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Proanthocyanidin profile and antioxidant capacity of Brazilian *Vitis vinifera* red wines

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ABSTRACT

The free flavan-3-ol and proanthocyanidin (PA) profile and the antioxidant capacity of wines *Vitis vinifera* L., 2006 and 2007 vintages, from the São Joaquim region, at southern Brazil, are reported here for the first time. Catechin and epicatechin were the two main monomers in the wine samples, followed by gallocatechin and epigallocatechin; and the PA B1 was the main dimer. The terminal units of the PAs were constituted mainly by catechin units, with the co-presence of epicatechin, gallocatechin, epigallocatechin and traces of epicatechin gallate. The epicatechin and epigallocatechin units were the main constituents of the extension units of PAs with the co-presence of catechin and epicatechin gallate. The values for the mean degree of polymerisation ranged from 4.9 to 9.8. The wine samples demonstrated effective scavenging activity against DPPH and ABTS radicals and against lipid peroxidation *in vitro*. A positive correlation existed between flavan-3-ol content and antioxidant capacity *in vitro*.

1. Introduction

The proanthocyanidins (PAs), also known as condensed tannins, are oligomers and polymers of flavan-3-ols, which are widely distributed in the plant kingdom. In particular, procyanidins consisting of catechin units [(+)-catechin and (−)-epicatechin], and prodelphinidins, based on gallocatechin units [(+)-gallocatechin and (−)-epigallocatechin], represent a ubiquitous group of plant phenolics (Prieur, Rigaud, Cheynier, & Moutounet, 1994; Souquet, Cheynier, Brossaud, & Moutounet, 1996). There is a lack of chemical studies on these groups, possibly due to the difficulties associated with determining tannins, given their polymeric nature and large structural diversity.

In wine, flavan-3-ols are one of the major classes of flavonoids present. They are found in grape skin and seeds from which they are extracted into the must during vinification (Souquet et al., 1996). These compounds are particularly important in terms of the sensory characteristics of wines, such as astringency and bitterness (Chira, Schmauch, Saucier, Fabre, & Teissedre, 2009), which are dependent on the structure and degree of polymerisation (Souquet et al., 1996). Moreover, it has been reported that PAs have high antioxidant capacity *in vitro* (Mattivi, Zulian, Nicolini, & Valenti, 2002; Raza & John, 2007; Rigo et al., 2000) and *in vivo* (Cirico & Omaye, 2006). Monomeric units of catechins, including catechin itself, epicatechin, gallocatechin, and gallate esters, for instance, have been shown to increase plasma antioxidant capacity and the resistance of low-density lipoproteins (LDL) to oxidation (Frankel, Waterhouse, & Teissedre, 1995).

São Joaquim is a new wine growing region located in the high plains of Santa Catarina State, in southern Brazil. It is known in Brazil as the coldest place in the country, and it lies at the highest altitude (1200–1400 m) in relation to other viticulture regions in Brazil (Falcão et al., 2008a). According to the Geoviticulture Multicriteria Climatic Classification System (Tonietto & Carbonneau, 2004) the weather in the São Joaquim – SC region is classified as "Cool, Cool nights and Humid": Huglin's heliothermal index-HI: 1714; cool night index: 12.1 °C; and dryness index – DI: 200 mm, humid. The summed GDD results for the period of the phenological cycle (budburst – harvest) of the grapevines characterised São Joaquim – SC as "Region I" (<1389 GDD), that is a "cold region" in terms of the Winkler Regions. It is believed that the São Joaquim regional characteristics (orographic, climate) are favourable for the cultivation of vines and consequently the production of high quality wines. Falcão, de Revel, Rosier, and Bordignon-Luiz (2008b) verified these characterisations, mainly through obtaining good results for the volatile composition of Cabernet Sauvignon wines produced in this region.

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In this study, an HPLC-DAD–MS method was developed to characterise and quantify the main monomers (catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin gallate), PA dimers (B1 and B2) and their phloroglucinol adducts in the Cabernet Franc, Merlot, Sangiovese and Syrah wines, from 2006 and 2007 vintages, from São Joaquim – SC, Brazil. The ability of these wines to scavenge DPPH and ABTS radicals and to inhibit lipid peroxidation *in vitro* (TBARS – thiobarbituric acid reactive substances) were also evaluated, as well as their correlation with the flavan-3-ol composition.

2. Materials and methods

2.1. Chemicals

All chromatographic solvents were HPLC grade and were purchased from Carlo Erba (Rodano, Italy). Pure, HPLC grade (+)-catechin (C), (−)-epicatechin (EC), (−)-gallocatechin (GC), (−)-epigallocatechin (EGC) and (−)-epicatechin gallate (ECG) were obtained from Sigma (Steinheim, Germany). The PAs B1 [(−)-epicatechin-(4β-8)-(+)-catechin] and B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin] were obtained from Extrasynthese (Genay, France). Phloroglucinol was purchased from Aldrich (Steinheim, Germany). Folin–Ciocalteau reagent, vanillin, 2-thiobarbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich Co. (St. Louis, USA).

2.2. Samples

Wines from the 2006 and 2007 vintages of the Cabernet Franc, Merlot, Sangiovese and Syrah varieties sampled from São Joaquim, Santa Catarina State (SC), Brazil, were analysed. Experimental plots of varieties were delimited in young commercial vineyards and used to make the wines. The region of São Joaquim is located in Santa Catarina State at altitudes of 1200–1400 m, coordinates 28° latitude and 49° longitude, and these are the highest altitudes of vineyards in Brazil. According to the USDA classifications the soil of this region is inceptisol, that is, a well drained soil with a soft friable consistency, a high capacity for water retention and absence of stones (Falcão et al., 2008a). The vineyards are located at 28° 15′ latitude, 49° 50′ longitude and 1290 m altitude. The vines of the varieties Cabernet Franc, Merlot, Sangiovese and Syrah were planted in 2003, and the clones used were 986, 181, VCR23 and VCR1, respectively. The rootstock used was Paulsen 1103 (*Vitis berlandieri Planch* × *Vitis rupestris Scheele*); the vertical shoot positioning trellis system training was used; the row and vine spacing was 3.0 × 1.2 m and the vineyard yield was between 6 and 7 t/ha.

2.2.1. Wine production

The wines were all produced under the same conditions in the commercial winery of São Joaquim – SC through a traditional skin-contact technique. The berries were separated from the stalks, crushed and maintained in a stainless steel vat. The maceration period was 15 days, with one or two daily pumping events at 22–28 °C. The must was separated from the solid parts and transferred to other stainless steel vats. Prior to initiating the alcoholic fermentation, a commercial sulphating agent (12 g 100 kg^{-1} of must, corresponding to 60 mg L^{-1} of free SO_2) (Noxitan, Pascal Biotech, Paris), *Saccharomyces cerevisae* strain (20 g 100 kg^{-1}) (Fermol Rouge, Pascal Biotech, Paris) and commercial enzymes with pectolytic activity (2–4 g h L^{-1}) (Pectinex SPL/Ultra, Pascal Biotech, Paris) were added to the musts. Malic acid consumption by lactic acid bacteria occurred spontaneously within 60–75 days. Once alcohol fermentation had finished the wines were stored in French oak wood for approximately 1 year. Before bottling, Noxitan (35 mg L^{-1} of free SO_2, on average) was added. The wine samples from 2007 and 2006 vintages were analysed after 1 and 2 years of aging in bottle, respectively. The wines were stored at 10 °C prior to analysis.

2.3. Sample preparation for HPLC-DAD–MS analysis

The wine was purified and concentrated using the method described by Pastor del Rio and Kennedy (2006) with the following modifications. Ten millilitres of wine, dealcoholised under reduced pressure at 30 °C, were applied on the C18-SPE cartridge (1 g, Waters, Milford, MA) previously activated with 4 mL of methanol followed by 10 mL of water. The applied sample was washed with 50 mL of water, eluted with 40 mL of methanol, evaporated, and then dissolved in 2 mL of methanol. The sample preparation and analysis were carried out in triplicate for each wine.

2.4. PA composition

The PA subunit composition, percentage of galloylation (%G), percentage of prodelphinidins (%P), and mean degree of polymerisation (mDP), were determined after acid-catalysis in the presence of excess phloroglucinol (phloroglucinolysis) (Kennedy & Jones, 2001). A solution of 0.2 N HCl in methanol, containing 100 g L^{-1} phloroglucinol and 20 g L^{-1} ascorbic acid, was prepared. One hundred microlitres of concentrated and purified wine sample (item 2.3) was reacted with 100 μL of the phloroglucinol reagent at 50 °C for 20 min and then combined with 1000 μL of 40 mm aqueous sodium acetate to stop the reaction. The final solutions were filtered through 0.22 μm, 13 mm PTFE syringe tip filters (Millipore, Bedford, MA) into LC vials and immediately injected into a HPLC-DAD–MS system.

2.5. HPLC-DAD–MS analysis

The HPLC-DAD–MS analysis was performed on a Waters 2690 HPLC system (Waters, Milford, MA, USA) equipped with a Waters 996 DAD and a Micromass ZQ electrospray ionisation-mass spectrometer (ESI-MS) in negative mode. The compound separation was performed using an Atlantis C18 column (5.0 μm, 4.6 × 250 mm; Waters, Manchester, UK) protected by a guard column containing the same material. The flow rate was 0.90 mL min^{-1} and the injection volume 10 μL. The mobile phases consisted of 2.5% acetic acid in H_2O (A) and methanol (B). The separation (Fig. 1) was carried out at 40 °C in 47 min, under the following conditions: linear gradients starting at 5% B, to 6% B in 5 min, to 18% B in 25 min, to 30% B in 1 min, and finally to 100% B in 16 min. The column was then washed with 100% of B for 1 min and afterwards equilibrated for 7 min prior to each analysis. The UV–Vis spectra were recorded from 210 to 400 nm, with detection at 280 nm. The MS detector operated at a capillary voltage of 3000 V, extractor voltage of 6 V, source temperature of 150 °C, desolvation temperature of 500 °C, cone gas flow (N_2) of 50 L h^{-1} and a desolvation gas flow (N_2) of 1200 L h^{-1}. ESI-MS spectra ranging from *m*/*z* 100 to 1500 were taken in the negative mode with a dwell time of 0.1 s.

The quantification of the flavan-3-ols and PA dimers was performed by MS with the external standard method using the molecular ions $(M-H)^-$, which were *m*/*z* 289.3 for catechin and epicatechin, *m*/*z* 305.3 for gallocatechin and epigallocatechin, *m*/*z* 441.4 for epicatechin gallate and *m*/*z* 577.5 for B1 and B2 dimmers. The optimal cone voltage (CV) for all ions was 30 V. The phloroglucinol adducts were identified on the basis of their retention times and of their molecular ion (*m*/*z* 413.3 for C and EC-phloroglucinol;

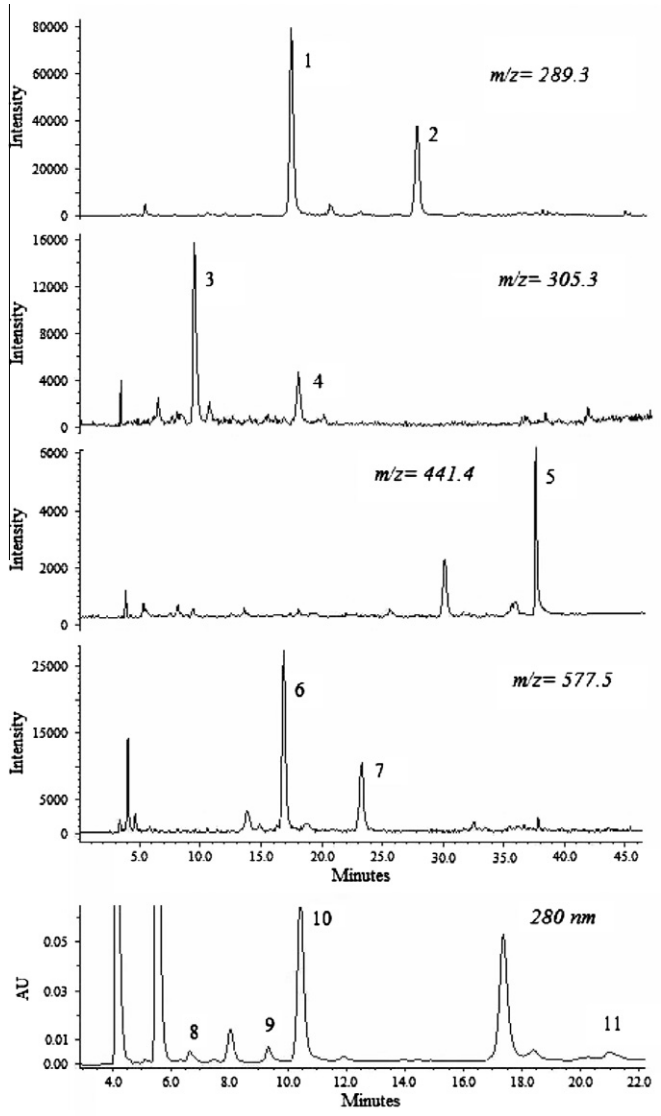

Fig. 1. HPLC-DAD–MS chromatograms of flavan-3-ols (MS) and phloroglucinol adducts (DAD). Peak numbering: 1, catechin; 2, epicatechin; 3, gallocatechin; 4, epigallocatechin; 5, epicatechin gallate; 6, B1; 7, B2; 8, epigallocatechin–phloroglucinol; 9, catechin–phloroglucinol; 10, epicatechin–phloroglucinol; 11, epicatechin gallate–phloroglucinol.

m/*z* 429.3 for EGC-phloroglucinol and *m*/*z* 565.5 ECG-phloroglucinol) and the main fragment by MS. Their quantification, as equivalents of their corresponding free flavan-3-ol (external standard method), was obtained by the UV signal at 280 nm, assuming the same molar absorptivity between each flavan-3-ol and its corresponding phloroglucinol adduct.

2.5.1. Method validation

The experimental limit of detection (LOD) and limit of quantitation (LOQ) for the HPLC–MS method were estimated at signal-to-noise ratios of 3 and 10, respectively. Method repeatability was assessed using one wine, and was based on 12 consecutive determinations with 12 purifications and concentration applied to the same wine. The distribution of the test results under repeatability conditions was estimated both for the direct HPLC–MS analysis of free flavan-3-ols and PA dimers, and for the HPLC-DAD–MS analysis of the proanthocyanidins after phloroglucinolysis.

2.6. Spectrophotometric analysis

Total phenols (TP) were directly measured using Folin–Ciocalteau reagent (Singleton & Rossi, 1965), and concentrations were determined by means of a calibration curve as gallic acid equivalents, mg L^{-1} of wine. The catechins and proanthocyanidins reactive to vanillin (PROC) were analysed according to Broadhurst and Jones (1978) and expressed as catechin equivalents per mg L^{-1} of wine. Also, the spectrophotometric analyses were performed in triplicate for each wine.

2.7. In vitro antioxidant activity

The free radical scavenging activity of the wine samples was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger method measured at 518 nm (Brand-Williams, Cuvelier, & Berset, 1995) and ABTS 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) according to Re et al. (1999), measured at 754 nm. Lipid peroxidation inhibition was assayed using the TBARS method, as described by Chen and Tappel (1996). Results were expressed as Trolox equivalents (mm TEAC). The analyses were carried out in triplicate.

2.8. Statistical analysis

Analysis of variance (ANOVA), the Tukey HSD Test and PCA were carried out using Statistica 7 (2006) (StatSotft Inc., Tulsa, OK) and $p < 0.05$ values were considered statistically significant.

3. Results and discussion

3.1. Method validation

The linear regression, the square of the correlation coefficient of the regression line, and the limits of detection and quantitation obtained from the calibration data for catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, PA B1 and PA B2 standards are shown in Table 1.

The % RSD obtained experimentally with 12 analyses of the wine sample were as follows: for free flavan-3-ols: catechin, 3.80%; epicatechin, 3.78%; gallocatechin, 4.04%; epigallocatechin, 2.87%; PA B1, 3.86%; and PA B2, 3.56%; for proanthocyanidins, terminal units: catechin, 4.71%; epicatechin, 4.07%; gallocatechin, 4.03%; epigallocatechin, 3.06%; and epicatechin gallate, 4.57%; and extension units: catechin, 6.75%; epicatechin, 3.17%; epigallocatechin, 1.87%; and epicatechin gallate 6.26%. All results were considered acceptable for research purposes.

3.2. Free flavan-3-ol composition

The flavan-3-ol monomers catechin (C), epicatechin (EC), gallocatechin (GC) and epigallocatechin (EGC) and PA dimers B1 and B2 were identified and quantified in wine samples of Cabernet Franc, Merlot, Sangiovese and Syrah, from 2006 and 2007 vintages, from São Joaquim – SC, Brazil (Fig. 1, Table 2). The main flavan-3-ol monomers found were catechin and epicatechin. These results are in agreement with those in the literature, since these are the main monomers in the skin and seeds of grapes (Chira et al., 2009; Mattivi, Vrhovsek, Masuero, & Trainotti, 2009; Prieur et al., 1994) and, consequently, in wine. Catechin was the main monomer in the wine samples evaluated, with the highest concentrations observed in all samples, representing, on the average, 60% of the total monomers, as also observed in other studies (Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Ricardo da Silva, 2003). The highest concentrations of catechin were observed in Merlot 2007

Table 1
Linear regression, R^2, limits of detection (LOD) and quantitation (LOQ) obtained for the standards of free flavan-3-ols and PAs B1 and B2.

Compound	Linear regression $y = ax + b$	R^2	LOD (mg/L)	LOQ (mg/L)
Catechin	$y = 905500x - 88{,}100$	0.9962	0.11	0.36
Epicatechin	$y = 89300x + 169{,}000$	0.9951	0.08	0.27
Gallocatechin	$y = 111000x + 21{,}800$	0.9956	0.27	0.93
Epigallocatechin	$y = 106000x + 39{,}800$	0.9932	0.32	1.06
Epicatechin gallate	$y = 109000x + 199{,}000$	0.9937	0.03	0.10
PA B1	$y = 43600x + 26{,}400$	0.9965	0.15	0.49
PA B2	$y = 40700x + 8440$	0.9948	0.17	0.58

Table 2
Content of monomeric and dimeric flavan-3-ols, total phenols (TP) and total proanthocyanidins (PROC) in wine samples.

Vintage	2006				2007			
	Cabernet Franc	Merlot	Sangiovese	Syrah	Cabernet Franc	Merlot	Sangiovese	Syrah
Catechin	12.25 ± 0.09^{a}	25.03 ± 0.37^{b}	20.38 ± 0.41^{c}	29.72 ± 0.78^{d}	22.03 ± 0.57^{c}	34.71 ± 0.28^{e}	13.72 ± 0.06^{a}	19.54 ± 0.54^{c}
Epicatechin	4.64 ± 0.19^{a}	10.44 ± 0.29^{b}	8.23 ± 0.18^{c}	11.89 ± 0.44^{d}	5.69 ± 0.18^{a}	16.08 ± 0.63^{e}	4.07 ± 0.09^{a}	9.14 ± 0.10^{c}
Gallocatechin	3.59 ± 0.06^{a}	3.64 ± 0.04^{a}	2.91 ± 0.06^{b}	3.28 ± 0.07^{c}	4.59 ± 0.09^{d}	4.07 ± 0.05^{e}	6.26 ± 0.06^{f}	1.92 ± 0.05^{g}
Epigallocatechin	1.47 ± 0.06^{a}	2.35 ± 0.05^{b}	1.91 ± 0.03^{c}	2.06 ± 0.02^{c}	1.89 ± 0.06^{c}	2.53 ± 0.03^{b,d}	2.66 ± 0.01^{d}	1.87 ± 0.07^{e}
PA B1	6.38 ± 0.14^{a}	17.19 ± 0.31^{b}	10.76 ± 0.23^{c}	20.64 ± 0.69^{d}	12.75 ± 0.33^{c}	35.47 ± 0.42^{e}	10.33 ± 0.38^{c}	24.30 ± 0.34^{f}
PA B2	2.37 ± 0.04^{a}	8.02 ± 0.14^{b,d}	5.63 ± 0.09^{c}	9.86 ± 0.25^{b}	7.29 ± 0.12^{c,d}	25.87 ± 0.37^{e}	5.58 ± 1.52^{c}	12.20 ± 0.11^{f}
Total monomers	21.95^{a}	41.46^{b}	33.44^{c}	46.95^{d}	34.20^{c}	57.40^{e}	26.71^{f}	32.47^{c}
Total dimers	8.75^{a}	25.20^{b}	16.40^{c}	30.49^{d}	20.04^{b}	61.34^{e}	14.92^{c}	36.51^{f}
TP	2680.4 ± 12.4^{a}	2692.1 ± 40.3^{a}	2287.6 ± 31.1^{b}	2732.2 ± 37.2^{a}	2691.4 ± 26.2^{a}	2813.7 ± 16.2^{c}	2732.1 ± 27.9^{a}	2790.5 ± 34.2^{a}
PROC	828.2 ± 11.8^{a}	894.9 ± 10.9^{b}	725.6 ± 19.2^{c}	860.6 ± 14.2^{b}	808.3 ± 16.3^{a}	1073.9 ± 31.1^{d}	867.9 ± 25.2^{b}	872.1 ± 24.3^{b}

Values in units of mg L^{-1} ± standard deviation over three replications in one wine sample. ANOVA to compare data; values with different letters within each row are significantly different (Tukey test, $p < 0.05$).

and Syrah 2006 samples. Epicatechin represented approximately 25% of the monomers quantified in the samples, with concentrations ranging from 4 to 16 mg L^{-1}, Merlot and Syrah being the varieties showing the highest concentrations. It was observed that concentrations of catechin in Merlot and Cabernet Franc varieties were higher in the 2007 vintage compared to the previous vintage, and the opposite was observed for Sangiovese and Syrah varieties. The same behaviour was observed for epicatechin.

In agreement with other studies (Prieur et al., 1994), gallocatechin and epigallocatechin were present at lower levels. The percentage of gallocatechin ranged from 6% to 23% of the total monomers. Among the studied samples Sangiovese 2007 and Cabernet Franc 2006 and 2007 variety samples contained ~23% and 15% of the total monomers as gallocatechin, respectively. Epicatechin gallate was responsible for ~6% of the free monomers quantified in this study.

Among the proanthocyanidins oligomers, dimers B1 and B2 are present at higher concentrations in grapes and, consequently, in wine (Monagas et al., 2003). PA B1 was the main dimer in the wine samples, contributing with more than 60% of the dimers, as also reported in other studies (Cosme, Ricardo-da-Silva, & Laureano, 2009). PA B1 is the main dimer in grape skins and during wine fermentation it is easier to extract than PA B2, present in high concentrations in seeds. Thus, for the varieties investigated, flavan-3-ols from grape skins contributed more to the wine flavan-3-ol composition, in agreement with results reported in the literature (Fernández, Kennedy, & Agosin, 2007).

Merlot and Syrah wine samples showed the highest values for the sum of total monomers and dimers flavan-3-ols, especially for Merlot 2007 (118 mg L^{-1}). Regarding percentage distribution, Cabernet Franc and Syrah wines, 2006 vintage, presented the highest monomer contents, followed by Sangiovese and Cabernet Franc, 2007 vintage, Merlot and Syrah, 2006 vintage, and Merlot and Syrah, 2007 vintage. The highest proportions of dimers were present in the samples of Merlot and Syrah from 2007 vintage (up to 51%), a finding previously observed in the Spanish wines Tempranillo, Graciano and Cabernet Sauvignon by Monagas et al. (2003). It is interesting to note that both the vintage and grape variety influenced the flavan-3-ol composition of the wines ($p < 0.05$), but with different behaviours according to the vintage. This was also observed by Chira et al. (2009), who evaluated, for two consecutive vintages, the tannin composition from the skin and seed extracts of Merlot and Cabernet Sauvignon grapes in Bordeaux, France.

3.3. Proanthocyanidin composition (PAs)

Grape and wine PAs are constituted of several oligomers and polymers with a very complex molecular structures. Phloroglucinolysis, which is the depolymerisation of PAs in an acid environment in the presence of phloroglucinol, gives access to important information regarding PA composition (Kennedy & Jones, 2001). Data on the PA structural composition of wine samples are shown in Table 3. Structurally, PAs present in wine samples comprised catechin, epicatechin, gallocatechin and epicatechin gallate as terminal and extension units, only gallocatechin not being detected as an extension unit (Table 3). Thus, the PAs of the wine samples analysed consisted of a mixture of procyanidins and prodelphinidins. The terminal units of wine samples were mainly comprised of catechin (from 55% to 66%), as also observed in other studies for both grape skin and seed (Mattivi et al., 2009; Pastor del Rio & Kennedy, 2006). Merlot 2007 and Syrah (2006 and 2007) wines showed the highest concentrations of the terminal unit catechin, followed by Cabernet Franc and Sangiovese wines. The highest proportions of this terminal unit were observed in all samples of 2006 vintage and in the Syrah 2007 wine. The epicatechin terminal unit had the second higher concentrations and proportions (from 22% to 41%). The Merlot 2007 wine presented the highest concentrations and proportions of epicatechin terminal units (40.8%), followed by Cabernet Franc and Syrah 2007.

The highest proportion of gallocatechin terminal units (10.8%) was found in the Sangiovese 2007 wine sample, followed by Syrah wines, in both vintages evaluated (2.5%), while the lowest was found in Merlot 2006 wine (0.6%). The highest percentage of epigallocatechin (8.2%) was, as for gallocatechin, found in Sangiovese

Table 3
Structural characteristics and composition (percent in moles) of PAs in wine samples.

Vintage	2006				2007			
	Cabernet Franc	Merlot	Sangiovese	Syrah	Cabernet Franc	Merlot	Sangiovese	Syrah
Terminal units								
Catechin	66.1[a]	66.0[a]	65.4[a]	66.0[a]	57.1[b]	54.3[c]	59.1[b]	64.1[d]
Epicatechin	27.9[a]	28.1[a]	28.2[a]	26.1[b]	36.1[c]	40.8[d]	22.1[e]	32.4[f]
Gallocatechin	1.2[a,c]	0.6[b]	1.0[c]	2.5[d]	1.2[a,c]	1.4[a]	10.4[e]	2.6[d]
Epigallocatechin	4.8[a]	5.3[a]	5.4[a]	5.4[a]	5.4[a]	3.4[b]	8.2[c]	1.0[d]
Epicatechin gallate	nd	nd	nd	nd	0.15[a]	0.13[a]	0.19[a]	0.11[a]
Extension units								
Catechin	0.6[a]	0.8[b]	0.7[a]	0.8[b]	0.5[c]	1.0[d]	0.4[c]	0.6[a]
Epicatechin	52.8[a]	55.3[b,c]	54.9[b]	56.1[c]	51.4[a]	61.2[d]	52.8[a]	44.9[e]
Epigallocatechin	44.6[a]	42.0[b]	41,6[b]	41.1[b,e]	46.4[c]	36.0[d]	44.9[c]	38.8 [e]
Epicatechin gallate	2.0	1.9	2.9	2.1	1.7	1.8	1.9	2.3
Total terminal units*	67.7[a]	49.0[b]	48.1[b]	76.7[c]	71.2[a,c]	94.6[d]	64.3[a]	73.2[a,c]
Total extension units*	261.0[a]	284.9[a]	215.9[b]	326.3[c]	349.7[c]	461.0[d]	568.3[e]	423.97[d]
mDP[A]	4.9[a]	6.9[b]	5.5[c]	5.2[c]	5.9[d]	5.8[d]	9.8[e]	6.8[b]
%P[B]	36.6[a]	36.7[a]	35.1[b]	34.8[b]	39.7[c]	30.5[d]	41.3[c]	33.7[e]
%G[C]	1.5[a,c]	1.6[a,b]	2.4[d]	1.7[b,c]	1.4[e]	1.5[a,c,e]	1.6[a,c]	2.0[f]

Values over three replications in one wine sample. ANOVA to compare data; values with different letters within each row are significantly different (Tukey test, $p < 0.05$). nd, Not detected.
* Values in units of mg L^{-1}.
[A] mDP, mean degree of polymerisation.
[B] %P, percentage of prodelphinidins (total, in terminal plus extension units).
[C] %G, percentage of galloylation (total, in terminal plus extension units).

2007. Epicatechin gallate was the only gallate-derivative found in terminal units, and only in samples from the 2007 vintage, corresponding to an average of 0.15% of the terminal units. Usually, concentrations of the gallates as terminal units in wines are low, or even undetectable (Fernández et al., 2007; Monagas et al., 2003). This finding has also been observed in grape skins (Chira et al., 2009; Mattivi et al., 2009).

The extension units present in lowest concentrations were catechin and epicatechin gallate (Table 3). The extension unit catechin represented up to 1.0% of the total extension units and epicatechin gallate up to 2.9%. Extension units of wine samples mainly comprised epicatechin and epigallocatechin, with a predominance of epicatechin, which represented more than 44% of the extension units. A similar profile has been observed by other researchers (Pastor del Rio & Kennedy, 2006; Prieur et al., 1994) with a small variation among varieties. Epicatechin represented 44.9–61.2% of all extension units, while epigallocatechin represented 36–46% of all extension units, suggesting a high contribution of proanthocyanidins from grape skins in the wine samples evaluated.

Comparing the two vintages, it was found that total PAs in the wine samples from the 2006 vintage was significantly lower than for the 2007 vintage. The total concentration of the extension units in the 2007 vintage was significantly higher than in wines of the previous vintage ($p \leqslant 0.05$). This is probably due to climatic differences observed between the two vintage years evaluated. In the 2007 the temperature and the GDD values observed were higher than in the previous year (data not shown). Many authors have confirmed that the sun exposure, temperature and GDD positively influence the PA concentration (Pastor del Rio & Kennedy, 2006). An alternative hypothesis, which cannot be ruled out, is the involvement of PAs in the polymerisation reactions generating – in older wines – new structures which are less hydrolysable by phloroglucinolysis.

The percentage of galloylation (%G) of the analysed wine samples (1.5–2.4%G) is in agreement with other published results (Fernández et al., 2007), although values higher than those presented in our study have also been reported (Cosme et al., 2009). The %G is relatively small in wine probably because, in general, higher concentrations of the gallate-derivatives are present in the seeds (Mattivi et al., 2009; Prieur et al., 1994), therefore the extraction of these compounds into wine is more difficult when compared with the PAs present in the skin. Also, according to Di Stefano, Cravero, and Guidoni (1990), the PAs of the grape seeds are a source of free gallic acid in the wine, which also decreases the concentration of gallate-derivatives of PAs in the wines.

In the present study, the percentage of prodelphinidin (sum of both terminal and extension units, %P) ranged from 30.2 to 41.3. Similar values have been observed in several studies (Cosme et al., 2009). The highest values were obtained for Sangiovese and Cabernet Franc samples, 2007 vintage, due to higher concentrations of gallocatechin and epigallocatechin in these samples. Merlot and Syrah, 2007 vintage, showed the lowest values of %P. The %P reveals the percentage of the contribution of gallocatechin and epigallocatechin and indicates the contribution of skin PAs in wines, since prodelphinidins are absent in the seeds.

The mDP reveals the polymerisation degree of PAs and can influence the flavan-3-ol bioavailability and bioactivity. The mDP values observed in our study ranged from 4.9 to 9.8, for Cabernet Franc 2006 and Sangiovese 2007, respectively. These results are in agreement with other reported values (Cosme et al., 2009; Monagas et al., 2003). It was also observed that the mDP values of the 2007 wine samples were higher than those of the 2006 vintage, due to the higher concentration of extension units in the 2007 vintage. These data agree with those of Drinkine, Lopes, Kennedy, Teissedre, and Saucier (2007) who evaluated different wines from various vintages from Bordeaux and found that the mDP values decreased with age. According to the results obtained for the mDP values, it can be concluded that, generally, the PAs of the wine samples are rich mainly in oligomers and short-chain polymers (mDP around 5–9).

The ANOVA analysis revealed significant differences ($p < 0.05$) for the flavan-3-ol composition of wine samples as a function of both variety and vintage factors, a finding which has been commonly reported. According to Mattivi et al. (2009) the biosynthesis of flavan-3-ols and PAs in grapes seems to be highly specific at the variety level. It is interesting to note that the composition of flavan-3-ols can vary significantly with grape variety and vintage and can also be influenced by environmental conditions (Mattivi et al., 2002). Wine composition is in constant evolution during winemaking, storage in barrels and aging in bottles. According to

Ribéreau-Gayon, Glories, Maujean, and Dubourdieu (1998), once a wine is bottled, transformations that occur are dominated by nonoxidative reactions. Nevertheless, according to Lopes, Saucier, Teissedre, and Glories (2006) wines are subjected to oxidative reactions if the bottle closure procedure allows oxygen ingress. Thus, all these changes influence the phenolic composition of wine and consequently of flavan-3-ols, which makes it very complex to study these compounds in wines.

Concentrations of free flavan-3-ols and PAs observed in wines produced in this new wine-producing region in southern Brazil are considered appropriate, being in agreement with those observed in several other studies (Cosme et al., 2009; Monagas et al., 2003; Pastor del Rio & Kennedy, 2006). This is of great importance since PAs will greatly influence the wine quality, affecting the wine colour through condensation with anthocyanins, and its sensory properties (Chira et al., 2009), besides having beneficial health effects, especially in terms of the potential antioxidant activity which is also essential to assure the chemical stability towards oxidation of red wines (Mattivi et al., 2002; Rigo et al., 2000).

3.4. Antioxidant activity

The *in vitro* antioxidant activity of the wines Cabernet Franc, Merlot, Sangiovese and Syrah, 2006 and 2007 vintages, were evaluated through the capacity to scavenge DPPH and ABTS radicals. Results are shown in Fig. 2, where an important antioxidant activity of the wine samples, ranging from 11.2 to 23.17 mm TEAC, can be observed. Samples from the 2007 vintage were found to be more effective, and this scavenging activity was estimated to be higher for the ABTS radical. The antioxidant activity of wine and its phenolic compounds has been widely studied, being considered partly responsible for the beneficial effects of moderate wine consumption (Frankel et al., 1995).

Lipid peroxidation is one of the most severe types of damage caused by an excess of free radicals in the organism. MDA is a important reactive aldehyde resulting from the peroxidation of biological membranes. Increased accumulation of MDA and conjugated dienes in the cell can result in cellular degradation, and biochemical and functional changes, which can eventually lead to cell death. In this study we evaluated the potential of wines in the inhibition of *in vitro* lipid peroxidation by the TBARS method. Fig. 2 shows the capacity of the wine samples to inhibit lipid peroxidation, which can be considered effective based in previous research of Filip and Ferraro (2003). These authors found that the antioxidant activity (inhibition lipid peroxidation – TBARS) of red wine was 8.85 mm TEAC and 7.78 mm TEAC for *Ilex brevicuspis* extract, a plant used in South America as tea-like beverage. The most significant values were observed for samples of Cabernet Franc, Merlot and Syrah from 2007 vintage. The ability of wine to inhibit lipid peroxidation has been observed in other studies (Frankel et al., 1995; Rigo et al., 2000) and has been ascribed to the ability of wine antioxidants to scavenge peroxy radicals.

Although it is well known that wine is a complex mixture of compounds which can act synergistically and be responsible for the antioxidant properties (Cirico & Omaye, 2006), it is also known that there are groups which can act more effectively as antioxidants, such as the proanthocyanidins. It is believed that the antioxidant potential of red wines is due, mainly, to their content of flavan-3-ols and PAs (Rice-Evans, Miller, & Paganga, 1996; Rigo et al., 2000). In this context, the influence of the flavan-3-ol and PA compositions on the *in vitro* antioxidant activity of our wine samples was assessed by principal components analysis (Fig. 3).

The first three principal components explained 82.02% of the total variance (Fig. 3). Factor 1 was negatively influenced by the main chemical and antioxidant analysis. C, EC, B1, B2, mDP, TBARS, DPPH and ABTS influenced negatively Factor 1 and B2 and %P influenced positively Factor 2. Fig. 3 shows that inhibition of lipid peroxidation, TBARS, and the ABTS radical scavenging were positively correlated with EC, B1, C, B2, EGC. Scavenging of the DPPH radical was strongly positively correlated with TP and PROC, these two being parameters also positively correlated with ABTS and TBARS.

In Fig. 3 it can also be observed that Factor 1 separated the wine samples into two distinct groups for each vintage. Wines from the 2006 vintage were all located on the right and positive side and wines from the 2007 vintage were located on the negative side. Wines from the 2007 vintage were associated with the major analysis carried out. This is probably due to higher concentrations of the compounds observed in the 2007 vintage, which also promoted, in general, higher antioxidant activity of the wines. The

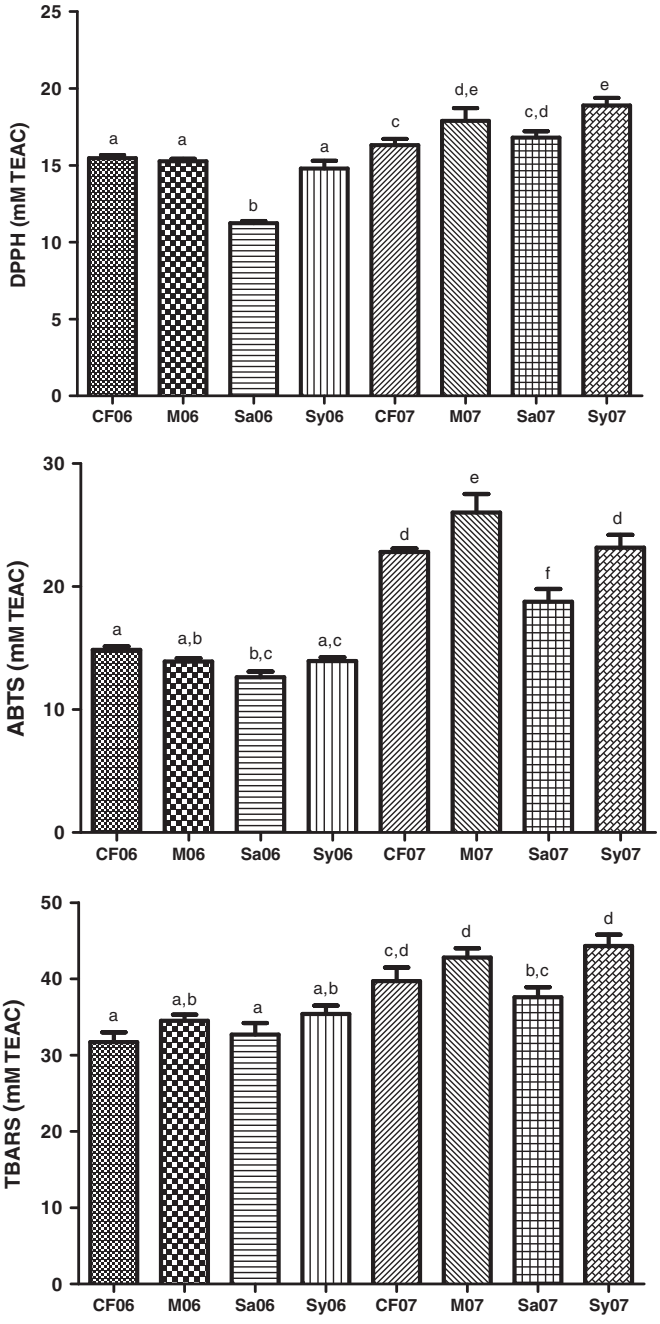

Fig. 2. DPPH, ABTS free radical scavenging activity and lipid peroxidation index (TBARS; mm TEAC) of Cabernet Franc (CF), Merlot (M), Sangiovese (Sa) and Syrah (Sy) wine samples from 2006 (06) and 2007 (07) vintages.

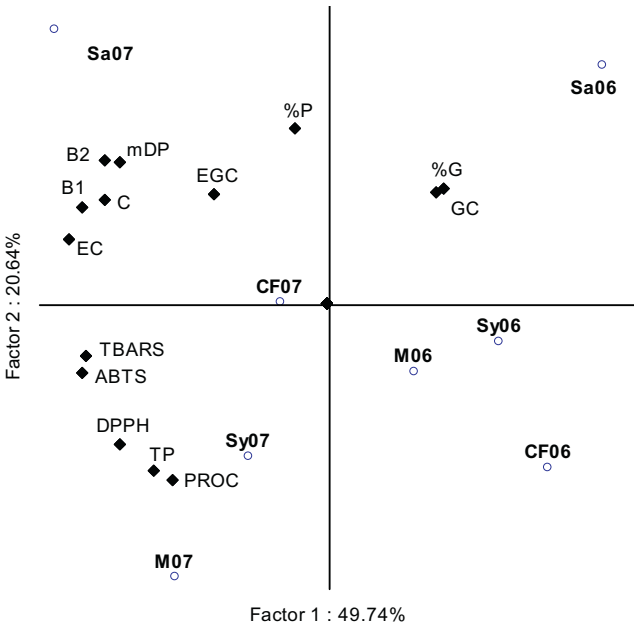

Fig. 3. Principal component analysis of the free flavan-3-ol profile, %P, %G, mDP, ABTS, PROC, TP, DPPH and TBARS. CF, Cabernet Franc; ME, Merlot; Sa, Sangiovese; Sy, Syrah.

Sangiovese 2006 wine was located in the upper quadrant and separated from other wines of the same vintage because of its higher %G. Wines from the 2007 vintage, Merlot and Syrah, were associated with TP and PROC values and with the TBARS, DPPH and ABTS analysis; Cabernet Franc and Sangiovese were associated with %P, C, EC, EGC, mDP, B1 and B2 values.

The high correlation between TP and PROC and *in vitro* antioxidant activity of wines has been reported by Rossetto et al. (2004). The observed flavan-3-ols antioxidant properties are probably due to the structure of these compounds. According to Rice-Evans et al. (1996), polyphenols with the ortho-dihydroxy structure in the B ring have the highest scavenging activities. The degree of polymerisation also influences the antioxidant activity of PAs (Rossetto et al., 2004), and in this study we found that mDP was positively correlated with TBARS and ABTS. This hypothesis is supported by the fact that the antioxidant activity of proanthocyanidins is, in part, dictated by the oligomer chain length. Flavan-3-ol monomers and dimers were found to inhibit more efficiently LDL oxidation than trimers and tetramers (Plumb, De Pascual-Teresa, Santos-Buelga, Cheynier, & Williamson, 1998).

According to some authors, the presence of prodelphinidin increases the antioxidant capacity of PAs due to the increase in the reactive hydroxyl number (Rice-Evans et al., 1996). In this study, high amounts of %P were observed and this probably contributed to the total antioxidant capacity observed, although this parameter has not been associated directly with antioxidant analysis. Esterification of position 3 with acid is another important factor that positively affects the scavenging capacity of grape PAs (Rice-Evans et al., 1996). This correlation was not found in our study probably due to the low concentrations of %G and GC observed in the wine samples.

Studies on flavan-3-ols as target compounds in research involving the antioxidant activity of wines are important since it has been proposed that these compounds can react with biomolecules and thus modify their metabolism and functions (Galati, Lin, Sultan & O'Brien, 2006). According to some authors the main function of catechins as antioxidants in the organism is the scavenging capacity of reactive oxygen and nitrogen species (Plumb et al., 1998), which can promote an increase in total antioxidant capacity in the organism and, as a consequence, improve the antioxidant defence system and reduce damage caused by these reactive species in the organism. Raza and John (2007) suggested that catechin and their derivatives may affect the metabolism of GSH *in vitro*, by conjugation of these compounds with GSH and through inhibition of enzymes such as GST and GPx. One report suggested the conjugation of EGCG with GSH under *in vivo* conditions (Galati et al., 2006).

4. Conclusions

In conclusion, this study presents the free flavan-3-ol and PA composition and *in vitro* antioxidant capacity of the wines Cabernet Franc, Sangiovese and Syrah, 2006 and 2007 vintages, from a new wine growing region in the south of Brazil. Until now, these analyses have never been studied in wines of this region. The quantitative method using HPLC-DAD–MS allowed the precise identification and quantification of the monomers catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin gallate, and the dimers B1 and B2 along with their adducts after phloroglucinolysis, giving access to the nature of the terminal and extension units of the PAs. Flavan-3-ol and PA concentrations were in line with those reported in the literature from the most renowned regions of premium wine production; the composition of these compounds correlated positively with the *in vitro* antioxidant activity of the wine samples, with differences among the varieties and vintages studied being evident. These interesting results further support the potential of the region to produce high quality wines.

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