Research Article

Received: 14 June 2011 Revised: 29 July 2011 Accepted: 1 August 2011 Published online in Wiley Online Library: 14 September 2011

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4638

Phenolic characterisation of red wines from different grape varieties cultivated in Mendoza province (Argentina)

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Abstract

BACKGROUND: Knowledge of the chemical composition of wine and its association with the grape variety/cultivar is of paramount importance in oenology and a necessary tool for marketing. Phenolic compounds are very important quality parameters of wines because of their impact on colour, taste and health properties. The aim of the present work was to study and describe the non-flavonoid and flavonoid composition of wines from the principal red grape varieties cultivated in Mendoza (Argentina).

RESULTS: Sixty phenolic compounds, including phenolic acids/derivatives, stilbenes, anthocyanins, flavanols, flavonols and dihydroflavonols, were identified and quantified using high-performance liquid chromatography with diode array detection coupled with electrospray ionisation mass spectrometry (HPLC-DAD/ESI-MS). Marked quantitative differences could be seen in the phenolic profile among varieties, especially in stilbenes, acylated anthocyanins and other flavonoids.

CONCLUSION: The polyphenolic content of Malbec wines was higher compared with the other red varieties. Dihydroflavonols represent a significant finding from the chemotaxonomic point of view, especially for Malbec variety. This is the first report on the individual phenolic composition of red wines from Mendoza (Argentina) and suggests that anthocyanins, flavanols and phenolic acids exert a great influence on cultivar-based differentiation.

Keywords: dihydroflavonols; Malbec; phenolics; red wine; variety

INTRODUCTION

Wine is considered one of the world's oldest beverages and constitutes an essential cultural component of many traditional producer countries. In the last few decades, its production has also spread to other countries. Argentina is a 'new world' wine producer and consumer in the southern hemisphere, with 228 575 ha of vineyards representing ∼3% of the global wine grape cultivation area. Mendoza province has ∼70% of all Argentinean vineyards with 160 704 ha, and the main red grape variety produced (29%) is Malbec (*Vitis vinifera* L.), considered the emblematic cultivar of Argentina. In addition to Malbec, the other five common red varieties used for winemaking in this country are Bonarda, Cabernet Sauvignon, Shiraz, Tempranillo and Merlot, accounting for 90% of red wine grapes produced in Mendoza.[1,2]

Knowledge of the chemical composition of wine and its association with the grape variety/cultivar is of paramount importance in oenology and a necessary tool for marketing. This has stimulated research on analytical methods to verify the authenticity of wines as well as other factors such as their geographical and technological origin. The differentiation of wines according to their variety has been carried out by analysing physicochemical parameters such as proteins,[3] amino acids and aroma compounds[4] or by DNA analysis.[5]

Phenolic compounds have also been suggested as chemical markers for the authentication and varietal differentiation of grapes and wines. In recent years the cultivar-characteristic profiles of monomeric anthocyanins have been widely used for the classification and differentiation of grape cultivars and monovarietal wines.[6–8] Other studies have demonstrated that flavonol profiles can also be used as a chemical indicator for the authenticity of both red and white grape cultivars and their

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corresponding single-cultivar wines.[9,10] Polyphenols are one of the most important quality parameters of wines and belong to two main groups of compounds, non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoids (anthocyanins, flavanols, flavonols and dihydroflavonols). These compounds contribute to the organoleptic characteristics of wines, such as colour, astringency and bitterness, are active in biochemical processes and have nutraceutical effects on human health, including antimicrobial, anticarcinogenic and antioxidant properties.[11] The phenolic profile of a wine depends mainly on the grape variety, the geographical location of the vineyard, factors that affect berry development (soil, weather, viticultural practices, etc.), grape maturity and the winemaking technique used.[12,13] Owing to its biological and agricultural importance, the genetics and biochemistry of the flavonoid biosynthetic pathway have been widely studied in different grape varieties.[14,15]

Numerous analytical methods have been used to detect and quantify phenolic compounds in wines, but high-performance liquid chromatography (HPLC) is the most widely employed technique for the analysis of individual compounds. The use of mass spectrometry (MS) techniques such as electrospray ionisation (ESI) coupled with HPLC to confirm the structure of the main phenolics and/or detect novel compounds is of great value in assessing the peculiar characteristics of different grape varieties, optimising oenological processes, obtaining wines with original and improved characteristics and achieving a better understanding of wine physiological properties.[16]

In order to improve the analytical information about wine composition and to assess wine authenticity, the development/employment of chemometric techniques has been of great value in obtaining reliable results. Several chemometric procedures have been used as the basis for discrimination of wines according to winemaking technology and classification according to region, type and variety. Various pattern recognition techniques such as principal component analysis,[17] cluster analysis[18] and discriminant analysis,[18,19] among others, have been used for this purpose.

To the best of our knowledge, there has been no report so far on the individual phenolic composition of Argentinean red wines. Considering this, the first aim of the present work was to study and describe the non-flavonoid and flavonoid composition of wines from the principal red grape varieties cultivated in Mendoza (Argentina). Taking into account the considerable number of chemical variables analysed, the second aim of the study was to obtain a classification model of red wine varieties by chemometric techniques of multivariate analysis.

EXPERIMENTAL

Wine samples

Thirty red wines produced on a commercial scale were collected in bottles (750 mL), at the end of malolactic fermentation, directly from the ten collaborating wineries in order to guarantee their varietal purity. The samples corresponded to five different wines for each of the six red varieties cultivated in Mendoza: Malbec (MB), Bonarda (BN), Cabernet Sauvignon (CS), Merlot (MT), Shiraz (SH) and Tempranillo (TP). All wines were pure monovarietals from the 2010 vintage. They were stored in darkness at 12–15 °C, and each wine bottle was opened immediately before the analyses. Owing to the time required for completing all analyses (about 1 month), the wine samples were transferred under a nitrogen gas stream to completely filled amber bottles to ensure their preservation.

Standards and reagents

Standards of gallic acid (149-91-7), syringic acid (530-57-4), caffeic acid (331-39-5), *p*-coumaric acid (501-98-4), ethyl gallate (831-61-8), tryptophol (526-55-6), (+)-catechin (7295-85-4), (−)-epicatechin (490-46-0), resveratrol (501-36-0), myricetin (529-44-2), kaempferol (520-18-3), quercetin-3-glucoside (21 637-25-2), *p*-dimethylaminocinnamaldehyde (6203-18-5) and polyvinylpolypyrrolidone (PVPP, 25 249-54-1) were purchased from Sigma-Aldrich (St Louis, MO, USA), tyrosol (501-94-0) from Fluka (St Louis, MO, USA) and protocatechuic acid (99-50-3), quercetin (117-39-5) and malvidin-3-glucoside chloride (7228-78-6) from Extrasynthese (Lyon, France). Sodium chloride and sodium metabisulphite were obtained from Anedra (Buenos Aires, Argentina). Ammonium iron(II) sulfate and butanol were purchased from Dalton (Mendoza, Argentina). Ethyl ether and ethyl acetate were acquired from Sintorgan (Buenos Aires, Argentina). Anhydrous sodium sulfate, gelatin, acetaldehyde, hydrochloric acid, acetic acid, formic acid, ethanol, chromatography-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade or superior. Ultrapure water was obtained from a RiO/Elix3-Sinergy185 purification system (Millipore, Sao Pablo, Brazil). Cellulose filters (3 μm pore size) and nylon membranes (0.45 μm pore size) were purchased from Microclar (Buenos Aires, Argentina). Nitrogen gas was supplied by Linde SA (Mendoza, Argentina).

Instrumentation

pH was measured using a TPX-1 digital pH meter (Altronix, Buenos Aires, Argentina). Centrifugation was performed in a CM4080 centrifuge (Rolco, Buenos Aires, Argentina). Absorbance measurements were made with a PerkinElmer Lambda 25 UV–visible spectrophotometer (PerkinElmer, Hartford, CT, USA). For quantification of individual phenolic compounds, a PerkinElmer Series 200 high-performance liquid chromatograph equipped with a diode array detector, a quaternary pump and an autosampler (HPLC-DAD; PerkinElmer, Shelton, CT, USA) was employed. The chromatographic system used for compound identification and confirmation consisted of a Hewlett-Packard Series 1100 high-performance liquid chromatograph equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface (HPLC-DAD/ESI-MS; Hewlett-Packard, Palo Alto, CA). A reverse phase Chromolith Performance C_{18} column (100 mm × 4.6 mm i.d., 2 μm) with a Chromolith guard cartridge (10 mm × 4.6 mm i.d.) (Merck, Darmstadt, Germany) was used for individual anthocyanin analysis. A reverse phase Nova-Pak C_{18} column (300 mm × 3.9 mm i.d., 4 μm; Waters Corp., Milford, MA, USA) was used for low-molecular-weight phenolic compound analysis.

Spectrophotometric characterisation

Total phenols were determined by direct reading of the absorbance of the samples (1 : 100 dilution) at 280 nm.[20] Total phenols were calculated from a calibration curve made with standard solutions of gallic acid (five replicates) in the range between 0 and 50 mg L^{-1} ($R^2 = 0.99$) and expressed as mg gallic acid equivalent (GAE) L^{-1}.

Total anthocyanins were measured by diluting the extract with 20 mL L^{-1} hydrochloric acid in ethanol and comparing spectrophotometric readings at 520 nm of single aliquots treated

with either sodium metabisulfite or water.[20] Total anthocyanins were expressed as mg malvidin-3-glucoside L^{-1}. Free and combined anthocyanins were calculated using the PVPP index.[21]

For total proanthocyanidins the analytical method applied was the acid butanol assay.[22] This method is based on the acid-catalysed oxidative cleavage of the C–C interflavanic bond of proanthocyanidins in butanol-HCl. Total proanthocyanidins were expressed as mg (+)-catechin L^{-1}.

Gelatin index (GI) was measured using the methodology described by Glories.[23] To two tubes with 10 mL of wine was added 1 mL of distilled water (total tannin) or 1 mL of 70 g L^{-1} gelatin solution (tannin precipitated with gelatin). After 3 days the samples were centrifuged at 2038 × *g* for 10 min. The supernatants were assayed to determine the tannin concentration.[22] GI (%) was expressed as the ratio between residual tannin (difference between total wine tannin and tannin after gelatin precipitation) and total tannin concentration.

Colour intensity (CI), percentage of yellow (%Yellow), percentage of red (%Red) and percentage of blue (%Blue) were estimated using the method described by Glories.[21,23] The CIELAB coordinates lightness (L^*), chroma or saturation (C^*), hue angle (h), redness/greenness (a^*) and yellowness/blueness (b^*) were determined according to Ayala *et al.*[24] and the data were processed with MSCV® software.[25] The total colour difference (ΔE^*) between two samples was obtained using the expression[26]

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The contribution of copigmented anthocyanins to the total wine colour at pH 3.6 (colour due to copigmentation, CC%) and the degree of anthocyanin polymerisation (colour due to polymeric pigments, CP%) were determined following the method described by Hermosín Gutiérrez.[27]

Other chemical parameters measured in the samples were molar concentration of flavanols by *p*-dimethylaminocinnamaldehyde assay[28] and pH, titratable acidity and ethanol content as described by Zoecklein *et al.*[29]

HPLC analysis of anthocyanins

A 2 mL aliquot of wine was filtered through a 0.45 µm pore size nylon membrane, then 100 µL of the filtrate was injected onto the column. Separation was carried out at 25 °C. A gradient consisting of solvent A (water/formic acid, 90 : 10 v/v) and solvent B (acetonitrile) was applied at a flow rate of 1.1 mL min^{-1} from 0 to 22 min and 1.5 mL min^{-1} from 22 to 35 min as follows: 96–85% A/4–15% B from 0 to 12 min, 85% A/15% B from 12 to 22 min and 85–70% A/15–30% B from 22 to 35 min; this was followed by a final wash with pure methanol and re-equilibration of the column. Diode array detection was performed from 210 to 600 nm and quantification was carried out by peak area measurements at 520 nm. Anthocyanin content was expressed using malvidin-3-glucoside chloride as standard for a calibration curve ($R^2 = 0.99$). ESI parameters were as follows: drying gas (N_2) flow, 11 L min^{-1}; temperature, 350 °C; nebuliser pressure, 380 Pa (55 psi); capillary voltage, 4000 V. The ESI was operated in positive mode scanning from *m/z* 100 to 1500 using the following fragmentation voltage gradient: 100 V from 0 to 15 min and 120 V from 15 to 35 min.[6]

HPLC analysis of low-molecular-weight phenolic compounds

A 50 mL aliquot of wine was mixed with 1 g of sodium chloride and extracted three times with 20 mL of ethyl ether and three times with 20 mL of ethyl acetate. The organic fractions were combined, dehydrated with 2.5 g of anhydrous sodium sulfate, filtered throughout a 3 µm pore size cellulose filter and evaporated to dryness under a gentle nitrogen gas stream at 35 °C. The solid residue was dissolved in 2 mL of methanol/water (1 : 1 v/v) and filtered through a 0.45 µm pore size nylon membrane, then 30 µL of the filtrate was injected into the HPLC system. Separation was performed at 25 °C. Two mobile phases were employed for elution: A (water/acetic acid, 98 : 2 v/v) and B (water/acetonitrile/acetic acid, 78 : 20:2 v/v/v). The gradient profile was as follows: 0–55 min, 100–20% A/0–80% B; 55–57 min, 20–10% A/80–90% B; 57–70 min, 10% A/90% B isocratic; 70–80 min, 10–0% A/90–100% B; 80–125 min, 0% A/100% B isocratic; this was followed by a final wash with pure methanol and re-equilibration of the column. The flow rate was 0.9 mL min^{-1} from 0 to 55 min and 1 mL min^{-1} from 55 to 125 min. Diode array detection was performed by scanning from 210 to 360 nm with an acquisition time of 1 s. ESI parameters were as follows: drying gas (N_2) flow, 11 L min^{-1}; temperature, 350 °C; nebuliser pressure, 380 Pa (55 psi); capillary voltage, 4000 V. The ESI was operated in negative mode scanning from *m/z* 100 to 3000 using the following fragmentation programme: from *m/z* 0 to 200 (100 V) and from *m/z* 200 to 3000 (200 V).[16] Quantitative determinations were made using the external standard method with commercial standards. The calibration curves were obtained by injection of standard solutions, under the same conditions as for the samples analysed, over the range of concentrations observed ($R^2 \geq 0.94$). Compounds for which no standards were available were quantified with the curves of quercetin (dihydroflavonols), quercetin-3-glucoside (quercetin and flavonol glycosides), myricetin (myricetin glycosides), resveratrol (*trans*- and *cis*-resveratrol glucoside), caffeic acid (fertaric, caftaric and coutaric acids), gallic acid (gentisic acid), ethyl gallate (methyl gallate) and (+)-catechin (procyanidins).

Statistical analysis

All analyses (including extractions) were carried out in triplicate. Statistical analysis was assessed with Statgraphics Plus Version 4.0 (Statistical Graphics Corp., Warrenton, VA, USA). All results were tested for homogeneity of variance using Cochran's test and subjected to one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. A $P < 0.05$ was considered to be statistically significant. Canonical discriminant analyses were performed to examine varietal differences in red wines from Mendoza using the individual phenolic parameters.

RESULTS AND DISCUSSION

General chemical composition

Table 1 presents the results for the general analytical parameters evaluated in the monovarietal red wines studied. Among all samples analysed, titratable acidity varied from 4.4 to 6.8 g L^{-1}, pH from 3.60 to 3.84 and ethanol content from 13.0 to 15.2%. These results show a considerable dispersion for these important parameters that influence not only the sensory quality of wine but also the colour intensity expression and microbiological stability.[29] MB wines presented significantly higher acidity and also reached higher ethanol content than the other varieties.

For all samples, total phenols ranged between 1585.6 and 4203.2 mg L^{-1}. On average, MB wines contained slightly higher phenolic levels than MT, CS, BN and TP, without significant

Table 1. General analytical parameters of monovarietal red wines from Mendoza

Parameter	Malbec	Bonarda	Cabernet Sauvignon	Merlot	Shiraz	Tempranillo
Titratable acidity (g tartaric acid L^{-1})	6.8 ± 0.3b	5.3 ± 0.2a	6.7 ± 0.3b	5.8 ± 0.4ab	4.4 ± 0.2a	5.6 ± 0.3ab
pH	3.60 ± 0.06a	3.80 ± 0.04a	3.73 ± 0.09a	3.73 ± 0.09a	3.80 ± 0.06a	3.84 ± 0.07a
Ethanol (% v/v)	15.2 ± 0.4d	13.0 ± 0.3a	14.5 ± 0.3bcd	14.5 ± 0.1cd	13.6 ± 0.2ab	13.8 ± 0.2bc
TA (mg malvidin-3-glucoside L^{-1})	1044.5 ± 88.2c	739.8 ± 55.0bc	681.8 ± 100.8b	644.1 ± 37.6b	301.4 ± 18.9a	717.6 ± 41.9b
FA (mg malvidin-3-glucoside L^{-1})	690.0 ± 139.6b	418.9 ± 33.3ab	417.2 ± 45.4ab	409.7 ± 45.2ab	226.1 ± 11.1a	407.1 ± 40.4a
CA (mg malvidin-3-glucoside L^{-1})	354.4 ± 55.3b	321.0 ± 28.0b	264.6 ± 56.9b	234.4 ± 14.5ab	75.2 ± 8.0a	310.5 ± 24.5b
CI ($A_{420\ nm}+A_{520\ nm}+A_{620\ nm}$) × 10	31.9 ± 1.3d	15.0 ± 1.4bc	15.2 ± 0.9bc	17.3 ± 0.5c	5.8 ± 0.1a	12.0 ± 0.7b
%Yellow	27.5 ± 0.1a	32.3 ± 0.7b	33.0 ± 0.9b	33.3 ± 0.9b	36.3 ± 0.3c	33.1 ± 0.3b
%Red	61.2 ± 0.1b	54.5 ± 1.1a	55.2 ± 1.3a	55.3 ± 1.1a	51.6 ± 0.4a	53.8 ± 1.0a
%Blue	11.3 ± 0.1a	13.2 ± 0.4a	11.8 ± 0.4a	11.3 ± 0.2a	12.1 ± 0.4a	13.1 ± 0.8a
L^*	22.3 ± 2.0a	40.5 ± 3.0b	40.7 ± 2.4b	38.0 ± 1.8b	68.5 ± 1.2c	46.5 ± 1.8b
C^*	57.6 ± 1.5c	52.7 ± 2.3bc	56.2 ± 1.5bc	58.6 ± 2.1c	32.1 ± 0.4a	49.8 ± 1.4b
h	21.6 ± 0.8c	9.0 ± 2.6a	10.8 ± 1.9ab	19.2 ± 1.9bc	3.6 ± 2.0a	4.9 ± 1.1a
a^*	53.5 ± 1.6b	51.9 ± 2.0b	55.2 ± 1.5b	55.4 ± 2.6b	32.0 ± 0.5a	49.6 ± 1.3b
b^*	21.1 ± 0.6c	8.4 ± 2.7ab	10.5 ± 1.9b	19.2 ± 1.1c	2.0 ± 1.1a	4.4 ± 1.0ab
CC (%)	49.2 ± 2.8b	24.1 ± 1.7a	20.3 ± 0.8a	22.1 ± 5.0a	22.0 ± 0.8a	25.2 ± 3.2a
CP (%)	11.0 ± 0.6a	25.4 ± 0.7b	27.4 ± 1.2b	26.6 ± 2.8b	23.1 ± 0.6b	21.3 ± 2.1b
TTP (mg GAE L^{-1})	4203.2 ± 412.8b	3372.1 ± 453.0b	3377.6 ± 369.6b	3447.5 ± 372.3b	1585.6 ± 50.6a	3137.4 ± 152.9b
PA (mg catechin L^{-1})	5013.0 ± 507.2b	3925.4 ± 556.3b	3860.7 ± 439.6ab	4439.9 ± 498.5b	1922.6 ± 160.1a	3200.7 ± 250.9ab
FL (mol catechin L^{-1})	$1.6 \times 10^{-3} \pm 6.3 \times 10^{-5}$b	$2.1 \times 10^{-3} \pm 1.8 \times 10^{-4}$bc	$2.7 \times 10^{-3} \pm 1.7 \times 10^{-4}$c	$2.2 \times 10^{-3} \pm 2.0 \times 10^{-4}$bc	$8.8 \times 10^{-4} \pm 3.7 \times 10^{-5}$a	$1.7 \times 10^{-3} \pm 6.8 \times 10^{-5}$b
GI (%)	83.7 ± 2.0a	78.3 ± 0.9a	79.1 ± 1.7a	79.2 ± 4.2a	75.1 ± 1.7a	80.9 ± 2.5a

Values are expressed as mean ± standard error ($n = 5$). Different letters in the same row indicate significant differences ($P < 0.05$) according to Tukey's HSD test.
TA, total anthocyanins; FA, free anthocyanins; CA, combined anthocyanins; CI, colour intensity; L^*, lightness; C^*, chroma; h, hue angle; a^*, redness/greenness; b^*, yellowness/blueness; CC, colour due to copigmentation; CP, colour due to polymeric pigments; TTP, total phenols; PA, proanthocyanidins; FL, molar concentration of flavanols; GI, gelatin index.

differences ($P > 0.05$) among the five varieties. By contrast, SH wines had the lowest values for this parameter. These results are comparable to those reported for Tempranillo wines from Spain,[30] Malbec wines from Argentina[2] and Shiraz, Cabernet Sauvignon and Merlot wines from Australia.[31]

Proanthocyanidins accounted for >70% of the total phenolic compounds in the red wines analysed. Among the six varieties, MB showed the highest values, with concentrations similar to those of a previous study.[2] Besides the influence of genetic and agroecological factors on the biosynthesis of these compounds, the high content of alcohol in MB wines could enhance the extraction of proanthocyanidins during the winemaking process. The astringency measured by GI showed a parallel behaviour to proanthocyanidins, with values between 75.1 and 83.7%.

Regarding colour parameters, statistically significant differences were observed among samples. MB wines presented significantly higher values of CI and *h* and lower L^* values (more dark colour) than the other varieties. This higher CI was principally due to the red component of the colour. When analysing the other cultivars, we found that BN, CS and MT had similar values for all colour components, TP showed slightly lower values and SH was the least coloured variety.

Table 2 shows ΔE^* values among wines of the different varieties. This parameter can be very important for the wine industry as it expresses the human eye's ability to discriminate between the colours of two wines. It is generally accepted that tasters can only distinguish the colours of two wines through the glass when $\Delta E^* \geq 5$ units.[26] In our study, ΔE^* values among all red wine varieties were greater than 5 units.

Parallel to what was observed for colour, total anthocyanin concentration ranged from 301.4 mg L^{-1} (SH) to 1044.5 mg L^{-1} (MB). This behaviour was similar for free and combined anthocyanins. These results are in agreement with those determined by other authors.[2,13,31,32] As for proanthocyanidins, the extracting effect of ethanol during maceration may be favoured by lower pH values in MB wines. A lower pH may also protect anthocyanins against oxidation.[21] The total anthocyanins determined by spectrophotometry were much higher than those obtained by HPLC. This is because spectrophotometric analysis overestimates the total anthocyanin concentration since it includes the contribution from other pigments,[33] whereas only monomeric anthocyanins are detected by the HPLC method.

Analysing the contribution of CC% to the total wine colour, we observed a higher proportion (49.2%) in MB wines, probably owing to the higher content of anthocyanins and other copigments. Conversely, the remaining red varieties, especially BN, MT and CS, had higher values for CP% than MB, which could be explained by higher levels of flavanols (Table 1). It is well known that the main reaction involving monomeric anthocyanins is the formation of polymeric red pigments through condensation with flavanols (especially oligomers), and this reaction can be mediated and accelerated by acetaldehyde.[34]

Finally, we note that the low concentration observed in SH wines for the phenolic parameters evaluated can be attributed mainly to the high yield of the vineyards from which they come. Peña-Neira *et al.*[35] studied the effect of cluster thinning in Shiraz grapes and observed a higher concentration of phenolic compounds in berries from low-yield plants. In general, the SH vines of our study yielded close to 20 000 kg ha^{-1}, about double compared with the other varieties.

Anthocyanins and pyranoanthocyanins

The identified and quantified compounds in the wine samples were grouped according to acylation (non-acylated glucosides, acetyl-glucosides and cinnamoyl-glucosides) and anthocyanidin (delphinidins, cyanidins, petunidins, peonidins and malvidins) characteristics. Cinnamoyl-glucosides included both *p*-coumaroyl and caffeoyl anthocyanins. Another group was formed by more complex anthocyanin-derived pigments (pyranoanthocyanins). The molecular ions $[M]^+$, the fragments corresponding to the anthocyanidins after cleavage of the glucose moiety, and the spectral information are shown in Table 3. All identified compounds were detected in all varieties studied, except cyanidin-3-(6″-*p*-coumaroyl)glucoside that was absent in CS and malvidin-3-glucoside acetate (vitisin B) that was absent in SH.

Table 4 summarises the concentrations of individual anthocyanins and pyranoanthocyanins in the different monovarietal red wines. Marked quantitative differences could be seen in the anthocyanin profile of the six varieties. Coincident with the results observed in the spectrophotometric determination of anthocyanins, MB wines had the highest content of total monomeric anthocyanins (587.2 mg L^{-1}), followed by TP, BN, MT, CS and SH. The mean proportions of some anthocyanic forms were different among varieties, but the relations between anthocyanic groups seem to be characteristic of them. The group of simple glucosides represented the highest proportion of all anthocyanins in all varieties, ranging from 54.8% (SH) to 75.8% (TP). These results are in agreement with those published by others authors for the same cultivars.[2,6,34,36,37] Acylated derivatives showed the largest differences among varieties. Considering the acetyl-glucosides, SH wines had the highest proportion, similar to CS and MT, followed by MB and BN, and the lowest values were observed in TP (26.5, 25.0, 22.5, 16.7, 14.3 and 8.7% respectively). However, the proportion of cinnamoyl-glucosides was very similar in SH and TP, higher than in BN, MB and MT, whereas in CS it was approximately half that of the other varieties (Table 4). These results are consistent with the literature.[2,6,34,36,38,39]

The ratio of acetyl- and coumaroyl-glucosides ($\sum$acetylated/$\sum$coumaroylated) was also calculated. It is related to the activity of enzymes of the anthocyanin synthesis pathway in grapes and is proposed by some authors for the verification of varietal authenticity in red wines.[8] The values obtained for the six varieties were significantly different ($P < 0.05$), i.e. 2.1 for MB, 1.6 for BN, 5.2 for CS, 2.9 for MT, 2.0 for SH and 0.8 for TP, in accordance with other studies.[2,6,36]

As shown in Fig. 1, the malvidin derivatives were the most abundant anthocyanins in all samples, while the cyanidin derivatives showed the lowest proportion, confirming its behaviour observed in previous studies.[2,36] In accordance with Roggero *et al.*,[40] the results of our study suggest that the enzyme activity involved in anthocyanin biosynthesis is different in each variety. MT and SH seem to have weaker flavonoid-3′-hydroxylase activity, favouring the accumulation of peonidin. MB, TP and BN seem to have less *o*-dihydroxyphenol-*O*-methyltransferase activity, allowing the accumulation of delphinidin and petunidin. Finally, CS seems to have strong activity of both enzymes, permitting the highest accumulation of malvidin.

Pyranoanthocyanins are of interest for winemakers because they show high stability during the aging of red wines, are more resistant to elevated pH values and bisulphite bleaching than anthocyanins and express more colour than other pigments at the typical pH of wine. Table 4 shows that MB wines contained the highest levels of all derived pigments, which seems to be

Table 2. Total colour differences (ΔE^*) among monovarietal red wines

Variety	MB	BN	CS	MT	SH	TP
MB	–	22.9 ± 2.5	21.6 ± 3.0	16.6 ± 0.6	54.5 ± 0.6	29.8 ± 1.4
BN	22.9 ± 2.5	–	10.1 ± 2.7	12.7 ± 1.9	35.1 ± 2.6	9.2 ± 2.1
CS	21.6 ± 3.0	10.1 ± 2.7	–	10.4 ± 1.5	37.3 ± 2.3	12.5 ± 3.0
MT	16.6 ± 0.6	12.7 ± 1.9	10.4 ± 1.5	–	42.1 ± 1.2	18.7 ± 1.5
SH	54.5 ± 0.6	35.1 ± 2.6	37.3 ± 2.3	42.1 ± 1.2	–	28.5 ± 1.4
TP	29.8 ± 1.4	9.2 ± 2.1	12.5 ± 3.0	18.7 ± 1.5	28.5 ± 1.4	–

Values are expressed as mean ± standard error ($n = 5$). MB, Malbec; BN, Bonarda; CS, Cabernet Sauvignon; MT, Merlot; SH, Shiraz; TP, Tempranillo.

Table 3. Anthocyanins identified by HPLC-DAD/ESI-MS in wines from *Vitis vinifera* L. cvs Malbec (MB), Bonarda (BN), Cabernet Sauvignon (CS), Merlot (MT), Shiraz (SH) and Tempranillo (TP)

Compound	λ (nm)	$[M]^+$ (*m/z*)	Fragment (*m/z*)	Wines					
				MB	BN	CS	MT	SH	TP
Delphinidin-3-glucoside	526	465	303	X	X	X	X	X	X
Cyanidin-3-glucoside	516	449	287	X	X	X	X	X	X
Petunidin-3-glucoside	528	479	317	X	X	X	X	X	X
Peonidin-3-glucoside	518	463	301	X	X	X	X	X	X
Malvidin-3-glucoside	528	493	331	X	X	X	X	X	X
Delphinidin-3-(6″-acetyl)glucoside	518	507	303	X	X	X	X	X	X
Cyanidin-3-(6″-acetyl)glucoside	518	491	287	X	X	X	X	X	X
Petunidin-3-(6″-acetyl)glucoside	530	521	317	X	X	X	X	X	X
Peonidin-3-(6″-acetyl)glucoside	520	505	301	X	X	X	X	X	X
Malvidin-3-(6″-acetyl)glucoside	530	535	331	X	X	X	X	X	X
Delphinidin-3-(6″-*p*-coumaroyl)glucoside	532	611	303	X	X	X	X	X	X
Cyanidin-3-(6″-*p*-coumaroyl)glucoside	526	595	287	X	X	–	X	X	X
Malvidin-3-(6″-caffeoyl)glucoside	526	655	331	X	X	X	X	X	X
Petunidin-3-(6″-*p*-coumaroyl)glucoside	532	625	317	X	X	X	X	X	X
Peonidin-3-(6″-*p*-coumaroyl)glucoside	524	609	301	X	X	X	X	X	X
Malvidin-3-(6″-*p*-coumaroyl)glucoside *cis*	540	639	331	X	X	X	X	X	X
Malvidin-3-(6″-*p*-coumaroyl)glucoside *trans*	535	639	331	X	X	X	X	X	X
Mlalvidin-3-glucoside pyruvate (vitisin A)	512	561	399	X	X	X	X	X	X
Malvidin-3-glucoside acetate (vitisin B)	492	517	355	X	X	X	X	–	X
Peonidin-3-glucoside pyruvate	520	531	369	X	X	X	X	X	X
Malvidin-3-(6″-acetyl)glucoside pyruvate	518	603	399	X	X	X	X	X	X
Malvidin-3-glucoside-ethyl-epicatechin	545	809	–	X	X	X	X	X	X
Malvidin-3-glucoside-4-vinylphenol	504	609	447	X	X	X	X	X	X

X, detected; –, not detected.

related to a larger concentration of its corresponding anthocyanin precursors. Moreover, the differences observed in levels of these compounds among the samples may be a varietal characteristic or due to different winemaking conditions.[6]

Low-molecular-weight phenolic composition

The identified and quantified low-molecular-weight phenolic compounds (non-anthocyanins) in the red wines analysed were grouped into non-flavonoids (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoids (flavanols, flavonols and dihydroflavonols). Table 5 shows the ESI-MS data of these compounds and their distribution in the six varieties studied.

The concentrations of low-molecular-weight phenolic compounds, individually and grouped, and the relative proportions of each group in the wine samples are presented in Table 6. Flavonoid compounds were the most abundant fraction (mean 75.5%) of non-anthocyanin phenolics compared with non-flavonoids (mean 24.5%) in the six varieties, as reported by other authors.[2,16,34] Coincident with the results observed for the general parameters evaluated (total phenols, proanthocyanidins, anthocyanins) and the monomeric anthocyanins determined by HPLC, MB wines had the highest concentration of total non-anthocyanin phenolic compounds (495.9 ± 37.9 mg L^{-1}), indicating their polyphenolic richness compared with the other varieties. Regarding the non-flavonoids, hydroxybenzoic acids/derivatives and phenolic alcohols were the most abundant groups found in our samples, ranging from 5.4% (MB) to 9.9% (BN) and from 7.4% (MB) to 13.4% (TP) of total phenolics quantified respectively. Gallic acid showed the highest concentration of all benzoic derivatives (mean 47.3%), especially in BN wines (mean 20.7 mg L^{-1}) compared with the other varieties. These results are in agreement with

Table 4. Anthocyanins (mg L^{-1}) quantified in different monovarietal red wines from Mendoza

Compound	Malbec	Bonarda	Cabernet Sauvignon	Merlot	Shiraz	Tempranillo
Delphinidin-3-glucoside	41.2 ± 2.4e	18.9 ± 1.5c	10.1 ± 1.0b	14.5 ± 1.1bc	2.7 ± 0.1a	26.5 ± 1.5d
Cyanidin-3-glucoside	5.48 ± 0.28c	2.44 ± 0.20b	2.10 ± 0.20b	2.46 ± 0.17b	0.66 ± 0.04a	2.42 ± 0.15b
Petunidin-3-glucoside	61.8 ± 2.3e	28.7 ± 2.0cd	14.4 ± 0.9b	23.2 ± 1.9c	6.0 ± 0.3a	35.0 ± 0.7d
Peonidin-3-glucoside	40.3 ± 2.4c	16.6 ± 1.2a	14.9 ± 1.3a	24.3 ± 2.3b	14.3 ± 1.0a	17.0 ± 1.0a
Malvidin-3-glucoside	257.0 ± 8.0d	146.2 ± 6.4bc	141.3 ± 12.2bc	119.3 ± 10.6b	73.7 ± 2.2a	162.0 ± 7.6c
Total glucosylated	405.8 ± 11.8d (69.1[a])	212.8 ± 10.1bc (69.2)	182.8 ± 14.8b (63.2)	183.8 ± 10.8b (62.5)	97.4 ± 3.3a (54.8)	242.9 ± 10.8c (75.8)
Delphinidin-3-(6″-acetyl)glucoside	11.08 ± 0.48d	4.42 ± 0.28b	4.06 ± 0.24b	6.00 ± 0.43c	1.68 ± 0.05a	3.12 ± 0.19b
Cyanidin-3-(6″-acetyl)glucoside	4.62 ± 0.05c	2.80 ± 0.24b	3.18 ± 0.38b	2.66 ± 0.20b	1.46 ± 0.05a	1.32 ± 0.09a
Petunidin-3-(6″-acetyl)glucoside	22.9 ± 1.8b	4.5 ± 0.3a	7.4 ± 0.4a	7.5 ± 0.5a	4.0 ± 0.2a	5.6 ± 0.3a
Peonidin-3-(6″-acetyl)glucoside	6.6 ± 0.3c	4.4 ± 0.4b	6.4 ± 0.5c	7.4 ± 0.4c	7.6 ± 0.5c	1.7 ± 0.1a
Malvidin-3-(6″-acetyl)glucoside	52.6 ± 0.9d	27.8 ± 1.9ab	51.1 ± 5.0d	42.7 ± 5.4cd	32.4 ± 1.7bc	16.0 ± 0.4a
Total acetylated	97.8 ± 2.3d (16.7)	43.9 ± 2.6ab (14.3)	72.1 ± 6.1c (25.0)	66.3 ± 5.9c (22.5)	47.1 ± 1.6b (26.5)	27.7 ± 1.0a (8.7)
Delphinidin-3-(6″-*p*-coumaroyl)glucoside	8.6 ± 0.5c	4.4 ± 0.2b	2.3 ± 0.1a	2.6 ± 0.1a	2.7 ± 0.1a	4.3 ± 0.1b
Cyanidin-3-(6″-*p*-coumaroyl)glucoside	0.84 ± 0.07c	0.58 ± 0.04b	ND	0.42 ± 0.05ab	0.32 ± 0.02a	0.60 ± 0.05b
Malvidin-3-(6″-caffeoyl)glucoside	1.46 ± 0.09c	0.82 ± 0.07b	0.76 ± 0.05b	0.72 ± 0.06b	0.32 ± 0.02a	1.42 ± 0.10c
Petunidin-3-(6″-*p*-coumaroyl)glucoside	5.18 ± 0.34e	1.98 ± 0.15c	0.26 ± 0.02a	1.16 ± 0.10b	0.62 ± 0.05ab	3.04 ± 0.04d
Peonidin-3-(6″-*p*-coumaroyl)glucoside	6.2 ± 0.4c	3.7 ± 0.2b	1.3 ± 0.1a	3.3 ± 0.3b	5.1 ± 0.3c	2.9 ± 0.1b
Malvidin-3-(6″-*p*-coumaroyl)glucoside *cis*	1.78 ± 0.22b	1.20 ± 0.10a	0.84 ± 0.10a	0.98 ± 0.08a	1.28 ± 0.04ab	1.78 ± 0.07b
Malvidin-3-(6″-*p*-coumaroyl)glucoside *trans*	23.5 ± 0.8c	16.1 ± 0.3b	9.2 ± 0.3a	14.4 ± 1.1b	13.5 ± 0.5b	21.9 ± 0.9c
Total coumaroylated	46.1 ± 2.0e (7.8)	28.0 ± 0.7c (9.1)	13.9 ± 0.5a (4.8)	22.9 ± 1.4b (7.8)	23.5 ± 0.5bc (13.3)	34.5 ± 1.0d (10.8)
Total cinnamoylated	47.6 ± 1.9e (8.1)	28.8 ± 0.7c (9.4)	14.7 ± 0.5a (5.0)	23.6 ± 1.4b (8.0)	23.8 ± 0.5bc (13.5)	35.9 ± 1.0d (11.2)
Vitisin A	12.8 ± 0.6d	5.6 ± 0.4bc	5.4 ± 0.3bc	6.4 ± 0.5c	2.0 ± 0.1a	4.0 ± 0.3b
Vitisin B	6.0 ± 0.4d	4.6 ± 0.3c	4.4 ± 0.2bc	3.3 ± 0.3ab	ND	2.3 ± 0.2a
Peonidin-3-glucoside pyruvate	5.24 ± 0.34c	2.90 ± 0.19b	2.44 ± 0.16b	2.46 ± 0.17b	1.32 ± 0.05a	2.10 ± 0.08ab
Malvidin-3-(6″-acetyl)glucoside pyruvate	3.9 ± 0.3c	3.3 ± 0.3bc	2.7 ± 0.3ab	3.0 ± 0.2bc	3.3 ± 0.1bc	1.9 ± 0.1a
Malvidin-3-glucoside-ethyl-epicatechin	7.5 ± 0.5d	4.6 ± 0.3c	4.2 ± 0.3bc	4.6 ± 0.4c	2.2 ± 0.1a	2.9 ± 0.2ab
Malvidin-3-glucoside-4-vinylphenol	0.60 ± 0.03a	0.94 ± 0.08b	0.62 ± 0.07a	0.62 ± 0.06a	0.48 ± 0.04a	0.60 ± 0.03a
Total pyranoanthocyanins	36.0 ± 0.9d (6.1)	21.9 ± 1.2c (7.2)	19.8 ± 0.9c (6.8)	20.4 ± 1.5c (6.9)	9.3 ± 0.3a (5.2)	13.8 ± 0.7b (4.3)
Total anthocyanins	587.2 ± 16.5c	307.4 ± 10.6b	289.4 ± 21.3b	294.1 ± 17.5b	177.6 ± 5.1a	320.3 ± 10.2b
∑glucosylated/∑acetylated	4.1	4.8	2.5	2.8	2.1	8.8
∑glucosylated/∑coumaroylated	8.8	7.6	13.2	8.1	4.1	7.0
∑coumaroylated/∑acetylated	0.5	0.6	0.2	0.3	0.5	1.2
∑acetylated/∑coumaroylated	2.1	1.6	5.2	2.9	2.0	0.8

Values are expressed as mean ± standard error ($n = 5$). Different letters in the same row indicate significant differences between varieties for each compound (Tukey's HSD test, $P < 0.05$). ND, not detected.
[a] Ratio (%) between anthocyanin derivatives by acylation and total anthocyanins.

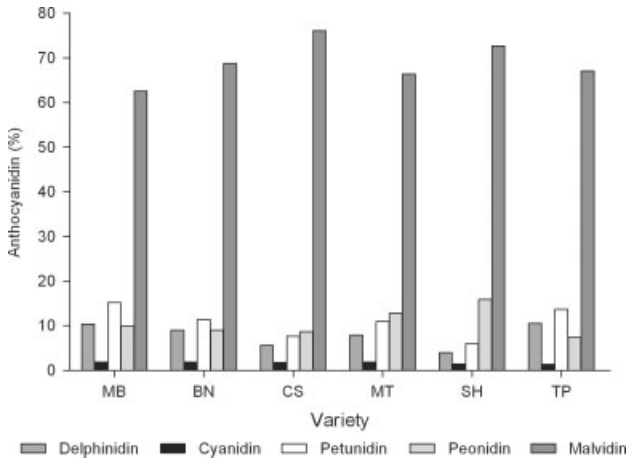

Figure 1. Anthocyanin distribution by anthocyanidins in Malbec (MB), Bonarda (BN), Cabernet Sauvignon (CS), Merlot (MT), Shiraz (SH) and Tempranillo (TP) wines.

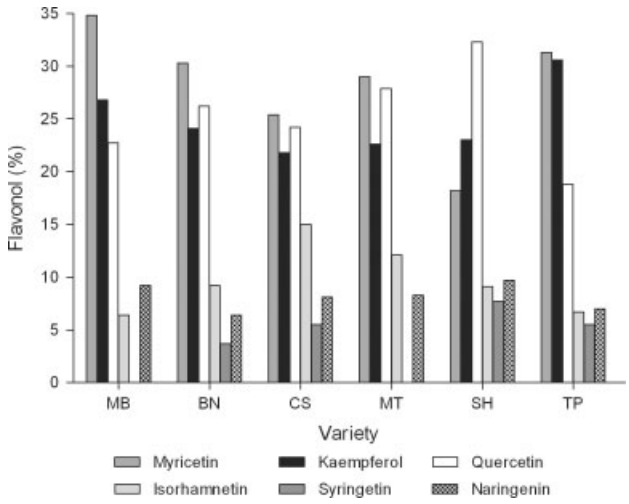

Figure 2. Flavonol distribution in Malbec (MB), Bonarda (BN), Cabernet Sauvignon (CS), Merlot (MT), Shiraz (SH) and Tempranillo (TP) wines.

other studies on Spanish red wines[16,41] and Malbec wines from Argentina.[2]

The hydroxycinnamic acids found in the wines studied were *trans*-caffeic and *trans*-*p*-coumaric acids, BN being the variety richest in these compounds (9.9 mg L^{-1}). Analysing their precursors (tartaric esters of hydroxycinnamic acids), we observed a higher content of these compounds than of the free acids in all wines evaluated, in accordance with Monagas *et al*.[16] and Hermosín Gutiérrez *et al*.[34] In addition, there were significant differences among varieties, with larger values of caftaric, coutaric and fertaric acids in SH wines, followed by MB, MT, CS, TP and BN.

Among non-flavonoids, stilbenes are the most important compounds related to nutraceutical properties. In this work we detected *trans*- and *cis*-resveratrol glucoside, with a greater abundance of the *trans* isomer, in accordance with other authors.[16,42] Of particular interest was the extremely low total concentration observed in CS wines (3.3 mg L^{-1}) compared with the other varieties, especially MB, which had the highest level (13.6 mg L^{-1}). These differences may be due not only to the grape variety but also to fungal infections, winemaking procedures and weather conditions.[42]

Flavanols were the major class of phenolic compounds present in the wines studied (mean 45.3%). When comparing the different cultivars, we found that BN, MB, CS and MT had similar values, whilst SH and TP showed much lower contents. In all wines analysed, (+)-catechin levels were higher than those of (−)-epicatechin. These results are in agreement with those found in the literature for the same varieties.[2,16,43,44] Considering the (+)-catechin/(−)-epicatechin ratio, we observed variations among cultivars (2.4 for MB, 1.7 for BN, 2.0 for CS, 1.4 for MT, 1.9 for SH and 1.6 for TP), confirming differences in the activity of enzymes involved in their biosynthesis. From these results it can be assumed that the enzyme leucoanthocyanidin reductase is more active than anthocyanidin reductase in the grapes of all varieties studied. On the other hand, the concentration of dimers and trimers detected was lower than that of monomers in all samples, with the exception of a compound called 'procyanidin dimer 2' that showed high levels (Table 6).

Concerning flavonols, their importance in red wines lies in their health properties and their contribution to colour via the phenomenon of copigmentation.[20] The total content in the samples ranged from 74.3 mg L^{-1} (CS) to 112.9 mg L^{-1} (MB). These elevated values for all varieties could be explained by the climatic conditions of Mendoza, characterised by high sunlight intensity during the ripening period of the grapes, which appears to be associated with an increased accumulation of flavonols.[45] The particularly higher concentration in MB can be related to the greater CC% observed in these wines, given the behaviour of the flavonols as copigments. Figure 2 shows the distribution pattern of flavonol structures in the red varieties evaluated. The main compound in the samples analysed was myricetin (mean 28.2%), followed by quercetin (mean 25.4%) and kaempferol (mean 24.8%), then, in descending order, isorhamnetin, naringenin and syringetin (means 9.7, 8.1 and 3.7% respectively). Looking at variety, we can see different proportions, in agreement with other authors,[9] indicating possible variations in activity of the enzymes flavonol synthase and/or methyltranferase in the different cultivars. The majority of the flavonols found in the six varieties were glycosides, mainly myricetin-3-glucoside, quercetin-3-glucuronide and quercetin-3-glucoside (Table 6), in accordance with Castillo-Muñoz *et al*.[46] From a biosynthetic point of view, the results suggest differences in substrate selectivity of the glycosyltransferase enzymes that might be involved in the biosynthesis of flavonol-3-glycosides. The 3-glucosides were the main flavonol derivatives synthesised in CS, MT, SH and TP, accounting for 66.3, 60.7, 63.4 and 68.7% of the overall flavonol-3-glycosides respectively. In the case of MB and BN the 3-galactosides and 3-glucuronides were the predominant derivatives, with 55.5 and 67.5% of the total respectively for both varieties.

Rounding out the flavonoids studied, we would like to highlight the presence of dihydroflavonols (flavanonols) in the wines analysed. These compounds play functional roles in plants, but there are few data on them in grapes and wines. In our experiment the flavanonols characterised by HPLC-DAD/ESI-MS and UV spectral information were dihydroquercetin-3-glucoside, dihydrokaempferol-3-glucoside and dihydroquercetin-3-rhamnoside (astilbin) (Table 5). The first two compounds were detected in MB, BN, CS, MT and SH, while the last one was only detected in MB. We found no dihydroflavonols in TP. According to the literature, astilbin and dihydrokaempferol-3-glucoside have been reported in white and red wines,[47–49] while dihydroquercetin-3-glucoside has only been detected in white grape varieties.[47,50,51] Dihydroquercetin-3-glucoside was the major compound among

the low-molecular-weight phenolics in the MB wines studied, representing 11.2% of the total phenolic content, while the other varieties had much lower concentrations of this compound, about 10% of that found in MB. The UV characteristics (λ_{max} 292 nm, $\lambda_{shoulder}$ 336 nm) and mass spectrum of dihydroquercetin-3-glucoside are shown in Fig. 3. The fragment ions obtained in our study are consistent with the fragmentation mechanism proposed by Abad-García *et al*.[52] Based on the results, we have observed the same profile behaviour in skin samples from Malbec grape berries compared with those of Cabernet Sauvignon from different zones of Mendoza (data not yet published). In addition, this finding confirms the results obtained in our previous study[2] and could represent a distinctive feature of this variety.

Classification of red wines according to variety

Since the phenolic compounds in a wine come primarily from grape berries, phenol profiling in a non-aged wine is determined to a great extent by the grape itself. In the present study the differences in phenolic composition observed among the wines analysed indicate that the biosynthesis of phenolic compounds depends largely on the genotypes of the grape cultivar rather than other factors (environmental conditions, viticultural practices and winemaking techniques). In order to classify the samples according to variety, we used the anthocyanin and non-anthocyanin profiles in separate multivariate analyses. The main reason for this choice is the high correlation and dependence between the two groups of variables (seen through an exploratory statistical analysis). Moreover, it is interesting to evaluate the potential for classification of both groups in order to simplify the determinations in future studies.

In multivariate analysis it is first necessary to select appropriate variables for sample classification. To achieve this goal, the elimination of redundant variables is required to avoid overfitting problems, by applying different methodologies of feature selection: forward selection, backward selection, principal component analysis or genetic algorithms.[53] Canonical discriminant analysis (CDA) with backward selection was carried out to provide a visualisation of the data in a reduced-dimension plot,

Table 5. Low-molecular-weight phenolic compounds identified by HPLC-DAD/ESI-MS in wines from *Vitis vinifera* L. cvs Malbec (MB), Bonarda (BN), Cabernet Sauvignon (CS), Merlot (MT), Shiraz (SH) and Tempranillo (TP)

Compound	λ (nm)	$[M-H]^-$ (*m/z*)	Fragment(s) (*m/z*)	Wines					
				MB	BN	CS	MT	SH	TP
Gallic acid	272	169	125	X	X	X	X	X	X
Protocatechuic acid	290, 260	153	109	X	X	X	X	X	X
Syringic acid	278	197	–	X	X	X	X	X	X
Gentisic acid	328 (sh), 292	153	125, 109	X	X	X	X	X	X
Methyl gallate	276	183	169, 125	X	X	X	X	X	X
Ethyl gallate	278	197	169, 125	X	X	X	X	X	X
trans-Caftaric acid	326, 298 (sh)	311	179	X	X	X	X	X	X
cis-Coutaric acid	313, 290 (sh)	295	163	X	X	X	X	X	X
trans-Coutaric acid	313, 290 (sh)	295	163	X	X	X	X	X	X
trans-Fertaric acid	292, 260	325	193	X	X	X	X	X	X
trans-Caffeic acid	320	179	135	X	X	X	X	X	X
trans-*p*-Coumaric acid	309	163	119	X	X	X	X	X	X
trans-Resveratrol-3-glucoside	318 (sh), 304	389	227	X	X	X	X	X	X
cis-Resveratrol-3-glucoside	285	389	227	X	X	X	X	X	X
Tyrosol	275	137	–	X	X	X	X	X	X
Tryptophol	279	160	–	X	X	X	X	X	X
(+)-Catechin	279	289	–	X	X	X	X	X	X
(−)-Epicatechin	279	289	–	X	X	X	X	X	X
Procyanidin dimers	280	577	425, 407, 289	X	X	X	X	X	X
Procyanidin trimers	280	865	713, 577, 289	X	X	X	–	X	X
Myricetin-3-glucuronide	350, 300 (sh), 262	493	317	X	X	X	X	X	X
Myricetin-3-galactoside	350, 300 (sh), 263	479	317	X	X	–	–	–	–
Myricetin-3-glucoside	350, 300 (sh), 264	479	317	X	–	X	X	X	X
Quercetin-3-glucuronide	354, 296 (sh), 256	477	301	X	X	X	X	X	X
Quercetin-3-glucoside	354, 292 (sh), 254	463	301	X	X	X	X	X	X
Quercetin-3-rhamnoside	348, 286 (sh), 266	447	301	X	X	X	X	X	X
Isorhamnetin-3-glucoside	360, 286 (sh), 254	477	315	X	X	X	X	X	X
Syringetin-3-glucoside	357, 304 (sh), 252	507	345	–	X	X	–	X	X
Naringenin	364, 300 (sh), 252	271	177, 151	X	X	X	X	X	X
Kaempferol	370, 302 (sh), 254	285	257	X	X	X	X	X	X
Dihydroquercetin-3-rhamnoside	336 (sh), 292	449	303	X	–	–	–	–	–
Dihydrokaempferol-3-glucoside	340 (sh), 292	449	287	X	X	X	X	X	–
Dihydroquercetin-3-glucoside	336 (sh), 292	465	303	X	X	X	X	X	–

X, detected; – , not detected; (sh), shoulder.

Table 6. Low-molecular-weight phenolic compounds (mg L^{-1}) quantified in different monovarietal red wines from Mendoza

Compound	Malbec	Bonarda	Cabernet Sauvignon	Merlot	Shiraz	Tempranillo
Non-flavonoid phenolics						
Gallic acid	11.6 ± 1.3a	20.7 ± 0.6b	15.9 ± 1.0a	15.1 ± 1.3a	11.2 ± 1.2a	11.1 ± 1.3a
Protocatechuic acid	1.4 ± 0.2a	1.1 ± 0.1a	1.2 ± 0.1a	2.7 ± 0.2b	1.5 ± 0.1a	1.1 ± 0.1a
Syringic acid	3.0 ± 0.4b	2.5 ± 0.3b	1.6 ± 0.1a	2.7 ± 0.2b	3.1 ± 0.4b	3.2 ± 0.2b
Gentisic acid	1.4 ± 0.3a	1.5 ± 0.2a	1.6 ± 0.1a	1.3 ± 0.1a	1.1 ± 0.2a	1.8 ± 0.3a
Methyl gallate	2.3 ± 0.2ab	2.6 ± 0.2ab	2.5 ± 0.1ab	2.5 ± 0.3ab	1.6 ± 0.1a	2.7 ± 0.2b
Ethyl gallate	7.0 ± 1.2ab	13.0 ± 0.8c	8.8 ± 0.7b	10.2 ± 0.8bc	4.7 ± 0.5a	3.8 ± 0.6a
Hydroxybenzoic acids/derivatives	26.7 ± 2.8ab (5.4[a])	41.4 ± 0.8c (9.9)	31.6 ± 1.9ab (8.7)	34.5 ± 2.6bc (8.6)	23.2 ± 0.8a (8.2)	23.7 ± 1.3a (9.0)
trans-Caftaric acid	5.6 ± 1.1c	1.4 ± 0.1a	3.6 ± 0.4bc	4.2 ± 0.5c	5.4 ± 0.4c	1.9 ± 0.3ab
cis-Coutaric acid	1.2 ± 0.2a	0.6 ± 0.1a	1.3 ± 0.2a	1.3 ± 0.1a	2.5 ± 0.2b	1.4 ± 0.3a
trans-Coutaric acid	3.9 ± 0.4b	2.0 ± 0.1a	2.3 ± 0.3a	3.6 ± 0.4b	6.1 ± 0.2c	3.1 ± 0.2ab
trans-Fertaric acid	3.55 ± 0.25bc	2.30 ± 0.04a	2.73 ± 0.25ab	3.64 ± 0.15c	2.50 ± 0.06ab	2.07 ± 0.03a
trans-Caffeic acid	1.7 ± 0.2a	5.6 ± 0.6b	3.3 ± 0.5a	3.3 ± 0.2a	2.2 ± 0.1a	1.8 ± 0.2a
trans-p-Coumaric acid	2.6 ± 0.1abc	4.3 ± 0.6c	3.3 ± 0.4bc	3.2 ± 0.2bc	2.1 ± 0.2ab	1.4 ± 0.4a
Hydroxycinnamic acids/derivatives	18.6 ± 1.8bc (3.8)	16.2 ± 1.1ab (3.9)	16.5 ± 0.8b (4.6)	19.2 ± 0.8bc (4.8)	20.8 ± 0.8c (7.4)	11.7 ± 0.4a (4.5)
trans-Resveratrol-3-glucoside	9.2 ± 1.0c	3.2 ± 0.4ab	2.1 ± 0.3ab	4.1 ± 0.6b	2.2 ± 0.4ab	1.9 ± 0.1ab
cis-Resveratrol-3-glucoside	4.4 ± 0.7bc	2.9 ± 0.4b	1.2 ± 0.2a	4.8 ± 0.4c	2.3 ± 0.1ab	2.7 ± 0.4ab
Stilbenes	13.6 ± 1.7c (2.7)	6.1 ± 0.5ab (1.5)	3.3 ± 0.5a (0.9)	8.9 ± 1.0b (2.2)	4.5 ± 0.4a (1.6)	4.6 ± 0.4a (1.8)
Tyrosol	24.3 ± 4.0a	24.9 ± 1.7a	22.3 ± 2.1a	30.1 ± 2.1a	24.0 ± 1.7a	20.9 ± 0.8a
Tryptophol	12.6 ± 2.3ab	10.2 ± 1.0ab	6.3 ± 0.5a	9.5 ± 1.3ab	6.5 ± 0.9a	14.4 ± 2.2b
Alcohols/related compounds	36.9 ± 6.2ab (7.4)	35.1 ± 1.2ab (8.4)	28.6 ± 2.5a (7.9)	39.6 ± 3.0b (9.8)	30.5 ± 2.6ab (10.8)	35.3 ± 1.6ab (13.4)
Total non-flavonoids	95.8 ± 8.9ab (19.3)	98.8 ± 1.9ab (23.7)	80.0 ± 4.4a (22.1)	102.2 ± 6.3b (25.4)	79.0 ± 3.4a (28.0)	75.3 ± 2.5a (28.7)
Flavonoid phenolics						
(+)-Catechin	52.7 ± 6.5bc	58.7 ± 5.4c	52.2 ± 3.7c	44.0 ± 3.5bc	28.5 ± 5.7ab	19.9 ± 0.3a
(−)-Epicatechin	21.8 ± 3.3ab	34.8 ± 3.0c	26.0 ± 1.4bc	31.7 ± 1.9bc	14.9 ± 3.6a	12.3 ± 1.4a
Procyanidin dimer 1	16.0 ± 1.5b	15.3 ± 1.9b	10.7 ± 1.1ab	11.9 ± 1.2ab	7.9 ± 1.5a	6.2 ± 0.6a
Procyanidin dimer 2	49.5 ± 1.1bcd	58.4 ± 3.2cd	62.8 ± 3.2d	44.6 ± 4.1abc	28.5 ± 3.8a	36.0 ± 1.8ab
Procyanidin trimer 1	15.6 ± 2.1b	14.6 ± 0.4b	9.9 ± 1.0ab	30.5 ± 2.6c	3.0 ± 0.3a	8.1 ± 0.4ab
Procyanidin trimer 2	11.3 ± 1.2ab	14.2 ± 1.1ab	11.6 ± 1.2ab	15.2 ± 1.6b	8.1 ± 1.0a	8.5 ± 0.6a
Procyanidin trimer 3	12.9 ± 0.8ab	17.9 ± 1.7b	12.2 ± 1.0a	12.5 ± 0.9a	12.6 ± 1.0ab	10.8 ± 0.2a
Procyanidin trimer 4	14.1 ± 2.8b	7.0 ± 0.7a	6.4 ± 0.6a	ND	6.0 ± 0.6a	4.7 ± 0.3a
Flavanols	193.9 ± 19.1b (39.1[a])	220.9 ± 14.1b (52.9)	191.8 ± 10.0b (53.0)	190.4 ± 13.3b (47.2)	109.5 ± 17.0a (38.8)	106.5 ± 1.8a (40.6)

Table 6. (*Continued*)

Compound	Malbec	Bonarda	Cabernet Sauvignon	Merlot	Shiraz	Tempranillo
Myricetin-3-glucuronide	12.4 ± 1.9b	9.3 ± 1.1ab	8.0 ± 1.1ab	8.8 ± 1.1ab	5.3 ± 0.7a	6.6 ± 0.1ab
Myricetin-3-galactoside	11.5 ± 1.4a	15.8 ± 0.4b	ND	ND	ND	ND
Myricetin-3-glucoside	15.6 ± 2.7abc	ND	10.8 ± 1.1a	19.9 ± 2.0c	9.8 ± 1.2a	18.7 ± 2.2bc
Quercetin-3-glucuronide	11.9 ± 1.4ab	9.2 ± 0.9ab	6.8 ± 0.7a	12.3 ± 1.8b	9.6 ± 0.9ab	5.9 ± 0.7a
Quercetin-3-glucoside	9.4 ± 1.3a	8.0 ± 1.1a	8.5 ± 1.0a	9.5 ± 1.4a	11.7 ± 1.9a	6.1 ± 1.1a
Quercetin-3-rhamnoside	4.6 ± 0.6abc	4.5 ± 0.4bc	2.8 ± 0.2a	5.6 ± 0.5c	5.5 ± 0.4c	3.2 ± 0.2ab
Isorhamnetin-3-glucoside	7.4 ± 1.2ab	7.6 ± 0.9ab	11.3 ± 1.4b	11.9 ± 1.2b	7.5 ± 1.2ab	5.4 ± 0.9a
Syringetin-3-glucoside	ND	3.1 ± 0.3a	4.1 ± 0.6ab	ND	6.4 ± 0.9b	4.3 ± 0.7ab
Naringenin	10.4 ± 1.4b	5.3 ± 0.9a	5.8 ± 0.4a	8.1 ± 1.0ab	8.0 ± 0.4ab	5.7 ± 0.8a
Kaempferol	29.7 ± 5.6b	19.9 ± 2.3ab	16.2 ± 1.3a	21.7 ± 1.3ab	18.9 ± 1.4ab	24.9 ± 3.0ab
Flavonols	112.9 ± 8.0b (22.8)	82.7 ± 3.5ab (19.8)	74.3 ± 5.0a (20.5)	97.8 ± 6.9b (24.3)	82.7 ± 3.7ab (29.3)	80.8 ± 7.5ab (30.8)
Dihydroquercetin-3-rhamnoside	4.7 ± 0.7a	ND	ND	ND	ND	ND
Dihydrokaempferol-3-glucoside	33.2 ± 1.8c	10.4 ± 0.3b	8.2 ± 0.3a	9.5 ± 0.2ab	8.6 ± 0.2ab	ND
Dihydroquercetin-3-glucoside	55.4 ± 0.5c	4.6 ± 0.3a	7.6 ± 0.9b	3.2 ± 0.3a	2.6 ± 0.1a	ND
Dihydroflavonols	93.3 ± 2.0c (18.8)	15.0 ± 0.5ab (3.6)	15.8 ± 0.9b (4.4)	12.7 ± 0.4a (3.2)	11.2 ± 0.2a (4.0)	ND
Total flavonoids	400.1 ± 29.1d (80.7)	318.6 ± 16.5cd (76.3)	281.9 ± 15.4bc (77.9)	300.9 ± 20.0c (75.0)	203.4 ± 19.7ab (72.1)	187.3 ± 8.0a (71.0)
Total non-anthocyanin phenolics	495.9 ± 37.9c	417.4 ± 17.4bc	361.9 ± 19.2ab	403.1 ± 25.7bc	282.4 ± 22.8a	262.6 ± 6.8a

Values are expressed as mean ± standard error ($n = 5$). Different letters in the same row indicate significant differences between varieties for each compound (Tukey's HSD test, $P < 0.05$). ND, not detected.
[a] Ratio (%) between phenolic groups and total phenolics.

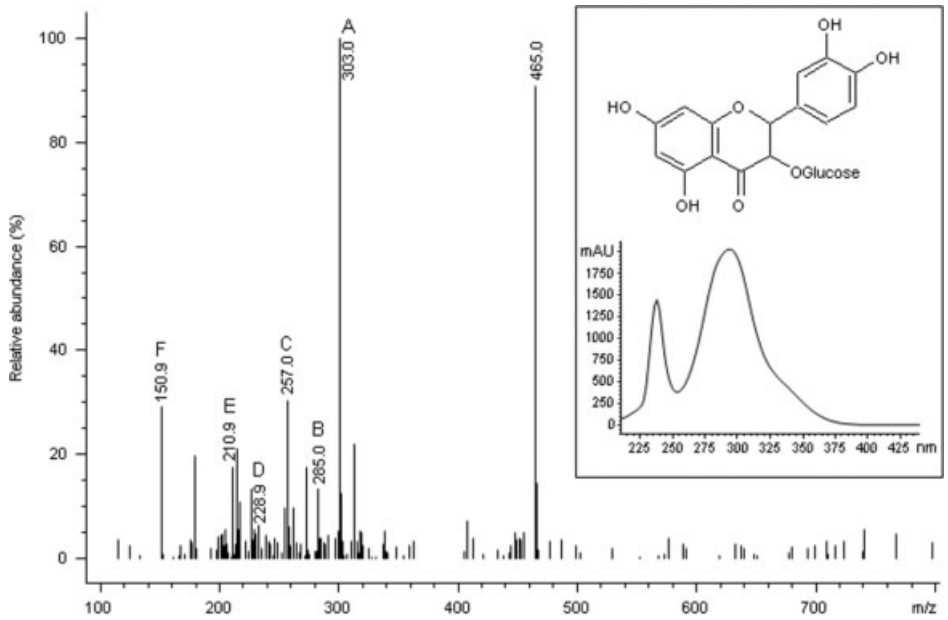

Figure 3. Negative ion ESI mass spectrum, chemical structure and UV spectrum of dihydroquercetin-3-glucoside. Fragments ions obtained according to Abad-García *et al.*[52]: A, *m/z* 303 (dihydroquercetin); B, *m/z* 285 (303 – H_2O); C, *m/z* 257 (303 – H_2O – CO); D, *m/z* 229 (303 – H_2O – 2CO); E, *m/z* 211 (303 – $2H_2O$ – 2CO); F, *m/z* 151 (303 – $C_8H_8O_3$).

using the information given in Tables 4 and 6. The first CDA, using individual anthocyanins as predictor variables, resulted in five discriminant functions (DFs) that accounted jointly for 100% of the total variance, with $P < 0.05$ and statistical significance at 95% confidence level (Table 7). The first function, assigned as DF1, accounted for 56.0% of the total variability, while DF2 accounted for 32.6%. The two functions showed Wilks' λ values of 3.6×10^{-9} and 1.5×10^{-6} respectively, indicating satisfactory discrimination. Table 7 shows the standardised coefficients for the predictor variables in the two DFs. The variables with higher incidence on DF1 were delphinidin-3-glucoside, malvidin-3-(6″-acetyl)glucoside and vitisin A in a positive way and petunidin-3-glucoside, cyanidin-3-(6″-acetyl)glucoside and malvidin-3-(6″-acetyl)glucoside pyruvate in a negative way, while DF2 was strongly influenced by delphinidin-3-glucoside and peonidin-3-glucoside pyruvate in a positive way and by delfinidin-3-(6″-acetyl)glucoside, petunidin-3-(6″-acetyl)glucoside, vitisin A and cyanidin-3-(6″-*p*-coumaroyl)glucoside in a negative way. Figure 4 depicts the distribution of red wine samples in the plane defined by DF1 and DF2. These two functions allowed the classification of 100% of the wines studied according to grape variety. MB, BN and TP wines showed a negative score on DF1, while SH, CS and MT presented a positive score. Regarding DF2, MB and MT showed a negative score and the other varieties presented a positive score.

In the same framework described for anthocyanins, a second CDA with low-molecular-weight phenolics as predictor variables also resulted in five discriminant functions, statistically significant at 95% confidence level (Table 8). DF1 accounted for 93.3% of the total variability, while DF2 accounted for 4.5%. A scatter plot of the wines in the plane defined by these two functions is presented in Fig. 5, where there was a perfect prediction (100%) of the samples and a clear differentiation of the six varieties. The main axis of differentiation (DF1) was strongly influenced by phenolic acids (*trans*-caftaric, *trans*- and *cis*-coutaric and gallic acids), tyrosol, *cis*-resveratrol-3-glucoside and procyanidin dimer 2. All samples were discriminated with this function, showing a negative score for MB and CS and a positive score for the other varieties. Additionally, DF2 was associated with flavanols ((+)-catechin and procyanidin dimers 1 and 2) as well as with phenolic acids (protocatechuic, fertaric and *trans*-caftaric acids), allowing the classification of MT, CS and SH in a positive way and MB, TP and BN in a negative way.

The discriminant analysis revealed that non-acylated delphinidin and petunidin, acetylated anthocyanins and pyranoanthocyanins as well as flavanols and phenolic acids exerted a profound influence on cultivar-based differentiation. However, the other phenolic groups had a rather minor impact. This performance is in agreement with other studies.[19,36] Given these results, we can select the phenolic variables for categorising future samples, taking into account the complexity, time and cost of the appropriate analytical technique.

CONCLUSIONS

The phenolic composition of wines from the principal red grape varieties cultivated in Mendoza (Argentina) is reported for the first time. Sixty phenolic compounds, including anthocyanins, phenolic acids/derivatives, stilbenes, flavanols, flavonols and dihydroflavonols, were identified and quantified using HPLC-DAD/ESI-MS. Some flavonoids detected in our study represent a significant finding from the chemotaxonomic point of view, especially for Malbec variety. This is the case of dihydroflavonols, because, as far as we know, only three (dihydroquercetin-3-rhamnoside, dihydromyricetin-3-rhamnoside and dihydrokaempferol-3-glucoside) have been described so far in *Vitis vinifera* L. red varieties. The compound dihydroquercetin-3-glucoside, not reported before in wines from the six red varieties studied and tentatively identified by ESI-MS in our research, needs to be isolated and finally characterised by molecular spectrometric techniques (MS/MS, NMR, IR).

The classification of red wine samples using chemical data and multivariate methods has been achieved successfully. It must be emphasised that, in spite of the small number of samples, both

Table 7. Results of canonical discriminant analysis for red wines using variety as discriminating factor. Standardised coefficients for anthocyanins in discriminant functions

	Discriminant functions				
	1	2	3	4	5
Eigenvalue	409.7[a]	238.0[a]	72.8	6.7	4.0
Variance (%)	56.0	32.6	10.0	0.9	0.5
Canonical correlation	0.9988	0.9979	0.9932	0.9324	0.8946
Wilks' λ	3.6×10^{-9}	1.5×10^{-6}	3.5×10^{-4}	2.6×10^{-2}	2.0×10^{-1}
P value	0.0000	0.0000	0.0000	0.0000	0.0009
Delphinidin-3-glucoside	22.3	4.8	20.1	3.6	5.1
Cyanidin-3-glucoside	−4.9	−0.5	−5.9	−2.8	−0.4
Petunidin-3-glucoside	−22.1	0.6	−12.3	−2.0	−5.0
Malvidin-3-glucoside	−2.9	−0.3	−1.1	−2.5	0.2
Delphinidin-3-(6″-acetyl)glucoside	3.9	−5.8	2.9	1.4	−0.2
Cyanidin-3-(6″-acetyl)glucoside	−5.6	0.8	7.4	−0.6	0.5
Petunidin-3-(6″-acetyl)glucoside	3.7	−3.4	−0.4	−2.1	1.1
Peonidin-3-(6″-acetyl)glucoside	0.7	1.5	−3.3	−0.7	0.6
Malvidin-3-(6″-acetyl)glucoside	5.5	−0.3	−1.5	0.6	−1.3
Delphinidin-3-(6″-*p*-coumaroyl)glucoside	−1.7	−0.6	1.3	1.1	1.0
Cyanidin-3-(6″-*p*-coumaroyl)glucoside	1.3	−2.3	0.3	2.0	0.1
Vitisin A	9.0	−3.7	−6.7	−0.4	0.9
Peonidin-3-glucoside pyruvate	−0.8	4.7	1.1	1.4	−1.0
Malvidin-3-(6″-acetyl)-glucoside pyruvate	−7.5	1.8	−4.7	−3.2	−1.4
Malvidin-3-glucoside-ethyl-epicatechin	2.2	−0.6	3.6	3.8	0.2

[a] First two discriminant functions used in the analysis.

Table 8. Results of discriminant analysis for red wines using variety as discriminating factor. Standardised coefficients for non-anthocyanin phenolics in discriminant functions

	Discriminant functions				
	1	2	3	4	5
Eigenvalue	20892.8[a]	1013.3[a]	419.4	60.3	11.5
Variance (%)	93.3	4.5	1.8	0.3	0.1
Canonical correlation	0.9999	0.9995	0.9988	0.9918	0.9592
Wilks' λ	1.5×10^{-13}	3.1×10^{-9}	3.1×10^{-6}	1.3×10^{-3}	8.0×10^{-2}
P value	0.0000	0.0000	0.0000	0.0000	0.0001
Gallic acid	22.1	−0.8	−1.3	−2.1	0.6
Protocatechuic acid	−8.3	10.5	2.4	1.1	−1.1
Syringic acid	5.7	−5.9	−3.1	0.0	0.1
Gentisic acid	2.0	−4.4	−1.8	1.1	−0.4
Methyl gallate	−8.5	−5.8	−7.2	−1.3	0.4
Ethyl gallate	−11.8	−2.2	3.6	2.6	0.1
trans-Caftaric acid	−32.6	−6.3	−9.0	−2.4	1.2
cis-Coutaric acid	15.3	−1.6	1.6	−0.3	−0.1
trans-Coutaric acid	32.1	4.4	8.7	2.1	0.0
trans-Fertaric acid	−9.1	12.2	5.1	−0.6	0.3
trans-Caffeic acid	5.1	3.9	0.2	−0.1	1.0
Tyrosol	−17.8	3.3	−8.7	−2.6	1.0
cis-Resveratrol-3-glucoside	16.5	−5.3	−3.2	−1.4	0.5
(+)-Catechin	2.4	−13.0	−8.7	0.1	−0.5
(−)-Epicatechin	4.6	4.5	6.4	1.2	0.8
Procyanidin dimer 1	4.8	−11.8	−11.3	0.5	−0.5
Procyanidin dimer 2	−22.0	8.1	10.8	1.1	−0.9
Procyanidin trimer 4	−8.5	5.7	7.3	−0.8	0.3
Quercetin-3-glucoside	5.2	3.8	2.2	−0.6	0.4

[a] First two discriminant functions used in the analysis.

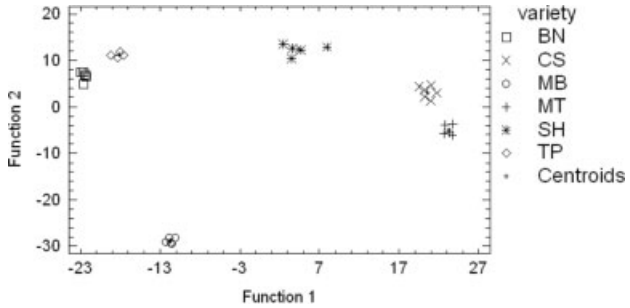

Figure 4. Discriminant plot of anthocyanins for red wines from Mendoza according to variety ($n = 30$): BN, Bonarda; CS, Cabernet Sauvignon; MB, Malbec; MT, Merlot; SH, Shiraz; TP, Tempranillo.

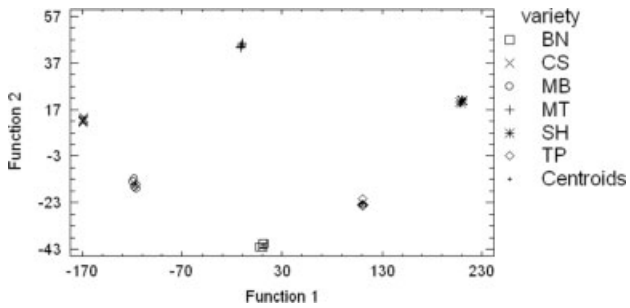

Figure 5. Discriminant plot of non-anthocyanin phenolics for red wines from Mendoza according to variety ($n = 30$): BN, Bonarda; CS, Cabernet Sauvignon; MB, Malbec; MT, Merlot; SH, Shiraz; TP, Tempranillo.

discriminant analyses yielded an unambiguous classification of samples according to grape variety without overlapping, which clearly demonstrates the high potential of phenol-based analysis for red wine differentiation. This outcome could be regarded as an additional criterion for studies pertaining to red wine quality control and authenticity.

The results are indicative of the polyphenolic richness of Malbec grapes compared with the other red varieties from Mendoza and their potential to produce quality wines. Future studies on wines from different geographical origins and obtained by different winemaking practices should be carried out to confirm these observations and to obtain products with identity.

ACKNOWLEDGEMENTS

The authors are grateful to the Instituto Nacional de Tecnología Agropecuaria (INTA), projects AETA282831 and PTRMZASJ510071, for the financial support of this work and to all ten wineries for providing the samples for the study. Special thanks are due to Mariana de la Cruz and Eugenia Turaglio for their technical assistance.

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