Antioxidant Activity of Commercial Ready-to-Drink Orange Juice and Nectar

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Abstract: Total antioxidant activity (TAA), total phenolic compounds (TPC), and physicochemical characteristics of ready-to-drink orange juice and nectar from the most consumed brands available in Brazil were evaluated. TPC ranged from 18.7 to 54.2 mg of gallic acid/100 mL, and TAA varied from 57.88 to 349.32 μmol TEAC/100 mL ready-to-drink orange juice and nectar. The ascorbic acid content was the only physicochemical parameter that showed strong variation among packages and brands. Correlation of TPC with TAA showed that the higher the level of TPC the higher the TAA. Correlation of ascorbic acid content with TAA is higher for ready-to-drink orange juice than nectar. The same was found for the correlation of ascorbic acid content with TPC. The results confirm the contribution of the TPC to TAA.

Keywords: ABTS, antioxidant activity, ascorbic acid, orange juice, ready-to-drink orange juice and nectar, total phenolic compounds

Introduction

Consumer demand for safe, functional, and fresh products, such as fruit juice, has been increasing as a consequence of the search for a healthier life. This contributes to the expansion of the orange juice industry in many countries particularly in Brazil, from which the most widely consumed beverages are ready-to-drink orange juice and nectar (ABIR 2009). According to the Brazilian legislation (Brazil 2009), industrially produced orange juice is a drink that is either directly obtained from the fruit, or reconstituted from concentrated juice until reaching the soluble solids contents of the original whole juice. If sugar is added, this must be listed on the label. On the other hand, the orange nectar is a diluted and sweetened drink, prepared from concentrated orange juice, plus sugar and additives. The maximum level of concentrated orange juice in nectars is 30% (m/v). Differently from Brazil, in the European Union fruit nectar made by orange is obtained by adding water and sugars and/or honey to the fruit juice, fruit juice from concentrate, concentrated fruit juice, and dehydrated/powdered fruit juice, to a minimum of 50% (m/v) (EU 2001).

Many substances in foods, especially fruits, such as ascorbic acid, vitamin E, beta-carotene, and phenolic compounds, are excellent antioxidants that are able to stabilize free radicals. The importance of these antioxidants in the maintenance of health and prevention of several pathologies, including different kinds of cancer, cardiovascular and neurological diseases, and aging-related disorders has been described (Sun and others 2002; Guarnieri and others 2007; Lim and others 2007). The protective effect of fruits on esophagus, oral cavity, larynx, pancreas, stomach, colorectal, and bladder cancer has also been reported by Block and others (1992).

Fruit juice, particularly orange juice, is a great source of antioxidants in the diet. Orange juice is rich in ascorbic acid, vitamin B, fiber, potassium, iron, and antioxidant compounds, mainly flavonoids. The beneficial properties of the fruits have been widely investigated. It was described that orange juice plays an important role in the protection of DNA against oxidative damage (Sun and others 2002; Liu and others 2009).

One of the main classes of natural antioxidants in plants, foods, and beverages is phenolic and polyphenolic compounds that are usually quantified using Folin-Ciocalteau assay (Singleton and others 1999; Franco 2002; Asami and others 2003). Several antioxidant activity methods have also been used to monitor and compare the antioxidant activity of foods.

Antioxidant activity has also been determined in food using several assays. The most used are the ABTS and DPPH that are based on the ability of the antioxidants to scavenge the radical cation ABTS and DPPH radical, respectively, resulting in a decreased in absorbance. The FRAP assay's principle is the reduction of ferric trypiridyl triazine complex to ferrous form at low pH, resulting in an increase in absorbance. The TRAP and ORAC are based on the reaction of peroxyl radicals, and β-carotene/linoleic acid method is based on the oxidative degradation of the linoleic acid products. Antioxidants can reduce radicals by single electron transfer and hydrogen atom transfer mechanisms. ABTS, DPPH, FRAP measure the single electron transfer, and ORAC and TRAP represents the hydrogen atom transfer mechanism (Prior and others 2005;Tabart and others 2009; Sariburun and others 2010). Their principles, reaction, mechanisms, and experimental conditions are different making it necessary to carry out different methods to estimate the antioxidant activity of the compounds.

The aim of this study was to investigate the total antioxidant activity (TAA) and the total phenolic compounds (TPC) of ready-to-drink orange juice and nectar from the most consumed brands available in Brazil.

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Materials and Methods

Samples

Ten brands of ready-to-drink orange juice and nectar available in supermarkets of Araraquara, SP, Brazil, were used: Del Valle (Sucos Del Valle Brazil, Americana, SP, Brazil), Mais (Mais Ind. Alimentares SA, Linhares, ES, Brazil), Maguary (Kraft Foods Brazil SA, Araguari, MG, Brazil), Su Fresh (Ind. Brasileira, Caçapava, SP, Brazil), Kiki (Santos Queiroz Industrial e Comercial de Bebidas e Alimentos Ltda, Artur Nogueira, SP, Brazil), Purity (Cocamar Cooperativa Agroindustrial, Maringá, PR, Brazil), Jal (Global Bebidas e Alimentos Ltda, Matão, SP, Brazil), Parmalat (Fábrica de Bebidas Parmalat, Jundiaí, SP, Brazil), Jandaia (Industrial e Comercial Jandaia Ltda, Pacajus, CE, Brazil), and Da Fruta (Da Fruta Ind. e Com. S/A, Aracati, CE, Brazil).

Three replicate packages from each brand with different date of production were analyzed.

Chemicals

The gallic acid and 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich (St.Louis, Mo., U.S.A.); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Sigma (Steinheim, Germany); ascorbic acid and ethanol from Merck (Darmstadt, Germany); sodium carbonate from Labsynth (Diadema, SP, Brazil); Folin-Ciocalteau reagent from Imbralab (Ribeirão Preto, SP, Brazil); potassium persulfate from Fluka (Steinheim, Germany); methanol from J.T. Baker (Phillipsburg, N.J., U.S.A.); glucose from Quimibrás (Rio de Janeiro, RJ, Brazil); sodium carbonate, sodium hidroxy, potassium sodium tartrate tetrahydrate, and cupric sulfate pentahydrate from Labsynth (Diadema, SP, Brazil); oxalic acid and 2,6-dichloroindophenol sodium salt hydrate from Vetec (Rio de Janeiro, RJ, Brazil).

Ready-to-drink orange juice and nectar brands survey

In order to identify the brands that occupied the biggest spaces on the shelves, they were surveyed at the supermarkets of Araraquara. The 10 most representative brands of ready-to-drink orange juice and nectar were chosen and analyzed in triplicate.

Extraction of phenolic compounds

The extraction was based on the procedure described by Asami and others (2003). Aliquots of 2 mL of ready-to-drink orange juice or nectar from all packages and brands were vortexed with 10 mL of methanol:water (80:20, v/v) for 1 min and then placed to an ultrasonic bath (15 min). Afterwards, the extracts were centrifuged at 23300 × *g* for 15 min at 20 °C. The supernatants were filtered through a Whatman #1 filter and analyzed. Extraction of the residues was repeated once using the same conditions.

Determination of TPC

TPC was determined using the Folin-Ciocalteau assay as reported by Franco (2002) and Asami and others (2003). In a 25-mL volumetric flask, 5.0 mL of water, 5.0 mL of ready-to-drink orange juice or nectar extracts, and 0.3 mL of Folin-Ciocalteau reagent were added, mixed and allowed to stand at room temperature for 8 min. After that, 10 mL of a sodium carbonate solution (7%, w/v) was added, and then the flask volume was adjusted with water, mixed, and heated at 40 °C for 30 min in a water bath. The absorbance was measured at 740 nm, using a Beckman DU® 640 Spectrophotometer (Fullerton, Calif., U.S.A.). Quantification was carried out using a gallic acid calibration curve (50 to 220 μg/mL) and the results were expressed as milligrams of gallic acid/100 mL ready-to-drink orange juice or nectar. Triplicate analyses were performed.

Determination of TAA by ABTS assay

The ABTS assay was performed according to Rufino and others (2009). The ABTS radical cation ($ABTS^+$) was obtained from the reaction between 5.0-mL ABTS (7 mM) with 88-μL potassium persulfate (140 mM), and was maintained in the dark, at room temperature for 16 h before use. Afterwards, 1.0-mL $ABTS^+$ solution was diluted with ethanol to the absorbance 0.7 ± 0.1 at 752 nm.

Ready-to-drink orange juice and nectar (10 mL) from all packages and brands were centrifuged at 23300 x *g* for 15 min at 20 °C and filtered through a Whatman no.1 filter. Three different solutions of ready-to-drink orange juice:ethanol and of nectar:ethanol were prepared with the supernatant (1:5; 3:5 v/v, and only supernatant). The 3 solutions were used to determine the TAA. The ready-to-drink orange juice and nectar solutions (30 μL) were added to 3.0-mL of the diluted $ABTS^+$ solution, and after 6 min the absorbance decrease was read at 752 nm, against a blank (ethanol). Triplicate analyses were performed.

Calibration curves for the Trolox equivalent antioxidant capacity (TEAC) and vitamin C equivalent antioxidant capacity (VCEAC) were built by plotting absorbance compared with concentration of Trolox standard solutions (100 to 1600 μM) and ascorbic acid standard solutions (2.58 to 18.48 mg/100 mL). Each point of the curve was performed in triplicate.

Physicochemical evaluation

Soluble solids (°Brix) (method 983.17), titratable acidity (method 942.15), reduced ascorbic acid (method 967.21), and total sugar (method 945.29) were determined according to AOAC (2005). Ratio, the soluble solids (°Brix)/titratable acidity rate, was calculated.

Statistical analysis

The ANOVA and Tukey test ($P \leq 0.05$) were carried out across the juices and nectars combined, and across the packages of each brand of juice and nectar using the Statistical Analytical System v. 6.12 (SAS Institute Inc., SAS Campus Drive: Cary, N.C., U.S.A.). Correlation analyses of ascorbic acid content compared with TPC, ascorbic acid content compared with TAA, and TPC compared with TAA were performed using Origin Microcal Software Inc. v. 7.0.

Results and Discussion

Determination of TPC

The Folin-Ciocalteau reaction is based on the principle that phenolic compounds reduce the mixture of phosphomolybdic–phosphotungstic acid to tungsten and molybdenum oxides under alkaline conditions, resulting in a blue complex that can be measured spectrophotometrically.

Absorption spectra of gallic acid solution and ready-to-drink orange juice and nectar extracts were compared in order to determine the maximum absorption lambda, 740 nm. Calibration curves (50, 90, 130, 170, and 220 μg gallic acid/mL) showed correlation coefficients greater than 0.999. Folin-Ciocalteau reaction was performed in triplicate for each packages of each brand and for each point of the calibration curve.

Correction factors were used to discount ascorbic acid and sugar, since they respond to Folin-Ciocalteau reaction. Ascorbic acid (12 to 70 mg/100 mL) and total sugar (2.5 to 10.0 g glucose/100 mL) standard solutions were prepared in concentrations corresponding to those found in the ready-to-drink orange juice and nectar, and submitted to Folin-Ciocalteau reaction. The results, expressed as milligram of gallic acid/ 100 mL juice or nectar, provided a gallic acid:ascorbic acid ratio of $2.4.10^{-3}$ and a gallic acid:sugar ratio of $1.93.10^{-4}$ that were deducted from the previous TPC values.

Table 1 shows the TPC results, which ranged from 18.7 to 54.2 mg/100 mL ready-to-drink orange juice or nectar. Significant differences ($P \leq 0.05$) were found between packages of each brand and between brands. Brands B and J showed the highest TPC levels; however, they did not differ ($P > 0.05$) from brands G and H.

TPC levels of this study are close to those described by Valverde and others (2000), for fruit juices (0.2 to 50 mg gallic acid/ 100 mL), although the juice from some varieties of orange showed higher levels (70 mg gallic acid/100 mL). Similarly, Franco (2002) determined 64.2 mg gallic acid/100 mL orange juice, and Sun and others (2002) reported 56.8 mg gallic acid/100 g orange (fresh weight of the edible part), while Lim and others (2007) reported 75 mg gallic acid/100 g Valencia orange from Australia. Rapisarda and others (1999), assessed TPC of orange juice prepared with different clones of fruits from Italy, which ranged from 0.36 to 1.15 mg ferulic acid/100mL fresh juice. TPC values of fresh, squeezed, pasteurized, concentrated, and frozen orange juice, expressed as mg ascorbic acid/serving portion (240 mL) (FDA 2009), varied from 50.8 to 219.8 (Gil-Izquierdo and others 2002). Gliszczynska-Swiglo and Tyrakowska (2003) showed TPC values for different commercial brands of apple juice ranging from 5.2 to 14 mg/100mL. The high TPC values in orange juice have been related to the flavanones hesperidin and narirutin, anthocyanins cyanidin 3–6″-malonyl glucoside and cyanidin 3-glucoside, and ferulic, caffeic, sinapic, coumaric, gallic and vanilic acids (Gil-Izquierdo and others 2002; Kelebek and others 2008; Xu and others 2008).

Determination of TAA by ABTS assay

The values of TEAC and VCEAC from ready-to-drink orange juice and nectar solutions were determined using these calibration curves, and are shown in Table 1. The TEAC values ranged from 57.88 to 315.42 μmol/100 mL ready-to-drink orange juice and from 87.59 to 349.32 μmol/100 mL nectar. All of the packages from each brand showed significant differences ($P \leq 0.05$), except for packages of brands F and G. No difference was found between the TAA of brands B and J that showed the highest TAA values, 315.43 μmol/100 mL and 349.32 μmol/100 mL, respectively (Table 1). One brand of ready-to-drink orange juice (B) and 2 of nectar (H and J) presented TEAC values close to that described by Cortés and others (2008) and Tabart and others (2009), for orange juice, 249 μmol/100 mL and 198 μmol/100 mL, respectively. Rapisarda and others (1999) found TAA values for different orange juice clones varying from 74 μmol/100 mL late Valencia to 705 μmol/100 mL Moro IV. There are few data in the literature on the TEAC of orange juice. Gliszczynska-Swiglo and Tyrakowska

Table 1– Total phenolic compounds[1] (mg gallic acid/100 mL), Trolox equivalent antioxidant capacity (TEAC μmol/100 mL), and vitamin C equivalent antioxidant capacity (VCEAC mg/100 mL) of ready-to-drink orange juice and nectar.

Brands		Package	Total phenolic compounds	TEAC	VCEAC
Juice	A	1	$27.44^{c}_{CDE} \pm 0.42$	$80.99^{c}_{CDE} \pm 2.91$	$10.31^{c}_{CDE} \pm 0.39$
		2	$42.27^{a}_{CDE} \pm 0.37$	$218.16^{a}_{CDE} \pm 1.70$	$31.13^{a}_{CDE} \pm 0.46$
		3	$33.41^{b}_{CDE} \pm 0.33$	$144.26^{b}_{CDE} \pm 3.25$	$20.85^{b}_{CDE} \pm 0.41$
	B	1	$50.25^{b}_{A} \pm 0.66$	$315.42^{a}_{AB} \pm 5.64$	$40.29^{a}_{AB} \pm 1.15$
		2	$49.45^{b}_{A} \pm 0.69$	$297.38^{b}_{AB} \pm 3.89$	$38.68^{a}_{AB} \pm 0.92$
		3	$52.52^{a}_{A} \pm 0.04$	$279.93^{c}_{AB} \pm 2.03$	$34.74^{b}_{AB} \pm 0.23$
	C	1	$18.68^{c}_{E} \pm 0.04$	$74.99^{b}_{EF} \pm 1.41$	$9.48^{b}_{E} \pm 0.16$
		2	$23.39^{a}_{F} \pm 0.76$	$96.21^{a}_{EF} \pm 2.37$	$12.20^{a}_{E} \pm 0.28$
		3	$21.89^{b}_{F} \pm 0.12$	$68.35^{c}_{EF} \pm 2.15$	$8.65^{c}_{E} \pm 0.29$
	D	1	$24.71^{b}_{EF} \pm 0.31$	$66.34^{b}_{F} \pm 2.47$	$9.39^{a}_{E} \pm 0.31$
		2	$29.56^{a}_{EF} \pm 0.91$	$57.88^{c}_{F} \pm 3.43$	$7.48^{b}_{E} \pm 0.44$
		3	$21.20^{c}_{EF} \pm 0.05$	$74.04^{a}_{F} \pm 3.06$	$9.39^{a}_{E} \pm 0.37$
Nectar	E	1	$32.84^{b}_{BCD} \pm 0.32$	$117.32^{b}_{DEF} \pm 4.05$	$14.89^{b}_{DE} \pm 0.52$
		2	$38.02^{a}_{BCD} \pm 0.19$	$186.35^{a}_{DEF} \pm 4.42$	$24.34^{a}_{DE} \pm 0.81$
		3	$38.21^{a}_{BCD} \pm 0.38$	$87.59^{c}_{DEF} \pm 3.96$	$11.22^{c}_{DE} \pm 0.51$
	F	1	$42.92^{a}_{BCD} \pm 0.14$	$186.34^{a}_{CD} \pm 3.46$	$23.85^{a}_{CD} \pm 0.43$
		2	$38.15^{b}_{BCD} \pm 0.55$	$182.86^{a}_{CD} \pm 2.63$	$23.32^{a}_{CD} \pm 0.47$
		3	$43.58^{a}_{BCD} \pm 0.37$	$180.30^{a}_{CD} \pm 7.29$	$23.13^{a}_{CD} \pm 0.89$
	G	1	$41.61^{b}_{ABC} \pm 0.30$	$178.23^{a}_{CD} \pm 3.58$	$22.66^{a}_{CD} \pm 0.39$
		2	$41.42^{b}_{ABC} \pm 0.44$	$181.92^{a}_{CD} \pm 5.02$	$23.26^{a}_{CD} \pm 0.60$
		3	$45.43^{a}_{ABC} \pm 0.21$	$175.56^{a}_{CD} \pm 2.64$	$22.59^{a}_{CD} \pm 0.24$
	H	1	$45.89^{b}_{AB} \pm 0.93$	$236.04^{a}_{BC} \pm 2.49$	$30.51^{a}_{BC} \pm 0.57$
		2	$41.06^{c}_{AB} \pm 0.30$	$211.31^{b}_{BC} \pm 3.24$	$27.24^{b}_{BC} \pm 0.44$
		3	$49.78^{a}_{AB} \pm 0.33$	$233.22^{a}_{BC} \pm 2.35$	$29.94^{a}_{BC} \pm 0.39$
	I	1	$26.34^{c}_{DEF} \pm 0.19$	$143.63^{b}_{CDE} \pm 4.29$	$18.36^{a}_{CDE} \pm 0.80$
		2	$31.27^{b}_{DEF} \pm 0.44$	$151.76^{ab}_{CDE} \pm 1.96$	$19.43^{a}_{CDE} \pm 0.25$
		3	$35.72^{a}_{DEF} \pm 1.35$	$154.09^{a}_{CDE} \pm 2.47$	$19.52^{a}_{CDE} \pm 0.42$
	J	1	$54.20^{a}_{A} \pm 0.14$	$343.35^{a}_{A} \pm 5.68$	$48.42^{a}_{A} \pm 1.49$
		2	$51.61^{b}_{A} \pm 0.33$	$349.32^{a}_{A} \pm 4.97$	$48.27^{a}_{A} \pm 0.82$
		3	$53.70^{a}_{A} \pm 0.87$	$321.16^{b}_{A} \pm 9.20$	$41.34^{b}_{A} \pm 1.84$

[1]Deduced from interferences.
Means with the same small letter in the same column did not differ significantly ($P \leq 0.05$) for the same brand.
Means with the same capital letter in the same column did not differ significantly ($P \leq 0.05$).
$n = 3$.

(2003) reported TEAC values of different commercial brands of apple juice from 97 μmol/100 mL to 191 μmol/100 mL. The VCEAC values ranged from 7.48 to 40.29 mg/100 mL ready-to-drink orange juice and 11.22 to 48.42 mg/100 mL nectar (Table 1). Only 3 brands (F, G, and I) did not show significant difference ($P > 0.05$) among the packages. The highest levels of VCEAC were from brands B (34.74 to 40.29 mg/100 mL) and J (41.34 to 48.42 mg/100 mL) ($P > 0.05$) that were similar to the values described by Arnao and others (2001), 40.3 mg/100 mL orange juice, and higher than those reported by Sun and others (2002), 17.61 mg/100 g orange.

TAA of orange juice was also evaluated using the DPPH assay. Studying different orange juice clones, Rapisarda and others (1999) described a SC_{50} range of 24.28 μL from Tarocco II orange juice to 80.01 μL for Sanguinello I orange juice. Sánchez-Moreno and others (2005) showed that there was no difference in the DPPH radical scavenge capacity of fresh (194.2 mL juice/g DPPH) and processed orange juice (184.7 to 197.8 mL juice/g DPPH), with the exception of pasteurized (90 °C/1 min) orange juice, with 206.9 mL juice/g DPPH. The influence of storage on the antioxidant activity of processed orange juice was studied by Plaza and others (2006). It was verified during storage at 4 °C that the orange juice treated by pulsed electric fields (168.94 to 251.57 mL juice/g DPPH) showed higher EC_{50} values than the pasteurized orange juice (low and high temperatures) (160.58 to 219.66 mL juice/g DPPH and 150.28 to 209.92 mL juice/g DPPH) that was higher than the freshly squeezed orange juice (156.83 to 178.98 mL juice/g DPPH), at the end of 40 days (Plaza and others 2006). TAA of orange juices submitted to different treatments was also evaluated (Gil-Izquierdo and others 2002). The values, expressed as VCEAC (milligram per serving portion) (240 mL), ranged from 97.4 to 150.1, without significant difference among orange juice treatments, due to the high L-ascorbic acid stability, according to the authors. The antioxidant activity differs depending on the assay principle and the experimental conditions that affect antioxidant compounds response.

Physicochemical evaluation

The soluble solids ranged from 11.5 to 13.5 °Brix for ready-to-drink orange juice and nectar, respectively (Table 2). There was no significant difference ($P > 0.05$) among the packages of brands A, B, E, F, and H, and among brands D, F, and H, which showed the highest values. Titratable acidity values ranged from 0.42 to 0.71 g citric acid/100 mL. Packages of all brands showed significant difference ($P \leq 0.05$). Brands D and E did not differ ($P > 0.05$) for titratable acidity. Ratio values (°Brix/titratable acidity) ranged from 19.9 to 27.5 for juices and from 17.1 to 35.2 for nectars, with no significant difference ($P > 0.05$) among brands A, B, F, G, H, I, and J. The total sugar values varied from 9.5 to 16.5 g glucose/100 mL for ready-to-drink orange juice and nectar. There was no significant difference ($P > 0.05$) among the packages of brands D, E, and J, and brands B, E, G, I, and J, as well as brands A, C, D, F, G, H, I, and J. The ascorbic acid content ranged from 8.2 to 57 mg/100 mL for juice and from 14.6 to 67.2 mg/100 mL for nectar. Significant difference ($P \leq 0.05$) was found among packages of all the brands (Table 2). It should be noted that there was a strong variation among the ascorbic acid content for some packages, but there was an even stronger variation

Table 2–Physicochemical parameters of ready-to-drink orange juice and nectar.

Brands		Package	Soluble solids (°Brix)	Titrable acidity (g citric acid/100 mL)	*Ratio*	Total sugar (g glucose/100 mL)	Ascorbic acid (mg/100 mL)
Juice	A	1	$12.1^{a}_{CD} \pm 0.10$	$0.44^{c}_{CD} \pm 0.00$	27.5_{AB}	$14.2^{a}_{AB} \pm 0.64$	$10.0^{c} \pm 0.17$
		2	$12.1^{a}_{CD} \pm 0.00$	$0.47^{a}_{CD} \pm 0.00$	25.8_{AB}	$11.9^{b}_{AB} \pm 0.10$	$39.4^{a} \pm 0.45$
		3	$12.1^{a}_{CD} \pm 0.00$	$0.46^{b}_{CD} \pm 0.00$	26.3_{AB}	$12.1^{b}_{AB} \pm 0.48$	$23.2^{b} \pm 0.09$
	B	1	$11.5^{a}_{D} \pm 0.12$	$0.42^{b}_{D} \pm 0.00$	27.4_{AB}	$10.1^{a}_{C} \pm 0.21$	$57.0^{a} \pm 0.35$
		2	$11.5^{a}_{D} \pm 0.12$	$0.44^{a}_{D} \pm 0.07$	26.5_{AB}	$9.5^{b}_{C} \pm 0.34$	$47.4^{b} \pm 2.54$
		3	$11.7^{a}_{D} \pm 0.23$	$0.44^{a}_{D} \pm 0.00$	26.9_{AB}	$9.6^{b}_{C} \pm 0.26$	$34.9^{c} \pm 0.25$
	C	1	$11.5^{b}_{D} \pm 0.00$	$0.54^{b}_{BC} \pm 0.00$	21.3_{BC}	$11.0^{b}_{ABC} \pm 0.15$	$19.5^{b} \pm 0.34$
		2	$11.5^{b}_{D} \pm 0.00$	$0.58^{a}_{BC} \pm 0.00$	19.9_{BC}	$11.1^{b}_{ABC} \pm 0.54$	$21.2^{a} \pm 0.16$
		3	$11.8^{a}_{D} \pm 0.12$	$0.51^{c}_{BC} \pm 0.00$	23.0_{BC}	$12.8^{a}_{ABC} \pm 0.22$	$13.3^{c} \pm 0.12$
	D	1	$13.2^{a}_{AB} \pm 0.12$	$0.60^{a}_{AB} \pm 0.00$	22.0_{BC}	$12.9^{a}_{AB} \pm 0.49$	$8.2^{b} \pm 0.18$
		2	$12.8^{b}_{AB} \pm 0.12$	$0.58^{b}_{AB} \pm 0.00$	22.3_{BC}	$12.0^{a}_{AB} \pm 0.65$	$9.5^{a} \pm 0.11$
		3	$12.9^{b}_{AB} \pm 0.00$	$0.56^{c}_{AB} \pm 0.00$	23.1_{BC}	$14.0^{a}_{AB} \pm 0.36$	$9.8^{a} \pm 0.06$
Nectar	E	1	$12.1^{a}_{CD} \pm 0.12$	$0.71^{a}_{A} \pm 0.01$	17.1_{C}	$11.9^{a}_{BC} \pm 0.94$	$30.9^{a} \pm 0.15$
		2	$12.1^{a}_{CD} \pm 0.12$	$0.61^{b}_{A} \pm 0.01$	19.8_{C}	$10.9^{a}_{BC} \pm 0.49$	$14.6^{c} \pm 0.09$
		3	$12.1^{a}_{CD} \pm 0.12$	$0.66^{a}_{A} \pm 0.00$	18.4_{C}	$10.8^{a}_{BC} \pm 0.22$	$16.1^{b} \pm 0.08$
	F	1	$13.1^{a}_{A} \pm 0.00$	$0.46^{b}_{BCD} \pm 0.01$	28.5_{AB}	$16.5^{a}_{A} \pm 0.23$	$38.0^{b} \pm 0.47$
		2	$13.1^{a}_{A} \pm 0.00$	$0.45^{b}_{BCD} \pm 0.01$	29.0_{AB}	$14.2^{b}_{A} \pm 1.18$	$38.6^{b} \pm 0.39$
		3	$13.1^{a}_{A} \pm 0.23$	$0.51^{a}_{BCD} \pm 0.00$	25.6_{AB}	$12.0^{c}_{A} \pm 0.72$	$44.2^{a} \pm 0.51$
	G	1	$12.0^{a}_{CD} \pm 0.12$	$0.47^{a}_{CD} \pm 0.00$	25.5_{AB}	$9.9^{a}_{BC} \pm 0.50$	$32.4^{a} \pm 0.43$
		2	$11.5^{b}_{CD} \pm 0.12$	$0.46^{b}_{CD} \pm 0.00$	25.2_{AB}	$9.9^{b}_{BC} \pm 0.09$	$29.6^{b} \pm 0.32$
		3	$12.1^{a}_{CD} \pm 0.12$	$0.47^{a}_{CD} \pm 0.00$	25.8_{AB}	$10.8^{a}_{BC} \pm 0.13$	$27.5^{c} \pm 0.24$
	H	1	$13.5^{a}_{A} \pm 0.00$	$0.51^{b}_{BCD} \pm 0.00$	26.7_{AB}	$11.7^{a}_{ABC} \pm 0.26$	$37.1^{a} \pm 0.12$
		2	$13.3^{a}_{A} \pm 0.00$	$0.51^{b}_{BCD} \pm 0.00$	26.0_{AB}	$12.2^{a}_{ABC} \pm 0.28$	$31.6^{b} \pm 0.36$
		3	$13.3^{a}_{A} \pm 0.00$	$0.52^{a}_{BCD} \pm 0.00$	25.5_{AB}	$11.3^{b}_{ABC} \pm 0.24$	$36.9^{a} \pm 0.31$
	I	1	$12.2^{a}_{BC} \pm 0.12$	$0.48^{b}_{D} \pm 0.00$	25.2_{A}	$10.9^{a}_{BC} \pm 0.06$	$27.8^{ab} \pm 0.21$
		2	$12.8^{b}_{BC} \pm 0.12$	$0.51^{a}_{D} \pm 0.01$	35.2_{A}	$11.3^{a}_{BC} \pm 0.18$	$28.3^{a} \pm 0.23$
		3	$12.2^{a}_{BC} \pm 0.12$	$0.45^{c}_{D} \pm 0.01$	27.1_{A}	$9.6^{b}_{BC} \pm 0.50$	$26.8^{b} \pm 0.61$
	J	1	$12.1^{b}_{BC} \pm 0.00$	$0.49^{b}_{BC} \pm 0.00$	24.7_{ABC}	$11.4^{a}_{BC} \pm 0.24$	$25.9^{b} \pm 0.07$
		2	$12.9^{a}_{BD} \pm 0.12$	$0.58^{a}_{BC} \pm 0.00$	22.2_{ABC}	$11.1^{a}_{BC} \pm 0.26$	$23.7^{c} \pm 0.12$
		3	$12.1^{b}_{BD} \pm 0.12$	$0.45^{c}_{BC} \pm 0.07$	27.0_{ABC}	$10.7^{a}_{BC} \pm 1.03$	$67.2^{a} \pm 0.34$

Means with the same small letter in the same column did not differ significantly ($P \leq 0.05$) for the same brand.
Means with the same capital letter in the same column did not differ significantly ($P \leq 0.05$).
$n = 3$.

among brands, indicating that the industry does not control the ascorbic acid content of ready-to-drink orange juice and nectar. The physicochemical parameters of ready-to-drink orange juice and nectar of some brands conformed to the requirements of quality and identity of the Brazilian regulations for orange juice, the values of which are minimum soluble solids of 10.5 °Brix, minimum ratio of 7.0, minimum ascorbic acid of 25 mg/100 mL, and maximum total sugar content of 13.0 g/100 mL (Brazil 2000). All of the brands conformed to the regulations for soluble solids and ratio of orange juice. One package of juice (brands A and D) and 2 packages of nectar (brand F) did not conform to the total sugar content. Two packages of nectar from brand E and 1 from brand J, 1 package of juice from brand A, and 2 from brands C and D did not conform to the ascorbic acid content. It should also be pointed out that the requirements of quality and identity of orange nectar have not yet been established (Brazil 2000).

Ascorbic acid, TPC, and TAA correlation

Correlations of TPC with TAA, ascorbic acid content with TAA, and TPC with ascorbic acid content were determined for ready-to-drink orange juice and nectar. The correlation coefficient between TPC and TAA was $r = 0.9574$ for ready-to-drink orange juice, very strong. Also, a positive and very strong correlation was obtained between ascorbic acid content and TAA, $r = 0.9545$. Positive and strong correlation occurred between TPC and ascorbic acid content ($r = 0.8610$). On the other hand, for orange nectar, positive and strong correlation was only obtained between TPC and TAA ($r = 0.8340$), while positive and weak correlations were obtained between ascorbic acid content and TAA ($r = 0.3490$), and TPC and ascorbic acid content ($r = 0.3807$). Ready-to-drink orange juice and nectar showed similar TAA and TPC values, but the ascorbic acid content affected TAA and TPC of ready-to-drink orange juice much more than those of nectar.

Positive and strong correlation was also obtained between TAA and TPC of orange juice ($r = 0.831$) and apple juice ($r = 0.940$) (Rapisarda and others 1999; Gliszczynska-Swiglo and Tyrakowska 2003). Also, positive and very strong correlation ($r \geq 0.991$) between TAA and L-ascorbic acid of high pressurized and thermally pasteurized orange juice stored at 5 °C during 111 d was described by Polydera and others (2004).

The results showed that the higher the level of TPC of ready-to-drink orange juice and nectar, the higher the TAA. The strong correlations between TAA and TPC of ready-to-drink orange juice and nectar confirm that TPC contribute to the TAA. The stronger correlation between ascorbic acid content and TAA of ready-to-drink orange juice compared to that shown by orange nectar suggests that ascorbic acid may play a more important role for the TAA of ready-to-drink orange juice than for nectar. It should be pointed out that orange nectar can be produced with less juice (minimum of 30%, w/w) than ready-to-drink orange juice (100% juice) and can have sugars and acids added. Nectar is allowed to be produced with lower amounts of juice plus sugar and acids (Brazil 2003). It was verified that the label of some brands of orange nectar showed ascorbic acid as part of the ingredients because it is permitted to add ascorbic acid to nectar, but it is not permitted to add ascorbic acid to ready-to-drink orange juice (Brazil 2003). A stronger correlation between ascorbic acid content and TPC values of ready-to-drink orange juice than nectar was also obtained.

Conclusion

Ready-to-drink orange juice and nectar showed high levels of TPC. Those with the highest levels of TPC also showed the highest antioxidant activity. Positive and strong correlations between TAA and TPC of ready-to-drink orange juice and nectar were obtained, showing that TPC may contribute effectively to the antioxidant activity. The positive and very strong correlation between ascorbic acid content and TAA of ready-to-drink orange juice rather than nectar suggests that the natural ascorbic acid present in ready-to-drink orange juice is also responsible for the higher antioxidant activity.

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