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From grape to wine: Changes in phenolic composition and its influence on antioxidant activity

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ABSTRACT

The evolution of phenolic compounds and their relationship with the antioxidant capacity (AC) of samples taken along the winemaking process of three *Vitis vinifera* L. cv., Syrah, Merlot and Cabernet Sauvignon grown in Argentina were studied. Forty-five compounds were identified by HPLC-PDA-MS/MS, while the AC was determined by FRAP, ABTS and DPPH assays. Results show that phenolic composition and AC vary along the winemaking process and between varieties. Multiple regression analysis showed a high correlation between phenolic composition and AC of samples, being anthocyanins the main family with significant contribution to AC. In addition, quantitative differences in specific phenolic compounds help to explain differences in AC observed between varieties. A high phenolic content and bioactivity still remain in pomaces which support its use as an inexpensive source of antioxidants.

1. Introduction

Epidemiological studies in humans have shown a positive correlation between the incidence of chronic diseases and the oxidative/nitrosative stress in underlying pathogenesis and dietary patterns. Despite the difficulties in establishing the effects of diet from other aspects of lifestyle, most authorities agree that the benefits of a diet rich in fruits and vegetables in human health may be related to the presence of bioactive compounds with antioxidant properties (Kondrashov, Ševčík, Benáková, Koštířová, & Štípek, 2009).

Among natural antioxidants, red grape (*Vitis vinifera* L.) and its product from the winemaking process: wine, have received much attention because of the high concentration and great variety of phenolic compounds, representing important sources of numerous bioactive dietary polyphenols (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004).

Red grape and red wine polyphenols are mainly flavonoid (anthocyanins, flavonols and flavanols) and non-flavonoid compounds (phenolic acids like hydroxycinnamic and hydroxybenzoic acids and stilbenes), all of them are well known for their strong biological actions (Monagas, Bartolomé, & Gómez-Cordovés, 2005a). According to some studies, besides their nutritional benefits, there is a close relation between high quality wines and high phenolic composition, as it contributes to the organoleptic characteristics of wines such as color, astringency, and bitterness (Fanzone et al., 2012; Langlois, Ballester, Campo, Dacremont, & Dominique, 2010). Several factors, including grape variety, grape ripeness, environmental factors and technological procedures used during winemaking, can qualitatively and quantitatively affect the phenolic composition of the grape, pomace and wine and, therefore, their nutritional and quality properties (Garrido & Borges, 2013).

Polyphenols are transferred from the grape into the wine during winemaking operations (crushing, maceration, and fermentation). As the major part of grape polyphenols comes from the solid grape parts (skin and seeds), a high proportion of polyphenols still remains in the solid waste: the pomace. Particular attention is currently being paid to the exploitation of this byproduct, as it is considered an inexpensive source for obtaining bioactive compounds with potential application as food antioxidants (Fontana, Antoniolli, & Bottini, 2013). Thus, the chemical characterization and antioxidant capacity (AC) of the winemaking byproducts constitute the first step to promote such applications.

Although there are many studies on the phenolic composition of red wines and the effects of the winemaking technology on its

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profile, the influence of changes of phenolic profile on AC has not been well elucidated. Several reports describe the correlation between different phenolic compounds and AC along vinification (Burns et al., 2001; Ginjom, ĎArcy, Caffin, & Gidley, 2011; Sun et al., 2011); however, most studies apply univariate statistical methods to determinate this relationship. The AC is the result of synergistic or antagonistic effects from interactions between the different polyphenols among each other and with other components of the food matrix or the organism like proteins, carbohydrates and lipids (Jakobek, 2015), therefore, a univariate statistical method does not provide indications of the relative contributions of each of these compounds to the AC. Taking this into account, the AC cannot be easily predicted by the content of a specific group of compounds, or by measuring a single substance.

Argentina ranks fifth among the ten main wine producing countries in the world, representing 5% of the world production (OIV (International Organization of Vine, 2014). To date and despite its economic importance, there has been little research on the antioxidant characteristics of Argentinean wines (Baroni, Di Paola-Naranjo, García-Ferreyra, Otaiza, & Wunderlin, 2012; Granato, Katayama, & Castro, 2011; Lotito, Renart, & Fraga, 2002). Furthermore, to the best of our knowledge, there are no research papers published on the evolution of a complete phenolic profile and its influence on antioxidant activity throughout the winemaking process for varieties cultivated in Argentina, nor on the chemical characterization and AC of pomaces as a potential source of bioactive compounds for the food industries (Antoniolli, Fontana, Piccoli, & Bottin, 2015). For this reason, more detailed information is necessary. Consequently, the present work was conducted to better understand the evolution of phenolic compounds of three *Vitis vinifera* L. red grapes varieties grown in Argentina along the winemaking process (from grape to wine), linking changes in the phenolic profile with their AC by multivariate statistics.

2. Materials and methods

2.1. Chemicals

Methanol (HPLC grade) and formic acid (puriss. p.a. for mass spectroscopy) were obtained from J. T. Baker (Edo. de México, México) and Fluka (Steinheim, Germany), respectively. Commercial standards of (+)-catechin, malvidin-3-glucoside and caffeic acid were obtained from Extrasynthese (Genay, France). Kaempferol and quercetin were purchased from Fluka (Dorset, U.K.). Myricetin, isoquercetin and *trans*-resveratrol were obtained from Sigma-Aldrich (Buenos Aires, Argentina), and gallic acid was purchased from Riedel-de-Hagën (Seelze, Germany). Filters (0.45 μm, HVLP04700) were obtained from Millipore (São Paulo, Brazil). ABTS (2,2′-azino-bis-(3-thylbenzothiazolne-6-sulfonic acid) diammonium salt), DPPH (1,1,-diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tripyridyl-S-triazine), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Buenos Aires, Argentina). All other reagents were of analytical grade.

2.2. Samples

Samples of monovarietal grapes of the species *Vitis vinifera* L. cv. Syrah, Merlot and Cabernet Sauvignon were harvested from San Juan province (central-west area of Argentina) in their optimal ripening stage (22–25 °Brix) from vineyard plots corresponding to the three grapevine varieties studied (Syrah, Merlot and Cabernet Sauvignon). After harvest, bunches were immediately transported to the winery for processing. A total of 10 bunches were randomly selected from the different trucks coming from vineyards. The grapes used for vinification were divided in three plastic bags for each variety and stored at 4–8 °C until analysis.

In order to verify differences in wines phenolic profile arising from the use of diverse grapes varieties, industrial scale fermentations were carried out. So, the vinification process of three wines was produced in the same winery "Antonio de la Torre" cellar (San Juan, Argentina), using 75,000 L steel tanks coated with epoxy vats and corresponded to the same vintage. Three independent fermentations were done per variety.

In the cellar, grapes were destemmed and crushed; gaseous sulfur dioxide was added immediately in a concentration of 30 mg/L. Later, pectinolytic enzymes (Endozym Rouge Liquid, France, 2 mL/hL) were incorporated to Syrah, Merlot and Cabernet Sauvignon musts and experienced a classical maceration for 2 days at a temperature of 23–25 °C. Subsequently, the starters were inoculated with a volume sufficient to obtain a cellular population of 10^7 cell/mL in the winery vat (Zymaflore XPURE, Laffort, Portugal) and fermentations were conducted at 24–26 °C. During this step, the pomace which floated on top of the tank, were pumped down twice by day. The alcoholic fermentation took place until there were traces of sugars (<2 g/L, analyzed by enologist), after which the wines obtained were separated from the pomace (devatting). At this point, wine (named as wine-1) and pomace samples were taken (one from each fermentation tank). After devatting, the wine obtained finished fermenting in tanks (malolactic fermentation). The wines were placed for stabilization in others refrigerated steel tanks coated with epoxy, being their alcohol content between 12% and 12.5% v/v. The final free sulfur dioxide contents were 29, 31 and 35 mg/L and the total contents were 103, 87 and 97 mg/L for Cabernet Sauvignon, Syrah and Merlot wine respectively. Wine samples (named wine-2) were obtained after stabilization (4–5 months after primary fermentation, 20–22 °C), filtration using bentonite and bottling in 750-mL dark glass bottles with cork plugs. All wines were made without oak contact. One bottle of wine-2, from each tank, was transported to the laboratory and dark stored upright at 4–8 °C until analysis. All analyses were performed during 2014 within 6 months.

2.3. Sample preparation

Extraction of phenolic compounds from whole grapes (previously selected, destemmed, washed with distilled water and dried with blotting paper) and pomaces (skins and seeds) was carried out as described by Poudel, Tamura, Kataoka, and Mochioka (2008) with minor changes. Briefly, grape and pomace samples were lyophilized (at 0.013 kPa till constant weight) and their moisture calculated by weight difference before and after freeze-drying. The moisture percentage ranged from 69 to 72% for grapes, and 49–52% for pomaces. After lyophilisation, samples were frozen using liquid nitrogen and grounded until obtaining a fine powder. A portion of 1 g of treated sample was extracted with 15 mL of acidified methanol (0.1% HCl v/v) in a blender (Ultra-Turrax T18; Ika-Labortechnik, Germany). The obtained homogenate was incubated with agitation for 2 h at 4 °C and then centrifuged at 2058g for 10 min. The supernatant was separated and the solid pellet re-extracted with 5 mL of acidified methanol as previously described. The combined extracts were filtered, fractionated in Eppendorf tubes and stored at −80 °C until phenolic and antioxidant activity determinations. The extraction procedure was carried out in triplicate.

Wine-1 (after alcoholic fermentation) and wine-2 (after stabilization) samples from the three varieties under study, were filtered using Whatman No. 1 filter paper (Whatman, UK), fractionated in 125 mL polyethylene bottles and stored at −80 °C until phenolic and antioxidant activity analysis determinations.

2.4. Determination of phenolic profile

All individual phenolic compounds including anthocyanins, flavonols, flavanols, phenolic acids and stilbenes were analyzed by HPLC-PDA-ESI-MS/MS, using an Agilent Series 1200 LC System (Agilent, Santa Clara, CA, USA), coupled to a PDA detector (Agilent Series 1200) in tandem with an ESI source, connected to a Micro-QTOF II (Bruker Daltonics, Billerica, MA, USA) mass spectrometer (MS and MS/MS). The HPLC system was equipped with a binary gradient pump, solvent degasser, and autosampler (Agilent Series 1200 L, Santa Clara, CA, USA).

HPLC analysis of all phenolic compounds were performed on a LUNA (Phenomenex, Torrance, CA, USA) C18 column (5 μm, 250 mm × 4.60 mm i.d.), at 35 °C and 0.4 mL min^{-1} flow rate, using 0.5% formic acid (solvent A) and 0.5% formic acid in methanol (solvent B). For the analysis of all the phenolic compounds the gradient program started with 20% B and changed to 50% B along 3 min, held for 5 min, followed by a second ramp to 70% B along 7 min, held for 5 min, a third ramp to 80% B along 1 min, and remained in this last condition for 9 min before the next run. The injection volume of samples was 40 μL. Wine-1 and wine-2 samples were injected diluted 1/25 v/v in ethanol 12%, meanwhile grape and pomace extractions were injected directly without dilution.

UV–Vis analyses of the individual phenolic compounds from different groups were carried out in the range of 200 and 600 nm. MS spectra were recorded in both negative (for analyses of phenolic acids, stilbenes, flavonols and flavanols) and positive ion modes (for the analysis of anthocyanins) between 80 and 1500 *m/z*. The working conditions for the ESI source were as follows: capillary voltage, 4500 V; nebulizer gas pressure, 4.0 bar; drying gas flow, 8.0 L min^{-1} and drying gas temperature 180 °C. Nitrogen and argon were used as nebulizer and collision gases, respectively. The MS detector was programmed to perform a MS/MS scan of the three most abundant ions, using collision energy of 13.0 eV. Data acquisition and processing were performed using Compass (V. 3.1) and Data Analysis (V. 4.0) software, respectively.

Polyphenols present in samples were identified according to their retention times, UV/Vis spectra, high resolution MS and MS/MS spectra, in comparison with pure compounds, when available, or by comparison with compounds reported in the literature (Castillo-Muñoz et al., 2009; de Villiers, Vanhoenacker, Majek, & Sandra, 2004; Monagas, Gómez-Cordovés, Bartolomé, Laureano, & da Silva, 2003; Mozetič, Tomažič, Škvarč, & Trebše, 2006; Vergara et al., 2012). MS analysis was used for quantification of phenolic compounds with specific external calibration plot, constructed by linear regression from available phenolic standards (malvidin-3-glucoside, myricetin, quercetin, kaempferol, isoquercetin, (+) catechin, *trans*-resveratrol, caffeic acid and gallic acid). The calibration curves were prepared by appropriate dilutions from stock solutions in methanol (concentration of 1000 mg L^{-1}). When reference compounds were not available, a calibration curve from structurally related compound was used. The limits of detection (LOD) and quantification (LOQ) of the method used to quantify the phenolic compounds were experimentally evaluated considering a signal-to-noise ratio of 3 and 10 respectively. Precision of the method was evaluated by calculating the coefficients of variation (CV) from a least nine determinations covering the specified range for the procedure. LOQ ranged from 0.0013 to 0.0500 mg L^{-1}. CV were below 13%. All samples under study, after appropriate dilution, and standards solutions were filtered (0.45 μm) and injected in the HPLC-PDA-ESI-MS/MS system. All injections were performed in triplicate.

2.5. Measurement of the antioxidant capacity (AC)

The AC of samples was measured with ferric reducing-antioxidant power (FRAP assay), and free radicals-scavenging activity (ABTS and DPPH assays). For all assays, results were obtained by interpolating the absorbance on a calibration plot, constructed by linear regression using Trolox (linear range between 0 and 0.02 mmol Trolox per L). Results are expressed in mmol Trolox equivalents per 100 g of sample (DW) in the case of grape and pomace extracts, and mmol Trolox equivalents per L of wine. All samples were analyzed in triplicate.

2.5.1. Ferric reducing-antioxidant power (FRAP)

FRAP assay was performed in according to Benzie and Strain (1996). Briefly, 100 μL of sample appropriately diluted were added to 3 mL of the FRAP reagent, measuring the absorbance at 593 nm after incubation at room temperature for 6 min in dark conditions.

2.5.2. Free radicals-scavenging activity (ABTS)

ABTS assay was performed in accordance with Re et al. (1999). Briefly, 100 μL of sample appropriately diluted was mixed with 3 mL of $ABTS^{\bullet+}$ (dissolved in methanol), measuring the absorbance at 734 nm after 4 min reaction.

2.5.3. Free radicals-scavenging activity (DPPH)

DPPH assay was performed in accordance with Villaño, Fernández-Pachón, Troncoso, and García-Parrilla (2006). Briefly, 100 μL of sample appropriately diluted were added to 3 mL of 60 μM $DPPH^{\cdot}$ (dissolved in methanol), incubated for 15 min in dark conditions and measured at 515 nm.

2.6. Statistical analysis

Data are expressed as mean ± SD. Analysis of variance (ANOVA) was performed with each variable to evaluate differences between varieties and between samples types and, in the case of significance ($p < 0.05$), a DGC (Di Rienzo, Guzmán & Casanoves) comparison test was performed to reveal paired differences between the means (Di Rienzo, Guzmán, & Casanoves, 2002). In all figures and tables, different letters mean statistically significant differences.

2.6.1. Stepwise multiple regression analysis (MRA)

MRA was used to assess the relationship between the polyphenolic profile and AC of the samples. We performed different stepwise analyses, including only a family of compounds in each test. From each of these analyses, the regression (*Beta*) coefficients were analyzed to evaluate key variables for the prediction of antioxidant activity in studied samples. The magnitude of these *Beta* coefficients allows comparing the relative contribution of each independent variable to the prediction of the dependent variable.

2.6.2. Principal component analysis (PCA)

PCA was applied to obtain biplot graphics, which summarize the association between variables (content of phenolic compounds and the FRAP, ABTS and DPPH values) and the three grape-wine varieties studied.

The statistical package Statistica 7.1 from StatSoft Inc. (2005) was used.

3. Results and discussion

3.1. Phenolic composition from grape to wine

To investigate the changes in the phenolic composition, grapes, pomaces, wine-1 and wine-2 samples were analyzed by HPLC-PDA-ESI-MS and MS/MS. A total of 45 compounds belonging to the family of anthocyanins, flavonols, flavanols, hydroxycinnamic and hydroxybenzoic acids and stilbenes were identified (See Supplementary data for identification parameters used). Figs. S1

and S2 of Supplementary data show extracted ion chromatogram of different compounds identified. As it can be seen from Figs. S1 and S2, there is co-elution of some of the identified compounds. However, the identification and quantification of co-eluting compounds was possible using the corresponding extracted molecular ion chromatogram (EMIC). Part of the phenolic profile has been recently reported (Lingua, Fabani, Wunderlin, & Baroni, 2016) additional data is presented in Tables 1–3.

3.1.1. Flavonoid phenolic compounds

3.1.1.1. Anthocyanins. Anthocyanins are located in the berry skin and are the main responsible compounds for the red color of grapes and wines (de Villiers et al., 2004; Núñez, Monagas, Gomez-Cordovés, & Bartolomé, 2004). As it can be seen from Table 1, the anthocyanin profile of all the samples under study was mainly formed by the related monoglucosides and acylated with either acetic, *p*-coumaric or caffeic acid from the five anthocyanidin structures commonly reported for *Vitis vinifera* L.: delphinidin, cyanidin, petunidin, peonidin, and malvidin. Anthocyanins were the most abundant components in grapes, representing from 50 to 60% in all varieties. Our results show that malvidin derivatives presented the highest content in grapes in accordance with many authors (Figueiredo-González, Martínez-Carballo, Cancho-Grande, Santiago, & Martínez, 2012; Núñez et al., 2004), being malvidin-3-glucoside and acetylgucoside the main anthocyanins in these samples, followed by coumaroylgucoside. On the other hand, cyanidin derivatives showed the lowest concentration probably because this anthocyanin is the precursor of all others (Núñez et al., 2004). After winemaking, a high proportion of anthocyanins still remain in the pomace, because they come from the grape skin. As it can be seen from Table 1, the total anthocyanins content in pomace samples was significantly lower ($p < 0.0001$) than in grape samples, being malvidin-3-coumaroylglucoside the main anthocyanin in pomace samples (Ruberto et al., 2007). Unexpectedly we observed that some anthocyanin compounds showed higher quantities in pomace samples than in their corresponding grapes, which is consistent with Barcia, Pertuzatti, Gómez-Alonso, Godoy, and Hermosín-Gutiérrez (2014) and Barcia, Pertuzatti, Rodrigues, et al. (2014). The latter result is most likely related to that the anthocyanin transfer during winemaking is not a simple process of solid/liquid partition and other physic-chemical processes have been suggested to modulate such transference (Barcia, Pertuzatti, Gómez-Alonso, et al., 2014; Barcia, Pertuzatti, Rodrigues, et al., 2014). In addition, three pyranoanthocyanins were present in pomace samples: pigment A and acetyl pigment A (both hydroxyphenyl-type pyranoanthocyanins, derived from the addition of *p*-coumaric acid to malvidin-3-glucoside and malvidin-3-acetylglucoside, respectively); and coumaroylvitisin B (vitisin-type pyranoanthocyan derived from addition of acetaldehyde to malvidin-3-coumaroylglucoside). These anthocyanin-derived pigments are formed during and after alcoholic fermentation, from the reaction of native grape anthocyanins with yeast metabolites, such as acetaldehyde, or with other phenolic wine constituents, such as hydroxycinnamic acids (Rentzsch, Schwarz, Winterhalter, Blanco-Vega, & Hermosín-Gutiérrez, 2010; Ruberto et al., 2007). As it was expected, from wine-1 to wine-2 samples, the content of native grape anthocyanins showed an important decreased ($p < 0.001$), while the pyranoanthocyanins showed a slight but significant increment ($p < 0.0001$) in all studied varieties, which is consistent with Ginjom et al. (2011) (Table 1). From the end of fermentation to the stabilized wine, changes in temperature, pH, formation of other compounds (e.g., ethanol, oxygen, other phenolics, etc) and the adsorption of the anthocyanins to yeast cell walls determine the final total anthocyanin content in the wine. In the present study, despite of the formation of pyranoanthocyanins, the changes in concentrations of anthocyanin compounds resulted in a decreased of more than 20% in the total anthocyanin content in all wine varieties which could be explained by hydrolysis, adsorption to yeast cell walls and/or to the formation of other anthocyanin-derived pigments that could not detected during this work (Barcia, Pertuzatti, Gómez-Alonso, et al., 2014; Barcia, Pertuzatti, Rodrigues, et al., 2014; Ginjom et al., 2011). In both wine-1 and wine-2, like in grapes and pomaces, malvidin derivatives showed the highest content, being malvidin-3-glucoside the main compound found, followed by acetyl and coumaroylglucoside derivatives (Ginjom et al., 2011).

Regarding the difference between varieties, it is commonly accepted that the anthocyanin profile of a given grape variety is closely linked to its genetic inheritance, although environmental factors may have some influence on this profile (de Villiers et al., 2004; Núñez, Monagas, Gomez-Cordovés, and Bartolomé, 2004). Quantitatively, we observed that the Syrah variety showed the highest content of anthocyanins in all analyzed samples along the winemaking process (Table 1).

3.1.1.2. Flavonols. These compounds are also found in *Vitis vinifera* L. grape berry skins (Castillo-Muñoz, Gómez-Alonso, García-Romero, & Hermosín-Gutiérrez, 2007). The flavonols detected during this work (Table 2) were formed by the six flavonoid structures commonly reported for *Vitis vinifera* L.: kaempferol, myricetin, syringetin, laricitrin, quercetin and isorhamnetin and their monoglycoside derivatives (Castillo-Muñoz et al., 2007). In addition, we noted the presence of the dihydroflavonol astilbin (dihydroquercetin-3-O-rhamnoside). Flavonol glycosides were the second most abundant phenolic compounds in grapes, representing 20–30% in all studied varieties. Our results showed that isoquercetin, followed by miricetyn-3-glucoside, presented the highest contents in grapes, in accordance with many authors (Castillo-Muñoz et al., 2007). In these samples the free aglycons represented trace amounts ($\leqslant$0.20%) because of their presence in grapes is considered an artifact of the extraction method under acidic conditions (Barcia, Pertuzatti, Rodrigues, et al., 2014). In contrast, byproducts and products of the winemaking process were generally characterized by a higher content of free aglycons (Table 2), possibly as the result of acid hydrolysis occurring in glycosilated flavonols during winemaking (Castillo-Muñoz et al., 2007; Monagas, Bartolomé, & Gómez-Cordovés, 2005). Along of this process, the content of flavonols decreased significantly ($p < 0.0001$) from grape to pomace samples (Table 2), probably as the result of their higher transfer from grape to wine during the maceration step. As it was expected, quercetin was the main flavonol present in pomace, wine-1 and wine-2 samples because of it comes of hydrolysis of the main flavonol glycoside found in grapes (Ruberto et al., 2007). From wine-1 to wine-2 samples, the content of glycosilated flavonols decreased significantly ($p < 0.0001$) as result of the hydrolysis of the sugar moiety (Table 2). A constant decreased of glycosilated forms during storage of monovarietal red wines from Greece has been observed by Bimpilas, Tsimogiannis, Balta-Brouma, Lymperopoulou, and Oreopoulou (2015) and they attributed this to hydrolysis of flavonol glycosides catalyzed by enzymes, like β-glucosidase, from *S. cerevisiae* yeast. In addition, we also observed that the total content of flavonols decreased significantly ($p < 0.0001$), which is in accordance with Bimpilas et al. (2015). These authors explained that the decreased of these compounds could be attributed to their oxidation through coupled reactions and/or they act as co-pigments with anthocyanins in co-pigmentations processes, although the adsorption to yeast cell walls could also be an explanation (Barcia, Pertuzatti, Gómez-Alonso, et al., 2014; Barcia, Pertuzatti, Rodrigues, et al., 2014).

Along the winemaking process, we did not see a variety characterized by the highest content in flavonol compounds.

Table 1
Content of anthocyanins in three *V. vinifera* L. red varieties along the winemaking process.

	Grapes#			Pomaces#			Wine-1			Wine-2#		
	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon
Dp-3-glc#	3.30 ± 0.15Bb	6.91 ± 1.92Bc	1.59 ± 0.36Ba	<LOD A	<LOD A	<LOD A	1.33 ± 0.02Bb	1.51 ± 0.01Bc	0.45 ± 0.02Ba	0.70 ± 0.02Ab	0.65 ± 0.12Ab	0.17 ± 0.01Aa
Cy-3-glc#	0.70 ± 0.27Ba	1.78 ± 0.06Bb	0.79 ± 0.15Ba	<LOD A	<LOD A	<LOD A	<LOQ	<LOQ	<LOD	<LOQ	<LOQ	<LOD
Pt-3-glc#	24.08 ± 5.41Bb	25.05 ± 3.59Bb	7.40 ± 1.46Ba	0.87 ± 0.22Ac	0.40 ± 0.04Ab	0.09 ± 0.01Aa	4.31 ± 0.07Bc	3.44 ± 0.04Bb	1.78 ± 0.03Ba	2.45 ± 0.04Ac	1.62 ± 0.28Ab	1.01 ± 0.03Aa
Pn-3-glc#	48.42 ± 17.62Bb	58.89 ± 5.10Bb	16.70 ± 0.49Ba	0.97 ± 0.19Ab	1.72 ± 0.16Ac	0.83 ± 0.06Aa	2.36 ± 0.02Bb	3.53 ± 0.12Bc	1.13 ± 0.02Ba	1.58 ± 0.03Ab	1.94 ± 0.31Ac	0.41 ± 0.01Aa
Mv-3-glc#	380.46 ± 26.50Bc	251.54 ± 22.34Ba	328.86 ± 24.43Bb	142.22 ± 10.15Ac	96.83 ± 26.25Ab	55.84 ± 8.14Aa	177.47 ± 21.32Bb	163.09 ± 4.57Bb	81.70 ± 6.93Ba	87.41 ± 0.70Ac	46.65 ± 2.14Aa	68.60 ± 8.75Ab
Dp-3-acglc#	1.86 ± 0.14Ba	3.39 ± 1.08Bb	1.28 ± 0.22Ba	<LOD A	<LOD A	<LOD A	0.51 ± 0.03Bb	0.67 ± 0.02Bc	0.14 ± 0.03Ba	0.15 ± 0.02 Ab	0.28 ± 0.03 Ac	<LOQ Aa
Cy-3-acglc#	0.18 ± 0.04Ba	0.65 ± 0.03Bb	0.17 ± 0.01Ba	<LOD A	<LOD A	<LOD A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pt-3-acglc#	17.68 ± 0.88Bb	19.92 ± 0.93Bc	11.62 ± 0.65Ba	0.86 ± 0.14Ac	0.39 ± 0.08Ab	0.03 ± 0.01Aa	1.24 ± 0.02Bb	1.24 ± 0.01Bb	0.43 ± 0.01Ba	0.65 ± 0.01Ac	0.52 ± 0.12Ab	0.22 ± 0.01Aa
Mv-3-acglc#	816.78 ± 58.22Bc	258.20 ± 26.86Ba	539.01 ± 25.80Bb	195.01 ± 16.59Ac	103.69 ± 23.53Ab	28.37 ± 2.21Aa	101.45 ± 10.33Bc	69.24 ± 4.80Bb	23.03 ± 0.74Ba	49.74 ± 2.54Ac	13.58 ± 3.38Aa	21.32 ± 0.94Ab
Pn-3-acglc#	72.53 ± 11.11Bc	47.79 ± 6.14Bb	32.08 ± 2.38Ba	1.83 ± 0.69Ab	3.31 ± 1.61Ac	0.25 ± 0.01Aa	4.23 ± 0.2Bb	4.88 ± 0.10Bc	1.60 ± 0.01Ba	2.05 ± 0.02Ab	2.11 ± 0.32Ab	0.82 ± 0.02Aa
Mv-3-cafglc#	2.46 ± 1.06Aa	0.47 ± 0.07Aa	0.16 ± 0.03Aa	23.77 ± 4.77Bc	3.43 ± 2.60Ba	2.60 ± 0.25Ba	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Dp-3-cmglc#	8.00 ± 0.92Aa	3.57 ± 1.13Ab	0.37 ± 0.03Ba	43.95 ± 3.47Bc	7.90 ± 4.81Bb	0.26 ± 0.05Aa	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pt-3-cmglc#	17.20 ± 2.18Ac	7.04 ± 1.39Ab	1.52 ± 0.62a	72.95 ± 1.22Bc	24.77 ± 10.73Bb	1.40 ± 0.51a	1.42 ± 0.10Bc	0.95 ± 0.04Bb	<LOQa	<LOQ A	<LOQ A	<LOD
Mv-3-cmglc#	251.70 ± 19.07c	49.58 ± 3.94Aa	71.08 ± 9.53b	238.94 ± 4.75c	142.79 ± 31.36Bb	67.54 ± 10.16a	37.30 ± 0.33Bc	19.55 ± 1.96Bb	7.95 ± 0.01Ba	8.88 ± 0.06Ab	4.25 ± 0.91Aa	3.79 ± 0.05Aa
Pn-3-cmglc#	63.86 ± 10.43Bc	23.02 ± 0.83b	13.08 ± 2.73Ba	42.71 ± 3.50Ac	24.62 ± 6.87b	1.62 ± 0.65Aa	3.03 ± 0.06Bb	3.58 ± 0.18Bc	1.55 ± 0.02Ba	1.18 ± 0.02Ab	1.14 ± 0.24Ab	0.18 ± 0.01Aa
Pigment A#	<LOD A	<LOD	<LOD A	0.54 ± 0.09Bb	<LOQa	12.79 ± 0.47Bc	2.02 ± 0.04Ac	<LOQ Aa	1.62 ± 0.02Ab	4.60 ± 0.20Bc	0.51 ± 0.01Ba	3.49 ± 0.10Bb
Acetyl Pig. A#	<LOD A	<LOD A	<LOD A	0.43 ± 0.06Bb	0.10 ± 0.04Ba	4.16 ± 0.56Bc	0.54 ± 0.06Ac	<LOQ a	0.40 ± 0.02Ab	1.34 ± 0.09Bc	<LOQ a	0.80 ± 0.07Bb
Coumaroylvit. B#	<LOD A	<LOD A	<LOD A	10.94 ± 2.13Bb	0.94 ± 0.47Ba	0.73 ± 0.04Ba	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Σ Ant-glc	456.97 ± 45.56Bb	344.17 ± 29.19Ba	355.33 ± 24.63Ba	144.05 ± 9.92Ac	98.94 ± 26.42Ab	56.76 ± 8.20Aa	185.48 ± 21.41Bb	171.56 ± 4.73Bb	85.06 ± 6.86Ba	92.15 ± 0.73Ac	50.87 ± 2.80Aa	70.19 ± 8.77Ab
Σ Ant-acglc	909.03 ± 50.40Bc	329.95 ± 33.40Ba	584.16 ± 28.80Bb	197.70 ± 17.29Ac	107.40 ± 25.22Ab	28.65 ± 2.22Aa	107.42 ± 10.09Bc	76.03 ± 4.91Bb	25.19 ± 0.72Ba	52.59 ± 2.53Ac	16.49 ± 3.85Aa	22.36 ± 0.97Ab
Σ Ant-cmglc	340.76 ± 25.34Ab	83.22 ± 6.74Aa	86.05 ± 12.71Ba	398.53 ± 10.27Bc	200.08 ± 52.95Bb	70.81 ± 11.36Aa	41.75 ± 0.39Bc	24.09 ± 2.18Bb	9.50 ± 0.01Ba	10.05 ± 0.05Ac	5.39 ± 1.15Ab	3.97 ± 0.05Aa
Σ Pyranoant.	<LOD A	<LOD A	<LOD A	11.90 ± 2.27Bb	1.04 ± 0.51Ba	17.68 ± 1.02Bc	2.56 ± 0.03Ac	<LOQ Aa	2.02 ± 0.03Ab	5.94 ± 0.16Bc	0.51 ± 0.01Ba	4.29 ± 0.17Bb
Σ Ant.	1709.21 ± 39.46Bc	757.81 ± 68.29Ba	1025.70 ± 61.96Bb	775.95 ± 19.41Ac	410.89 ± 106.89Ab	176.49 ± 22.86Aa	337.21 ± 31.30Bc	271.68 ± 7.46Bb	121.81 ± 7.38Ba	160.77 ± 2.23Ac	73.27 ± 7.66Aa	100.81 ± 9.53Ab

Abbreviations: wine-1 (wine obtained after alcoholic fermentation); wine-2 (wine obtained after stabilization). Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; glc, glucoside; ac, acetyl; caf, caffeoyl; cm, coumaroyl; Ant, anthocyanin; LOD, limit of detection; LOQ, limit of quantification. Anthocyanin compounds were quantified as malvidin-3-glucoside. Contents are reported in mg kg^{-1} DW for grape and pomace, and mg L^{-1} for wine samples. Different uppercase letters indicate significant differences ($p < 0.05$) from grape to pomace and from wine-1 to wine-2 in each variety. Different lowercase letters indicate significant differences ($p < 0.0001$) in each sample types among the three varieties. # Data adapted from Lingua et al. (2016).

Table 2
Content of flavonols and flavanols in three *V. vinifera* L. red varieties along the winemaking process.

	Grapes#			Pomaces#			Wine-1			Wine-2#		
	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon
Kaempferol#	0.05 ± 0.04Ac	<LOQAa	0.02 ± 0.01Ab	9.83 ± 0.80Ba	34.23 ± 5.57Bc	13.85 ± 0.67Bb	0.60 ± 0.12b	0.78 ± 0.01Bc	0.28 ± 0.01Ba	0.48 ± 0.12c	0.42 ± 0.01Ab	<LOQ Aa
Myricetin#	2.33 ± 0.22b	0.61 ± 0.06Aa	0.58 ± 0.14Aa	2.17 ± 0.14a	2.45 ± 0.26Ba	7.25 ± 0.42Bb	13.97 ± 0.24Ab	8.78 ± 1.07Ba	13.76 ± 0.25Bb	19.25 ± 2.22Bc	4.98 ± 0.10Aa	8.43 ± 1.63Ab
Laricitrin#	0.08 ± 0.01Ac	<LOQ Aa	0.07 ± 0.01Ab	0.30 ± 0.03Bc	0.14 ± 0.01Ba	0.26 ± 0.02Bb	1.61 ± 0.16Bb	<LOQa	2.03 ± 0.50Bc	<LOQ A	<LOQ	<LOQ A
Syringetin#	0.09 ± 0.01Ac	0.05 ± 0.01Aa	0.07 ± 0.01Ab	0.39 ± 0.02Bb	0.20 ± 0.02Ba	0.49 ± 0.03Bc	<LOQa	<LOQa	1.76 ± 0.23b	<LOQ	<LOQ	<LOQ
Quercetin#	0.38 ± 0.16Aa	2.28 ± 0.39Ab	3.18 ± 0.41Ac	92.98 ± 29.47Ba	251.06 ± 65.60Bc	163.56 ± 31.91Bb	42.19 ± 0.91Ab	40.00 ± 0.91a	42.03 ± 2.13Bb	56.85 ± 2.34Bc	39.66 ± 1.29b	31.51 ± 1.54Aa
Isorhamnetin#	0.23 ± 0.11Ab	0.02 ± 0.01Aa	0.03 ± 0.01Aa	16.07 ± 1.70Bb	12.46 ± 1.47Ba	20.52 ± 3.30Bc	12.54 ± 0.23Ac	6.52 ± 0.24Aa	8.80 ± 0.35Ab	28.36 ± 0.17Bc	10.70 ± 0.25Bb	10.25 ± 0.57Ba
Isoquercetin#	278.51 ± 89.62Bb	174.76 ± 18.18Ba	336.17 ± 26.41Bc	26.53 ± 1.41Ac	16.05 ± 0.80Aa	21.77 ± 1.37Ab	25.23 ± 1.75Bc	16.64 ± 0.36Bb	7.77 ± 0.20Ba	2.62 ± 0.02Aa	4.34 ± 0.12Ac	3.15 ± 0.19Ab
Myr-3-glc#	209.61 ± 30.73Bb	75.92 ± 8.02Ba	198.93 ± 29.59Bb	11.37 ± 0.71Ac	6.13 ± 0.46Ab	3.56 ± 0.38Aa	27.28 ± 0.30Bc	12.18 ± 0.27Bb	9.15 ± 0.61Ba	14.20 ± 0.94Ac	8.56 ± 0.15Ab	8.02 ± 0.49Aa
Myr-3-glcr#	1.06 ± 0.05Aa	1.57 ± 0.40Bb	3.96 ± 0.13Bc	1.80 ± 0.14Bc	0.47 ± 0.08Aa	0.60 ± 0.07Ab	0.61 ± 0.04b	0.38 ± 0.02Ba	0.34 ± 0.02Ba	0.59 ± 0.05b	0.27 ± 0.11Aa	0.32 ± 0.01Aa
Astilbin#	2.04 ± 1.05Aa	2.06 ± 0.05Aa	4.32 ± 0.37Bb	7.57 ± 0.22Bc	2.46 ± 0.32Ba	3.75 ± 0.32Ab	7.55 ± 0.38Bc	3.62 ± 0.02Ba	6.17 ± 0.51b	6.36 ± 0.33Ab	2.90 ± 0.01Aa	6.51 ± 0.63b
Lar-3-glc#	4.32 ± 0.43Ab	2.21 ± 0.15Aa	5.62 ± 0.28Bc	6.37 ± 0.23Bb	2.91 ± 0.19Ba	2.95 ± 0.27Aa	8.48 ± 0.18Bc	2.98 ± 0.07Ba	4.34 ± 0.19Bb	8.17 ± 0.02Ac	2.66 ± 0.01Aa	2.97 ± 0.14Ab
Quer-3-glcr#	10.95 ± 3.66Aa	35.18 ± 9.44b	46.79 ± 15.97b	81.42 ± 3.44Bc	31.92 ± 3.58a	38.32 ± 2.76b	15.74 ± 0.81Bb	10.99 ± 0.78Ba	11.92 ± 0.37Ba	14.48 ± 0.08Ab	8.29 ± 0.42Aa	8.19 ± 0.18Aa
Kp-3-glc#	0.14 ± 0.07Ba	0.20 ± 0.06Ba	0.52 ± 0.22Bb	<LOD A	<LOD A	<LOD A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Syr-3-glc#	25.38 ± 4.40Bb	7.16 ± 0.77Ba	29.86 ± 2.60Bc	4.90 ± 0.20Ab	4.17 ± 0.72Aa	11.97 ± 0.62Ac	11.38 ± 0.75Ac	4.66 ± 0.14Aa	8.50 ± 0.67Bb	13.25 ± 0.77Bb	5.32 ± 0.44Ba	5.31 ± 0.28Aa
Isorh-3-glc#	50.24 ± 20.02Bc	11.41 ± 3.04Ba	34.46 ± 6.75Bb	7.73 ± 0.31Ac	1.96 ± 0.28Aa	2.88 ± 0.28Ab	11.33 ± 0.19Bc	4.31 ± 0.31Bb	2.52 ± 0.31Ba	2.24 ± 0.01A	2.20 ± 0.25A	2.14 ± 0.23A
Σ Flavonol aglycones	3.16 ± 0.53Aa	2.70 ± 0.90Aa	3.93 ± 0.31Ab	121.74 ± 30.36Ba	300.54 ± 66.34Bc	205.93 ± 30.78Bb	70.91 ± 0.87Ac	56.07 ± 1.75a	66.39 ± 2.08Bb	104.93 ± 0.17cB	55.77 ± 1.13b	50.18 ± 3.74Aa
Σ Flavonol glycosides	582.23 ± 137.64Bb	295.44 ± 59.95Ba	660.63 ± 74.64Bb	147.70 ± 3.94Ac	66.07 ± 5.84Aa	85.80 ± 4.41Ab	107.59 ± 2.71Bc	55.76 ± 0.81Bb	50.50 ± 0.18Ba	61.91 ± 0.08Ac	34.53 ± 0.10Aa	36.62 ± 0.73Ab
Σ Flavonols	585.39 ± 138.12Bb	298.14 ± 60.83Aa	664.57 ± 74.80Bb	269.45 ± 30.27Aa	366.61 ± 69.26Bb	291.73 ± 31.87Aa	178.50 ± 1.83Bc	111.83 ± 0.94Ba	116.89 ± 2.26Bb	166.84 ± 0.25Ac	90.30 ± 1.23Ab	86.81 ± 4.47Aa
Procyanidin dime#r	13.12 ± 1.35Bb	<LODAa	19.71 ± 1.71Bc	10.05 ± 1.14Aa	24.62 ± 3.02Bb	8.99 ± 1.66Aa	18.14 ± 0.60Ab	19.34 ± 1.63Ab	16.29 ± 0.53Aa	19.95 ± 0.28Ba	25.31 ± 0.44Bb	28.81 ± 1.28Bc
Procyanidin dimer monogallate#	4.04 ± 0.48Ba	5.97 ± 1.34Ab	3.58 ± 0.21Aa	2.59 ± 0.27Aa	13.64 ± 1.99Bb	17.25 ± 1.75Bc	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
(+)-Catechin#	28.70 ± 1.77Bb	20.85 ± 1.50Aa	28.37 ± 1.69Bb	21.78 ± 1.67Aa	89.73 ± 9.64Bb	19.62 ± 1.83Aa	34.27 ± 0.84Aa	46.93 ± 2.11Ab	33.59 ± 1.12Aa	41.94 ± 2.07Ba	59.45 ± 2.48Bb	74.95 ± 2.00Bc
(-)-Epicatechin#	74.80 ± 4.99Bc	68.09 ± 3.16Ab	57.45 ± 2.88Ba	27.19 ± 4.03Ab	112.76 ± 8.21Bc	17.29 ± 1.66Aa	39.89 ± 2.12b	52.37 ± 0.37Ac	31.82 ± 1.20Aa	40.44 ± 1.24a	55.57 ± 0.78Bb	65.57 ± 2.10Bc
Epicatechin gallate#	25.39 ± 2.29Ba	75.30 ± 27.86Bb	6.81 ± 0.38Aa	14.72 ± 2.84Ab	45.62 ± 6.69Ac	10.49 ± 1.15Ba	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Σ Flavanols	146.04 ± 8.72Bb	166.56 ± 29.15Ab	115.93 ± 4.40Ba	76.3 ± 6.82Aa	286.37 ± 20.44Bb	73.65 ± 4.34Aa	92.30 ± 3.56Ab	118.64 ± 0.85Ac	81.70 ± 5.63Aa	102.33 ± 3.03Ba	140.33 ± 3.71Bb	169.33 ± 2.82Bc

Abbreviations: wine-1 (wine obtained after alcoholic fermentation); wine-2 (wine obtained after stabilization). Myr, myricetin; Lar, laricitrin; Quer, quercetin; Kp, kaempferol; Syr, syringetin; Isorh, isorhamnetin; glc, glucoside; glcr, glucuronide; LOD, limit of detection; LOQ, limit of quantification. Quantification: Myr, Lar and Syr compounds as myricetin; Quer and Isorh compounds as quercetin; Kp compound as kaempferol. Flavonol glycosides compounds as isoquercetin; Flavanols compounds as (+)-catechin. Contents are reported in mg kg^{-1} DW for grape and pomace, and mg L^{-1} for wine samples. Different uppercase letters indicate significant differences ($p < 0.05$) from grape to pomace and from wine-1 to wine-2 in each variety. Different lowercase letters indicate significant differences ($p < 0.01$) in each sample types among the three varieties. # Data adapted from Lingua et al. (2016).

Table 3
Content of non-flavonoid phenolic compounds in three *V. vinifera* L. red varieties along the winemaking process.

	Grapes#			Pomaces#			Wine-1			Wine-2#		
	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon
Caftaric acid#	154.73 ± 15.30Bc	100.23 ± 4.78Ba	127.55 ± 21.26Bb	1.58 ± 0.24Ab	1.80 ± 0.12Ac	0.25 ± 0.15Aa	67.38 ± 1.72Bb	69.92 ± 6.44Bb	20.15 ± 0.56Aa	28.28 ± 1.38Aa	29.87 ± 1.12Ab	35.60 ± 0.37Bc
Coutaric acid#	136.05 ± 6.82Bc	39.00 ± 2.37Ba	84.86 ± 3.21Bb	5.41 ± 0.33Ab	5.10 ± 0.53Ab	1.38 ± 0.26Aa	17.04 ± 0.73Bc	14.85 ± 1.19Bb	6.36 ± 0.30Aa	7.90 ± 0.11Aa	8.94 ± 0.16Ab	11.02 ± 0.77Bc
Caffeic acid#	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	7.02 ± 1.06Ab	1.21 ± 0.13Aa	19.14 ± 2.50Bc	8.38 ± 0.35Bc	1.75 ± 0.27Ba	3.50 ± 0.46Ab
Fertaric acid#	2.62 ± 1.39B	2.72 ± 1.34B	3.62 ± 1.44B	<LOD A	<LOD A	<LOD A	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Σ HCA	293.40 ± 20.61Bc	136.39 ± 17.80Ba	216.03 ± 21.98Bb	6.99 ± 0.40Ab	6.90 ± 0.58Ab	1.64 ± 0.39Aa	91.45 ± 3.51Bb	85.98 ± 7.76Bb	45.65 ± 0.98Aa	44.56 ± 1.85Ab	40.56 ± 1.01Aa	50.12 ± 0.68Bc
Gallic acid#	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	41.67 ± 2.48Aa	45.76 ± 1.52Ab	48.89 ± 0.50Ac	62.85 ± 1.91Ba	63.58 ± 2.40Ba	75.29 ± 1.54Bb
Ethyl gallate#	<LOD A	<LOD A	<LOD A	28.99 ± 4.14Ba	50.49 ± 1.21Bb	53.04 ± 3.02Bb	78.38 ± 0.08Ab	52.15 ± 1.38Aa	165.63 ± 20.67Ac	155.25 ± 8.16Ba	172.03 ± 0.24Bb	197.24 ± 20.16Bc
Σ HBA	<LOD A	<LOD A	<LOD A	28.99 ± 4.14Ba	50.49 ± 1.21Bb	53.04 ± 3.02Bb	120.04 ± 2.40Ab	97.92 ± 0.14Aa	214.52 ± 25.90Ac	218.10 ± 6.25Ba	235.61 ± 2.16Bb	272.52 ± 21.70Bc
trans-Resveratrol#	0.08 ± 0.06Ba	6.99 ± 1.51Bb	0.47 ± 0.06Ba	<LOD A	<LOD A	<LOD A	0.710 ± 0.005Bb	5.76 ± 0.39Bc	0.30 ± 0.02Aa	0.498 ± 0.004Aa	1.16 ± 0.03Ac	0.61 ± 0.05Bb

Abbreviations: wine-1 (wine obtained after alcoholic fermentation); wine-2 (wine obtained after stabilization). HCA, hydroxycinnamic acids; HBA, hydroxybenzoic acids; LOD, limit of detection; LOQ, limit of quantification. Quantification: HCA compounds as caffeic acid; HBA compounds as gallic acid; *trans*-resveratrol compound as *trans*-resveratrol. Contents are reported in mg kg^{-1} DW for grape and pomace, and mg L^{-1} for wine samples. Different uppercase letters indicate significant differences ($p < 0.05$) from grape to pomace and from wine-1 to wine-2 in each variety. Different lowercase letters indicate significant differences ($p < 0.0001$) in each sample types among the three varieties. # Data adapted from Lingua et al. (2016).

3.1.1.3. Flavanols. Flavan-3-oles or flavanols include to the monomeric, oligomeric and polymeric forms (the latter two forms are also known as proanthocyanidins or condensed tannins), and are found in the solid parts of the berry, with seeds having the highest concentration (Monagas et al., 2003). As it can be seen from Table 2, the flavanol profile in grapes was mainly formed by the monomers (−)-epicatechin, (+)-catechin and epicatechin gallate. In addition, we detected two proanthocyanidins: procyanidin dimer and procyanidin dimer monogallate. (−)-Epicatechin was the most abundant flavanol in Syrah and Cabernet Sauvignon grapes, while epicatechin gallate was present in higher amounts in Merlot. From grape to pomace samples, we observed a significant drop ($p < 0.0001$) in the content of flavanols perhaps as a consequence of its higher transfer from grape to wine during the maceration and alcoholic fermentation steps, except for the Merlot variety (Table 2). This last result could be due to that transfer rate of phenolics during winemaking is not a simple solid/liquid partition and other physic-chemical processes have been suggested to modulate such transference (Barcia, Pertuzatti, Gómez-Alonso, et al., 2014; Barcia, Pertuzatti, Rodrigues, et al., 2014) or to an increase in the proportion of seeds present in the Merlot pomace during sampling. This last assumption is supported by previous reports, for instance, Ivanova et al. (2011) showed that the Merlot grape from R. Macedonia had a higher content of flavanols than the Vranec variety, being this exceeding content stored in seeds. From wine-1 to wine-2 samples, the flavanols showed a significant increment ($p < 0.0001$), which is probably as consequence of the hydrolysis that suffer their polymeric and galloylated precursors during stabilization to obtained the finished wines. For example, in this study the increment observed in (−)-epicatechin and procyanidin dimer could be probably a consequence of the hydrolysis from their galloylated precursors, like epicatechin gallate and procyanidin dimer monogallate respectively (Table 2). In addition, this result is consistent with the statically increment ($p < 0.0001$) observed in gallic acid from wine-1 to wine-2.

Among varieties, we observed that Merlot showed the highest content of flavanols in grape, pomace and wine-1 samples, while Cabernet Sauvignon did among wine-2 samples.

3.1.2. Non-flavonoid phenolic compounds

3.1.2.1. Hydroxycinnamic acids (HCA). The expected hydroxycinnamoyl tartaric acids, also known as caftaric (from caffeic acid), coutaric (from *p*-coumaric acid), and fertaric acids (from ferulic acid), were found in grapes (Monagas et al., 2005a). Caftaric acid was the most abundant cinnamate, followed by coutaric and fertaric acids, with this latter at relatively very low levels (Barcia, Pertuzatti, Rodrigues, et al., 2014). As with previous families of phenolic compounds, HCA significantly decreased ($p < 0.0001$) from grape to pomace (Table 3) as the result of their higher transfer from grape to wine during the maceration and alcoholic fermentation steps. The aforementioned HCA, with the exception of fertaric acid, was also quantified in pomace, wine-1 and wine-2 samples. It is known that hydroxycinnamoyl tartaric acids suffer hydrolysis during the winemaking process (Ginjom, ĎArcy, Caffin, and Gidley, 2011), consequently, free caffeic acid, from hydrolysis of the main HCA present in grapes, was found in wine-1 and wine-2 samples. Furthermore, the absence of *p*-coumaric acid could be associated with the increase in anthocyanin-derived pigments, as pigment A and acetyl pigment A. As it was observed in grapes, caftaric acid was the predominant HCA in wine-1 and wine-2 samples (Ginjom et al., 2011). Among samples studied along the winemaking process we did not get evidence of a variety characterized by its highest content in HCA.

3.1.2.2. Hydroxybenzoic acids (HBA). As it can be seen in Table 3, we did not find HBA in grapes probably due to the detection limit,

however we found ethyl gallate in pomace samples. From wine-1 to wine-2, we found that gallic acid and ethyl gallate increased significantly ($p < 0.0001$) their concentrations which are in accordance with Ginjom et al. (2011) and Monagas et al. (2005b). Gallic acid is extracted from the seeds after the hydrolysis of gallate esters from the flavanols under the action of esterases during the maceration and fermentation processes, and its esterification with ethanol to form ethyl gallate, also appears to occur during fermentation and aging conditions (Monagas et al., 2005b), which explain the presence of ethyl gallate in pomace, wine-1 and wine-2 samples.

Among pomace samples the content of HBA was higher in Cabernet Sauvignon and Merlot varieties. Respect to wine-1 and wine-2 samples, these compounds exhibited higher content in Cabernet Sauvignon.

3.1.2.3. Stilbenes. These are one of the minor non-flavonoid phenolic classes of compounds in grapes and can be found in the skin of grapes but not in seeds. Resveratrols are the main compounds belonging to this class of polyphenols (Monagas et al., 2005a). In this study *trans*-resveratrol was the only detectable stilbene in samples examined. As it can be seen in Table 3, this compound was present in small amounts in grape samples, but absent in pomace probably due to its high ratio transfer to wine (Barcia, Pertuzatti, Rodrigues, et al., 2014). From wine-1 to wine-2 this compound did not follow a common trend for the three varieties studied. In case of Syrah and Merlot, we observed that the content of t*rans*-resveratrol decreased significantly ($p < 0.0001$) in accordance with Monagas et al., 2005b. In contrast, the t*rans*-resveratrol content was significantly increased ($p < 0.0001$) in Cabernet Sauvignon. The above results suggest that t*rans*-resveratrol is extracted from grape during the alcoholic fermentation however in the later steps of winemaking it undergoes changes that alter its content. After alcoholic fermentation, its absorption by the yeast cell walls has been observed (Barcia, Pertuzatti, Rodrigues, et al., 2014). In addition, the hydrolysis of its glucoside and *cis*/*trans* isomerization have also been observed to occur during the winemaking process and aging in bottle (Monagas et al., 2005b). Therefore, according to these factors, the first could be explaining the reduction of t*rans*-resveratrol content in the finished wines (wine-2) of Syrah and Merlot varieties. In case of Cabernet Sauvignon, the second factor would be prevailing over the first.

As with the other phenolic compounds, concentrations are governed by the grape variety, although external factors such as environmental conditions and winemaking process can affect them. Quantitatively, we detected that the Merlot variety exhibited the highest content of *trans*-resveratrol in all samples where it was found (grape, wine-1 and wine-2).

3.2. Antioxidant activity

The effects of the different stages of the winemaking process on the antioxidant activities of samples are shown in Table 4. The three different assays (FRAP, ABTS and DPPH) gave similar trends. Antioxidant activity of Syrah and Merlot pomaces was higher ($p < 0.001$) than their grapes while Cabernet Sauvignon pomace was lower ($p < 0.0001$) than its grape. From wine-1 to wine-2, the AC was stable or slightly increased. Regarding to the differences in AC among varieties there was not a variety that stood out for its AC along the winemaking process, being dependent on sample type (Table 4).

Differences found in AC between samples and varieties are probably due to distinct phenolic profiles owning to the effect of winemaking on this compounds and proper differences due to the grape variety.

Table 4
Antioxidant Capacity in three red varieties of *V. vinifera* L. along the winemaking process.

	Grapes			Pomaces			Wine-1			Wine-2		
	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon	Syrah	Merlot	Cabernet Sauvignon
FRAP	5.2 ± 0.4Aa	8.9 ± 0.8Ab	10.1 ± 0.9Ac	11.4 ± 1.1Bc	10.2 ± 0.8Bb	7.3 ± 0.7Ba	8.7 ± 0.3	9.0 ± 0.8	9.6 ± 0.6B	8.5 ± 0.2b	9.0 ± 0.1c	8.2 ± 0.4Aa
ABTS	14.5 ± 1.4Aa	19.9 ± 1.0Ab	23.6 ± 2.1Ac	24.8 ± 2.6Bb	27.4 ± 1.8Bc	12.8 ± 1.1Ba	14.2 ± 0.9A	13.1 ± 1.1A	12.7 ± 1.4A	17.3 ± 0.3Bb	18.5 ± 0.5Bc	14.1 ± 1.0Ba
DPPH	10.2 ± 0.7Aa	17.1 ± 0.9Ab	22.2 ± 2.6Ac	21.3 ± 2.1Bb	23.8 ± 1.4Bc	15.0 ± 0.7Ba	11.6 ± 1.1	12.1 ± 0.7	11.1 ± 0.6	12.8 ± 1.6b	11.9 ± 0.8a	11.9 ± 1.0a

Wine-1 (wine obtained after alcoholic fermentation); wine-2 (wine obtained after stabilization). FRAP, ABTS and DPPH values: in mmol Trolox 100 g^{-1} DW for grape and pomace; in mmol Trolox L^{-1} for wine samples. Different uppercase letters indicate significant differences ($p < 0.05$) from grape to pomace and from wine-1 to wine-2 in each variety. Different lowercase letters indicate significant differences ($p < 0.05$) in each sample types among the three varieties.

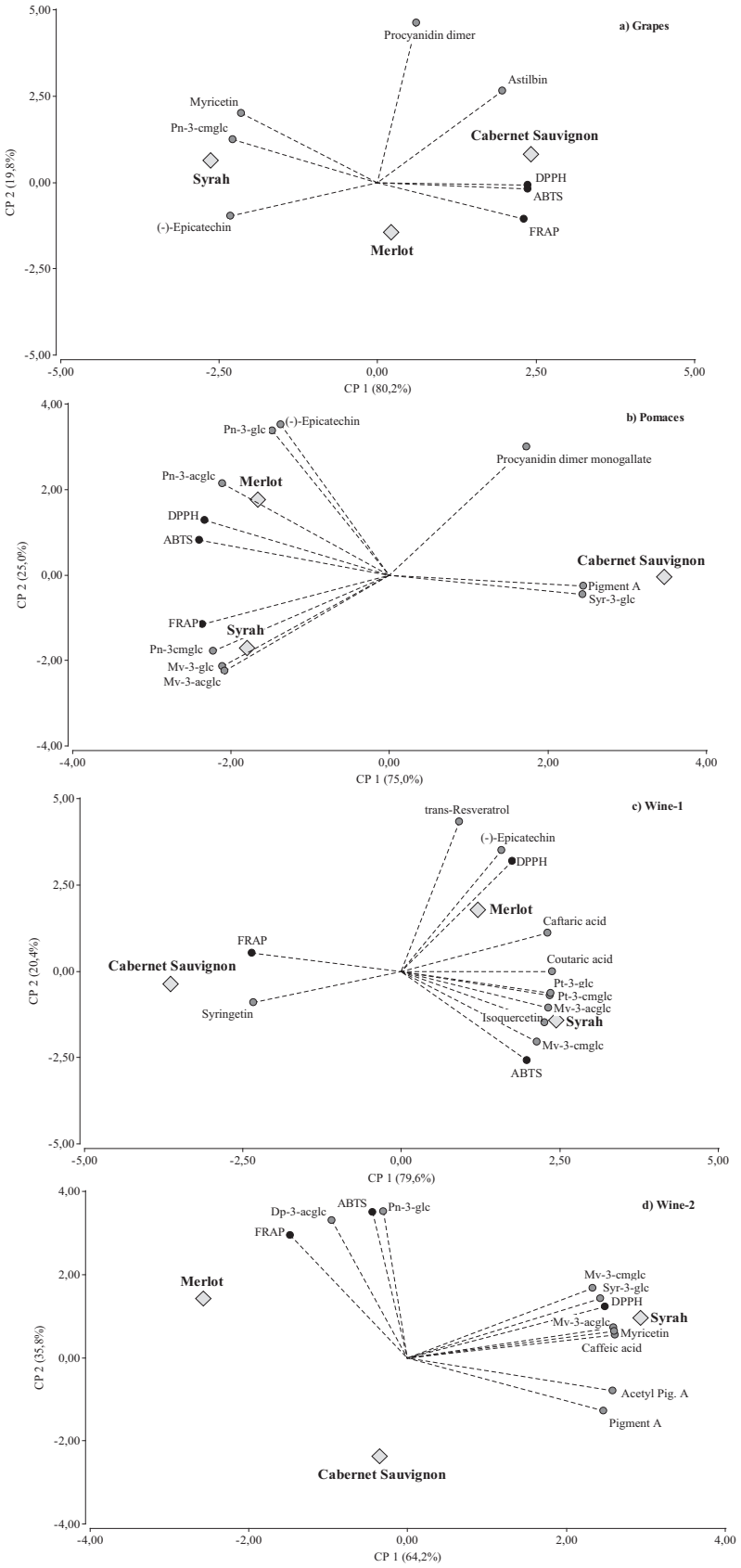

Fig. 1. Biplot graphics representing the association of phenolic compounds selected as key variables for the prediction of antioxidant capacity of three *V. vinifera* L. red varieties along the winemaking process. (a) Grapes, (b) pomaces, (c) wine-1, and (d) wine-2.

3.3. Correlation of antioxidant activity with phenolic profile

Since we observed differences in phenolic profile as well as in antioxidant activity along winemaking process and between varieties, we were interested in evaluate the relationship between them. With this objective, we applied Multiple Regression Analysis (MRA), considering qualitative and quantitative differences observed in the content of phenolic compounds. For all samples, stepwise multiple regression analysis was used to correlate FRAP, ABTS and DPPH assays with the phenolic compounds grouped by family. *Beta* coefficient of each compound was analyzed in order to study its relative contribution, being compounds with *Beta* absolute values higher than 0.40 ($p < 0.05$) those with the highest contribution to AC. In all samples (grape, pomaces, wine-1 and wine-2) a high correlation with phenolic profile was observed (r higher than 0.60, $p < 0.05$), even though key compounds with highest contribution to the AC were different in each case.

In case of grapes, astilbin and procyanidin dimer were compounds with highest positive contribution to the FRAP, ABTS and DPPH value, while peonidin-3-coumaroylglucoside, (-)-epicatechin and myricetin were the ones with highest negative contribution. Fig. 1 shows the biplot graphic, where it is represented the association of these key polyphenols with FRAP, ABTS and DPPH values in three varieties studied. As it can be seen from Fig. 1a, the content of astilbin and procyanidin dimer were highest in Cabernet Sauvignon while, peonidin-3-coumaroylglucoside, (-)-epicatechin and myricetin showed the highest content in Syrah. These results would be explaining the highest and lowest AC in Cabernet Sauvignon and Syrah grapes, respectively.

In contrast, in pomaces, differences were observed in compounds that contributed to the different antioxidant assays. FRAP correlated positively with malvidin-3-glucoside, malvidin-3-acetylglucoside, peonidin-3-coumaroylglucoside and negatively with procyanidin dimer monogallate. On the other hand, ABTS and DPPH were correlated significantly with five selected compounds: peonidin-3-glucoside, peonidin-3-acetylglucoside and (−)-epicatechin contributed positively to these assays, while pigment A and syringetin-3-glucoside were the compounds with higher negative contribution. As it is shown in Fig. 1b, compounds with a positive contribution to the AC were in higher concentration in Syrah and Merlot with respect to Cabernet Sauvignon. Conversely, compounds with negative contribution were in higher concentrations in Cabernet Sauvignon (Fig. 1b). These results are in accordance with the higher activity of Syrah and Merlot pomaces respect to Cabernet Sauvignon.

Differences were also observed in wine-1, FRAP assay was negatively correlated with petunidin-3-glucoside, malvidin-3-acetylglucoside, petunidin-3-coumaroylglucoside, isoquercetin and coutaric acid and positively correlated with syringetin. On the other hand, ABTS assay was correlated with malvidin-3-cumaroylglucoside, (−)-epicatechin and coutaric acid as positive contributors, but with caftaric acid and *trans*-resveratrol as negative contributors. DPPH assay was correlated significantly with petunidin-3-coumaroylglucoside as positive contributor, while malvidin-3-coumaroilglucoside showed a negative contribution (Fig. 1c).

Finally in wine-2 samples, FRAP and ABTS assays were correlated significantly with peonidin-3-glucoside and delphinidin-3-acetylglucoside as positive contributors, while pigment A, myricetin and caffeic acid were negative contributors. Results from DPPH assay correlated positively with malvidin-3-acetylglucoside, malvidin-3-coumaroylglucoside, acetyl pigment A and syringetin-3-glucoside. Merlot variety showed the highest content of peonidin-3-glucoside and delphinidin-3-acetylglucoside but the lowest content of pigment A, myricetin and caffeic acid, explaining the higher AC of this varietal (Fig. 1d). Likewise, Syrah variety showed the highest content of malvidin-3-acetylglucoside, malvidin-3-coumaroylglucoside, acetyl pigment A and syringetin-3-glucoside (Fig. 1d).

According to these results along the winemaking process and taking in account the observed changes in phenolic profile, flavonol glycosides (specifically astilbin) were the main contributors to AC in raw material, meanwhile anthocyanins were the most important ones in wine samples. In the case of pomace, (−)-epicatechin together with anthocyanins were pointed out as important for its antioxidant capacity. It is worth to remark that in general, different compounds were selected to correlate with the different *in vitro* assays. It is known, that according to the chemical structure of compounds, they will react differently in the *in vitro* assays according to the different mechanisms involved (hydrogen atom transfer, single electron transfer, reducing power, and metal chelation, among others) (Ginjom et al., 2011).

Polyphenols are compounds that participate in redox reactions, so they may act as antioxidant or pro-oxidant depending on their concentration and environment (Braicu, Ladomery, Chedea, Irimie, & Berindan-Neagoe, 2013; Chedea, Braicu, & Socaciu, 2010; Choueiri, Chedea, Calokerinos, & Kefalas, 2012; Samra, Chedea, Economou, Calokerinos, & Kefalas, 2011). They can either act as scavengers of reactive oxidant species or generate more oxidative stress. It has been shown that compounds with strong scavenging capacities are oxidised at relative low potentials. However, the most powerful reducing agents are phenolics with low reducing potential and they can, by autooxidation, exert pro-oxidant activity (Samra, Chedea, Economou, Calokerinos, Kefalas, 2011). *In vitro* assays with cell culture suggest that polyphenols may have unpredictable effects that may be concentration and cell type-specific dependent (Halliwell, 2008). Thus, the balance between antioxidative and pro-oxidative effects is very delicate (Samra, Chedea, Economou, Calokerinos, Kefalas, 2011). For example Samra, Chedea, Economou, Calokerinos, Kefalas (2011) and Choueiri et al. (2012) evaluated the antioxidant activity of different mixtures of polyphenols, founding that depending on the mixture and the relative concentration of each standard the effect observed was antioxidant or pro-oxidant. In this sense, the mixture of catequin and quercetin show a pro-oxidant activity compared to each antioxidant alone. Furthermore different proportions of the mixture of standards give different results, showing less or higher antioxidant activity. So, taking in account the possibility of polyphenols of acting as antioxidant or pro-oxidant, and analyzing results obtained by MRAs, polyphenols with positive contribution to AC are probably having synergism between them and therefore high antioxidant effect, on the other hand those compounds which showed negative contribution, are showing antagonistic effects and they probably have pro-oxidant effects in the samples analyzed. Results obtained in the antioxidant assays are the sum of the antioxidant/pro-oxidant effect of each compound.

4. Conclusions

The results presented in this study highlight that the phenolic composition of grapes is greatly affected by the winemaking process. The measurement of individual compounds at the different stages helped explain and understand the systematic changes caused by alcoholic fermentation and wine production to the phenolic composition. The main changes observed were hydrolysis of glycosidated flavonols, hydroxycinnamoyl tartaric acids and gallate esters, and also the formation of anthocyanin-derived pigments. In addition, our study evidenced quantitative differences in phenolic profile among varieties which indicate that genotype affects phenolic profile. Results from MRA showed a high correlation between the phenolic composition and AC of samples. The

key compounds that contributed to this bioactivity depended on the type of sample analyzed. Anthocyanins were pointed out as the main group, with significant, and in general positive, contribution to this property. In addition, differences in antioxidant activity between varieties could be explained by quantitative differences in phenolic compounds pointed out by MRA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.04.009.

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