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Original Research Article

Phenolic profile and effect of regular consumption of Brazilian red wines on *in vivo* antioxidant activity

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ABSTRACT

In this study, *Vitis vinifera* L wines cv. Cabernet Franc, Merlot, Sangiovese and Syrah, 2006 and 2007 vintages, produced in São Joaquim, a new wine-producing region in southern Brazil, were evaluated. As phenolic compound content is one of the most important parameters in assessing wine quality and is possibly partially responsible for the beneficial health properties of wines, in this paper the levels of the main anthocyanins, flavonols, hydroxycinnamic acid and hydroxybenzoic acid (HPLC-DAD and HPLC-DAD–MS analysis) and the *in vivo* antioxidant activity in mice are reported. The antioxidant capacity of plasma was assessed through the reduction of ferric iron (FRAP). Lipid peroxidation (TBARS), carbonyl protein (CP), reduced glutathione (GSH) levels and the catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity were determined in livers of the test animals. The results for the phenolic compounds content of the wine samples were considered appropriate for quality red wines, and the wine consumption promoted a significant increase in FRAP and decreases in the TBARS and CP levels and in the CAT, SOD and GPx activity. Moreover, the phenolic content of the wines was positively correlated with the *in vivo* antioxidant capacity promoted by regular wine consumption.

1. Introduction

A number of epidemiological studies have demonstrated the correlation between an unbalanced diet and coronary heart diseases, some types of cancer and diabetes. Epidemiologists have observed that a diet rich in polyphenolic compounds may provide a positive effect due to antioxidant properties (Renauld and Lorgeril, 1992; Frankel et al., 1995). Wine is an important component in Mediterranean dietary traditions, and it is rich in antioxidant compounds. These compounds have a functional role, acting against free radicals, as well as a physiological role; in fact, they can increase the antioxidant capacity of the human body following red wine consumption (Renauld and Lorgeril, 1992). Furthermore, phenolic compounds constitute one of the most important wine quality parameters since they contribute to the organoleptic characteristics, particularly color, astringency and taste (Vrhovsek, 1998).

The flavonoid composition of red wines includes anthocyanins, catechins, and flavonols. The main flavonols are myricetin, quercetin, kaempferol, syringetin and laricitrin (Mattivi et al., 2006). Anthocyanins are the main phenolic compounds associated with the color of red wines and are antioxidants (Rice-Evans et al., 1996; Rossetto et al., 2004). These compounds are present in *Vitis vinifera* grapes as glycosylated anthocyanidins with glucose attached to the 3-hydroxyl position, which can be esterified by different organic acids (Mazza, 1995; Rice-Evans et al., 1996).

The main non-flavonoid compounds in wine are the phenolic acids (various benzoic and cinnamic acid derivatives). They play a primary role in defining the sensorial characteristics of wines, giving the "oak wood" taste typical of long-aged products, besides being largely responsible for the astringency and bitterness of young wines (Somers et al., 1987; Vrhovsek, 1998; Monagas et al., 2005). Hydroxycinnamic acids and their tartaric esters are the

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main class of non-flavonoid phenolics in red wines. They are involved in the browning reactions of must and wine and are precursors of volatile phenols (Vrhovsek, 1998). Gallic acid is the main hydroxybenzoic acid in red wine, which is formed mainly through the hydrolysis of flavonoid gallate esters. Wines matured in oak present high levels of hydroxybenzoic acid derivatives, mainly ellagic acid.

The chemical characterization of wine phenolics is currently an issue for several reasons: it can aid the evaluation of the authenticity of regional products and the prediction of the sensory properties and oxidative stability of wine (Lopes et al., 2006). Moreover, phenolics are used as markers of the wine processing technology or wine aging (Ribéreau-Gayon et al., 1998; Vrhovsek, 1998).

São Joaquim is a recent wine-making region in southern Brazil. This region is situated in the high plains of Santa Catarina State and is known as the coldest place in the country, at altitudes ranging from 1200 to 1400 m. Recent papers have reported interesting results that support the potential of the region to produce high quality wines. Falcão et al. (2008a,b) reported important results regarding the sensory profile and the main aroma-impact components of Cabernet Sauvignon wines from this region. These authors established a positive relationship between vineyard altitude and pyrazine content. Gris et al. (2011a) verified that flavan-3-ol and proanthocyanidin concentrations were in line with those reported in the literature for wine from the most renowned production regions. Moreover, the results presented by Gris et al. (2011b) showed that some wines from this region have an unusually high content of stilbenes. Furthermore, the consumption of these wines by laboratory animals submitted to a hypercholesterolemic diet reduced significantly the hypercholesterolemia and hypertriglyceridemia, and also decreased the atherogenic level and increased significantly the HDL level.

However, data on the detailed phenolic composition and *in vivo* antioxidant capacity (in Swiss mice) have not previously been reported. The aim of this study was therefore to evaluate the association between *in vivo* antioxidant capacity and phenolic contents. To this end the concentration of the main flavonoid and non-flavonoid compounds and their contribution to the antioxidant activity in Cabernet Franc, Merlot, Sangiovese and Syrah wines, of the 2006 and 2007 vintages, produced in São Joaquim, Santa Catarina State, southern Brazil were investigated.

2. Materials and methods

2.1. Standards and reagents

All chromatographic solvents were HLPC grade and were purchased from Carlo Erba (Rodano, Italy). Pure, HPLC grade myricetin, quercetin, laricitrin, kaempferol, isorhamnetin, syringetin and malvidin 3-glucoside chloride, were purchased from Extrasynthèse (Genay, France); ellagic acid, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid and *trans*-*p*-coumaric acid were purchased from Sigma Chemical Co. (Steinheim, Germany); *trans*-caftaric acid, *trans*-coutaric acid and *trans*-fertaric acid were isolated from Grenache grapes as described by Meyer et al. (1998); 2,5 dihydroxybenzoic acid, vanillic acid, and ferulic acid were purchased from Fluka (Steinheim, Germany); trichloroacetic acid, 2,4-dinitrophenylhydrazine (DNPH), 2-thiobarbituric acid (TBA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), butylated hydroxytoluene (BHT), epinephrine, hydrogen peroxide, *tert*-butyl hydroperoxide, 2,4,6-tripyridyl-s-triazina (TPTZ), TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), β-nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase (GR) and reduced glutathione (GSH) were purchased from Sigma–Aldrich Co.

2.2. Characterization of the wine-growing region

São Joaquim is located in the high plains of Santa Catarina State at altitudes ranging from 1200 to 1400 m, and is the viticulture region situated at the highest altitude in Brazil. The soil of this region is of the type Inceptisol according to U.S.D.A. classifications and the climate of the São Joaquim region is "Cool, Cool nights and Humid" according to the Geoviticulture Multicriteria Climatic Classification System, and as "Region I" (<1389 GDD), a "cold region", in terms of the Winkler Regions (Gris et al., 2011a,b).

2.3. Samples

2.3.1. Grapes

The wines were produced from Cabernet Franc, Merlot, Sangiovese and Syrah grapes, 2006 and 2007 vintages, from young commercial vineyards in São Joaquim, Santa Catarina State, Brazil. These vineyards are located at an altitude of 1290 m, latitude of 28° 15′ and longitude of 49° 50′. The vines investigated in this study were planted in 2003 and the clones used were 986, 181, VCR23 and VCR1, respectively. The rootstock was Paulsen 1103 (*Vitis berlandieri Planch* × *Vitis rupestris Scheele*), the vertical shoot positioning trellis system was used, the row and vine spacing were 3.0 and 1.2 m, respectively, and the vineyard yield was between 6 and 7 t/ha. The grapes were harvested at the stage of technical maturity and had sugar readings of 19.1–24.2°Brix, 0.57–0.81 mg L^{-1} titratable acidity and pH values between 3.31 and 3.58.

2.3.2. Wine samples

The wines were all produced under the same conditions in a commercial winery in São Joaquim as described by Gris et al. (2011a,b). The wine samples from the 2007 and 2006 vintages were analyzed after one and two years of aging in the bottle, respectively. The wines were stored at 10 °C prior to analysis.

2.4. Determination of phenolic compounds

2.4.1. Spectrophotometric analysis of total phenols (TP)

Total phenols (TP) were directly measured using the Folin-Ciocalteu reagent method (Singleton and Rossi, 1965) and concentrations were determined by means of a calibration curve as mg gallic acid/L of wine.

2.4.2. Flavonols content

The flavonols (myricetin, quercetin, laricitrin, kaempferol, isorhamnetin and syringetin) contents of the wine samples were determined by HPLC after acid hydrolytic cleavage of the flavonol conjugates (Mattivi et al., 2006). HPLC separation and quantification of flavonols was carried out according to Mattivi et al. (2006) using a Waters 2695 HPLC system equipped with a Waters 2996 DAD (Waters, Milford, MA), using a Purospher RP18 reversed-phase column of 250 mm × 4 mm (5 μm), with a precolumn (Merck, Germany). The following solvents were used: (A) 0.3% $HClO_4$ in water; (B) methanol. The linear gradient was from 40 to 90% B in 30 min and the flow rate was 0.45 mL/min. The column equilibration time was 5 min and the injection volume was 5 μL. The presence of flavonol aglycons was confirmed by co-injection with the corresponding standards. Each flavonol was quantified at 370 nm and expressed as mg mL^{-1} of wine by means of calibration with external standards.

2.4.3. Free anthocyanins content

The sample preparation for the determination of the main free anthocyanins in the wine samples was carried out according to Rossetto et al. (2004). Based on Mattivi et al. (2006), the samples

were filtered through 0.22 μm, 13 mm PTFE syringe tip filters (Millipore) into LC vials and immediately injected employing the same system, column and eluents used for the HPLC-DAD analysis of flavonols. Separation of the 15 main free anthocyanins was obtained at 40 °C with a flow rate of 0.45 mL/min. The binary gradient was applied as follows: from 27 to 44.5% of B in 32 min, then to 67.5% of B in 13 min, to 100% B in 2 min, isocratic 100% B for 3 min; total analysis time, 50 min. Delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, and their relevant acetic acid and *p*-coumaric acid esters were identified according to Castia et al. (1993) and quantified at 520 nm using a calibration curve with malvidin 3-glucoside chloride.

2.4.4. Hydroxycinnamic acids (HCAs)

Sample preparation and HPLC separation and quantification of HCAs (with modifications) were performed according to Vrhovsek et al. (2004). The same system and column used for the flavonols were used for the determination of HCAs. The following solvents were used: (A) 0.5% formic acid in water and (B) 2% formic acid in methanol. The gradients were as follows: from 16% to 19% B in 7 min, from 19% to 53% B in 12 min, from 53% to 100% B in 0.1 min, 100% B for 5 min, back to 16% B in 0.1 min. The column was equilibrated for 7 min prior to each analysis. The flow rate was 0.4 mL/min and the injection volume 10 μL. The detection of HCAs was carried out at 320 nm. Each compound was quantified as mg mL^{-1} of wine by means of calibration with an external standard. The %RSD values obtained experimentally from six consecutive determinations of the same wine were as follows: *cis*-caftaric acid, 0.89%; *trans*-caftaric acid, 0.64%; *cis*-cutaric acid, 0.57%; *trans*-cutaric acid, 0.32%; fertaric acid, 0.43%; *trans*-caffeic acid, 0.64%; *trans*-*p*-coumaric acid, 0.53%; and *trans*-ferulic acid, 0.82%.

2.4.5. Hydroxybenzoic acids

Sample preparation for *p*-hydroxybenzoic, syringic and vanillic acids was carried out as follows: 10 mL of wine and 1.0 mL of internal standard (2,5-dihydroxybenzoic acid, 100 mg L^{-1}) were concentrated to approximately 7 mL using rotary evaporation and reduced pressure at 35 °C. After concentration, the sample pH was adjusted to 8.0 with NaOH and the sample was made up to the initial volume (10 mL) with distilled water. An aliquot of 5 mL was added to a C18-SPE cartridge (1 g, Waters), previously activated with methanol (5 mL) and water (10 mL). The eluate was collected, its pH value was adjusted to 2.7 with formic acid and the volume was completed to 10 mL with distilled water. The final sample was filtered through 0.22 μm, 13 mm PTFE syringe tip filters (Millipore, Bedford, MA) into LC vials and immediately injected into the HPLC-DAD.

2.4.6. HPLC-DAD analysis of p*-hydroxybenzoic, syringic and vanillic acids*

The HPLC system consisted of a Waters 2695 HPLC system equipped with a Waters 2996 DAD (Waters, Milford, MA). Chromatographic separations were performed on a Gemini RP18 (Phenomenex) column (250 mm × 2.0 mm, 5 μm), protected by a precolumn. Solvent A was 1% formic acid in water and solvent B was acetonitrile. The linear gradient was as follows: from 0 to 20% B in 40 min, 20 to 100% B in 0.10 min, 100% B for 2 min, back to 0% B in 0.1 min. The column equilibration time was 5 min, the injection volume was 10 μL and the temperature was 40 °C. The compounds detected were identified at 280 nm by comparing their retention times with those of pure standards and quantified by means of the external standard method (results were corrected on the basis of recovery of the internal standard).

2.4.7. HPLC-DAD–MS analysis of ellagic, gallic and protocatechuic acids

The wine samples, without prior preparation, were filtered through PTFE syringe tip filters (0.22 μm pore size, 13 mm; Millipore, Bedford, MA) prior to injection into the Waters 2690 HPLC system (Waters, Milford, MA, USA) equipped with a Waters 996 DAD and Micromass ZQ electrospray ionization-mass spectrometer. 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 3 V, source temperature of 105 °C, desolvation temperature of 200 °C, cone gas flow (N_2) of 61 L h^{-1} and desolvation gas flow (N_2) of 460 L h^{-1}. Electrospray ionization-mass spectrometry (ESI-MS) was carried out from *m*/*z* 100 to 1500 with a residence time of 0.1 s.

The same chromatographic separation conditions used for *p*-hydroxybenzoic, syringic and vanilic acids were applied in the determination of ellagic, gallic and protocatechuic acids. The identification was performed on the basis of their retention time, molecular ion and main fragment by MS through comparison with the values for the pure standards. The optimal coefficient of variation (CV) for all ions was 20. The molecular ions $(M-H)^-$ for ellagic acid (*m*/*z* 301.19) and gallic acid (*m*/*z* 169.12) as well as the $(M+H)^+$ for protocatechuic acid (*m*/*z* 153.12) were used for the quantification based on external standard calibration curves.

2.4.8. Method repeatability

Method repeatability was based on six consecutive determinations (ellagic, gallic and protocatechuic acids) by six SPE purifications (*p*-hydroxybenzoic, syringic and vanillic acids) applied to the same wine. The %RSD values obtained were the following: ellagic acid 6.75%; gallic acid 1.26%; protocatechuic acid 1.61%; p-hydroxybenzoic acid 4.32%; syringic acid 3.23%; and vanillic acid 4.76%.

2.4.9. Detection and quantification limits (phenolic acids)

The experimental limit of detection (LOD) and limit of quantification (LOQ) for the HPLC-DAD–MS method were estimated at a signal-to-noise ratio of 3 and 10, respectively, and the values obtained were as follows: ellagic acid 0.031 and 0.098 mg L^{-1} (R^2 = 0.9956); gallic acid 0.172 and 0.568 mg L^{-1} (R^2 = 0.9945); protocatechuic acid 0.148 and 0.490 mg L^{-1} (R^2 = 0.9953); *p*-hydroxybenzoic acid 0.039 and 0.130 mg L^{-1} (R^2 = 0.9993); syringic acid 0.027 and 0.084 mg L^{-1} (R^2 = 0.9989); and vanillic acid 0.034 and 0.113 mg L^{-1} (R^2 = 0.9986), respectively. All results were considered acceptable for research purposes.

2.5. In vivo *antioxidant activity*

2.5.1. Animals

All animal used in this study received humane care in accordance with the principles of the legal requirements appropriate for the species (Guiding Principles for the Care and Use of Laboratory Animals, NIH publication #85.23, revised in 1985) and with the approval of Institutional Ethics Committee of University of Santa Catarina (PP005422010/CEUA/UFSC). Male Swiss mice weighing 22 ± 2 g were housed under controlled conditions (12-h light–dark cycle, 22 ± 2 °C, 60% air humidity) and had free access to standard laboratory chow and water.

Mice were randomly divided into 10 groups (n = 6 each group) as detailed below: one control group, treated with water daily; one control ethanol group, treated with vehicle (hydroalcoholic solution 12%) daily and eight test groups, treated with one of the 8 wine samples daily. The treatment (7.0 mL kg^{-1}, ~20 mg kg^{-1} day^{-1} total polyphenols) was performed by gavage for 30 days and on the 31st day the animals were euthanized by cervical dislocation.

2.5.2. Oxidative stress biomarkers – in vivo *antioxidant activity*

The total antioxidant capacity (FRAP) of the animals plasma was determined and liver samples were analyzed to evaluate the endogenous lipid peroxidation (TBARS), oxidative damage to proteins by carbonylation (PC) and catalase activity (CAT) as well as the reduced glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) content.

The total antioxidant capacity (FRAP) was determined using the animals plasma according to Benzie and Strain (1996). In this analysis the antioxidant present in the sample reduces Fe^{2+} to Fe^{3+} which is chelated by 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and the blue color is monitored at 593 nm. Peroxidation of hepatic tissue lipids, *in vivo*, was measured by the method described by Ohkawa et al. (1979). The amount of thiobarbituric acid-reactive substances (TBARS) was expressed as nmoles of TBARS formed per mg of protein, using a molar coefficient (ε) of 153 mmol L^{-1} cm^{-1}. Oxidative damage of proteins was quantified as carbonyl protein content (CP) according to Levine et al. (1990). This method is based on spectrophotometric detection of the end product of the reaction of 2,4-dinitrophenylhydrazine with carbonyl protein to form protein hydrazones detected at 370 nm. The results were expressed as nmoles of carbonyl group per mg of protein, using ε = 22 mmol L^{-1} cm^{-1}. Liver GSH levels were measured by a spectrophotometric method (Anderson, 1985), in acid hepatic homogenates combined with a disodium hydrogen phosphate and DTNB solution, and the yellow chromophore formed was detected and quantified at 412 nm using a molar coefficient (ε) of 14.1 mmol L^{-1} cm^{-1}. The results were expressed in mmol mg^{-1} of protein. CAT activity was determined by measuring, at 240 nm, the decrease in H_2O_2 in a freshly prepared 10 mM hydrogen peroxide solution and expressed in mmol min^{-1} mg $protein^{-1}$ and ε = 40 mmol L^{-1} cm^{-1} (Aebi, 1984). SOD activity in homogenates was determined by measuring the inhibition of the rate of autocatalytic adrenochrome formation and expressed in U SOD mg $protein^{-1}$ (Misra and Fridovich, 1972). GPx activity was quantified by a coupled assay with glutathione reductase (GR)-catalyzed oxidation of NADPH. Measurements were taken at 340 nm and expressed in mmol min^{-1} mg $protein^{-1}$ (Flohé and Gunzler, 1984). Protein was measured by the method of Lowry et al. (1993) using bovine serum albumin as the standard.

2.6. Statistical analysis

Analysis of variance (ANOVA), Tukey HSD Test and principal components analysis (PCA) were carried out using Statistica 7 (2001) (StatSoft Inc., Tulsa, OK, USA). Values of $p \leq 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Chemical analysis

3.1.1. Flavonoid phenolic compounds

Table 1 shows the results for the flavonol contents of the wine samples. The six flavonols determined in the present study, after acid hydrolysis, included all aglycons expected on the basis of the diagram for the biosynthesis of grape flavonols proposed by Mattivi et al. (2006). In general, the main flavonols in these wine samples were quercetin and myricetin. These compounds were especially high in Sangiovese 2007 (quercetin) and Syrah 2007 (myricetin). Lower concentrations of laricitrin, kaempferol, isorhamnetin and syringetin were found. However, despite their low concentrations, the identification and quantification of such compounds is important since they allow a better characterization for this family of compounds. Moreover, according to Mattivi et al. (2006) all of these flavonols are required for a more detailed classification of red and white grape varieties on the basis of their flavonol profile. The concentrations of total flavonols (sum of the individual contents) in the wine samples ranged from 20.81 to 46.79 mg L^{-1}, and are similar to those reported for regions renowned for wine production (Simonetti et al., 1997; Monagas et al., 2005).

The levels of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside and their respective derivatives (esters of acetic and *p*-coumaric acids) in the wine samples are presented in Table 2. The mean total anthocyanins content (sum of the individual contents) was 47.43 mg L^{-1}, and ranged from 12.94 to 125.60 mg L^{-1}. Of the anthocyanins evaluated, malvidin-3-glucoside presented the highest concentrations (as expected since this is the main anthocyanin in grapes and wines) and the relative amount of this anthocyanin in the cultivars studied ranged from 33.4% (Cabernet Franc, 2006 vintage) to more than 47% (Syrah, 2007 vintage). In general, acetylated anthocyanins were the derivatives found in highest concentrations in both vintages, with relative amounts ranging from 17% to 25% of the total anthocyanins. The highest values were found for Cabernet Franc 2007 (22.83 mg L^{-1}). The *p*-coumarated anthocyanin content corresponded to 4.3–11% of the total anthocyanins.

Two-way ANOVA analysis revealed that the total contents of flavonols and anthocyanins were influenced by two factors: variety and vintage ($p < 0.05$). It is known that the biosynthetic pathways involved in flavonoids production in plant tissues are greatly influenced by many climatic factors including sunlight exposure, temperature and UV exposure. Thus, in this study, the differences found between the two vintages were to be expected since significant differences were observed between climates in which they were grown (Gris et al., 2010). However, an alternative hypothesis to explain the differences observed in the phenolic compounds content of the two vintages is the time of wine aging. Since wine composition is in constant evolution, the aging in bottles also seems to contribute to changes in the flavonols content, through the interaction of flavonols with other constituents (Ribéreau-Gayon et al., 1998).

It was found that the effect of vintage was significantly more pronounced on the anthocyanins than on the flavonols, the anthocyanins concentration in the 2006 vintage being significantly lower for all varieties when compared to the later vintage ($p < 0.05$). This can be explained by the fact that, although weather conditions have a strong influence on the anthocyanins concentration, according to Monagas et al. (2005) these are one of the groups of phenolic compounds that present major losses during wine aging in the bottle. This is attributed to their participation in numerous chemical reactions which, in general, lead to the disappearance of monomeric anthocyanins and the formation to more stable oligomeric and polymeric pigments. Moreover, Rossetto et al. (2004) also affirm that the polymerization process which occurs during wine aging causes the disappearance of the free form of anthocyanins. Once a wine is bottled, changes are determined mainly by non-oxidative reactions (Ribéreau-Gayon et al., 1998). However, recent studies indicate that wines are also subjected to oxidative reactions (Lopes et al., 2006). Considering the non-oxidative conditions present in the bottle, the direct condensation reaction of anthocyanins with other phenolic compounds, and hydrolytic and degradative reactions (Somers and Evans, 1986) are most probably responsible for the decrease in the monomeric anthocyanins concentration during aging in the bottle. Thus, a combination of these factors can influence the phenolic composition of wine and, consequently, the levels of anthocyanins and flavonols.

Regarding the significant differences observed in the contents of flavonoid phenolic compounds of the different varieties, it is

Table 1
Content of main flavonols and total phenols (TP) in wine samples.

Vintage	2006				2007			
	Cabernet Franc	Merlot	Sangiovese	Syrah	Cabernet Franc	Merlot	Sangiovese	Syrah
Myricetin	14.49 ± 0.18	11.59 ± 0.23	9.33 ± 0.13	10.42 ± 0.06	13.83 ± 0.13	14.04 ± 0.10	11.83 ± 0.21	16.47 ± 0.14
Quercetin	22.59 ± 0.23	19.01 ± 0.23	7.49 ± 0.20	14.82 ± 0.19	17.80 ± 0.17	20.50 ± 0.23	27.44 ± 0.22	13.88 ± 0.16
Laricitrin	3.39 ± 0.14	2.40 ± 0.12	1.47 ± 0.12	2.32 ± 0.10	2.86 ± 0.11	2.50 ± 0.09	1.67 ± 0.08	3.41 ± 0.13
Kaempferol	1.65 ± 0.08	1.25 ± 0.06	0.23 ± 0.05	0.43 ± 0.05	0.38 ± 0.08	0.76 ± 0.04	0.74 ± 0.07	0.69 ± 0.05
Isorhamnetin	2.72 ± 0.04	2.02 ± 0.06	0.74 ± 0.02	2.12 ± 0.07	2.00 ± 0.08	2.00 ± 0.07	0.48 ± 0.08	3.28 ± 0.10
Syringetin	1.94 ± 0.08	2.06 ± 0.04	1.57 ± 0.09	1.73 ± 0.04	2.29 ± 0.09	1.99 ± 0.06	0.60 ± 0.05	2.21 ± 0.08
Total flavonols	46.79 ± 0.9[a]	38.32 ± 1.4[b]	20.81 ± 0.7[d]	31.85 ± 0.3[e]	39.15 ± 0.2[b]	41.79 ± 0.3[b,c]	42.76 ± 0.4[a,c]	39.93 ± 0.3[b,c]
Total phenols	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]

Values in units of mg L^{-1} ± standard deviation over three replications in one wine sample. Different letters (total contents) within each row are significantly different (Tukey's test, $p \leq 0.05$).

known that, although the environmental conditions where the fruit is grown influence greatly the synthesis of phenolic compounds, their nature and different relative amounts are consequences of a genetic determinant, specific for each variety (Mazza, 1995).

3.1.2. Non-flavonoid phenolic compounds

The results for the determination of hydroxycinnamic and hydroxybenzoic acids in the wine samples are presented in Table 3. In wines, the most abundant hydroxycinnamic acids are present in the conjugated form (tartaric esters; hydroxycinnamates), as found in this study (Table 3). The presence of free forms of these acids in wines, since they are not present in grapes (Vrhovsek, 1998), according to Somers et al. (1987), is due to the hydrolysis of hydroxycinnamates by cinnamoyl esterases.

A two-way ANOVA carried out considering two factors, variety and vintage, revealed that these factors influence the total content of hydroxycinnamic acids ($p < 0.05$). Regarding the varieties, Cabernet Franc presented the highest content of hydroxycinnamic acid in both vintages analyzed ($p < 0.05$). It was verified that the total hydroxycinnamic acid content was higher for the 2007 vintage ($p < 0.05$).

It was found that the values for *trans*-caftaric and *cis* and *trans*-coutaric acids in the wines of the 2006 vintage were lower than those found for the 2007 vintage, with the opposite being observed for the *trans*-caffeic and *trans*-*p*-coumaric acids. One hypothesis which may explain this observation is the constant evolution of the wine composition and the several reactions that occur during wine production, storage in barrels and aging in bottles. This trend was also verified by Monagas et al. (2005) who noted that the increase in free acids in wines may originate not only from the hydrolysis of the respective tartaric esters (Somers et al., 1987), but also from the hydrolysis of *p*-coumaroyl-acylated anthocyanins during aging in the bottle and that the disappearance of acylated anthocyanins during wine aging is, in part, due to the hydrolysis of the acylated group. This explanation is also consistent with the lower concentrations of these anthocyanic derivatives verified in the 2006 compared with the 2007 vintage (Table 2).

The results for the hydroxybenzoic acids concentrations are presented in Table 3. Of these acids, gallic acid was predominant, representing, on average, 76% of all hydroxybenzoic acids. The presence of high amounts of gallic acid in red wines is to be expected since it is formed mainly through the hydrolysis of flavonoid gallate esters. The variety and vintage influenced the total hydroxybenzoic acids as determined by two-way ANOVA ($p < 0.05$). The total hydroxybenzoic acids content (sum of the individual contents) of the 2007 vintage was higher than that of the 2006 vintage ($p < 0.05$) for all varieties, except for Syrah 2007. The individual analysis of hydroxybenzoic acids showed that the contents varied among vintages and varieties ($p < 0.05$). The evolution of such acids during aging in bottles is very variable. According to Monagas et al. (2005), although hydroxybenzoic acids are susceptible to changes during enological

Table 2
Content of main anthocyanins and derivatives (esters of acetic and *p*-coumaric acids) in wine samples.

Vintage	2006				2007			
	Cabernet Franc	Merlot	Sangiovese	Syrah	Cabernet Franc	Merlot	Sangiovese	Syrah
Delphinidin-3-glucoside	1.77 ± 0.07	2.16 ± 0.06	1.50 ± 0.06	2.01 ± 0.06	14.83 ± 0.34	11.23 ± 0.29	2.69 ± 0.16	7.51 ± 0.06
Cyanidin-3-glucoside	0.49 ± 0.07	0.69 ± 0.05	0.46 ± 0.05	0.55 ± 0.03	2.74 ± 0.05	2.72 ± 0.04	2.76 ± 0.03	1.07 ± 0.06
Petunidin-3-glucoside	1.48 ± 0.05	1.74 ± 0.06	1.24 ± 0.05	1.94 ± 0.03	13.26 ± 0.25	8.49 ± 0.18	3.30 ± 0.08	9.52 ± 0.25
Peonidin-3-glucoside	1.32 ± 0.03	1.49 ± 0.08	1.12 ± 0.08	1.71 ± 0.06	10.50 ± 0.10	6.61 ± 0.08	2.58 ± 0.06	6.91 ± 0.06
Malvidin-3-glucoside	5.58 ± 0.06	6.02 ± 0.08	4.53 ± 0.07	7.62 ± 0.08	53.48 ± 1.01	25.25 ± 0.78	8.45 ± 0.47	41.54 ± 1.02
Delphinidin-3-glucoside-acetate	0.38 ± 0.04	0.34 ± 0.04	0.16 ± 0.02	0.34 ± 0.01	2.39 ± 0.05	2.07 ± 0.06	0.21 ± 0.03	1.35 ± 0.05
Cyanidin-3-glucoside-acetate	0.12 ± 0.03	0.17 ± 0.06	0.07 ± 0.014	0.09 ± 0.02	0.84 ± 0.03	0.74 ± 0.03	0.16 ± 0.03	0.26 ± 0.04
Petunidin-3-glucoside-acetate	0.17 ± 0.06	0.35 ± 0.03	0.17 ± 0.03	0.22 ± 0.02	2.52 ± 0.13	1.48 ± 0.08	0.22 ± 0.03	1.40 ± 0.03
Peonidin-3-glucoside-acetate	0.74 ± 0.03	0.72 ± 0.03	0.47 ± 0.03	0.58 ± 0.06	3.39 ± 0.09	1.52 ± 0.04	1.78 ± 0.08	2.20 ± 0.03
Malvidin-3-glucoside-acetate	2.82 ± 0.05	2.37 ± 0.06	1.89 ± 0.10	2.73 ± 0.07	13.68 ± 0.49	6.27 ± 0.18	4.62 ± 0.21	9.74 ± 0.24
Delphinidin-3-glucoside-cumarate	0.19 ± 0.03	0.06 ± 0.02	0.17 ± 0.04	0.16 ± 0.03	0.68 ± 0.06	0.41 ± 0.03	0.35 ± 0.05	0.51 ± 0.06
Cyanidin-3-glucoside-cumarate	0.42 ± 0.03	0.27 ± 0.04	0.20 ± 0.04	0.37 ± 0.06	1.07 ± 0.08	0.73 ± 0.05	n.d.	0.97 ± 0.06
Petunidin-3-glucoside-cumarate	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.11 ± 0.02	0.46 ± 0.03	0.33 ± 0.03	0.07 ± 0.01	0.38 ± 0.04
Peonidin-3-glucoside-cumarate	0.32 ± 0.02	0.29 ± 0.04	0.23 ± 0.05	0.29 ± 0.03	1.57 ± 0.06	0.31 ± 0.03	0.30 ± 0.02	1.81 ± 0.06
Malvidin 3-glucoside-cumarate	0.85 ± 0.07	0.85 ± 0.06	0.68 ± 0.05	1.01 ± 0.08	4.17 ± 0.12	2.41 ± 0.06	0.46 ± 0.06	3.18 ± 0.03
Total glucoside	10.65 ± 0.3[a]	12.09 ± 0.3[a]	8.85 ± 0.3[a]	13.84 ± 0.3[a]	94.81 ± 0.5[b]	54.31 ± 1.2[c]	19.78 ± 0.8[d]	66.54 ± 1.3[e]
Total acetylated anthocyanins	4.23 ± 0.08[a]	3.96 ± 0.06[a]	2.76 ± 0.1[a]	3.97 ± 0.9[a]	22.83 ± 0.1[b]	12.09 ± 0.6[c]	6.99 ± 0.07[d]	14.95 ± 0.7[e]
Total p-coumarated Anthocyanins	1.83 ± 0.03[a]	1.51 ± 0.02[a]	1.33 ± 0.05[a]	1.94 ± 0.04[a]	7.96 ± 0.07[b]	4.19 ± 0.06[c]	1.19 ± 0.09[a]	6.86 ± 0.05[d]
Total anthocyanins	16.71 ± 0.5[a,b]	17.56 ± 0.9[a,b]	12.94 ± 0.2[b]	19.75 ± 0.9[a]	125.60 ± 2.1[c]	70.58 ± 1.8[d]	27.96 ± 1.4[e]	88.35 ± 2.3[f]

Values in units of mg L^{-1} ± standard deviation over three replications in one wine sample. Different letters (total contents) within each row are significantly different (Tukey's test, $p \leq 0.05$). LOD of cyanidin-3-glucoside-cumarate = 0.09 mg L^{-1}.

Table 3
Content of main hydroxycinnamic and hydroxybenzoic acids in wine samples.

Vintage	2006				2007			
	Cabernet Franc	Merlot	Sangiovese	Syrah	Cabernet Franc	Merlot	Sangiovese	Syrah
cis-Caftaric acid	2.00 ± 0.03	2.37 ± 0.01	1.84 ± 0.02	2.05 ± 0.01	2.14 ± 0.01	2.36 ± 0.02	1.41 ± 0.021	2.80 ± 0.01
trans-Caftaric acid	92.16 ± 3.17	73.59 ± 3.20	68.68 ± 1.48	73.82 ± 2.19	150.97 ± 4.79	92.30 ± 2.97	99.85 ± 3.61	69.76 ± 3.11
cis-Coutaric acid	4.21 ± 0.18	3.61 ± 0.29	3.61 ± 0.23	4.54 ± 0.21	6.61 ± 0.23	5.79 ± 0.14	17.89 ± 1.50	11.67 ± 1.04
trans-Coutaric acid	20.94 ± 1.87	17.58 ± 0.95	14.69 ± 1.01	19.11 ± 0.90	32.49 ± 1.80	18.66 ± 0.93	35.28 ± 1.51	27.49 ± 1.49
Fertaric acid	3.39 ± 0.11	3.09 ± 0.15	2.97 ± 0.09	3.03 ± 0.12	5.21 ± 0.15	3.61 ± 0.13	4.04 ± 0.10	1.46 ± 0.10
trans-Caffeic acid	9.47 ± 0.32	8.55 ± 0.30	8.09 ± 0.25	9.20 ± 0.23	8.66 ± 0.13	9.16 ± 0.21	4.32 ± 0.12	10.48 ± 0.12
trans-*p*-Coumaric acid	6.61 ± 0.11	7.73 ± 0.23	6.54 ± 0.11	6.30 ± 0.20	5.59 ± 0.13	5.31 ± 0.12	2.73 ± 0.06	5.86 ± 0.19
trans-Ferulic acid	2.60 ± 0.03	2.78 ± 0.11	2.32 ± 0.07	2.61 ± 0.10	2.75 ± 0.11	2.96 ± 0.08	2.06 ± 0.08	2.39 ± 0.09
Gallic acid	40.48 ± 1.31	41.60 ± 1.61	34.53 ± 0.49	39.94 ± 0.54	45.03 ± 1.41	54.44 ± 1.19	32.85 ± 0.83	39.33 ± 0.79
Protocatechuic acid	0.87 ± 0.08	1.43 ± 0.08	1.91 ± 0.06	2.92 ± 0.09	9.73 ± 0.22	5.74 ± 0.17	12.66 ± 0.13	10.12 ± 0.25
p-Hydroxybenzoic acid	n.d.	1.26 ± 0.06	0.59 ± 0.08	0.86 ± 0.09	n.d.	n.d.	1.71 ± 0.08	2.10 ± 0.11
Vanillic acid	3.48 ± 0.070	2.13 ± 0.04	1.66 ± 0.08	3.67 ± 0.05	3.50 ± 0.11	3.78 ± 0.10	2.98 ± 0.07	3.22 ± 0.08
Syringic acid	1.48 ± 0.06	1.23 ± 0.08	1.15 ± 0.05	3.53 ± 0.08	4.11 ± 0.08	0.99 ± 0.17	1.56 ± 0.09	4.22 ± 0.11
Ellagic acid	0.93 ± 0.08	0.89 ± 0.07	0.76 ± 0.09	0.50 ± 0.08	3.19 ± 0.06	0.13 ± 0.04	3.86 ± 0.07	0.11 ± 0.02
Total hydroxycinnamic acids	141.40 ± 4.7^{a}	119.32 ± 4.3^{b,c}	108.78 ± 3.8^{b}	120.63 ± 3.4^{b,c}	214.48 ± 7.9^{e}	140.27 ± 4.8^{a,d}	167.57 ± 6.2^{f}	131.86 ± 3.7^{a,c,d}
Total hydroxybenzoic acids	47.23 ± 2.2^{a}	48.55 ± 2.7^{a}	40.59 ± 1.7^{b}	51.41 ± 1.6^{a}	65.56 ± 2.2^{c,d}	65.08 ± 1.8^{c}	55.62 ± 1.6^{a}	50.10 ± 2.1^{d}

Values in units of mg L^{-1} ± standard deviation over three replications in one wine sample. Different letters (total contents) within each row are significantly different (Tukey's test, $p \leq 0.05$). n.d. = not detected. LOD of *p*-hydroxybenzoic acid = 0.039 mg L^{-1}.

practices, it seems that their evolution profile during wine aging in the bottle does not change significantly.

3.2. Antioxidant activity

The results for the *in vivo* study on the antioxidant activity of wine samples are presented in Fig. 1. It was found that the ethanol control group did not differ significantly from the water control group, indicating that the consumption of ethanol did not modify the parameters of the *in vivo* antioxidant activity. The effect of ethanol on an organism is still very controversial. Epidemiological evidence suggests that moderate consumption of alcoholic beverages (20–30 g alcohol per day), such as wine, is associated with a reduced risk of death from cardiovascular disease (Renauld and Lorgeril, 1992). However, the results of some studies suggest that ethanol consumption promotes toxic effects such as the generation of damaging free radical species in various tissues (Mantle and Preedy, 1999) and an increased activity of CAT and SOD enzymes (Rodrigo et al., 2002).

The levels of plasma antioxidant capacity (FRAP) are presented in Fig. 1. When compared to the control groups, the FRAP increased with administration of red wine samples, with the exception of the group treated with the Sangiovese 2006 wine ($p < 0.05$). Increases in the plasma antioxidant activity ranged from 17.8% to 70.7% with significant differences found only among varieties and not among vintages (two-way ANOVA; $p < 0.05$). Increases in plasma antioxidant capacity due to wine consumption have been reported in several studies (Gris et al., 2011a,b; Rodrigo et al., 2005). In this regard, it should be noted that the FRAP indicates the reducing ability of the plasma, but it is not exactly equivalent to the whole antioxidant effect of plasma, since in the method the chelating ability is not measured.

Wine consumption promoted a significant decreasing in the TBARS and CP levels when compared to the control groups ($p < 0.05$), demonstrating the protective effect of wine consumption against lipoperoxidation and proteic carbonylation. It was also observed that the variety and vintage influenced both the TBARS and the CP values (two-way ANOVA; $p < 0.05$). In the case of the TBARS levels, the most significant decreases were found for Syrah (43.2%) and Merlot (27.6%) from the 2007 vintage. Cabernet Franc and Syrah varieties presented the highest CP decreases for both vintages evaluated, causing an inhibition that ranged from 30.5 to 42.9%.

The increase in FRAP probably occurred due the absorption of wine phenolic compounds, which are known to have reducing power (Burin et al., 2011; Baroni et al., 2012). Another possibility is that the wine consumption promoted an increase in the levels of urates in the plasma, one of the most abundant free radical scavengers in humans, and this could lead to protective antioxidant effects and an increased antioxidant potential of the plasma (Modun et al., 2008). Rodrigo et al. (2005) suggested that wine-induced increases in the antioxidant capacity of plasma contribute to the enhancement of the antioxidant defense systems of organs like the kidneys, liver and lungs, due to their well-known high perfusion rate. It has been postulated that the increase in plasma antioxidant capacity and inhibition of lipoperoxidation and proteic carbonylation, as observed in the present study, promoted by wine ingestion is due to the high capacity of phenolic compounds to offer protection against free radicals (Frankel et al., 1995). The phenolic compounds can act as metal chelants which, theoretically, prevent iron-dependent lipid peroxidation in membranes by rendering iron inactive and by scavenging chain-initiating peroxyl radicals at the liquid-aqueous interface.

In this study, a significant difference in hepatic GSH levels of the different experimental groups ($p < 0.05$) was not observed, suggesting that wine ingestion did not affect the homeostasis of the animal organism. This is an interesting result since an increase in GSH levels may suggest an antioxidant response to an increase in stressing agents. Similar results have been reported by other authors (Gris et al., 2011a,b; Ferreira et al., 2010).

In relation to the activity of SOD, CAT and GPx enzymes (Fig. 1), it was found in this study that wine ingestion decreased significantly the expression of scavenger enzymes when compared to control groups ($p < 0.05$). Statistical analysis (two-way ANOVA) revealed that the vintage influenced the SOD and CAT activity, but presented no significant effect for GPx ($p < 0.05$). However, conversely, GPx was influenced by the variety, while the other enzymes (CAT and SOD) did not present a significant association with this factor ($p < 0.05$).

The SOD activity decreased by around 15% in the wines of the 2006 vintage, particularly for Syrah ($p < 0.05$). This decrease was more pronounced for the wines of the 2007 vintage (43%), with no significant difference among varieties ($p < 0.05$). Wine consumption caused a decrease in CAT activity ranging from 12.1 to 36% for Merlot 2006 and 2007, respectively. Cabernet Franc and Merlot wines of the 2007 vintage were more effective at decreasing the CAT activity when compared to wines of the 2006 vintage

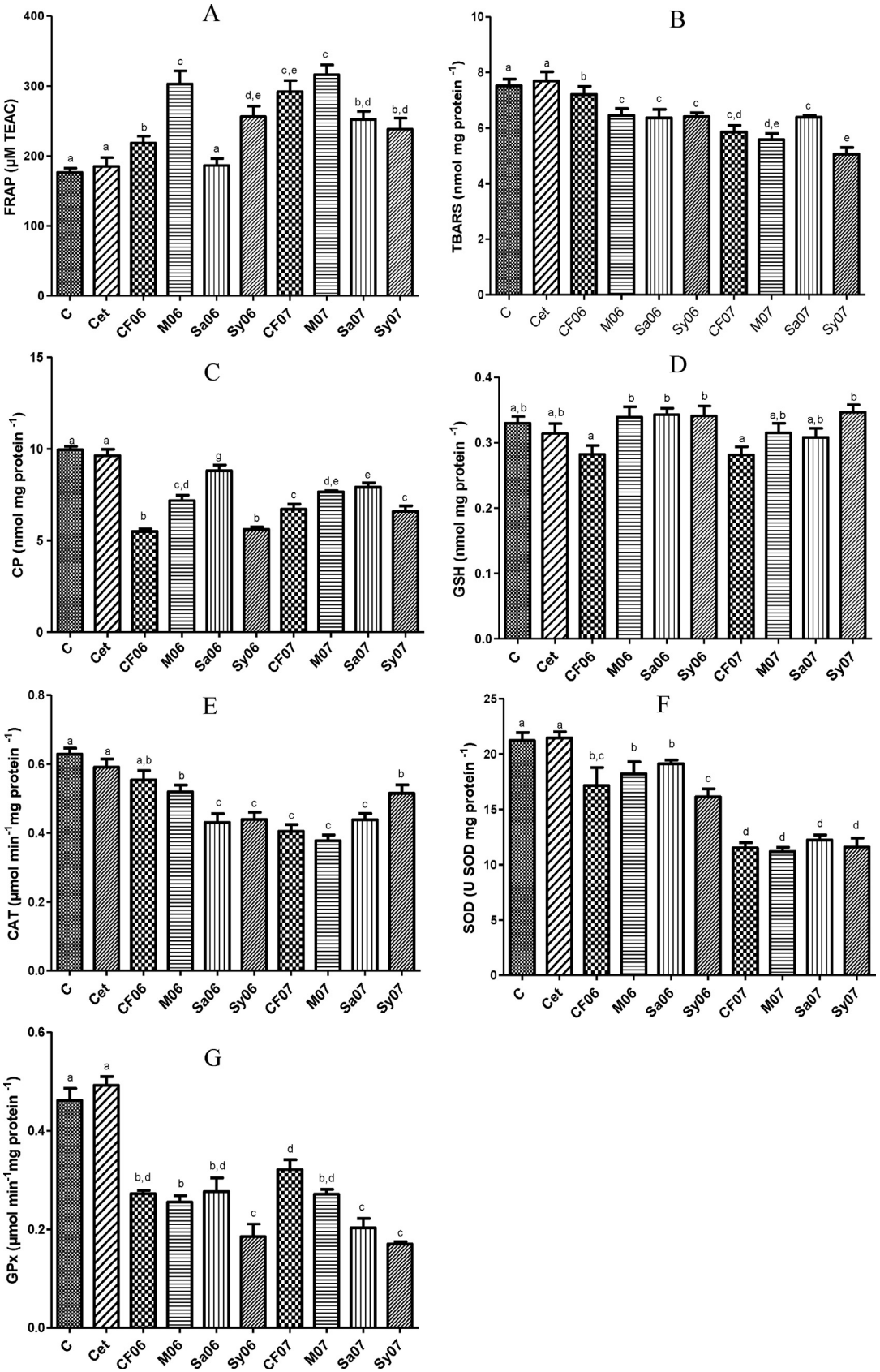

Fig. 1. Values for total antioxidant capacity of mice plasma (A – FRAP, TEAC μM), lipid peroxidation (B – TBARS, nmol mg protein^{-1}), carbonyl protein (C – CP, nmol mg protein^{-1}), hepatic GSH (D – nmol mg protein^{-1}) and catalase (E – CAT, μmol min^{-1} mg protein^{-1}), superoxide dismutase (F – SOD, SOD U mg protein^{-1}) and glutathione peroxidase (G – GPx; μmol min^{-1} mg protein^{-1}) activity in mice liver. Control groups – C: water; Cet: 12% hydroalcoholic solution. Test groups: CF06: Cabernet Franc 2006 wine; M06: Merlot 2006 wine; Sa06: Sangiovese 2006 wine; Sy06: Syrah 2006 wine; CF07: Cabernet Franc 2007 wine; M07: Merlot 2007 wine; Sa07: Sangiovese 2007 wine; Sy07: Syrah 2007 wine. ANOVA to compare data; values with different letters within each column are significantly different (Tukey test, $p \leq 0.05$).

($p < 0.05$). The opposite effect was observed for the Syrah variety ($p < 0.05$). Suppression of GPx activity ranged from 34.7 to 65.4%. Syrah (2006 and 2007) and Sangiovese (2007) presented the most significant decrease in GPx activity (62.4 and 65.4%, respectively).

These observations are of great interest since the main enzymatic antioxidant defense system is composed of the cellular enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). This represents the first line of defense in the neutralization of endogenous ROS, through which the cells attempt to maintain lower quantities of $O_2^{\bullet -}$ and H_2O_2 and thus avoid the formation of $^{\bullet}OH$ which, although short-lived even in very low concentrations, is extremely reactive and damaging to cells (Halliwell and Gutteridge, 1999).

CAT and GPx are the two enzymes that have the ability to convert cell H_2O_2 to H_2O and O_2 (Halliwell and Gutteridge, 1999). Since CAT and GPx are also involved in the elimination of hydroperoxides, the decline in TBARS levels observed in this study might be related to the observed decrease in CAT and GPx activity. The decrease in the activity of these enzymes is probably due to suppression of the ROS mediated trigger of antioxidant enzymes at the transcriptome level, due to the presence of wine phenolic compounds.

3.3. Association between antioxidant activity and concentration of phenolic compounds

Under physiological conditions, ROS are eliminated by enzymatic and nonenzymatic antioxidant defense systems. The scavenging of ROS, binding of metal ions and degradation of peroxides are common mechanisms to prevent ROS-induced damage propagation. An increase in ROS production and a deficient antioxidant status may cause severe oxidative stress in cells, leading to several diseases and toxicity. In this context, ingestion of red wine has been the target of several studies and represents an area of intensive research in preventing disorders related to oxidative stress. In this study it was verified that wine consumption increased the plasma antioxidant capacity and decreased the TBARS and CP levels. A reduction in the antioxidant activities of the enzymes CAT, SOD and GPx was also observed. Thus, the influence of the main phenolic compounds on the *in vitro* antioxidant activity of the wine samples studied was assessed by principal components analysis (Fig. 2), since it is believed that the antioxidant potential of red wine is related to its phenolic compounds content.

The first three principal components explained 83.27% of the total variance (Fig. 2). Factor 1 was negatively influenced by the main chemical and antioxidant analysis. GSH, GPx, kaempferol and *trans*-*p*-coumaric and *p*-hydroxybenzoic acids positively influenced Factor 1. Factor 2 was negatively influenced by CAT and SOD antioxidant activity, and by total hydroxycinnamic acid (THCA), total hydroxybenzoic acid (THBA) and total flavonols (TFLA). Some hydroxycinnamic and hydroxybenzoic acids, cyanidin and quercetin also negatively influenced Factor 2.

Fig. 2 shows that increases in the total antioxidant capacity (FRAP) and inhibition of lipid peroxidation (TBARS) were associated with many of the phenolic compounds evaluated, such as TP, TFLA, THBA, vanillic acid, myricetin and the majority of anthocyanins. The inhibition of proteic carbonylation (CP) was associated with t-ferulic, *trans*-caftaric, syringic and gallic acids as well as isohramnetin, laricitrin, myricetin and TP.

With regard to the antioxidant enzymes, Fig. 2 shows that a decrease in the SOD activity was associated with THCA, THBA, TFLA, *trans*-caftaric acid, protocatechuic acid and the main anthocyanins. A decrease in the CAT activity was associated with fertaric, protocatechuic, *cis*-cutaric, *trans*-caftaric and elagic acids, cyanidin, quercetin and THCA.

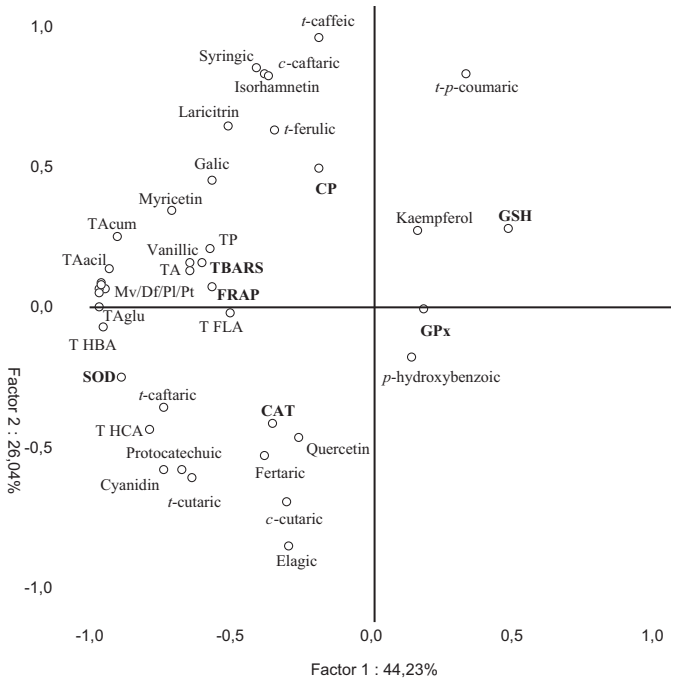

Fig. 2. Principal component analysis of antioxidant analysis and the phenolic compounds. THBA: total hydroxybenzoic acids; THCA: total hydroxycinnamic acids; TFLA: total flavonols; TA: total anthocyanins; TAacil: total acetylated anthocyanins; TAglu: total glucoside; TAcum: total *p*-coumarated anthocyanins; TP: total phenols; Mv: malvidin-3-glucoside; Df: delphinidin-3-glucoside; Pl: perlagonidin-3-glucoside; Pt: petunidin-3-glucoside; PC: protein carbonylation inhibition; TBARS: lipid peroxidation inhibition; FRAP: total antioxidant capacity; SOD: decrease activity of superoxide dismutase; CAT: decrease activity of catalase; GPx: decrease activity of glutathione peroxidase; GSH: reduced glutathione.

In general, the association between phenolic compounds and *in vivo* antioxidant activity found in this study was to be expected since these compounds are mainly responsible for the antioxidant activity in red wine. However, the mechanism of action of these compounds in the organism is still not fully understood requires further study.

The correlation/association between antioxidant activity and phenolic compounds has been verified by many authors (Gris et al., 2011a,b; Ferreira et al., 2010; Alén-Ruiz et al., 2009; Di Majo et al., 2008). The antioxidant properties of phenolic compounds are probably due to the structure of these compounds. *In vitro*, the degree and position of hydroxyl and methyl groups in the B ring of the flavonoids affect their stability and reactivity. In general, higher antioxidant capacity is observed in compounds presenting the ortho-dihydroxy structure in the B ring, with these compounds being effective hydrogen donors. The antioxidant activity of phenolic acids and their esters is dependent on the number of hydroxyl groups in the molecule that would be strengthened by steric hindrance. The electron-withdrawing properties of the carboxylate group in benzoic acids have a negative influence on the H-donating abilities of the hydroxy benzoates, thus, theoretically, hydroxylated cinnamates are more effective antioxidants than benzoate (Rice-Evans et al., 1996).

The beneficial effects of the ingestion of phenolic compounds, especially in wines, have frequently been reported. However, data concerning the absorption mechanism and bioavailability of polyphenols in organisms are scarce, since the issue of the biological destination of flavonoids or their dietary glycoside forms is highly complex and dependent on a large number of processes. Phenolic compounds, such as some hydroxycinnamic and hydroxybenzoic acids, quercetin, kaempferol and malvidin-3-glucoside,

have been detected and quantified in human biological fluids after both acute and sustained wine ingestion (Pignatelli et al., 2006; Nardini et al., 2009). It appears that after wine ingestion polyphenols undergo a rapid and extensive metabolism resulting in trace amounts of unchanged phenols in the circulatory system. The majority of polyphenols absorbed are present in plasma and urine in conjugated forms (methylated, glucuronated and sulfated) indicating an extensive first-pass intestinal/hepatic metabolism of the ingested primary phenolic compound forms. Thus, the biological activity of polyphenols can be attributed to their metabolites (Pignatelli et al., 2006; Vanzo et al., 2007; Nardini et al., 2009; Garcia-Alonso et al., 2009; Sancho and Pastore, 2012).

Initially, it was considered that only free flavonoids without a sugar molecule (aglycones) were able to pass through the intestine wall and be absorbed (Griffiths, 1982). However, more recent studies contest this assumption. Apparently, quercetin glucosides from the diet are mostly hydrolyzed to their aglycones, followed by conjugation to glucuronides and/or sulfates (Garcia-Alonso et al., 2009; Wu et al., 2011). Chang et al. (2005) found that quercetin-3-O-glucoside could be rapidly absorbed and transformed into glucuronidated quercetin. According to reports in the literature, anthocyanins are absorbed in the glycosylated form after oral ingestion (Nielsen et al., 2003; Garcia-Alonso et al., 2009), and the stomach seems to be the site of absorption for these compounds (Passamonti et al., 2003). A recent study demonstrated that hydroxycinnamic acids in white wine are absorbed in the gastrointestinal tract and circulate in the blood after being largely metabolized to glucuronide and sulfate conjugates in humans (Nardini et al., 2009).

It should be noted that although the antioxidant activity of wine is accepted as one of the main biological mechanisms related to red wine-derived phenolic compounds, many others have been proposed including nitric oxide-mediated vasorelaxation (Diebolt et al., 2001; Dell'Agli et al., 2005), estrogenic activity (Klinge et al., 2003; Varadinova et al., 2009), inhibition of platelet aggregation (Corder, 2008) and modulation of lipid metabolism (Frankel et al., 1993; Rouanet et al., 2010).

4. Conclusions

The results of this study showed that the wines under study had adequate phenolic content and composition, demonstrating the potential of this region of southern Brazil to produce high quality wines. Significant *in vivo* antioxidant activity was also verified after wine ingestion, through increased FRAP and decreased TBARS and PC levels, and the suppression of CAT, SOD and GPx enzymes. In general, the antioxidant activity promoted by wine ingestion was associated with the main phenolic compounds quantified, suggesting the possible involvement of these compounds, or their metabolites, in the mechanism of action. Therefore, the data presented in this study provide evidence that red wine ingestion contributes to increasing the *in vivo* antioxidant activity, and suggest that this effect can be attributed to the phenolic composition of the wine. Our results would thus seem to support the recommendation that moderate wine consumption may be beneficial for health.

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