O*-gallate (E-3893) and 0.45 μm pore size membranes were acquired from Sigma Chemical Company (St Louis, MO, USA). Vanillin 99% (V-8510), trifluoroacetic acid, ethyl acetate, high-performance liquid chromatography (HPLC)-grade acetonitrile 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). Phosphate-buffered saline solution (Code 5608-02) was acquired from J.T. Baker (Ecatepec, Mexico).

Instrumentation
Absorbances were measured using a Shimadzu UV-1700 UV–visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisted of a G1315B photodiode array detector, a Quat G1311A pump and an ALS G1329A autosampler. A reverse phase Nova Pak C$_{18}$ column (4 μm, 3.9 mm i.d. × 300 mm; Waters) was used for HPLC diode array detection (HPLC-DAD) analysis of individual phenolic compounds. To obtain grape pomaces, a Defranceshi CD 80 stainless steel membrane press (Bolzano, Italy) measuring 4.7 m × 2.2 m × 2.3 m, with a maximum capacity of 32.2 tons and a maximum pressure of 8 bar, was used.

Pomace samples
Grape pomaces were prepared from each of four grapevine (*V. vinifera* L.) varieties grown at the Viña de Santa Alicia vineyard located in the Maipo Valley, Chile (33° 40′ 21.1″ S, 70° 32′ 25″ W). The white grape varieties Sauvignon Blanc (SB) and Chardonnay (CH) and the red grape varieties Cabernet Sauvignon (CS) and Carménère (CA) were planted in 2004 in the same soil type using similar cultural practices. All analyses were performed in triplicate at the three times the press was emptied (beginning, middle and end).

The must obtained from pressing the white grapes was fermented for 20 days at temperatures ranging from 12 to 18 °C. The red grapes were subjected to maceration at 8 °C for 3 days after de-stemming. Fermentation was conducted at 26–28 °C for 8 days and then the solid matter was separated from the liquid. To obtain grape pomace, pressing was conducted prior to the winemaking process in the case of white grapes and after the winemaking process in the case of red grapes (3 cycles for 10 min at 0–0.25 bar,

2 cycles for 10 min at 0.25–0.6 bar, 2 cycles for 10 min at 0.6–0.8 bar and 2 cycles for 10 min at 0.8–1.6 bar; total time 90 min). The samples were transported to the laboratory in the dark at 2 °C, where they were ground and kept frozen at −20 °C for further analyses. For extracting phenolic compounds, 10 g of grape pomace was macerated for 60 min at 20 °C with 100 mL of methanol/water solution (80:20 v/v) adjusted to pH 3 with HCl. Solids were separated by filtration through a sieve and the liquid fraction was saved. Solids were macerated again for 1 h with 100 mL of acetone/water solution (80:20 v/v) adjusted to pH 3. After filtration, the two liquid fractions were mixed, evaporated at 30 °C to eliminate methanol and acetone, adjusted to 100 mL of water, centrifuged (1000 × *g*, 10 min) and filtered through a 0.45 μm pore size membrane.

Spectrophotometric characterization
Total phenol content was determined by UV absorption spectroscopy at 280 nm[19] using gallic acid as a standard. Total anthocyanins were measured by diluting the extracts with acidified ethanol (2 mL of HCl in 100 mL of ethanol) and comparing spectrophotometric readings of single aliquots treated with either sodium metabisulfite or water.[20] Colour intensity and hue were determined by visible absorption spectroscopy at 420, 520 and 620 nm.[21] To determine total proanthocyanidins, 1 mL of pomace extract and 3 mL of methylcellulose solution (0.4 mg mL^{-1} in distilled water) were combined, stirred and left to rest for 2–3 min. Then 2 mL of ammonium sulfate solution (434.7 mg mL^{-1}) and 4 mL of distilled water were added. After stirring, the mixtures were allowed to rest for 10 min at 20 °C. Control samples were processed in a similar way but with no addition of methylcellulose. Finally, the tubes were centrifuged (750 × *g*, 5 min) and absorbances were measured at 280 nm. Results are expressed in epicatechin equivalent units.[22]

Antioxidant capacity
Different aliquots of the four pomace extracts were transferred into volumetric flasks (0.25, 0.50, 0.75 and 1.25 mL for SB; 0.25, 0.75, 1.25 and 1.50 mL for CH; 0.10, 0.50, 0.75 and 1.25 mL for CS and CA) and the volumes were adjusted to 10 mL with distilled water. Subsequently, 100 μL of each diluted sample was mixed with 3.9 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (20 mg L^{-1}). For the 'target', 100 μL of each sample was added to 3.9 mL of methanol, while 100 μL of methanol mixed with 3.9 mL of DPPH solution served as the 'control'. After placing the tubes in the dark for 30 min, absorbances were measured at 517 nm and the % discoloration of each sample was calculated as

$$\{[1 - (\text{sample absorbance} - \text{target absorbance})] / \text{control absorbance}\} \times 100$$

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a standard. Results are expressed as Trolox equivalent antioxidant capacity (TEAC) g^{-1} dry pomace.[23]

HPLC-DAD analysis of individual phenolic compounds
Extracts of grape pomace 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 methanol (0.5 mL mL^{-1} water) and membrane filtered (0.45 μm pore size). Aliquots (50 μL) of the final solution were subjected to reverse phase chromatographic separation at 20 °C using a Nova Pak C$_{18}$ column. The photodiode array detector was set at 280 nm.

Two mobile phases were used: A, water/acetic acid (98:2 v/v); B, water/acetonitrile/acetic acid (78:20:2 v/v/v). A two-step gradient was carried out at a constant flow rate of $1\,mL\,min^{-1}$: 0–55 min, 100–20% A; 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 by comparing both retention time and absorption spectrum (from 210 to 360 nm) against those of pure standards. Polyphenols for which standards were unavailable were assigned by retention time and spectral parameters as described in previous reports.[7,8,24] Quantitative determinations were made using the external standard method and commercial standards.

Fractionation of proanthocyanidins into monomers, oligomers and polymers

Each grape pomace extract (10 mL) was vacuum dried at 30 °C, resuspended in 20 mL of phosphate buffer solution (pH 7), filtered and loaded onto C-18 and tC-18 cartridges containing 10 mL of methanol, 20 mL of distilled water and 10 mL of phosphate-buffered saline solution (pH 7). Next, 10 mL of phosphate-buffered saline solution diluted in water (1:8 v/v) was added to each cartridge. These were dried for 2 h with gaseous nitrogen and the monomeric (FI) + oligomeric (FII) fractions were eluted by adding 25 mL of ethyl acetate. The polymeric fraction (FIII) was then eluted with 15 mL of methanol. The FI + FII fractions were vacuum dried at 30 °C, redissolved in 10 mL of phosphate-buffered saline solution (pH 7) and loaded again into reconditioned cartridges, which were then dried with gaseous nitrogen. Finally, F1 was eluted with 25 mL of ether and FII was eluted with 15 mL of methanol.[25]

Total content of preoanthocyanidins in monomer, oligomer and polymer fractions

The vanillin assay was performed as described by Sun *et al*.[25] A 2.5 mL aliquot of H_2SO_4/methanol solution (1:3 v/v) and 2.5 mL of vanillin solution ($10\,mg\,mL^{-1}$ methanol) were mixed with 1 mL of sample. The tubes were incubated at 30 °C for either 15 min (F1 fraction) or for a period of time long enough to allow maximal reaction (FII and FIII fractions). Absorbances were read at 500 nm. A blank was prepared by replacing the vanillin solution in the reaction mix with methanol. Results are expressed as mg monomer, oligomer or polymer g^{-1} dry pomace according Sun *et al*.[25]

Statistical analysis

Minitab Release 13.32 (Minitab Inc., State College, Pennsylvania, USA) and Tukey's *t* test were applied to contrast quantitative variables with a 95% confidence interval.

RESULTS

Pomace dry weight

A 10 g sample of each grape pomace was dried and separated into seeds and skins. Figure 1 shows that SB and CH had statistically higher seed weights than CS and CA. Conversely, the red grape varieties showed higher skin weights than the white grape varieties. Additionally, SB and CH showed similar skin weight/seed weight ratios, whereas this metric was higher in CS than in CA.

Global phenolic composition and antioxidant capacity

Figures 2 and 3 show data derived from spectrophotometric analyses of pomace extracts of different grape varieties. In this
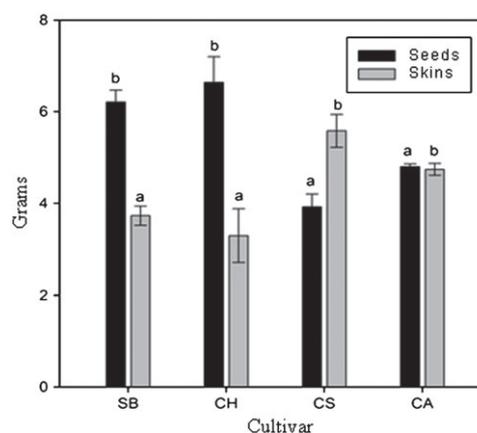

Figure 1. Skin and seed weights of Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace. Different letters above bars indicate statistically significant differences between cultivars (Tukey test, $P < 0.05$).

study the white grape varieties showed higher levels of total phenols and proanthocyanidins compared with the red grape varieties. Similarly, SB showed statistically higher levels of total phenols and proanthocyanidins than the other grape varieties. Furthermore, this pattern was similar to that observed for the antioxidant capacity (Fig. 2). In contrast, CS and CA displayed higher levels of total anthocyanins and deeper colour intensities than CH and SB, with CS being the variety with the highest values (Fig. 3).

Quantification of low-molecular-weight phenols using HPLC-DAD

Table 1 shows the low-molecular-weight phenolic compounds identified and quantified in the pomace extracts. These compounds included gallic acid (GA), protocatechuic acid (PA), caftaric acid (CA), procyanidins B3 (PB3), B1 (PB1), B4 (PB4) and B2 (PB2), procyanidin trimer 1 (PT1), (+)-catechin (C), vanillic acid (VA), (−)-epicatechin (EC), epicatechin-3-*O*-gallate (ECG), tryptophol (T), flavonols (Fs), procyanidins (Ps) and procyanidin gallates (PGs). Overall, (+)-catechin, (−)-epicatechin and procyanidin gallates were the most abundant compounds in the extracts. CS was the variety showing the highest concentration of flavonols. Comparatively, pomaces from the white grape varieties showed significantly higher contents of low-molecular-weight polyphenols than pomaces from the red grape varieties. In particular, SB showed the highest levels and CS the lowest levels of most of those polyphenols.

Flavan-3-ol subfractions

Figure 4 shows the contents of monomeric, oligomeric and polymeric flavan-3-ol subfractions in grape pomaces. In the majority of varieties the polymeric subfraction was the most abundant, whereas the monomeric subfraction was without exception the least abundant. Comparatively, the white grape varieties had significantly higher monomer contents, whereas no significant differences were observed among different varieties with respect to the oligomeric and polymeric subfractions.

DISCUSSION

Phenolic compounds are secondary metabolites associated with sensory, taxonomic, pharmacological and nutritional properties of
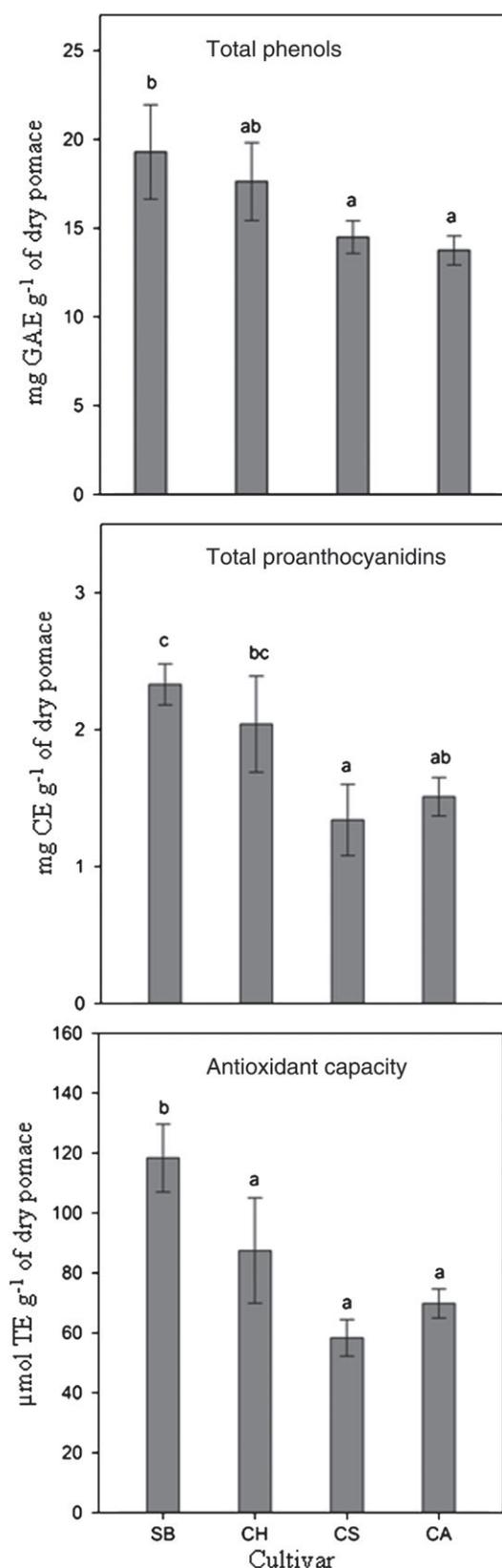
Figure 2. Total phenols, total proanthocyanidins and antioxidant capacity of Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace. Different letters above bars indicate statistically significant differences between cultivars (Tukey test, $P < 0.05$). GAE, gallic acid equivalent; CE, (+)-catechin equivalent; TE, Trolox equivalent.
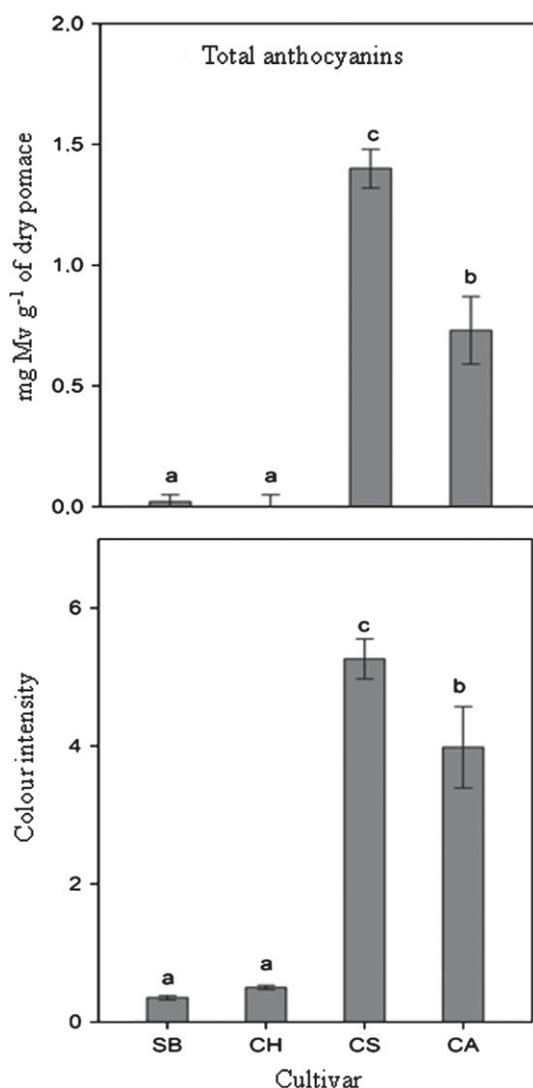

Figure 3. Total anthocyanins and colour intensity of Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace. Different letters above bars indicate statistically significant differences between cultivars (Tukey test, $P < 0.05$). Mv, malvidin-3-glucoside.

food.[1−4] In the case of wine grapes, seeds and skins are important sources of these compounds, which are extracted during the winemaking process.[10] Therefore winemaking residues likely represent a significant reservoir of important polyphenols owing to incomplete extraction of these compounds during winemaking.[12] However, grape pomace availability and phenolic composition can vary owing to a number of factors involved in berry development, including soil, geographical location, weather conditions, winemaking technology and grape variety.[9] Therefore we characterized the phenolic composition and antioxidant capacity of pomaces extracted from four *V. vinifera* L. grape varieties.

Initially, each sample of grape pomace was weighed, dried and separated into seeds and skins. For red grapes, the sum of seed and skin weights decreased by approximately 5% with respect to the wet weight, whereas pomaces from white grape varieties maintained their initial weight. Likewise, weights of the red grape pomace skins were higher than those from SB and CH, whereas the opposite was observed for seed weights. These observations are consistent with previously described differences

Table 1. Extractable low-molecular-weight phenolic compounds (mg kg^{-1}) of Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace

Polyphenol	SB	CH	CS	CA
Gallic acid	25.9 ± 2.3b	13.2 ± 0.5b	10.5 ± 2.4a	19.9 ± 5.3ab
Protocatechuic acid	4.2 ± 1.9b	0.9 ± 0.2a	0.5 ± 0.2a	0.9 ± 0.2a
Caftaric acid	ND	ND	2.6 ± 0.1a	2.9 ± 0.2a
Procyanidin B3[a]	40.8 ± 4.9c	21.1 ± 3.6b	9.2 ± 3.4a	18.8 ± 3.9ab
Procyanidin B1[a]	22.5 ± 3.6b	14.8 ± 2.4a	10.6 ± 0.9a	11.9 ± 2.3a
(+)-Catechin	477.2 ± 36.8b	194.8 ± 21.1a	87.7 ± 3.3a	178.3 ± 22.2a
Vanillic acid	ND	ND	8.4 ± 0.6a	8.7 ± 1.6a
Procyanidin trimer 1[a]	24.2 ± 4.1a	17.9 ± 3.5a	ND	ND
Procyanidin B4[a]	59.1 ± 7.7c	33.7 ± 7.4b	17.0 ± 1.0a	17.7 ± 3.0a
Procyanidin B2[a]	80.7 ± 6.4a	66.3 ± 10.5a	ND	ND
(−)-Epicatechin	506.1 ± 67.0b	409.0 ± 67.1b	68.4 ± 5.1a	130.9 ± 22.5a
Epicatechin-3-O-gallate[a]	39.3 ± 14.9	ND	ND	ND
Tryptophol	ND	ND	12.7 ± 2.4a	6.6 ± 1.7a
Flavonols[b]	ND	ND	121.1 ± 4.1b	74.6 ± 9.9a
Other procyanidins[a]	19.3 ± 2.9a	51.8 ± 12.0b	45.3 ± 3.5b	13.9 ± 3.9a
Procyanidin gallates[c]	450.7 ± 69.6c	228.9 ± 1.2b	61.7 ± 2.9a	74.3 ± 9.9a

Values are mean ± standard deviation of triplicate measurements. Different letters within a row indicate statistically significant differences between cultivars (Tukey test, $P < 0.05$). ND, not detected.
[a] Expressed as (+)-catechin equivalent.
[b] Expressed as quercetin equivalent.
[c] Expressed as gallic acid equivalent.

between the weights of seeds and skins from white and red grape varieties.[26] Additionally, the higher skin/seed ratio observed in the red grape varieties may account for the increased presence of certain polyphenols in red wines in comparison with white wines, such as anthocyanins and flavonols.[7,27]

As to polyphenols, pomaces from white grape varieties showed higher concentrations of total phenols and proanthocyanidins than pomaces from red grape varieties, which is consistent with a more complete extraction of polyphenols from berries during the red winemaking process.[12,14] In contrast, white wine production is performed without solid matter,[28] thus resulting in higher concentrations of polyphenols left in white grape skins. Similarly, in this study we observed that antioxidant capacity was higher in the pomaces from the white varieties, with SB showing the highest value. This phenomenon is likely linked to the total levels of phenols and proanthocyanidins, which were shown to be associated with antioxidant properties of V. vinifera L. grapes.[15,27,29]

It should also be noted that the total phenol and proanthocyanidin contents of CH pomace were lower than those reported in other studies.[28] In contrast, the antioxidant capacity value in our report was slightly higher than that previously observed by other authors in the same variety.[28] Also, in our study the levels of total phenols and total proanthocyanidins in the CS pomace were higher than those observed by Yi et al.[30] and González-Paramás et al.[31] respectively. Finally, the average antioxidant capacity values observed in this study were below those reported by Cataneo et al.[29] and above those reported by Yi et al.[30] and Poudel et al.[32]

In this study we also found that both SB and CH pomaces lacked anthocyanins. This observation has also been reported by other researchers, who mention that pomaces of white grape varieties do not contain those compounds.[15] This observation is also consistent with the observed values for colour intensity, which were lower than those observed in red grape varieties. In this same regard, we also noted that total anthocyanin levels in the

CS pomace were twice as high as those in the CA pomace, which is likely related to higher concentrations of these compounds in the grape skins prior to the winemaking process.[6] Values for total anthocyanins and colour intensities in this study agree with those observed in other studies involving pomace skins.[15,33]

With respect to the flavan-3-ol subfractions, we found that the polymeric subfraction was the most abundant in most of the wine grape pomaces. However, proportions of the flavan-3-ol subfractions in pomaces of different grape varieties were roughly similar. Significant differences between pomaces from different grape varieties were observed punctually in the monomeric subfraction. Interestingly, concentrations of all three subfractions in grape pomaces in this study were found to be much lower than those observed previously in skins and seeds of grapes of the corresponding varieties.[6−9,14] This observation can be entirely accounted for by a significant transfer of those subfractions from solid matter to must during maceration and fermentation.[12] Considering the reported levels of those subfractions in berries, the polymeric subfraction is the one experiencing the largest decrease from the pomace components, considering that concentrations of polymeric polyphenols as high as 900 mg g^{-1} have been reported in skins and seeds.[6,8] Concentrations of this polyphenol subfraction are higher in wine than in grapes, but both wine and grapes show higher levels of that subfraction than the ones observed in the pomace.[6−9,14]

As to low-molecular-weight phenols, the various compounds identified in white and red grape pomaces were the same as those observed in extracts of V. vinifera L. seeds and skins.[6−8,12,34] With respect to non-flavonoid polyphenols, we found that GA was the most abundant of these compounds and that its concentrations in white grape pomaces were higher than in CS pomace. This observation is consistent with several studies indicating that seeds and skins are an important source of GA.[6,8,12,34] Interestingly, both SB and CA showed GA concentrations twice as high as
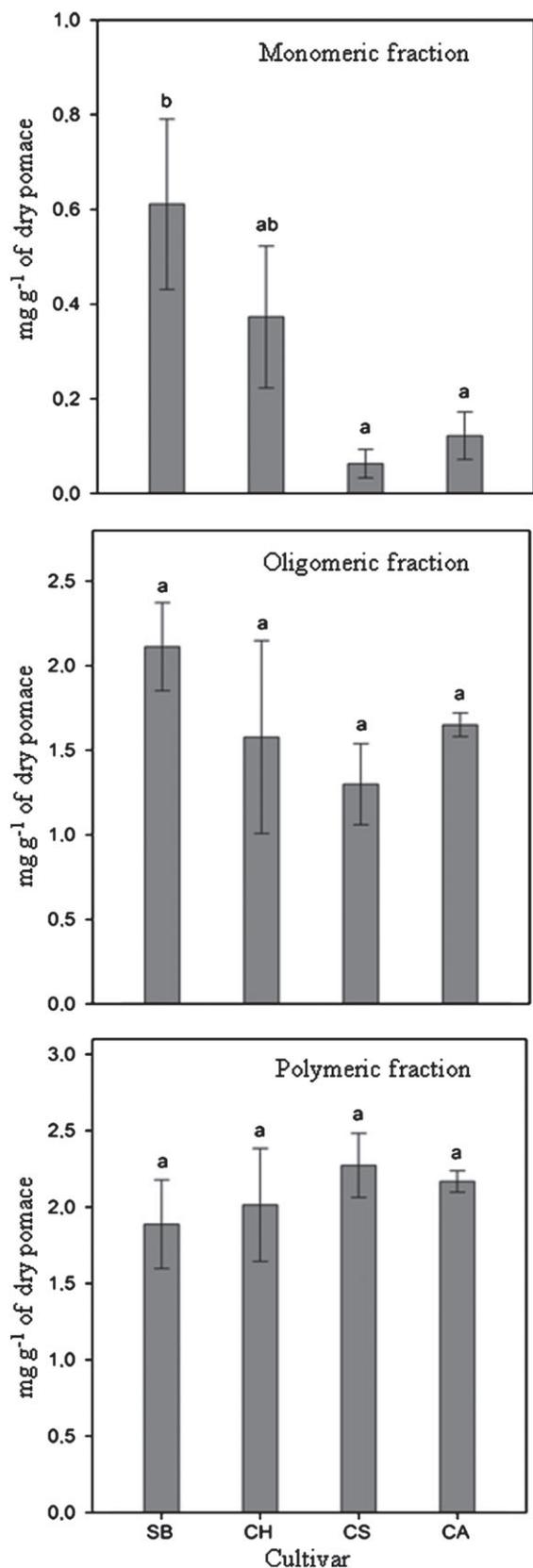
Figure 4. Proanthocyanidin content of monomeric, oligomeric and polymeric fractions of Sauvignon Blanc (SB), Chardonnay (CH), Cabernet Sauvignon (CS) and Carménère (CA) grape pomace. Different letters above bars indicate statistically significant differences between cultivars (Tukey test, $P < 0.05$).

those found in CH and CS respectively. This trend may be highly relevant, since GA has been linked to important sensory properties such as bitterness and astringency.[5,9] In this regard, we have recently described that GA displays growth-inhibitory effects on *Helicobacter pylori*, a common pathogenic gut bacterium.[35]

Finally, in this study we observed that C and EC were the most abundant flavonoid polyphenols in pomaces from both white and red grape varieties, which is consistent with previous observations in *V. vinifera* L. grape seeds and skins.[5,6,34] It is important to recall that in this study white grape pomaces showed higher concentrations of both compounds compared with red grape pomaces. In the present study a similar trend was observed in the levels of procyanidin dimers (B1, B2, B3 and B4). These findings strongly suggest that the levels of these compounds are associated with the extraction processes used during red winemaking.[36] It is also important to note that in our study some non-flavonoids (e.g.VA and T) and flavonols were found only in red grape pomaces, which may be due to the high concentrations of these compounds in red grapes[7] and/or to the absence of these compounds in white grapes.[34] In addition, T is a polyphenol that is synthesized during fermentation and produced from deamination and decarboxylation of tryptophan. Therefore only red grape pomaces can contain this compound, as fermentation is performed with both solid and liquid constituents in red wine production.[36]

Considering these findings and earlier studies, we conclude that pomaces from the SB, CH, CS and CA wine grape varieties have significantly different polyphenolic compositions and antioxidant capacities. Those differences are likely due either to differences in the availability of certain compounds among varieties or to differences in the extraction processes used in red and white winemaking. Accordingly, grape pomace represents a diverse and potentially important source of polyphenols, which could be used advantageously for either nutritional or pharmacological purposes.

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REFERENCES

1 Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B and Weil JA, Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* **84**:551–562 (2004).

2 López-Vélez M, Martínez-Martínez F and Del Valle-Ribes C, The study of phenolic compounds as natural antioxidants in wine. *Crit Rev Food Sci Nutr* **43**:233–244 (2003).

3 Monagas M, Bartolomé B and Gómez-Cordovés C, Updated knowledge about the presence of phenolic compounds in wine. *Crit Rev Food Sci Nutr* **45**:85–118 (2005).

4 Soto ML, Moure A, Domínguez H and Parajó JC, Recovery, concentration and purification of phenolic compounds by absorption: a review. *J Food Eng* **105**:1–27 (2011).

5 Obreque-Slier E, López-Solís R and Peña-Neira A, Differential interaction of seed polyphenols from grapes collected at different maturity stages with the protein fraction of saliva. *Int J Food Sci Technol* **47**:1918–1924 (2012).

6 Obreque-Slier E, Peña-Neira A, López-Solís R, Cáceres-Mella A, Toledo-Araya H and López-Rivera A, Phenolic compositions of skins from four Carmenet grape varieties (*Vitis vinifera* L) during ripening. *LWT – Food Sci Technol* **54**:404–413 (2013)

7 Obreque-Slier E, Peña-Neira A, López-Solís R, Zamora-Marín F, Ricardo da Silva JM and Laureano O, Comparative study of the phenolic

composition of seeds and skins from Carménère and Cabernet sauvignon grape varieties (*Vitis vinifera* L) during ripening. *J Agric Food Chem* **58**:3591–3599 (2010).

8 Obreque-Slier E, López-Solís R, Castro-Ulloa L, Romero-Díaz C and Peña-Neira A, Phenolic composition and physicochemical parameters of Carménère, Cabernet Sauvignon, Merlot and Cabernet Franc grape seeds (*Vitis vinifera* L) during ripening. *LWT – Food Sci Technol* **48**:134–141 (2012).

9 Monagas M, Gómez-Cordovés C, Bartolomé B, Laureano O and Ricardo da Silva JM, 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* **51**:6475–6481 (2003).

10 Matějíček D, Mikeš O, Klejdus B, Štěrbová D and Kubáň V, Changes in contents of phenolic compounds during maturing of barrique red wines. *Food Chem* **90**:791–800 (2005).

11 Lachman J, Šulc M and Schilla M, Comparison of the total antioxidant status of Bohemian wines during the wine-making process. *Food Chem* **103**:802–807 (2007).

12 Rockenbach II, Rodrigues E, Gonzaga LV, Caliari V, Genovese MI, Gonçalves ADS, *et al.*, Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L and *Vitis labrusca* L) widely produced in Brazil. *Food Chem* **127**:174–179 (2011).

13 Lachman J, Hejtmánková A, Hejtmánková K, Horníčková Š, Pivec V, Skala O, *et al.*, Towards complex utilisation of wine-making residues: characterisation of grape seeds by total phenols, tocols and essential elements content as a by-product of wine-making. *Ind Crops Prod* **49**:445–453 (2013).

14 Sun B, Ricardo da Silva JM and Spranger MI, Quantification of catechins and proanthocyanidins in several Portuguese grapevine varieties and red wine. *Ciência Téc Vitiv* **16**:23–34 (2001).

15 Deng Q, Penner MH and Zhao Y, Chemical compounds of dietary fiber and polyphenol of five different varieties of wine grape pomace skins. *Food Res Int* **44**:2712–2720 (2011).

16 Laufenberg G, Kuntz B and Nystroem M, Transformation of vegetable waste into value added products: (A) the upgrading concept, (B) practical implementations. *Bioresour Technol* **87**:167–198 (2003).

17 Alonso AM, Guillén DA, Barroso CG, Puertas B and García A, Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *J Agric Food Chem* **50**:5832–5836 (2002).

18 Ping L, Pizzi A, Guo ZD and Brosse N, Condensed tannins extraction from grape pomace: characterization and utilization as wood adhesives for wood particleboard. *Ind Crops Prod* **34**:907–914 (2011).

19 Glories Y, La couleur des vins rouges, 2° partie mesure, origine et interpretation. *Connaiss Vigne Vin* **18**:253–271 (1984).

20 Ribéreau-Gayon J and Stonestreet E, Le dosage des anthocyanes dans le vin rouge. *Bull Soc Chim Fr* **9**:2649–2652 (1965).

21 Ribéreau-Gayon P, Pontallier P and Glories Y, Some interpretations of colour changes in young red wines during their conservation. *J Sci Food Agric* **34**:505–516 (1983).

22 Mercurio MD, Dambergs RG, Herderich MJ and Smith PA, High throughput analysis of red wine and grape phenolics: adaptation and

23 Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* **28**:25–30 (1995).

24 Peña-Neira A, Cáceres A and Pastenes C, Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L) in the Maipo Valley (Chile): effect of clusters thinning and vineyard yield. *Food Sci Technol Int* **13**:153–158 (2007).

25 Sun B, Ricardo Da Silva JM and Spranger I, Critical factors of vanillin assay for catechins and proanthocyanidins. *J Agric Food Chem* **46**:4267–4274 (1998).

26 Bordiga M, Travaglia F, Locatelli M, Coïsson JD and Arlorio M, Characterisation of polymeric skin and seed proanthocyanidins during ripening in six *Vitis vinifera* L. cv. *Food Chem* **127**:180–187 (2011).

27 Anastasiadi M, Pratsinis H, Kletsas D, Skaltsounis AL and Haroutounian SA, Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: evaluation of the antioxidant activities of their extracts. *Food Res Int* **43**:805–813 (2010).

28 González-Centeno MR, Jourdes M, Femenia A, Simal S, Rosselló C and Teissedre PL Characterization of polyphenols and antioxidant potential of white grape pomace byproducts (*Vitis vinifera* L). *J Agric Food Chem* **61**:11579–11587 (2013).

29 Cataneo CB, Caliari V, Gonzaga LV, Kuskoski EM and Fett R, Atividade antioxidante e conteúdo fenólico do resíduo agroindustrial da produçao de vino. *Semina Ciências Agrárias* **29**:93–102 (2008).

30 Yi C, Shi J, Kramer J, Xue S, Jiang Y, Zhang M, *et al.*, Fatty acid composition and phenolic antioxidants of winemaking pomace powder. *Food Chem* **114**:570–576 (2009).

31 González-Paramás AM, Esteban-Ruano S, Santos-Buelga C, De Pascual-Teresa S and Rivas-Gonzalo JC, Flavanol content and antioxidant activity in winery byproducts. *J Agric Food Chem* **52**:234–238 (2004).

32 Poudel PR, Tamura H, Kataoka I and Mochioka R, Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native in Japan. *J Food Compos Anal* **21**:622–625 (2008).

33 Gómez-Plaza E, Miñano A and López-Roca JM, Comparison of chromatic properties, stability and antioxidant capacity of anthocyanin-based aqueous extracts from grape pomace obtained from different vinification methods. *Food Chem* **97**:87–94 (2006).

34 Rodríguez Montealegre R, Romero Peces R, Chacón Vozmediano JL, Martínez-Gascueña J and García-Romero E, Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *J Food Compos Anal* **19**:687–693 (2006).

35 Díaz-Gómez R, López-Solís R, Obreque-Slier E and Toledo-Araya H, Comparative antibacterial effect of gallic acid and catechin against *Helicobacter pylori*. *Food Sci Technol* **54**:331–335 (2013).

36 Cheynier V, Moutounet M and Sarni Machado P, Los compuestos fenólicos, in *Enología, Fundamentos Teóricos y Tecnológicos*, ed. by. Mundi Prensa Libros S.A., A. Madrid Vicente, Ediciones pp. 114–136 (2000).