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Brazilian fortified wines: Chemical composition, chromatic properties and antioxidant activity

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ABSTRACT

The phenolic compounds, minerals and higher alcohols content, carbon isotope ratio, colour and antioxidant activity were determined for samples of white and red fortified wines produced in Brazil, in the regions of the Planalto Catarinense, Serra Gaúcha and Carbonífera Region. The analysis was performed using spectrophotometry, colorimetry, high performance liquid chromatography, inductively coupled plasma mass spectrometry, capillary zone electrophoresis, gas chromatography and isotopic ratio mass spectrometry. The results showed that white and red fortified wines produced in Brazil are active in the scavenging of DPPH and ABTS radicals and in iron reduction. The results for the antioxidant activity of samples of Brazilian fortified wines correlated significantly ($p < 0.05$) with the content of total polyphenols, *ortho*-diphenols, tartaric esters, flavonols, total monomeric anthocyanins, total tannins, gallic acid, *trans*-resveratrol, catechin, caffeic acid, coumaric acid and ferulic acid. The analytical determinations combined with principal components analysis showed the separation of white and red fortified wine samples according to region of origin, with contributions from the variables antioxidant activity, tartaric esters, flavonols, non-polymerized polyphenols, *ortho*-diphenols, anthocyanins, tannins, total polyphenols, *trans*-resveratrol, gallic acid, potassium, ethanal, iron, cadmium, sodium, calcium and magnesium. The results obtained showed that fortified red wines produced in the Serra Gaucha show a high concentration of potassium, *trans*-resveratrol and phenolic compounds along with a high antioxidant activity, while the wines produced in the Planalto Catarinense are notable for their contents of caffeic acid, coumaric acid, cobalt, isoamyl alcohol and isobutanol and their relatively low carbon isotope ratio. White wines produced with the Goethe variety in the Carbonífera Region had low levels of phenolic compounds and minerals.

1. Introduction

The winemaking procedure used to produce fortified wines involves stopping the fermentation of the must by adding grape brandy when around half the concentration of sugar has been converted to alcohol (Esteves, Lima, Lima, & Duarte, 2004). To obtain quality fortified wines different oenological practices which alter the wine composition and sensory properties are applied (Gómez-Míguez et al., 2007). In Brazil, the practice of adding sugar during the fermentation of must is allowed in order to increase the ethanol content by up to 3%, or sugar can be added after fermentation to give a maximum of 10% over the final volume of the product, in order to sweeten the fortified wine according to the required degree of sweetness. The alcohol content allowed for fortified wines is 14 to 18% (Brasil, 1988).

In Brazil, fortified wines are produced in the South Region, predominantly in three sub-regions: Planalto Catarinense, Serra Gaúcha and Carbonífera Region. The Planalto Catarinense, with an average altitude of 1200 m, has a cold (heliothermic index = 1714) and wet (dry index = 200) climate with cold nights (cold night index = 12.1). In this region the fortified wines are produced by only a few wineries, but they are nationally known. The Serra Gaúcha region, with an average altitude of 640 m above sea level, is classified as a warm-temperate region (heliothermic index = 2365) with a wet climate (dry index = 200) and temperate nights (cold night index = 16.1.) The Carbonífera region, with an average altitude of 50 m, is classified as a hot/warm temperate region (heliothermic index > 2400) (Tonietto & Carbonneau, 2004). This region is notable for the production of "Goethe" white grapes, with the Geographical Indication of Valley of Goethe Grape (Brasil, 2011).

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For the characterization of fortified wines it is essential to determine the content of several chemical compounds, such as minerals and volatile compounds, the carbon isotope ratio and the phenolic composition. The mineral content of wines, for example, provides information regarding its origin and authenticity (Paneque, Álvarez-Sotomayor, Clavijo, & Gómez, 2010), since it is the result of several factors including the grape variety, soil characteristics and solubility of inorganic compounds present in it, environmental conditions, agricultural practices, climate and the winemaking process employed (Grindlay, Mora, Maestre, & Gras, 2008).

The wine aroma is dependent on factors such as the grape variety, production region, climatic conditions, winemaking practices and ageing process, among others (Gil, Cabellos, Arroyo, & Prodanov, 2006). The higher alcohols represent more than half of the volatile compounds of wines and their production is influenced by the yeasts, pH, content and sources of nitrogen and sugars, and temperature and oxygen content during fermentation. Also, the sugaring and the pressing process increase the synthesis of higher alcohols (Jackson, 2008).

The ratio of stable carbon isotopes $^{13}C/^{12}C$ ($\delta^{13}C$) is used to determine the presence of sugar and ethanol derived from cane sugar in beverages such as wine, and is applied in the control of the authenticity and the denomination of origin (Cabañero, Recio, & Rupérez, 2008). This analysis provides information on the climate, distance from the sea, altitude, latitude and winemaking practices. Therefore, it can be used to determine the origin of food products and to reveal whether a compound has been replaced by another chemically identical molecule (Rummel, Hoelzl, Hom, Rossmann, & Schlicht, 2010).

The characteristics and concentration of the phenolic compounds in wine are influenced by the chemical composition of the grapes, determined by the variety, maturation stage, atmospheric conditions during maturation and harvesting and soil type. The techniques used during the winemaking process employed to produce fortified wine and the maturation and ageing conditions are also significant factors (Roussis et al., 2008). Phenolic aldehydes, benzoic acids, hydroxycinnamic acids and their esters, flavanols, flavonols and anthocyanins are extracted from the grapes during the winemaking process. Some phenolic compounds can be extracted from wood during the aging stage and oxidation reactions may also occur, increasing the stability of the wine and its pleasant sensorial characteristics (Bravo, Silva, Coelho, Vilas Boas, & Bronze, 2006).

The antioxidant activity and the colour characteristics of wines are often associated with the total polyphenol content and with the composition of individual phenolic compounds, being of interest in many research studies. Some phenolic compounds found in wines are antioxidants, contributing to a reduction in the risk of cardiovascular diseases, while others have been recognized for their activity against allergies, inflammation, hypertension, arthritis and carcinogens (Bravo et al., 2006; Granato, Katayama, & de Castro, 2011).

The aim of this study was to determine the chemical composition and the colour parameters and to evaluate the *in vitro* antioxidant activity of white and red fortified wines produced in Brazil. This research may also contribute to the listing of technical support information for the Geographic Indications of Origin of Brazilian wines, since it is the first detailed study on Brazilian fortified wines.

2. Material and methods

2.1. Samples

In Brazil, the production of fortified wines is an activity which has experienced a recent increase, particularly in the past decade. Although there are few vineyards that produce fortified wines, these products are representative of the producing regions and, particularly, the varieties of grapes used in the winemaking. The commercial samples of white and red fortified wines analysed in this study came from different producers, each sample being prepared by a different vineyard. The samples represented 80% of the fortified wines produced in Brazil. They were produced in three different wine producing regions of Brazil, from different varieties of grapes: red grapes (CSME1 to CSME3 samples) Cabernet Sauvignon/Merlot, Serra Gaúcha; (CSME 4) Cabernet Sauvignon/Merlot, Planalto Catarinense; (CSTA1 and CSTA2) Cabernet Sauvignon/Tannat, Serra Gaúcha; (TNAC1) Touriga Nacional, Planalto Catarinense; and white grapes (GOET1) Goethe, Carbonífera Region; (MOSC1 and MOSC2) Moscato Giallo, Serra Gaúcha; (MOSC3) Moscato Giallo, Planalto Catarinense. Red Port wine, category vintage, from the Douro region, was used as the reference sample (PORT1). The fortified wine derived from the Carbonífera Region (GOET1) is prepared by an association of producers and, therefore, is the only example of the fortified wine Goethe. In this region only white fortified wines are produced using grapes of the Goethe variety.

2.2. Reagents

All reagents used were of analytical grade. The water employed was distilled and subsequently deionized to a resistivity of 18.2 MΩ cm in a Millipore Milli-Q system (Bedford, MA, USA). Nitric acid (Carlo Erba, Milan, Italy) was bidistilled below its boiling point in a quartz Kürner Analysentechnik system (Rosenheim, Germany). Multielement ICP IV stock solutions were used (Merck, Darmstadt, Germany) and the internal standard was a stock solution of Rh 1000 mg L^{-1} (Merck). The standards of ethanal, ethyl acetate, methanol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 4-methyl-2-pentanol were obtained from Merck (Darmstadt, Germany). Acetonitrile, methanol and ethyl acetate of HPLC grade were obtained from Merck (Darmstadt, Germany). All solvents used as the mobile phase had been previously filtered through a 0.45 mM membrane (Millipore) and degasified before use. The SPE cartridges used were: Spe-ed C_{18}/18% (500 mg of sorbent mass and 6 mL of volume of the reservoir) supplied by Applied Separations (Allentown, United States). The standards of caffeic acid, ferulic acid and quercetin were obtained from Fluka (Steinheim, Germany). The standards of *p*-coumaric acid, catechin, *trans*-resveratrol and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All standards were dissolved in a synthetic wine matrix prepared with 18% (v/v) ethanol and 5 g L^{-1} of tartaric acid in ultra pure water, adjusted to pH 3.5 with NaOH 1 N.

2.3. Determination of mineral elements for ICP-MS and CZE

The determination of ten mineral elements was performed on a mass spectrometer with inductively coupled plasma, Elan model 6000 (Perkin Elmer-Sciex, Thornhill, ON, Canada) using pneumatic nebulization with a cross-flow nebulizer (Perkin Elmer). The argon used was of 99.996% purity (White Martins, São Paulo, SP, Brazil). The instrument was optimized (daily performance) to give maximum sensitivity for M^+ ions and the double ionization and oxides were monitored by means of the ratios between Ba^{2+}/Ba^+ and Ce^+/CeO^+, respectively, these always being less than 3%.

The calibration was performed using an ICP VI multielement solution and Rh in the concentration of 10 μg L^{-1} was used as the internal standard. The fortified wine samples were diluted 1:10 in bidistilled water and introduced directly into the ICP-MS where the following elements were determined according to the methodology of Catarino, Curvelo-Garcia, and Bruno de Sousa (2006): aluminium (Al), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), cadmium (Cd), thallium (Tl) and zinc (Zn).

The determinations of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were performed by capillary zone electrophoresis, in Agilent Technologies equipment model $HP^{3D}CE$ (Palo Alto, CA, USA) equipped with detector array diodes (DAD). The electrophoretic separation was performed on a fused-silica capillary with an external coating of polyamide (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 32.5 cm (24 cm up to the detector) × 50 μm

internal diameter × 375 μm external diameter. For the acquisition and processing of data the software HP Chemstation was used. The standard solutions and samples were injected hydrodynamically with a pressure of 0.5 psi for 5 s. The separation of analytes was carried out at a positive voltage of 30 kV, within a time of <0.7 min with indirect detection at 214 nm and at a controlled and stabilized temperature of 25 °C. The electrolyte solution was composed of imidazole and acetic acid.

2.4. Determination of higher alcohols for GC-FID

The pretreatment of the samples of fortified wine consisted of distillation through vapour in a Gibertini electronic distiller, model DEE (Gibertini, Novate Milanese, Italy). Subsequently, 100 μL of 4-methyl-2-pentanol was added to 5 mL of the distillate, as the internal standard. Aliquots of 1 μL were injected into the gas chromatograph Varian Star 3400 CX with a FID detector (Varian, Palo Alto, USA). The Varian column (30 m × 0.25 mm internal diameter, 0.25 μm) was operated at an initial temperature of 40 °C, increasing gradually by 5 °C per minute up to 200 °C. The injector temperature was 220 °C and the detector temperature was 250 °C, and hydrogen at 1 ml min^{-1} was used as the carrier gas. The identification and quantification of higher alcohols were performed using the standards of ethanal, ethyl acetate, methanol, isobutanol (2-methyl-1-propanol) and of isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-ol). Calibration curves were prepared for each standard in four different concentrations and the regression coefficients obtained were >0.99.

2.5. Determination of the ratio of stable carbon isotopes by IRMS

The measurement of the $\delta^{13}C$ ratio in ethanol was performed using an isotopic-ratio mass spectrometer (Thermo Electron Finnigan-MAT, model Delta ^{Plus} XL, Bremen, Germany) coupled to a Flash EA 1112 elemental analyser, consisting of oxidation and reduction furnaces, desiccant perchlorate of magnesium ($Mg(ClO_4)_2$) and a Porapack Q chromatographic separation column (25 m × 0.32 mm internal diameter). The sample (1.5 mL) was distilled under cryogenic action at −196 °C under vacuum (10^{-2} mbar) for around 25 min and then 100 μL of distillate were packaged in capsules of pure tin. The carbon of the sample underwent combustion inside the elemental analyser at 950 °C, under a continuous flow of oxygen at 1 mL min^{-1}. The generated gases passed through a reduction column of reduced copper at 680 °C and, after water retention, were separated through a chromatographic column at a temperature of 43 °C, before reaching the ion source of the spectrometer. The mass spectrometer contains a triple ion collector in order to measure simultaneously the *m/z* corresponding to the masses 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{12}C^{16}O^{12}C_2^{17}O$) and 46 ($^{12}C^{16}O^{18}O$) of the CO_2 formed by combustion of the samples.

The $\delta^{13}C$ values for the samples were obtained in relation to the international standard PDB (American Pee Dee Belemnite, of the Formation Pee Dee of South Carolina, USA), expressed in ‰ and calculated according to the equation $\delta^{13}C = \left(\frac{R_{sample} - R}{R_{standard}}\right)1000$, where R is the stable isotope ratio $^{13}C/^{12}C$. The samples were analysed in triplicate and the standard deviation for the analysis was ≤0.2‰.

2.6. Determination of phenolic compounds by spectrophotometry

All spectrophotometric measurements were performed in a Hitachi spectrophotometer model U2010 (Tokyo, Japan). The total polyphenol content was determined by the Folin–Ciocalteau method (Singleton & Rossi, 1965) using gallic acid as the standard. The absorbance values at 320 and 360 nm were used to estimate the content of tartaric esters and flavonols, respectively, by the method of Glories (1978) adapted by Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999). The non-polymerized polyphenols were determined by determining the vanillin index and the *ortho*-diphenols by the Arnow reaction, according to the methodologies of Paronetto (1977). For the determination of total tannins and of the monomeric (catechins), oligomeric (proanthocyanidins with a degree of polymerization of 2 to 12–15) and polymeric (proanthocyanidins with degree of polymerization >12–15) fractions the method of Sun, Leandro, Ricardo da Silva, and Spranger (1998) was used. The total monomeric content of anthocyanins was determined using the differential pH method according to Giusti and Wrolstad (2001).

2.7. Determination of phenolic compounds by HPLC

The identification and quantitation of phenolic compounds (gallic acid, catechin, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin) were carried out using a Shimadzu liquid chromatograph, equipped with a quaternary system of pumps (model 10-AT), a vacuum degasser (model DGU-14A), a UV–Vis detector (SPD-10AV) and a Rheodyne loop of 20 μL. The set was controlled by the CLASS VP 6.1 software, with a model SCL-10A communicator. The stationary phase was composed of a reverse phase column Hichrom (Berkshire, UK) of 250 mm length and 4.6 mm internal diameter with particles of 5 μ. A guard column was used to protect the analytical column.

All samples were treated prior to the injection into the chromatograph according methodology of Arcari, Burin, Costa, Ogliari, and Luiz (2012). The sample pretreatment was performed by solid phase extraction: 5 ml of the sample was applied to a cartridge SPE C_{18} with 500 mg of sorbent mass and 6 mL of reservoir volume of the Applied Separations (Allentown, United States) pre-conditioned with 2 mL of ethyl acetate, 2 mL of methanol and 2 mL of 0.01 N HCl. The cartridge was washed with 3 mL of 0.01 N HCl. The non-coloured phenolic compounds were eluted with 20 mL of ethyl acetate at a flow rate of 0.06 mL s^{-1} and concentrated in a rotate evaporator at 30 °C. The residue obtained was dissolved in 2 ml of methanol, filtered through a Millipore membrane (0.45 μm) and analysed in HPLC–UV–ViS. For the separation of the compounds, the methodology of Burin, Arcari, Costa, and Bordignon-Luiz (2011) was employed.

The determination of *trans*-resveratrol was carried out according to the methodology of Souto et al. (2001) with some modifications of the parameters: the mobile phase was a solution of water and acetonitrile (75:25) with the pH adjusted to 2.4 with concentrated H_3PO_4 (Merck, Darmstadt, Germany), flow of elution of 1.2 mL min^{-1} and monitoring of the signal at 306 nm. All samples were pretreated before injection into the chromatograph using the methodology of liquid-liquid extraction described by Bravo et al. (2008). The identification of *trans*-resveratrol was performed by comparing the peak retention times of the samples and standards and the measurement was carried out by external standardization.

Calibration curves were constructed with seven concentration levels, the first corresponding to the limit of quantification for each compound. The limits of detection and limits of quantification obtained were, respectively, 0.05 and 0.16 for gallic acid, 0.04 and 0.12 for catechin, 0.02 and 0.06 for caffeic acid, 0.08 and 0.24 for coumaric acid, 0.04 and 0.13 for ferulic acid, 0.05 and 0.15 for quercetin, and 0.05 and 0.10 for *trans*-resveratrol.

2.8. Colour determinations

The absorbance values for the wine samples were determined in a spectrophotometer Hitachi U2010 (Tokyo, Japan) using a cuvette of 1 mm, as described by Glories (1984). The colour intensity (CI) was calculated by the sum of the absorbances at 420, 520 and 620 nm. The Konica Minolta colorimeter model Chroma Meter CR 400 (Mettler Toledo, Ohio, USA) was used to determine the coordinate values L*, a* and b*. The values of C* and h were calculated according to the equations $C^* = \left[(a^*)^2 + (b^*)^2\right]^{\frac{1}{2}}$ and $h = \arctan\left(b^*/a^*\right)$, respectively.

2.9. Antioxidant activity

The antioxidant activity was determined by the ABTS method (2,2-acid-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) according to the methodology described by Re et al. (1999), DPPH (2,2-diphenyl-1-picrylhydrazyl) with the methodology of Brand-Williams, Cuvelier, and Berset (1995), modified by Kim, Lee, Lee, and Lee (2002), and FRAP according to the methodology of Benzie and Strain (1996), modified by Arnous, Makris, and Kefalas (2002).

2.10. Statistical analysis

All of the fortified wines samples were analysed in triplicate and the results were expressed as mean ± pooled standard deviation. The Kolmogorov–Smirnov test was used to check the normality of the results and showed that for all variables tested the distribution was normal, which allowed for comparison using statistical parametric data. Analysis of variance (ANOVA) and Tukey's test at a significance level $p < 0.05$ were used to determine the statistical differences between the samples analysed. The analytical data were also processed using the linear correlation matrix and principal components analysis techniques. The linear correlation matrix method was used to determine the correlations between the phenolic compounds content and antioxidant activity of the wines studied and was expressed by the Pearson's correlation coefficient (r). Principal components analysis was applied to all samples of white and red fortified wines, considering the variables mineral content, higher alcohols content, carbon isotope ratio, phenolic composition and antioxidant activity. The principal components were determined and in order to group the samples for similarity components that accounted for over 70% of the total variation were considered. Scatter plots were obtained to visualize the dispersion of the treatments according to the scores of the principal components. All statistical analysis was performed in the Statistica 7.0 software (Statsoft Inc., Tulsa, Okla., USA).

3. Results and discussion

3.1. Profiles for minerals, higher alcohols and ratio of stable carbon isotopes

The concentrations of minerals were determined in fourteen samples of Brazilian fortified wines (Table 1) and only cadmium and thallium were found at levels below the quantification limit (in five of the samples analysed). The presence of aluminium in wine is associated with the use of pesticides, contact with surfaces of aluminium and some winemaking products such as bentonites, tannins and filtration aids (Catarino, Curvelo-Garcia, & Bruno de Sousa, 2008). Aluminium levels of 84 to 1086 mg L^{-1} were observed in the samples analysed. The highest levels of aluminium were found in the samples of white wines from the Serra Gaúcha, which may indicate a greater use of clarifiers and filtration products.

The manganese concentration ranged from 678 to 2890 μg L^{-1}, the highest values being observed for fortified wines produced in the Planalto Catarinense (CSME4, TNAC1 and MOSC3). The manganese content is characteristic of the soil of origin and is also associated with the use of phytosanitary products containing manganese salts, conservation in stainless steel containers, and the use of bentonites and pectolytic enzymes (Álvarez et al., 2007; Catarino et al., 2008; Rizzon, Salvador, & Miele, 2008).

The presence of iron in wine is related to the nature of the soil, different types of equipment used in the winemaking processes and some technological additives. This element plays an important role in the oxidation and ageing phenomenon, and its concentration is related to the stability of the wine (Catarino et al., 2008). The iron content of the wines analysed ranged from 192 to 3335 μg L^{-1}.

Cobalt and nickel are usually present in low concentrations and originate from the contact of must and wine with stainless steel equipment, the use of bentonites and of phytosanitary products applied in vineyards and atmospheric pollution (Catarino et al., 2008).

Table 1
Mineral composition, content of higher alcohols and $^{13}C/^{12}C$ stable isotope ratio of ethanol of white and red fortified wines produced in Brazil.

Analyte	Samples												
	Red wines								White wines				PSD[a]
	CSME1	CSME2	CSME3	CSME4	CSTA1	CSTA2	TNAC1	PORT1	GOET1	MOSC1	MOSC2	MOSC3	
Minerals													
Al (μg L^{-1})	252	369	235	98	261	84	227	268	198	1086	727	304	277.06
Mn (μg L^{-1})	1805	1856	1279	2094	1452	1823	2095	1781	678	1151	1119	2890	562.84
Fe (μg L^{-1})	1013	3335	876	353	1464	192	355	756	445	1752	1541	279	873.62
Co (μg L^{-1})	3.61	3.74	2.05	5.03	2.28	2.37	7.61	6.60	1.17	4.50	4.03	7.98	2.15
Cu (μg L^{-1})	112	62.22	41.41	88.10	1023	62.59	101	50.51	85.66	1396	574	37.38	441.77
Pb (μg L^{-1})	8.66	9.54	12.06	7.32	43.91	45.44	3.38	7.40	10.62	24.09	49.36	8.23	16.59
Ni (μg L^{-1})	14.20	22.50	11.39	11.93	15.00	10.37	26.90	42.19	6.47	14.49	31.20	14.85	10.06
Zn (μg L^{-1})	946	854	335	863	786	778	464	615	287	728	1721	889	359.60
Cd (μg L^{-1})	1.56	1.00	1.36	<0.02	1.17	0.45	<0.02	0.51	<0.02	0.80	0.77	<0.02	0.55
Tl (μg L^{-1})	2.19	1.11	0.59	<0.4	0.85	1.84	0.83	<0.4	<0.4	1.40	1.40	0.80	0.69
Na (mg L^{-1})	19.8	70.4	47.8	4.8	41.2	29.5	6.7	5.55	6.5	82.8	97.9	24.1	31.37
K (mg L^{-1})	1532	1480	1120	1096	1656	1364	1461	1115	1117	759.2	1108	836.0	270.46
Ca (mg L^{-1})	102.4	230.2	145.3	50.8	98.5	57.4	43.0	48.0	56.1	277.6	316.7	52.4	95.03
Mg (mg L^{-1})	158.2	304.6	179.3	100.4	186.3	115.8	80.8	91.50	78.0	316.1	370.2	90.7	99.97
Higher alcohols													
Ethanal (mg L^{-1})	85.83	91.70	107.66	108.64	92.34	80.45	89.95	43.70	194.95	146.21	147.21	139.16	38.91
Ethyl acetate (mg L^{-1})	240.30	13.15	10.49	32.32	25.54	70.80	44.82	117.79	101.91	30.66	17.18	28.39	64.30
Methanol (mg L^{-1})	96.21	127.96	137.79	66.28	80.97	81.02	70.62	39.69	86.32	77.70	56.46	42.67	28.98
Isobutanol (mg L^{-1})	92.97	106.92	76.88	154.24	220.03	103.52	247.74	123.88	28.62	160.36	54.83	226.60	68.09
Isoamilic alcohols (mg L^{-1})	206.62	206.62	153.52	316.61	343.64	213.40	398.04	292.83	30.87	222.16	147.18	429.93	110.70
Carbon isotope ratio													
$\delta^{13}C$ (‰)	−21.62	−21.95	−19.68	−26.52	−24.86	−19.50	−27.23	−27.39	−17.47	−23.33	−21.06	−27.40	3.36

[a] PSD = Pooled standard deviation.

Nickel was detected in the range of 6.47 to 42.19 μg L^{-1} and for cobalt concentrations of 1.17 to 7.98 μg L^{-1} were determined.

The copper content of the wines examined ranged from 37.38 to 1396 μg L^{-1}. The lowest concentration was found in the white wine Moscatto Giallo from the Planalto Catarinense (MOSC3). Copper is an important constituent of must, necessary for fermentation as a growth factor for yeasts (Rizzon et al., 2008). In must, copper may originate from phytosanitary treatments carried out in the vineyard or they may be naturally derived from soil (Álvarez et al., 2007). During conservation, the copper content can increase through contact of the wine with copper materials and in fortified wines it may be present in the grape brandy added (Catarino et al., 2008).

The most important source of lead in wine is atmospheric pollution and the relationship between the lead content of grapes grown in proximity to roads has been proven (Catarino et al., 2008). The lead contents determined in this study ranged from 3.38 to 49.36 μg L^{-1}. The results are consistent with the characteristics of these regions: the highest levels of lead were found in wines from the Serra Gaúcha, a region of considerable industrial production and heavy vehicle traffic and the lowest levels of lead were found in samples from the Planalto Catarinense, typically an agricultural region, consisting of small towns and large agricultural areas.

Zinc is a natural constituent of grapes, must and wine, and is always present in small amounts. An increase in zinc content may arise from contact with materials based on metal alloys, the application of fungicides in vineyards and the use of oenological products that contain zinc in their composition. Longer maceration periods during wine production lead to higher concentrations of zinc (Álvarez et al., 2007; Rizzon et al., 2008). The samples analysed in this study had zinc concentrations of 287 to 1721 μg L^{-1}.

The highest concentration of cadmium (1.56 μg L^{-1}) was observed in the sample produced in the Serra Gaúcha from Cabernet Sauvignon/Merlot grapes (CSME1). The presence of this element in wine is associated with atmospheric pollution, phytosanitary products and contact with stainless steel materials (Catarino et al., 2008). The highest levels of cadmium were found in wines from the Serra Gaucha region with notable industrial activity and the highest pollution levels of the regions studied.

The levels of thallium in uncontaminated wine are usually in the range of 2 to 8 μg L^{-1} and this element originates from soil and fertilizers (Catarino et al., 2008). The highest content of thallium was determined in the sample CSME1 (2.19 μg L^{-1}).

The sodium content of wines is related to the geographical origin and the addition of oenological products (Rizzon et al., 2008). In the wines analysed concentrations ranging from 4.8 mg L^{-1} to 97.9 mg L^{-1} were detected. Samples with lower sodium concentrations were those from the Planalto Catarinense and Carbonífera Region, which may indicate the use of oenological products in smaller proportions. Potassium was found in the wines analysed in concentrations ranging from 759.2 to 1656 mg L^{-1}, the lowest concentration being observed in the white wine sample MOSC1 and the highest in the red wine sample CSTA1. In general, the potassium content is higher in red wines due to the process of maceration used in their production. However, no statistical differences were found between some white and red wine samples (CSME3, CSME4, PORT1, GOET1, MOSC1, MOSC2 and MOSC3), mainly because in Brazil fortified white wines are produced with maceration of the grape skins, the calcium concentration of wines being mainly related to the techniques of vinification (Rizzon et al., 2008). For the wines analysed values of 43.0 to 316.7 mg L^{-1} were observed. Magnesium was present in levels of 78.0 mg L^{-1} (GOET1) to 370.2 mg L^{-1} (MOSC1). Magnesium contributes to the typicity characteristics, participating in the stability of sensorial aspects and certain wine alterations. It is an important element for the multiplication and metabolism of yeasts (Rizzon et al., 2008).

In relation to our study, higher levels of aluminium and iron were found by Paneque et al. (2010) and Paneque, Álvarez-Sotomayor, and Gómez (2009) in Spanish fortified wines. Dugo, La Pera, Pellicanó, Di Bella, and D'imperio (2005) found higher values of cadmium, zinc and lead in Italian fortified wines. Similar concentrations of manganese, potassium and copper were detected by Rizzon et al. (2008) in Brazilian wines.

Ethanal is the main aldehyde formed during vinification and its concentration is directly dependent on the amount of sulfur dioxide and ethanol, which combine to form acetals. Only the free ethanal has a significant aroma and at low levels it contributes to the fruity aroma, while high concentrations (>200 mg L^{-1}) contribute to the reduced flavour to the wine (Gil et al., 2006). The samples analysed had concentrations of ethanal varying from 43.70 to 194.95 mg L^{-1}.

Ethyl acetate is produced by the enzymatic esterification of acetic acid and of ethanol. Concentrations higher than 150–200 mg L^{-1} are considered to have a negative effect on the flavour of wines and are usually associated with contamination of the grape, must or wine by acetic bacteria, indicating the existence of flaws in the vinification and/or conservation process (Gil et al., 2006). In the samples analysed values of 10.49 to 240.30 mg L^{-1} were observed, the highest content of ethyl acetate being observed for the CSME1 sample, indicating the possibility of the bacterial contamination of this sample.

Methanol was determined in the samples analysed at concentrations ranging from 39.69 to 137.79 mg L^{-1}. Methanol is produced during maceration through the hydrolysis of the must pectins and does not affect the organoleptic properties of wines. After ingestion methanol is oxidized producing formaldehyde and formic acid, both toxic to the central nervous system. The level which represents a risk to humans is around 350 mg L^{-1} (Jackson, 2008).

Isobutanol was detected in concentrations of 28.62 to 247.74 mg L^{-1} and for isoamyl alcohol values of 30.87 to 429.93 mg L^{-1} were obtained. Studying the volatile compounds of Spanish fortified wines, Moreno, Zea, Moyano, and Medina (2005) found the content of isoamyl alcohols to be above 380 mg L^{-1}.

The determination of the $\delta^{13}C$ value for wines was performed based on the ethanol obtained in the fermentation and the ethanol added in the fortification step. The $\delta^{13}C$ values for the samples analysed varied from −27.40 to −17.47‰ for the white fortified wines and from −27.39 to −19.50‰ for the red fortified wines (Table 1). The highest value (−17.47‰) was observed for a white wine produced in the Carbonífera Region (GOET1), while the lowest value (−27.40‰) was obtained for a white wine produced in the Planalto Catarinense (MOSC3). For the red wines, the highest $\delta^{13}C$ values of −19.50‰ and −19.68‰ were observed for wines produced in the Serra Gaúcha from Cabernet Sauvignon/Tannat (CSTA2) and Cabernet Sauvignon/Merlot (CSME3) grapes, respectively, and the lowest value of −27.39‰ for the sample PORT1, which was statistically equal the samples CSME4 and TNAC1 produced in the Planalto Catarinense.

The $\delta^{13}C$ value mainly reflects the botanical origin and the photosynthetic pathway, but also provides information regarding the geographical origin. C3 plants are predominantly cultivated in high latitudes and C_4 plants are more common in the tropics and hot climate regions in which plants are cultivated under water stress. The $\delta^{13}C$ value is higher for C_3 plants cultivated in the region of the equator and decreases in plants cultivated towards the poles, where the climates are colder (Rummel et al., 2010). The results observed for the samples analysed, in which lower $\delta^{13}C$ values were found in wines originating from the colder region (Planalto Catarinense) and higher values were detected in samples produced in warmer regions, can be attributed to the temperature-dependence of the isotopic fractionation during photosynthesis. However, when variations in $\delta^{13}C$ are very high (greater than 2–3‰), the climate is not the only factor that has an effect on the isotopic fractionation. The most likely reason for the variations observed in the samples analysed (~9.9‰) is the addition of sugar or ethanol derived from C4 plants, such as cane sugar (Cabañero et al., 2008). The presence of derivatives of C4 plants may be the result of the process of adding cane sugar before or after

fermentation, or due to the addition of ethanol derived from cane sugar in the wine fortification step (Pissinatto, Martinelli, Victoria, & Camargo, 1999). This result indicates that the grapes produced in the Carbonífera Region and the Serra Gaúcha have a lower amount of total soluble solids compared to samples produced from grapes grown in the Planalto Catarinense. The lower content of total soluble solids determines the need to add sugar to obtain alcohol in wines. In this case, it was noted that in the region of the Planalto Catarinense grapes with higher sugar content are produced and it is not necessary to add sugar or ethanol from sugar cane to the fortified wines, which means that the wines have only the characteristics of compounds associated with the grapes and the fermentation.

3.2. Phenolic compounds, colour and antioxidant activity

The results for the determinations of phenolic compounds and colour are presented in Table 2. The total polyphenols content of the samples analysed was higher in red wines than in white wines, variations of 894.09 to 3241.82 mg L^{-1} and 277.00 to 427.45 mg L^{-1} being observed for fortified red and white wines, respectively. Similar results (201 to 446 mg L^{-1}) were found by Villaño, Fernández-Pachón, Troncoso, and García-Parrilla (2004) for fortified white wines produced in southern Spain. The concentration of flavonols ranged from 55.85 to 240.97 mg L^{-1} for the fortified red wines and from 25.74 to 67.33 mg L^{-1} for the fortified white wines. Tartaric esters were quantified in the range of 43.22 to 114.10 mg L^{-1} and 108.86 to 360.83 mg L^{-1} in the samples of fortified white and red wines, respectively. The observed concentrations of non-polymerized phenolic compounds were in the order of 737.66 to 1728.10 mg L^{-1} in the fortified red wines and 401.12 to 555.84 mg L^{-1} in the fortified white wines. The concentration of *ortho*-diphenols ranged from 67.10 to 525.39 mg L^{-1} for the red wines and from 3.84 to 40.28 mg L^{-1} for the white wines. Total monomeric anthocyanins were found in concentrations of 2.24 to 50.46 mg L^{-1} in the samples of fortified red wines while in samples of fortified white wines these compounds were not detected. The polymeric fraction of tannins was predominant in the samples analysed, followed by the oligomeric and monomeric fractions, with the exception of the CSME4 and TNAC1 samples, produced in the Planalto Catarinense, which presented higher concentrations of monomers in relation to oligomers. The CSME4 sample from the Planalto Catarinense presented the highest content of total tannins (665.45 mg L^{-1}) and the CSME3 sample from the Serra Gaúcha presented the lowest content (52.84 mg L^{-1}). The lowest monomeric fraction was observed in the sample of port wine (PORT1), with 2.78 mg L^{-1}, equivalent to 2% of the total tannins and the highest concentration of monomers was found in the TNAC1 sample from the Planalto Catarinense (26.81 mg L^{-1}), equivalent to 7% of the total tannins. The highest proportion of oligomers was detected in the CSME1 sample from the Serra Gaúcha, with 72.39 mg L^{-1}, corresponding to 19.6% of total tannins, while the lowest concentration of oligomers was verified in the CSME4 sample from the Planalto Catarinense. The polymers were predominant in all samples analysed and the CSME4 sample from the Planalto Catarinense had the highest proportion of polymers (96%).

Catechin was one of the major phenolic compounds detected in the fortified wine samples, with an average content of 15.05 mg L^{-1}. In fortified white wines produced in southern Spain, values of 3.55 mg L^{-1} (Fernández-Pachón, Villaño, Troncoso, & García-Parrila, 2006) and 25.7 mg L^{-1} (Ortega, Mayen, & Medina, 2008) have been detected. Concentrations in the range of 2.20 to 7.50 mg L^{-1} were found in fortified red wines produced in Italy (La Torre et al., 2008).

The phenolic compound with the highest concentration in the samples analysed was gallic acid (average content of 64.65 mg L^{-1}), and the highest content of this acid (137.98 mg L^{-1}) was observed in the CSME2 sample of fortified red wine from the Serra Gaúcha. The lowest concentrations of gallic acid were observed in samples of fortified

Table 2
Phenolic compounds and chromatic characteristics of white and red fortified wines produced in Brazil.

Analytes	Samples												
	Red wines								White wines				PSD[a]
	CSME1	CSME2	CSME3	CSME4	CSTA1	CSTA2	TNAC1	PORT1	MOSC1	MOSC2	MOSC3	GOET1	
Total polyphenols (mg acid gallic L^{-1})	2512.27	3241.82	894.09	1900.91	2473.64	1916.82	1316.82	2020.36	305.64	501.54	427.45	277.00	974.24
Tartaric esters (mg acid caffeic L)	282.24	360.83	108.86	240.04	221.24	319.03	249.04	237.04	45.50	77.12	114.10	43.22	105.88
Flavonols (mg quercetin L^{-1})	159.04	240.97	67.33	181.13	125.21	192.31	137.23	128.56	26.16	36.48	55.85	25.74	69.74
Non-polymerized phenolic compounds (mg catechin L^{-1})	927.23	1063.10	737.66	890.77	1034.64	940.39	804.52	1728.10	639.42	344.22	555.84	401.12	354.70
Ortho-Diphenols (mg catechin L^{-1})	244.95	525.39	67.10	190.29	341.20	215.64	132.85	239.40	4.04	37.11	40.28	3.84	152.23
Total monomeric anthocyanins (mg malvidin-3-glucoside L^{-1})	38.62	44.35	2.24	33.20	31.45	22.55	50.46	25.73	n.d.	n.d.	n.d.	n.d.	18.79
Monomeric fraction of tannins (mg L^{-1} catechin)	23.46	11.43	12.96	18.52	25.78	24.07	26.81	2.78	n.d.	n.d.	n.d.	n.d.	8.49
Oligomeric fraction of tannins (mg L^{-1} procyanidin B1)	72.39	45.29	13.77	6.93	53.26	33.70	21.81	7.24	n.d.	n.d.	n.d.	n.d.	23.72
Polymeric fraction of tannins (mg L^{-1} proanthocyanidins)	306.31	441.44	26.1263	641.44	530.18	96.49	326.13	126.12	n.d.	n.d.	n.d.	n.d.	188.92
Total tannins (mg L^{-1})	402.15	498.16	52.86	665.45	609.22	154.26	374.75	136.15	n.d.	n.d.	n.d.	n.d.	247.14
Gallic acid (mg L^{-1})		102.30	137.98	94.55	58.50	106.31	55.24	67.68	109.65	14.99	5.54	16.36	6.65
Caffeic acid (mg L^{-1})	19.71	0.79	0.66	27.07	5.60	1.64	20.88	20.07	5.98	6.93	8.12	2.62	8.91
p-coumaric acid (mg L^{-1})	8.62	0.64	0.72	2.69	1.68	1.09	5.08	7.81	1.60	2.56	4.24	1.51	2.67
Ferulic acid (mg L^{-1})	0.95	0.63	0.78	0.85	2.44	2.40	1.99	2.78	0.85	0.96	0.96	1.24	0.75
(+) Catechin (mg L^{-1})	10.22	16.39	11.98	12.01	21.83	21.69	22.34	1.57	5.39	9.31	5.01	3.86	11.42
Quercetin (mg L^{-1})	10.67	14.34	11.57	10.73	26.42	22.62	9.06	2.71	3.01	8.38	8.71	1.64	7.77
Trans-resveratrol (mg L^{-1})	9.11	8.08	6.78	7.94	9.97	10.94	5.90	2.33	<0.19	<0.19	<0.19	<0.19	4.37
Colour intensity	12.49	13.96	2.76	14.05	7.17	12.59	10.88	7.04	0.28	0.17	0.37	0.25	5.83
L^*	17.62	13.50	19.40	13.11	18.71	18.11	16.80	9.99	37.50	38.69	36.12	38.11	11.02
a^*	1.18	1.26	9.07	1.27	4.28	3.09	2.05	5.87	0.41	−0.56	1.16	0.84	2.72
b^*	0.30	1.31	4.08	−0.99	0.90	0.62	0.82	1.84	13.79	12.52	14.79	12.36	6.17
C^*	1.23	1.84	9.91	1.63	4.38	3.06	2.18	6.15	13.79	12.54	14.84	12.39	5.32
H	15.88	45.85	24.31	32.54	12.32	12.33	22.37	17.42	88.07	92.69	85.25	85.80	33.37

n.d. = not detected.
[a] PSD = pooled standard deviation.

white wines with values of 5.54 to 16.36 mg L^{-1}. Lower concentrations of this compound were found by Fernández-Pachón et al. (2006) and Ortega et al. (2008) in fortified white wines and by Ho, Hogg, and Silva (1999) and La Torre et al. (2008) in fortified red wines. In Brazilian wines values of 13.88 to 52.94 mg L^{-1} have been detected (Granato et al., 2011). The main source of gallic acid is grape seeds. High concentrations of this compound in wines indicate the use of practices that increase their extraction from the grapes, such as extensive maceration, high fermentation temperatures and aggressive pressing techniques (Bravo et al., 2006) and may also result from their extraction from wood during aging or the degradation of tannins (Ho et al., 1999). The white fortified wines had the lowest concentrations of gallic acid probably because these wines are not subjected to aggressive pressing techniques and are fermented at lower temperatures.

Caffeic acid was found in concentrations ranging from 2.62 to 8.12 mg L^{-1} in samples of fortified white wines and from 0.66 to 27.07 mg L^{-1} in fortified red wines. Caffeic acid was detected, in lower concentration, in fortified wines by Fernández-Pachón et al. (2006), Ortega et al. (2008), Ho et al. (1999) and La Torre et al. (2008), and in Brazilian wines by Granato et al. (2011).

p-coumaric acid is a phenolic acid naturally present in wine which acts as a substrate for enzymes that synthesize resveratrol and is released during fermentation through the action of the esterase activity of enzymes (Salameh, Brandam, Medawar, Lteif, & Strehaiano, 2008). Concentrations ranging from 0.64 to 8.62 mg L^{-1} of *p*-coumaric acid were quantified in the samples analysed. Lower levels of *p*-coumaric acid have been detected in European fortified wines (Fernández-Pachón et al., 2006; Ho et al., 1999; La Torre et al., 2008; Ortega et al., 2008). In contrast, higher concentrations were determined in Brazilian wines (6.17 to 10.73 mg L^{-1}) by Granato et al. (2011).

Ferulic acid was detected in the range of 0.63 to 2.78 mg L^{-1} in the samples of fortified wines analysed. This hydroxycinnamic acid can be converted to 4-vinylguaiacol when the fortified wine is matured in oak barrels and thus the lowest concentrations of this compound are found in aged wines (Ho et al., 1999). In Brazil, where fortified wines are produced from a blend of wines from different vintages and are not subjected to the aging process in oak higher levels of ferulic acid are found. In this case, the differences between the samples can be attributed to the composition of the grapes with which the wines were produced (Bravo et al., 2006).

In the samples of fortified white wines quercetin concentrations in the range of 1.64 to 26.42 mg L^{-1} were detected. The biosynthesis of phenolic compounds, especially flavonols, is largely influenced by the exposure to sunlight and high temperatures and therefore a higher content of flavonols, such as quercetin, is usually found in wines produced from grapes cultivated in warmer regions (Rastija, Srecnik, & Marica-Medic-Saric, 2009). From the samples of red wines analysed, the CSTA1 from the Serra Gaúcha presented a higher content of quercetin in relation to the samples produced in the Planalto Catarinense, known as the coldest region of Brazil.

The *trans-resveratrol* stilbene was detected in all samples analysed, however, in white wines levels were below the quantification limit established by the analytical method (0.19 mg L^{-1}). Concentrations of 2.33 to 10.94 mg L^{-1} of *trans*-resveratrol were detected in the fortified red wine samples. These values are higher than those reported by La Torre et al. (2008) for fortified wines and by Souto et al. (2001) and Granato et al. (2011) for non-fortified wines.

The greatest colour intensity was detected in a sample of fortified wine produced in the Planalto Catarinense from Cabernet Sauvignon/Merlot grapes (CSME4). In fortified white wines colour intensity values of 0.17 to 0.37 were observed. The CIELAB coordinates represent the qualitative and quantitative components of the colour. L^* is the measure of lightness; $L^* = 0$ corresponds to black and $L^* = 100$ to white (Recamales, Hernanz, Álvarez, González-Miret, & Heredia, 2007). The fortified white wines were more transparent, with L^* values varying from 36.12 to 38.11 units. The fortified red wines presented L^* values in the range of 9.99 to 19.40 units. Other CIELAB parameters are related to the red colour ($+a^*$) or green ($-a^*$) and yellow ($+b^*$) or blue ($-b^*$). Significant differences were observed for the a^* and b^* parameters of the samples analysed. The MOSC2 sample from the Serra Gaúcha presented a green colour, indicated by the negative a^* value (-0.56), unlike the other samples, which obtained positive a^* values. Concerning the b^* values, in the CSME4 sample produced in the Planalto Catarinense, a negative value (-0.99) corresponding to a blue colour was observed, while in the other samples there were positive values, indicative of a yellow colour. The highest values for a^* and b^* were detected for the CSME3 sample, corresponding to an orange-brown colour. C^* values in the range of 1.23 to 9.91 units were observed for the fortified red wines and 12.39 to 14.84 units for the fortified white wines. These values indicate a greater absorption of white light and the predominance of slightly saturated colours (Recamales et al., 2007). There was a big dispersion observed in the h results for the samples analysed. For the fortified white wines values of 85.25 to 92.69° were observed, indicating the predominance of a yellow hue. For red fortified wines, a red hue prevailed, with results ranging from 12.32 to 45.85° (Recamales et al., 2007).

The samples of the fortified red wines presented higher antioxidant activity than those of the fortified white wines (Fig. 1). In the samples of fortified white wines significant differences were verified at the $p < 0.05$ level only on applying the FRAP method. The values obtained for antioxidant activity were in the range of 0.68 to 1.62 mg L^{-1} for the fortified white wines. Fernández-Pachón et al. (2006) observed antioxidant activity values lower than those obtained in this study while researching European fortified white wines.

The highest value for antioxidant activity was obtained through the DPPH method for the CSME1 sample produced in the Serra Gaúcha, with 20.11 mg L^{-1} of TEAC. In the ABTS method, the highest antioxidant activity values were obtained for samples of fortified wines produced in the Serra Gaúcha (CSTA1 with 19.44 mg L^{-1} of TEAC and CSME2 with 17.69 mg L^{-1} of TEAC). In the FRAP method antioxidant activity values of 2.46 to 11.55 mg L^{-1} of TEAC were observed for fortified red wines, especially for samples CSME2 and CSTA1. The discrepancies observed in the results obtained with the three methods used can be attributed to differences in the electrons and in the hydrogen transfer ability of phenolic compounds (Roussis et al., 2008).

The polyphenols content showed significant positive correlation ($p < 0.05$) with the antioxidant activity measured through the ABTS ($r = 0.96$), DPPH ($r = 0.82$) and FRAP ($r = 0.98$) methods. Also, classes of phenolic compounds with similar chemical structures were found to influence the antioxidant activity, with significant positive correlations (r) between antioxidant activity and: (a) *ortho*-diphenols, 0.89 (ABTS), 0.69 (DPPH) and 0.94 (FRAP); (b) tartaric esters, 0.88 (ABTS and FRAP) and 0.77 (DPPH); (c) flavonols, 0.86 (ABTS and FRAP) and 0.74 (DPPH); (d) total tannins 0.86 (ABTS), 0.85 (DPPH) and 0.83 (FRAP); (e) total monomeric anthocyanin 0.86 (ABTS), 0.87 (DPPH) and 0.85 (FRAP). Furthermore, high positive correlations (significance level of $p < 0.05$) were observed between antioxidant activity and gallic acid and *trans*-resveratrol contents, with *r* values of 0.84 (ABTS), 0.71 (DPPH), 0.87 (FRAP), and 0.83 (ABTS), 0.70 (DPPH), 0.75 (FRAP), respectively. Significant correlation correlations ($p < 0.05$) were also observed between antioxidant activity and catechin, 0.64 (ABTS), 0.39 (DPPH) and 0.70 (FRAP), caffeic acid 0.62 (DPPH), coumaric acid 0.51 (DPPH) and ferulic acid, 0.34 (ABTS and FRAP). Granato et al. (2011) studying South American red wines found significant positive correlations between antioxidant activity and the contents of phenolic compounds, total flavonoids, quercetin, rutin, myricetin, gallic acid, catechin, ferulic acid and kaempferol.

According to Cheynier (2006) and Granato et al. (2011), the antioxidant activity of phenolic compounds, especially flavonoids, is due to both the number and acidity of their phenolic hydroxyl groups and to the resonance ring, which increases electron delocalisation

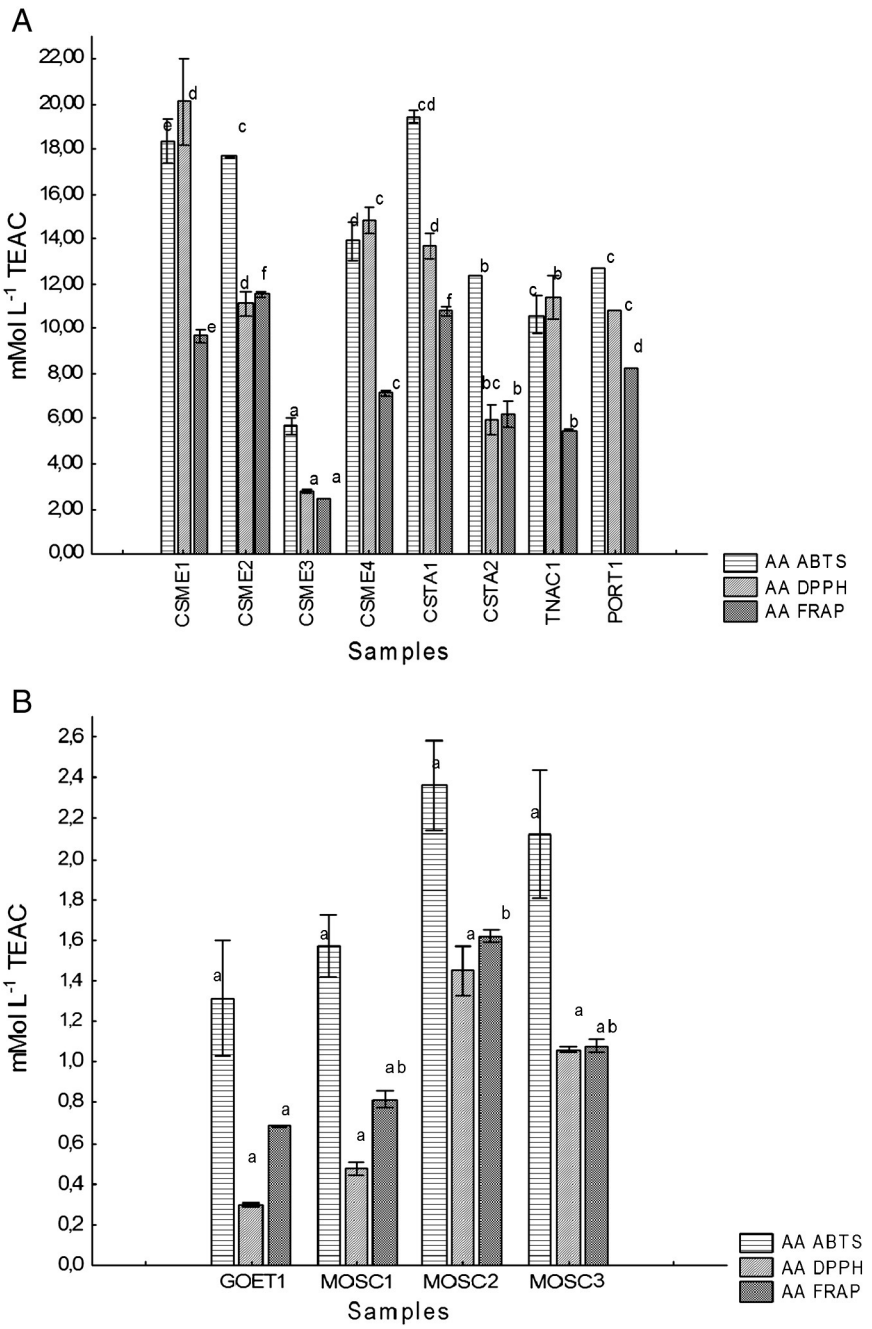

Fig. 1. Antioxidant activity (ABTS, DPPH and FRAP) of fortified red (A) and white (B) produced in Brazil. Different letters in columns indicate difference significant at $p < 0.05$ between samples.

between the pair of free electrons on the phenolic oxygen, and the benzene confers a partial negative charge leading to the nucleophilic character of the substitution position adjacent to the hydroxyl group.

3.3. Principal components analysis

The application of principal components analysis to the samples of Brazilian fortified wines, using variables related to mineral content, higher alcohols, carbon isotope ratio, phenolic composition and antioxidant activity resulted in 26 principal components and demonstrated that four first principal components can explain more than 70% of the variation obtained for the samples. The importance of each principal component (PC) is evaluated by the percentage of total variance that it explains. Table 3 shows that PC1 explains 35.78% of the total variance and that PC2 explains 18.51%, these being the components of greatest importance in this analysis.

Factor analysis showed that the variables with the highest contribution to the separation of the samples in relation to the first principal

Table 3
Estimation of eigenvalues and proportion of variance explained by the principal components obtained by the analysis of minerals, higher alcohols, carbon isotope ratio, phenolic composition and antioxidant activity in samples of Brazilian fortified wines.

Principal component	Eigenvalue	% Total variance	Cumulative eigenvalue	Cumulative %
1	13.23	35.76	13.23	35.76
2	6.81	18.43	20.05	54.20
3	4.75	12.85	24.81	67.05
4	3.13	8.48	27.95	75.54

component, with marked factorial loading >0.70, were antioxidant activity (ABTS, DPPH and FRAP), tartaric esters, flavonols, non-polymerized polyphenols, *ortho*-diphenols, anthocyanins, tannins, total polyphenols, *trans*-resveratrol, gallic acid, potassium and ethanal. For the second principal component, the variables with the greatest contribution were iron, cadmium, sodium, calcium and magnesium. In the third principal component the variables with greatest contribution in terms of explaining the data were cobalt, nickel and isoamyl alcohols and the principal component 4 was quercetin.

The variables that contributed little to the study of the composition of Brazilian fortified wines from different regions were the carbon isotopic ratio, aluminium, manganese, copper, lead, zinc, thallium, catechin, caffeic acid, coumaric acid, ferulic acid, ethyl acetate, methanol and isobutanol.

Scatter plots of the scores were obtained only in the case of the first two principal components, highlighted as the most important for the separation of the samples according to region of origin (Fig. 2).

The samples of fortified red wines, with the exception of the CSME3, were found to be negatively correlated with PC1, while the samples of fortified white wines were found to be positively correlated with PC1. Three groups of samples from Brazil could be distinguished: all samples of fortified wines produced in the Serra Gaúcha were found to be negatively correlated with PC2, samples from the Planalto Catarinense were found to be positively correlated with PC2 and the sample of Carbonífera Region was found to be positively correlated with PC1 and PC2. The sample from the Douro Region, used as a reference sample, was found to be positively correlated with PC2 and negatively with PC1, with close proximity to the samples from the Planalto Catarinense.

The samples of fortified white wines MOSC1 and MOSC2, located in the lower right quadrant, exhibited characteristics commonly associated with high concentrations of aluminium, copper, zinc, lead, calcium, sodium, magnesium and a high carbon isotope ratio. On the other hand, the fortified red wines produced in the region of the Serra Gaúcha presented high concentrations of potassium, *trans*-resveratrol, total polyphenols and antioxidant activity. From the samples produced in this region, CSME1 and CSTA2 showed high values for ethyl acetate, gallic acid and tartaric esters, CSTA1 and CSTA2 had high levels of quercetin and catechin, CSME2 revealed high concentrations of iron, *ortho*-diphenols and gallic acid, and CSME3, located in the central region of the principal components graph, presented intermediate values for the variables analysed. The samples of fortified red wines produced in the Planalto Catarinense region (CSME4 and TNAC1) and the sample of port wine (PORT1) were located in the upper left quadrant, with

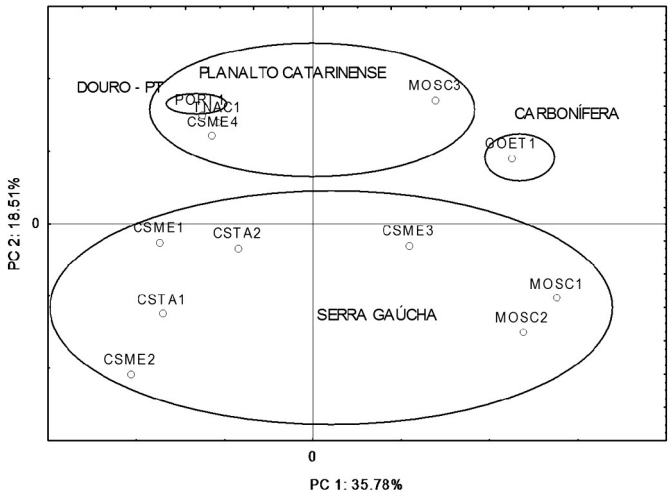

Fig. 2. Principal components analysis of the determinations of minerals, higher alcohols, carbon isotope ratio, phenolic composition and antioxidant activity for samples of Brazilian fortified wines. Variance explained: 35.78% (PC1) and 18.51% (PC2).

notable levels of cobalt, caffeic acid, coumaric acid, manganese, nickel, isoamyl alcohols and isobutanol. The sample of fortified white wine originating from the same region (MOSC3) was characterized by high contents of manganese, coumaric acid, caffeic acid, cobalt, isoamyl alcohols and isobutanol. The sample GOET1 located in the upper right quadrant was separated from the other samples due to its non-similarity in the results, revealing a high content of ethanal and low levels of phenolic compounds and minerals.

4. Conclusions

The results of this study showed that the white and red fortified wines produced in Brazil are active in the scavenging of DPPH and ABTS radicals and in the reduction of iron (FRAP). The fortified red wines showed higher antioxidant activity and higher levels of phenolic compounds than the fortified white wines. It was observed that the antioxidant activity of fortified wines is positively correlated with the total polyphenol content, *ortho*-diphenols, tartaric esters, flavonols, total monomeric anthocyanins, total tannins, gallic acid, *trans*-resveratrol, catechin, caffeic acid, coumaric acid and ferulic acid. Using principal components analysis it was possible to group the Brazilian samples of fortified wines according to the region of origin, the significant variables in relation to this separation being the antioxidant activity (ABTS, DPPH and FRAP), tartaric esters, flavonols, non-polymerized polyphenols, *ortho*-diphenols, anthocyanins, tannins, total polyphenols, *trans*-resveratrol, gallic acid, potassium, ethanal, iron, cadmium, sodium, calcium and magnesium.

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