

Physicochemical characterisation, bioactive compounds and *in vitro* antioxidant activities of commercial integral grape juices

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Abstract

Integral grape juice is an unfermented drink prepared from the edible part of grapes, with no water or sugar added. Besides commercial interest, several nutritional benefits for consumers' health have been associated with the regular consumption of grape juice which helps to consolidate its popularity among Brazilian consumers. In the present work, ten different commercial integral grape juices, eight from red grape and two from white grape were analysed for their physicochemical properties, including pH, °Brix, density, colour, total phenolic content, anthocyanins, and ABTS and DPPH antioxidant activities. All samples had a pH of 3.02 to 3.27, 13.9 to 15.2°Brix, and a density of around 1.06. However, there were significant differences in colour intensity, which varied from 5.89 to 22.42 AU (red grape juices) and from 0.333 to 0.395 AU (white grape juices). Phenolic contents of 0.790 to 1.774 mg GAE/mL were detected in red grape juices, and 0.193 to 0.343 mg GAE/mL from white grape juices. While anthocyanins were not identified in white grape juices, their values ranged from 19.87 to 101.61 mg/L in red grape juices. High antioxidant activities were found in all samples, but white grape juices showed significantly less activity as compared to red grape juices. Red grape juices showed DPPH values of 52.46–103.98 and 11.57–17.09 mM TEAC/mL, and ABTS values of 201.30–423.94 and 60.96–79.87 mM TEAC/mL for red and white grape juices, respectively. Based on these results, it is possible to conclude that grape juices, especially from red grapes, can provide important quantities of natural antioxidant molecules, such as polyphenols, including anthocyanins, which strongly act to neutralise free radicals.

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Keywords

Antioxidant Activity

Anthocyanins

Integral Grape Juices

Polyphenols

Physicochemical Analyses

Introduction

According to the Brazilian Institute for Wine (IBRAVIN), integral grape juice is defined as an unfermented drink prepared from edible part of grapes, with no water or sugar addition (Bruch, 2012). Its production involves a delicate process of thermal extraction, followed by maceration, but with no crushing of the grains, which avoids a bitter taste in the juice. The production of grape juice in Brazil centralised in “Serra Gaúcha”, located in the southern region of Rio Grande do Sul, but due to their favourable climate for growing grapes, some other states, for instance, São Paulo and Minas Gerais, also produce the product. Despite the intense cultivation

of grapes for wine production, a substantial fraction of grapes has been used for juice production in southern Brazil, which is responsible for the export of concentrated grape juice to many countries (Rizzon and Miele, 2012).

According to the Integrated System of Viniculture Declaration (SisDeclar), a databank for registration of Brazilian viniculture productions, the consumption of integral grape juices has risen about 30% per annum. From 2007 to 2012, it increased by around 400%, from ten million to fifty million litres. Eighty percent of the wine producers have also been producing integral grape juice to attend to this growing demand (Avindima, 2016).

In addition to commercial interest, several

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nutritional health benefits have been associated with the consumption of integral grape juice, contributing to consolidating its popularity among Brazilian consumers. Part of this preference can be attributed to the belief that grape juices possess a high content of phenolic compounds, such as anthocyanins and tannins, which are molecules associated with several prominent and beneficial biological activities. Antioxidant activity is one characteristic that is most studied and strongly associated with human benefits (Angelo and Jorge, 2007; Mitić *et al.*, 2011; Lima *et al.*, 2014; Lima *et al.*, 2015).

Free radicals are unstable and highly reactive molecules that have an unpaired electron in their atomic orbital, which allows them to attack and damage other important and functional molecules in the body such as DNA, proteins and lipids. The formation of free radical molecules and concomitant body's low ability to neutralise such molecular species, solely by endogenous antioxidant production, promote a phenomenon called oxidative imbalance/oxidative stress. The final consequence of this free radical chain reaction is the accumulation of cell damage, which in turn helps to promote the development of metabolic, cardiovascular, neurodegenerative and other chronic diseases. However, there is substantial evidence indicating that regular dietary consumption of phenolic compounds may contribute to reducing the incidence of chronic diseases linked with the formation of free radicals and consequently, ameliorating the oxidative imbalance. Polyphenols can act as antioxidants by various mechanisms; but one of the most important and well-described is their ability to neutralise free radicals by donation of a hydrogen atom (Valko *et al.*, 2007; Lobo *et al.*, 2010; Carocho and Ferreira, 2013; Pisoschi and Pop, 2015; Newsholme *et al.*, 2016).

Although many papers report the physicochemical characteristics and antioxidant activities in red and white wines (Stratil *et al.*, 2008; Xanthopoulou

et al., 2010; Leahu *et al.*, 2014), for commercial grape juices, such analyses are currently under-documented. In this context, the present work was aimed to characterise the physicochemical properties of different types of commercial integral grape juices, to quantify the bioactive compounds and to evaluate their *in vitro* antioxidant activities.

Materials and methods

Materials

Reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox[®]), methyl alcohol, ethyl alcohol, sodium acetate, potassium chloride, and ammonium persulfate were obtained from Sigma-Aldrich[®] (St. Louis, MO, USA). Folin-Ciocalteu phenol reagent was obtained from Merck[®] KGaA (Darmstadt, Germany). All other reagents were of analytical grade.

Sample selection

Commercial grape juices were obtained from local stores in the city of Rio de Janeiro, Brazil. Ten different types of grape juices, including eight prepared from red grapes and two from white grapes, were selected. Only two white grape juices were included in the present work due to two reasons: firstly, a general comparison of their results with the red grape ones was initially intended, and secondly, there is less availability of commercial white grape juice samples. Of the eight red grape juices, half were bottled in amber glass bottles and the other half in transparent ones. The two white grape juices were in amber glass bottles. Samples were coded from A1 to A10. Table 1 lists the general characteristics of the selected samples reported in the respective product labels.

Table 1. General information and characteristic of the grape juice samples analysed.

Samples	Lot	Grape type	Additives	Bottle glass colour	Region of origin
A1	511191	Red	INS 202 e 220	Transparent	RS/Brazil
A2	012/15	Red	NL*	Transparent	RS/Brazil
A3	22	Red	INS 220	Amber	GO/Brazil
A4	22/06/16 [#]	Red	NL*	Amber	RS/Brazil
A5	516	Red	NL*	Transparent	RS/Brazil
A6	070/26	Red	INS 202 e 220	Transparent	RS/Brazil
A7	CAT2961	Red	NL*	Amber	RS/Brazil
A8	31/08/16 [#]	Red	NL*	Amber	RS/Brazil
A9	1747	White	INS 202 e 220	Amber	RS/Brazil
A10	CAT29816	White	NL*	Amber	RS/Brazil

*NL: No information on the label. Preservative additives: INS 202 (potassium sorbate); INS 220: (sulphur dioxide). [#]In these samples, fabrication date was used as lot number.

Determination of pH, refraction index, °Brix, and relative density

The juice quality was physicochemically analysed in accordance with previously described methods (Santana *et al.*, 2008; Theuma *et al.*, 2015). pH values were measured by a potentiometer pH analyzer (MS Tecnopon® model mPA-210); Refraction index (RI) and °Brix values were determined using an Abbe refractometer (model 2WA-J Biobrix®) under a controlled temperature of $20 \pm 1^\circ\text{C}$. Specific gravity or relative density was established by theoretical calculations using °Brix values as shown in the following formula (Beechum, 2009; Sánchez-Sánchez *et al.*, 2017):

$$\text{Relative density} = (^\circ\text{Brix}/[258.6 - (^\circ\text{Brix}/258.2) \times 227.1]) + 1$$

Dry matter content

The dry matter content or mass percentage (m/v) of the grape juice samples was determined according to the European Pharmacopoeia. In triplicate, 1.0 mL grape juice sample, placed in a glass dish of about 100 mm in diameter, was allowed to evaporate in an oven at 105°C for 2 h. After total water evaporation, the dish was cooled in a desiccator over silica gel until weight stabilisation (three weighings, with intervals of 30 min to achieve the state of constant weight). Dry matter content was calculated as a mass percentage, as follows:

$$\text{Residual weight (g)} = \text{final weight of dish}_{(\text{after evaporation})} - \text{initial weight of dish}_{(\text{with no sample})}$$

$$\text{Dry matter content (m/v)} = (100 \times \text{residual weight}) / \text{volume (mL)}$$

Colour measurements

Colour analyses were performed as described previously by Ivanova *et al.* (2012) and Revilla *et al.* (2016), which were based on Somers (1978) and Glories (1984), who standardized the method using wine samples. In this method, colour intensity (CI) is directly related to the content of anthocyanins and can be defined as a sum of the absorbances measured at 420, 520, and 620 nm. Colour density (CD) is a sum of the absorbances at 420 and 520 nm. The hue or redness is defined as the ratio of absorbances at 420/520 nm. Red juice samples were pre-diluted with distilled water in a volumetric ratio of 1:5, due to the threshold of the equipment. Immediately, spectrophotometric measurements of the grape juices

at 420, 520, and 620 nm were recorded using a 1 cm optical path, and the CI, CD and hue (H°) were determined.

Determination of total phenolics

The total phenolic compounds present in the ten different integral grape juices were determined spectrophotometrically using the Folin–Ciocalteu method (Singleton and Rossi, 1965). The absorbance values measured at 765 nm were compared with a calibration curve of gallic acid (10–100 $\mu\text{g/mL}$) ($y = 4.3752x + 0.0076$; $R^2 = 0.999$), and the results were expressed as gallic acid equivalents (mg GAE/mL).

Determination of monomeric anthocyanins

The total monomeric anthocyanins were verified using the pH-differential method based on Fuleki and Francis (1968), with adaptations reported by Giusti and Wrolstad (2001) and Mitić *et al.* (2011). Juice samples were diluted with separate buffer solutions of KCl (0.025 M, pH = 1.0) and $\text{CH}_3\text{-COONa}$ (0.4 M, pH = 4.5), and absorbances (A) were recorded at 520 and 700 nm on a UV/Vis spectrophotometer and calculated as follows:

$$A = (\lambda_{520} - \lambda_{700})_{\text{pH}1.0} - (\lambda_{520} - \lambda_{700})_{\text{pH}4.5}$$

The monomeric anthocyanin concentration in the integral grape juices was calculated as follows:

$$\text{Monomeric anthocyanin} = (A \times M \times DF \times 1000) / \varepsilon \times l$$

where M = molar mass of cyanidin-3-glucoside (449.2 g/mol), which is the anthocyanin pigment most found in nature (Lee *et al.*, 2005); ε = molar extinction coefficient for cyanidin-3-glucoside (26.900 L/cm mol); DF = dilution factor, and l = cuvette optical path length (1 cm). The concentrations of anthocyanin pigments in the integral grape juices were expressed as cyanidin-3-glucoside equivalents in mg/L.

Antioxidant activity by DPPH

This assay, which estimates the capacity of antioxidant molecules present in the samples to scavenge DPPH^\cdot , was conducted as originally reported by Blois (1958) with adaptations by Brand-Williams *et al.* (1995). The DPPH^\cdot radical absorbs light at 517 nm, and antioxidant activity is observed by monitoring the decrease in this absorbance, in the presence of antioxidant molecules (Antolovich *et al.*, 2002).

A methanolic solution of DPPH^\cdot (100 μM) was prepared. For each sample, 0.05 mL of each grape juice sample was mixed with 1.95 mL 100 μM

DPPH'. The mixture was kept in the dark for 30 min, and then the absorbance was read at 517 nm. The results were interpreted using the calibration curve of Trolox® (0.0–2.0 mM) ($y = -0.4834x + 0.7653$; $R^2 = 0.987$), as a standard antioxidant, diluted in methanol. DPPH values were expressed as both mM of Trolox equivalent antioxidant capacity (TEAC/mL of grape juice) and as a percentage of free radical scavenging activity (antioxidant activity total [AAT]) relative to DPPH' solution with no antioxidants (control), calculated using the following equation:

$$\text{AAT\%} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}) / \text{Abs}_{\text{control}} \times 100$$

Antioxidant activity by ABTS

The ability of the commercial grape juices to reduce the blue/green radical ABTS^{•+}, causing its decolorisation, was spectrophotometrically measured at 734 nm, as detailed by Re *et al.* (1999). The results were assessed relative to the reactivity of Trolox®, used as a standard antioxidant, in different concentrations (0.0–2.0 mM) diluted in ethanol, under the same conditions as the samples.

The ABTS^{•+} radical monocation was previously prepared by reacting 1.0 mL ABTS stock solution with 44 µL 140 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 16 h before use. Next, a working solution was prepared by diluting ABTS^{•+} in ethanol until obtaining a maximum absorbance of ±700 at 734 nm. An aliquot (10 µL) of each grape juice sample diluted in distilled water at 1:4 (v/v) or ethanol solution of Trolox® (0.0–2.0 mM) was mixed with 1.99 mL 7 mM ABTS^{•+} ethanolic solution. The mixture was thoroughly vortexed. After incubation in the dark for 6 min, the absorbance was measured at 734 nm against a blank of ethanol without ABTS^{•+}.

Results were calculated according to the equation obtained from the calibration curve for Trolox® ($y = -0.1461x + 0.7093$; $R^2 = 0.997$). ABTS values were expressed as both mM of TEAC/mL grape juice, and as a percentage of free radical reduction (AAT) relative to ABTS^{•+} with no antioxidants (control), calculated using the following equation:

$$\text{AAT\%} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{samples}}) / \text{Abs}_{\text{control}} \times 100$$

Statistical analysis

All analytical experiments were performed in triplicate, where mean and standard deviation were further determined, except for pH, Brix°, RI, and

density. Differences in the values were analysed by analysis of variance (ANOVA), followed by Tukey's multiple comparison test to compare each two samples pair-to-pair. Associations between total phenolic compounds, anthocyanins, and antioxidant capacities were determined using Pearson's correlation. Statistical significance was set at $p < 0.05$.

Results and discussion

Physicochemical characteristics

Table 2 provides the physicochemical characteristics presented by the ten selected samples analysed in the present work. °Brix, RI, and dry matter are parameters related to the soluble solid quantities contained in juices. Among these parameters, °Brix is commonly used for analysing juice quality because it represents the amount of sucrose in juices. It means that high °Brix values may reveal extra sugar addition and low °Brix values may represent water dilution (e.g., reconstituted juices) (Rizzon and Miele, 2012) or even sugar fermentation by microorganisms (Nonga *et al.*, 2014). In both cases, other analyses are necessary to confirm such alterations during juice production.

In Brazil, the legislation for juice quality establishes a minimum value of 14 °Brix measured under a controlled temperature of 20°C. For integral juice, this value represents only sugars naturally present in the fruit (Brasil, 2000). Among the samples analysed in the present work, only one showed °Brix value below 14.0, sample A8 (13.9 °Brix). In general, the °Brix values found in most of the samples analysed agreed with other authors, although slight variations have been reported. Santana *et al.* (2008) recorded 14.21–17.30 °Brix from three different brands of grape juice commercialised in the city of Lavras, Minas Gerais, Brazil. Rizzon and Miele (2012) noted 16.2, 16.5 and 14.0 °Brix for whole, sweetened, and reprocessed juices, respectively. Munhoz *et al.* (2016) documented 11.01–17.14 °Brix, for juice samples obtained from different preparations (integral, organic, and handmade production). Gurak *et al.* (2008) also analysed eight different integral grape juices commercialised in the city of Rio de Janeiro, Brazil, and reported °Brix values varying from 14.0 to 16.9.

Another important parameter affecting juice quality is pH. This parameter represents the acidity of grape juices and is related to the flavour perception and sensory attributes of the juice. In these systems, only malic acid and tartaric acid are the effective acidic components. The pH is also responsible for stabilisation of the soluble solids (e.g., monomeric

Table 2. Physicochemical characteristics, colour analyses, and bioactive compounds of different types of integral grape juices.

Samples	Physicochemical characteristics					Colour analyses			Bioactive compounds	
	pH	°Brix	Relative density	Refraction index	Dry matter* (g/mL)	Colour intensity (AU**)	Colour density (AU)	Hue (AU)	Total phenolics (mgGAE/mL)	Monomeric anthocyanins (mg/L)
A1	3.22	14.3	1.060	1.3545	0.145±0.001	6.913±0.05	6.421±0.07	1.410±0.03	0.969±0.016 ^a	19.87±0.68
A2	3.15	14.6	1.062	1.3550	0.141±0.002	22.424±0.32	18.083±0.14	1.142±0.01 ^c	1.602±0.011 ^b	101.64±0.42
A3	3.25	14.6	1.062	1.3550	0.147±0.001	17.773±0.19	14.524±0.07	1.052±0.01 ^{de}	1.722±0.015 ^c	83.16±1.70
A4	3.20	14.3	1.060	1.3545	0.145±0.002	9.926±0.13 ^a	8.555±0.04 ^b	1.037±0.01 ^d	0.790±0.011	64.12±2.36
A5	3.14	14.8	1.063	1.3551	0.134±0.002	14.523±0.05	12.148±0.07	1.173±0.02 ^c	1.298±0.005	51.54±1.37 ^e
A6	3.26	14.2	1.060	1.3541	0.140±0.004	5.898±0.04	5.728±0.04	1.144±0.03 ^c	1.774±0.007 ^c	48.87±1.40 ^e
A7	3.24	14.1	1.059	1.3543	0.137±0.001	12.413±0.07	10.693±0.02	1.006±0.02 ^d	1.603±0.012 ^b	75.26±1.97
A8	3.22	13.9	1.058	1.3533	0.136±0.001	10.047±0.26 ^a	8.678±0.08 ^b	1.077±0.01 ^e	0.995±0.011 ^a	58.78±2.06
A9***	3.27	14.2	1.060	1.3544	0.136±0.001	0.395±0.00 [§]	0.395±0.00 [§]	3.293±0.00 [§]	0.343±0.002	ND [#]
A10***	3.02	15.2	1.064	1.3560	0.146±0.002	0.333±0.00 [§]	0.333±0.00 [§]	4.203±0.00 [§]	0.193±0.006	ND [#]

* Dry matter values are expressed as mean ± SD ($n = 3$). **AU: Absorbance units. Values are expressed as mean ± SD ($n = 3$). Same letters indicate no significant differences and no letter indicates values significantly different by pairwise comparison of grape juices at the 5% level (Tukey's test). ***White grape juices. §White grape juices were not analysed by statistic tests for colour analyses. ND[#]: Not detected (white grape juices).

anthocyanins), colour, stability against precipitation of solids, and in some cases, pH exerts some function by controlling microbial growth (Kodur, 2011; Manns *et al.*, 2015). Similar to °Brix values, pH values also vary among different juice samples, particularly when they are produced in different regions, or obtained by different production processes. In the present work, the minimum pH value was measured in sample A10 (pH 3.02), a white grape juice, and the maximum pH value was found in sample A6 (3.26), a red grape juice. Many other authors have found pH values for grape juices mostly around 3.0, although pH's as low as 2.4 and as high as 4.0 have been stated for some samples produced under specific conditions (Gurak *et al.*, 2008; Santana *et al.*, 2008; Carpen and Torezan, 2009; Manns *et al.*, 2015; Munhoz *et al.*, 2016). Brazilian legislation does not specify pH values for grape juices in its Standard for Quality and Identification of Juices (Brasil, 2000). However, pH values are often used in food industries to control the quality of their products.

Colour analysis

The colour of grape juices is an important parameter related to pigments extracted and presented in the samples, mainly those prepared from red grapes. According to Somers (1978), compounds, such as anthocyanins and other minor phenolics, are responsible for not only the colour of the products produced from grapes but also for other sensory attributes, like astringency and visual appearance. In the present work, a significant correlation was noticed between CI and anthocyanin contents in red grape juice samples (Pearson's $r^2 = 0.8380$, $p = 0.009$). In this context, some investigators have

considered the colour of grape juice samples as also another important indicator of quality, which in turn informs the quality of the raw material used, along with their production (Vilas Boas *et al.*, 2014). The colour analysis of grape juices can be spectrophotometrically measured because yellow, red and blue compounds absorb light at 420, 520, and 620 nm, respectively (Analytik Jena Ag, 2009). The H° or tint (also called redness) is the ratio between the yellow–orange and red pigment contents. A high H° value represents white grape-derived products, whereas, red grape-derived products show a low H° value (Analytik Jena Ag, 2009).

As monomeric anthocyanins are major components related to the redness of grape juices (Celotti and De Prati, 2005), alterations in their content may be caused by non-efficient extraction process, degradation post-aging, or even precipitation of such compounds during storage. All these events may subsequently contribute to altering colour characteristics of juices. For spectral evaluation of grape juices, there are no previous or defined values, which means that all results must be interpreted based on comparisons among different samples or even based on correlations with anthocyanin quantities. Although, as mentioned above, colour analysis based on spectral measures are commonly used for products prepared from red grapes (juices or wines), here it was also applied to white grape juices, to emphasise the differences that occur when anthocyanins are present or absent in the samples. Figure 1 shows the differences in colour appearances among the ten different grape juice samples analysed in the present work. It is possible to correlate each colour with their respective spectral values presented in Table 2.

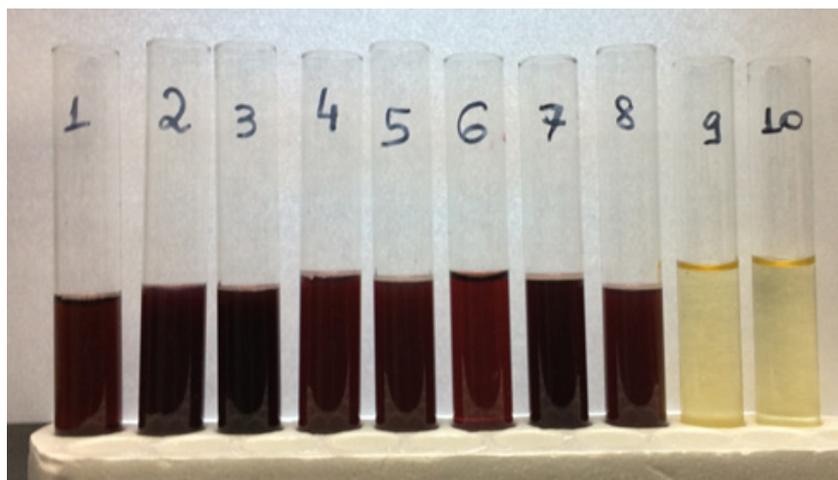


Figure 1. Colour appearances of the different samples analysed in the present work.

Theuma *et al.* (2015) stated that CI depends not only on anthocyanin content but also on pH values because the more acidic the pH, the more monomeric anthocyanins are present in soluble forms. This interaction was also confirmed by Manns *et al.* (2015). Here, samples A2 (pH 3.15) and A5 (pH 3.14) showed more CI, 22.42 and 14.52, respectively, than the other samples, corroborating the values found by Theuma *et al.* (2015) for grape wine varieties. As there is no standard process for extraction of juice from grapes, which in turn could reduce the difference in the content of pigment compounds present in the juice (e.g., anthocyanins), colour variations will always be expected when different samples are compared.

Phenolic compounds and monomeric anthocyanins

Table 2 reveals the contents of phenolic compounds and monomeric anthocyanins in the juices. Corroborating the colour values, it was also observed that samples A2 and A7 both showed a high monomeric anthocyanin content. These results may have contributed to the colour intensities also found in such samples.

The mean values obtained for total phenolics and monomeric anthocyanins were significantly different for almost all juice samples ($p < 0.05$), according to one-way ANOVA, followed by Tukey's multiple comparison test. Although phenolic compounds can be extracted from pulp, skin, and seed of the grapes (Burin *et al.*, 2010), the differences found in the present work express a wide variation in the content of these molecules. These variations may be a result of the different processes used for juice production, along with no standardised methods for extraction of the juice from grapes; and may also interfere in the bioactive compounds found in the juices. Similar results showing the same variations are also documented in the literature. Lima *et al.* (2014)

found phenolic contents varying from 779 to 2712 mg GAE/L in juices from new cultivars of Brazilian grape planted in São Francisco's Valley, Petrolina, PE, Brazil. For seven commercial juices produced in the state of Santa Catarina, Brazil, the total phenolics varied from 1117 to 2286 mg GAE/L (Burin *et al.*, 2010) while grape juices commercialised in the metropolitan region of Belo Horizonte, MG, Brazil, contained total phenolic values varying from 0.60 to 2.41 g GAE/L (Malacrida and Motta, 2005). In different samples of integral grape juices produced in various states of Brazil (Rio Grande do Sul, São Paulo e Santa Catarina) but commercialised in the state of Rio de Janeiro, Brazil, the phenolics ranged from 1.07 to 2.62 g/L (Gurak *et al.*, 2008). As expected, there was less polyphenolic content in white than red grape juices, respectively (Dani *et al.*, 2007; Mullen *et al.*, 2007), which was also verified in the present work (Table 2).

Similar variations are also found for the values of monomeric anthocyanins. Interestingly, in the present work, a weak and non-significant association existed between the total phenolic compounds and anthocyanin content (Pearson's $r^2 = 0.4861$, $p = 0.222$). The presence of anthocyanins is substantially dependent on the process used for juice production. Therefore, different quantities of anthocyanins can be expected in different types of commercial juices. Previously, anthocyanin contents varying from 44.2 to 164.9 mg/L for integral grape juices produced in different regions of Brazil (Gurak *et al.*, 2008), 283.7 and 184.8 mg/L for whole and reprocessed grape juices, respectively (Rizzon and Miele, 2012), and 194.54 and 261.84 mg/L for commercial organic juices produced from an authentic purple grape variety in Brazil (Granato *et al.*, 2015), have been described. Some of these values are higher than those found in the present work, but the production

of such juices may have occurred under controlled and known conditions, which may produce samples containing much more anthocyanins.

Despite the different contents of bioactive molecules found in the samples used in the present work, it is known that grape-derived products, especially those produced from red or purple grapes, are rich in polyphenols, including anthocyanins and other minor phenolics. Therefore, the regular consumption of food rich in polyphenols helps to protect the humans from deleterious effects caused by oxidative stress, thereby preventing the development of chronic diseases (Mullen *et al.*, 2009; Burin *et al.*, 2010; Pisoschi and Pop, 2015; Newsholme *et al.*, 2016). It is especially true for consumption of food or drinks containing anthocyanins since they have been shown to be rapidly absorbed by the gastrointestinal tract (Prior and Wu, 2006).

Antioxidant activities

Figure 2A and 2B illustrate the antioxidant activity represented by the ability of polyphenols present in the juice samples to scavenge DPPH[•] radicals *in vitro*. In the DPPH test, antioxidant activity was highly associated with the total phenolic compounds (Pearson's $r^2 = 0.988$, $p < 0.0001$) than with the anthocyanins content (Pearson's $r^2 = 0.423$, $p = 0.296$). The results were expressed in mM TEAC/mL and as a percentage of the oxidation inhibition or AAT, respectively. TEAC values reflect the relative ability of hydrogen or electron-donating antioxidants to scavenge or neutralise free radicals (ABTS^{•+} or DPPH[•]) as compared to that of Trolox[®] used as a standard antioxidant (Antolovich *et al.*, 2002). Although antioxidant activities showed similar results in both figures, it was decided to express both graphs because the white grape juices were tested

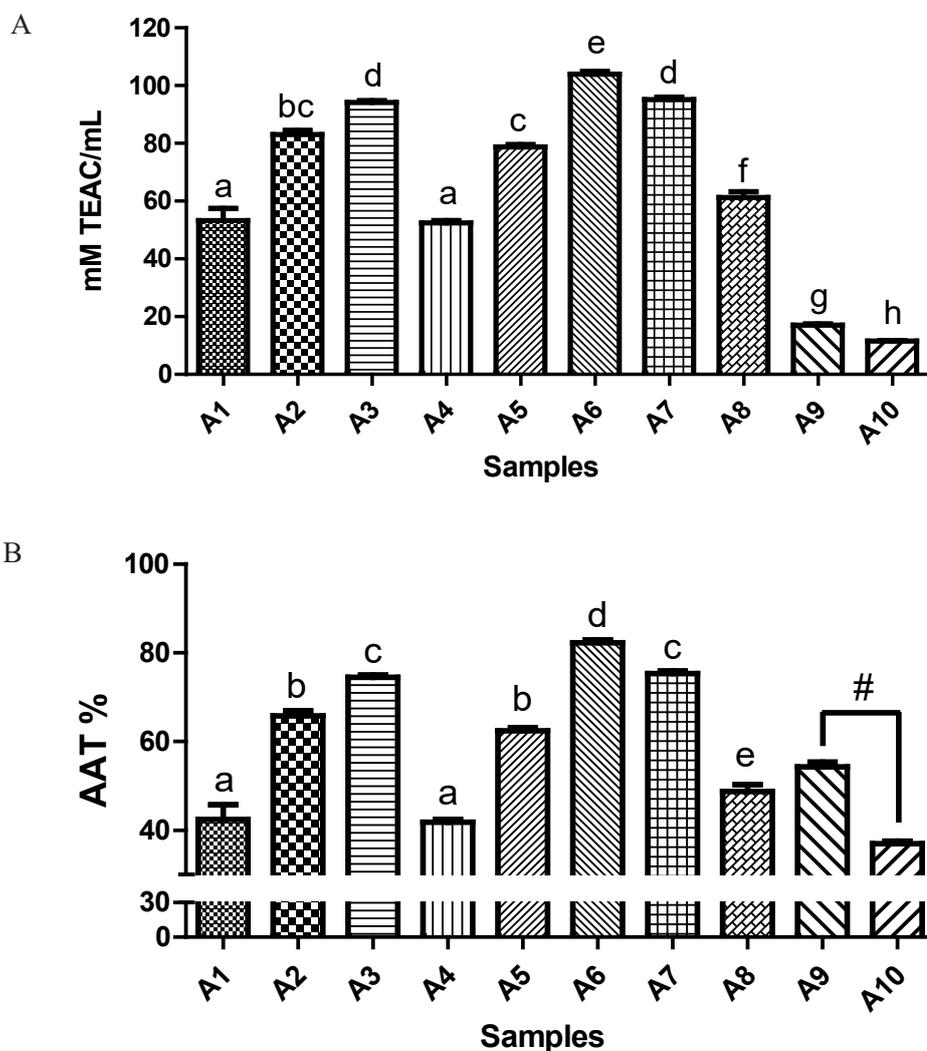


Figure 2. (A) DPPH antioxidant activity (mM TEAC/mL) of the commercial grape juices. (B) Antioxidant activity (%) of the commercial grape juices expressed as a percentage relative to the control (DPPH solution without antioxidants). Samples A1 to A8 from red grape juices were diluted in distilled water at 1:5 (v/v). Samples A9 and A10 from white grape juices were not diluted, and they were separately compared. Different letters or symbols on the bars indicate significantly different values analysed by Tukey's Multiple Comparison test with confidence interval (CI) of 95% for each pair ($p < 0.05$).

without previous dilution, whereas the red grape ones were diluted five-fold before testing. Thereby, results expressed in mM TEAC allow for normalisation of the values and comparing them with other grape juices, whether prepared from red or white grapes. Conversely, values expressed as a percentage (AAT%) not only allow comparison between samples under the same conditions of analysis, but they can be better compared with results from other authors. Based on results obtained in the present work, it can be inferred that anthocyanins, which were absent in white grape juices, may be responsible for the potent antioxidant activities found in red grape juices. Anthocyanins are compounds that contain many hydroxyl groups, which account for their ability to act as antioxidants, by providing hydrogen atoms, which neutralise the instability of free radicals, thereby interfering with the propagation of the oxidative chain reaction (Kähkönen and Heinonen, 2003; Prior and Wu, 2006; Ge and Ma, 2013).

As expected, based on the polyphenol content present in the juice samples, a wide, but highly correlated (Pearson's $r^2 = 0.988$) variation in the antioxidant activities was apparent. However, all juice samples, except the white grape juices, displayed strong DPPH scavenging activities. It is well known that white grape juices or wines have less antioxidant activity as compared to red grape juices or wines (Stratil *et al.*, 2008; Vargas *et al.*, 2008; Xanthopoulou *et al.*, 2010; Leahu *et al.*, 2014). Disparities in the values of antioxidant activity are prevalent, especially when researchers are working with commercial samples, as found in the present work. Burin *et al.* (2010) analysed seven commercial samples of red grape juices and reported DPPH values varying from 2.51 to 11.05 mM TEAC, which help to exemplify the extensive variation that can be found in such type of analysis.

When juice samples were tested for their ability to scavenge ABTS⁺, the observed results (Figure 3A and 3B) were very similar to those obtained by the DPPH test. Also, the ABTS antioxidant activity showed a higher association with the total phenolic compounds (Pearson's $r^2 = 0.972$, $p < 0.0001$) than the anthocyanins content (Pearson's $r^2 = 0.382$, $p = 0.349$). The high correlation between total phenolic compounds and antioxidant activity has already been reported (Sério *et al.*, 2014). However, it is important to use more than one approach to measure the antioxidant activity because different methods present distinct performances. For instance, the ABTS method presents an extra advantage in that it can be used at different pH's, unlike the DPPH procedure that

is more sensitive to acidic pH (Shalaby and Shanab, 2013). In this context, it is worth to keep in mind that grape juices often present low pH (2.0 to 4.0). Nevertheless, the similarity in the results obtained from both tests (Pearson's $r^2 = 0.9916$, $p < 0.0001$) has been confirmed by other authors, such as Gil *et al.* (2000), who compared the antioxidant activities of pomegranate juices. Shalaby and Shanab (2013) also noticed a similar DPPH and ABTS behaviour of aqueous and methanol extracts of *Spirulina platensis*, although the aqueous extract showed slightly lower ABTS activity. Villanueva-Tiburcio *et al.* (2010) analysed the skin of the Amazonian native fruit "camu-camu" harvested at different maturation times and mentioned that the percentage of DPPH and ABTS radical inhibition followed the same trend to each other, although the DPPH percentages were higher, which is consistent with the present work, for almost all the different samples. In contrast, Lima *et al.* (2014) detected slightly higher ABTS than DPPH antioxidant activities in various grape juice samples produced from new Brazilian grape varieties planted in Northeast Brazil, as well as in grape juices produced by different maceration processes (Lima *et al.*, 2015).

Irrespective of the methodological approach taken, it is worthwhile to emphasise the nutritional importance represented by the regular consumption of integral red grape juices which are rich in beneficial compounds. While some controversy exists about the regular intake of alcoholic beverages commonly prepared from grapes (e.g., wine and its derived products) for the prevention of cardiovascular and other chronic diseases, which is based on the "French paradox" (Mullen *et al.*, 2009; Xanthopoulou *et al.*, 2010; Biagi and Bertelli, 2015), red grape juices represent an optimal choice. Grape juices contain no alcohol and maintain most of the principal bioactive compounds present in wine, such as polyphenols, including anthocyanins. Full comprehension of the role of dietary phenolics in disease prevention remains unclear since their bioavailability is not yet totally established (Mullen *et al.*, 2009). Nevertheless, insights regarding the absorption, metabolism, and bioavailability of anthocyanins, as reviewed by Prior and Wu (2006), and other valuable information about natural and synthetic compounds acting as antioxidants or prooxidants can be obtained, by reading Carocho and Ferreira (2013). The body of evidence accumulated emphasises the importance of the consumption of the integral grape juices, contributing to the maintenance of a healthy condition.

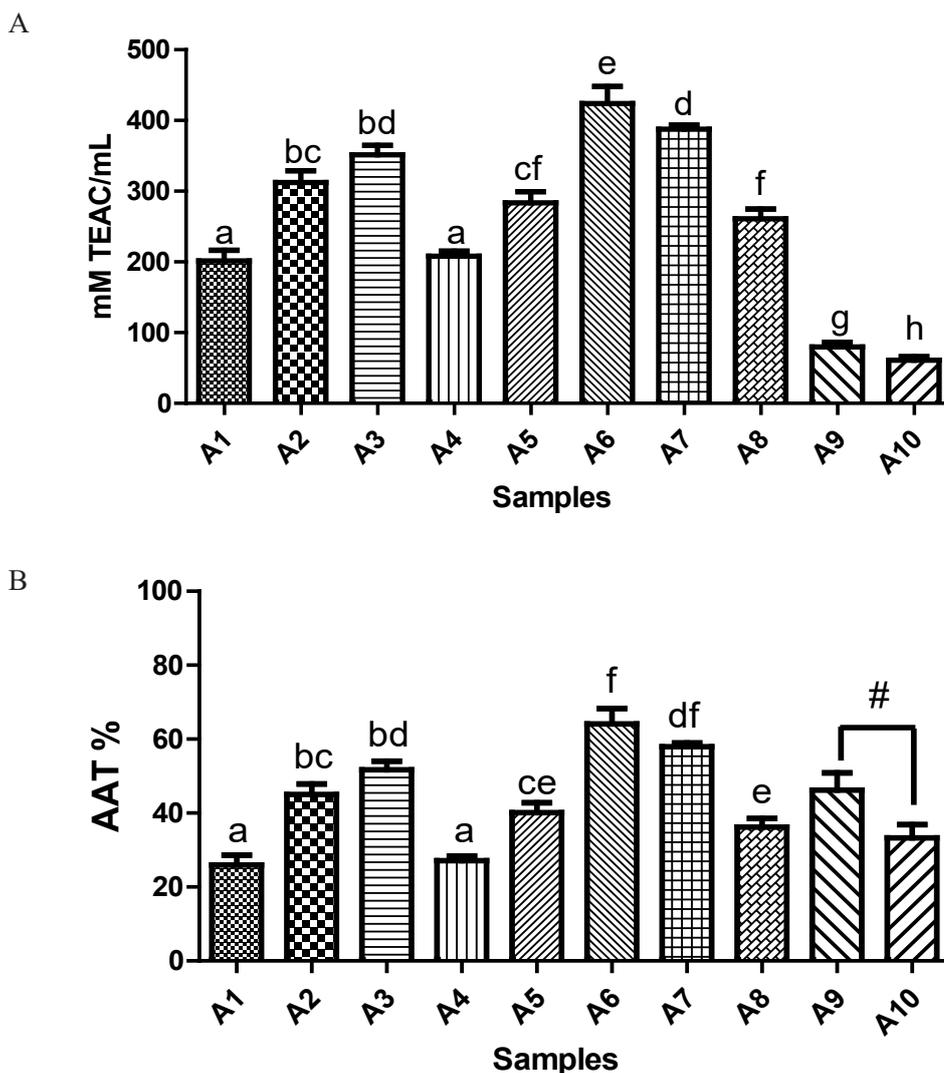


Figure 3. (A) ABTS antioxidant activity (mM TEAC/mL) of the commercial grape juices. (B) Antioxidant activity (%) of the commercial grape juices expressed as a percentage relative to the control (ABTS+• solution without antioxidants). Samples A1 to A8 from red grape juices were diluted in distilled water at 1:5 (v/v). Samples A9 and A10 from white grape juices were not diluted, and they were separately compared. Different letters or symbols on the bars indicate significantly different values analysed by ANOVA followed by Tukey's Multiple Comparison test with confidence interval (CI) of 95% for each pair ($p < 0.05$).

Conclusion

Based on the results obtained in the present work, it was possible to conclude that integral grape juices represent an important source of bioactive compounds. These compounds, such as polyphenols, including anthocyanins, which are mainly present in red grape juices, can act as potent antioxidants. The consumption of red grape juice can provide the body with high amounts of phenolic compounds and anthocyanins, which are associated with many beneficial effects to humans. White grape juices also contain valuable amounts of polyphenols, although anthocyanins are absent, which in turn reduces their antioxidant capacities as compared to red grape juices.

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