

Bioactive compounds of juices from two Brazilian grape cultivars

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Abstract

BACKGROUND: Grape juice consumption may prevent several chronic diseases owing to the presence of phenolic compounds, which have an important role in the reduction of oxidative stress. This study investigated the polyphenol content and antioxidant activities of grape juices from two cultivars: BRS-Cora and Isabella. Total polyphenol content (TPC), anthocyanins, antioxidant capacity (oxygen radical absorbance capacity, ferric reducing antioxidant power and 1,1-diphenyl-2-picrylhydrazyl), and phenolic profile (high-performance liquid chromatography with diode array and fluorescence detection – HPLC-DAD-FLD) were determined.

RESULTS: BRS-Cora grape juice showed higher concentrations of total polyphenols and anthocyanins, as well as higher antioxidant potential, than those of Isabella grape juice. A significant positive correlation was found in TPC or anthocyanin contents when correlated with the remaining antioxidant assays. In addition, HPLC-DAD-FLD showed a higher total phenolic content in BRS-Cora grape juice compared to Isabella.

CONCLUSION: The present results show BRS-Cora as a promising cultivar for grape juice production with an improved functional potential.

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Keywords: Isabella grape; BRS-Cora grape; grape juice; polyphenols; antioxidant potential; bioactive compounds

INTRODUCTION

Grapes, grape juice and wine have shown both *in vitro* and *in vivo* potential to prevent several chronic diseases. The benefits stem from their high polyphenol content, which are secondary metabolites produced by plants as a defense mechanism against environmental stress.^{1–4} The protective effects against health problems such as cancer and cardiovascular diseases may be attributed to their antioxidant activity and antiproliferative properties.⁵ Furthermore, interest in polyphenols has increased owing to their potential activity as antitoxic and cytoprotective molecules with a modulatory action in inflammatory processes and neurodegenerative diseases.^{1–3,6,7}

The search for natural sources of bioactive compounds is a strong trend nowadays. At the same time, an increase in the consumption of grapes and their products was noted, focusing not only on nutrition but also on their beneficial health effects. Grapes are sources of polyphenols belonging to different subclasses such as monomeric flavan-3-ols, proanthocyanidins, flavonoids, anthocyanins, phenolic acids and the stilbene resveratrol.^{8–11}

Several factors such as geographic origin, cultivar and environmental conditions may influence polyphenol concentration.^{9,12–15} Different agronomic strategies have been employed to develop new crops, focusing on sensory quality, field production yield and

resistance to pests and diseases,^{16,17} which, in turn, may influence the biosynthesis of phenolic compounds.¹²

Red wine is the most famous grape product, which has been correlated with the 'French paradox', in which low coronary disease death rates have been observed in France, despite the high amount of dietary cholesterol and saturated fatty acids. However, several studies have also reported improvements on health aspects with grape juice intake. Supplementation with Concord grape juice (8 mL kg⁻¹ body weight d⁻¹) for 14 days positively altered the endothelial function in adults with coronary artery

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disease.¹⁸ Castilla *et al.*¹⁹ found greater antioxidant capacity and high-density lipoprotein cholesterol, as well as a reduction in low-density lipoprotein (LDL) cholesterol in plasma of healthy adults and adults in hemodialysis subsequent to the daily ingestion of concentrated grape juice (100 mL for 14 days). In another study, supplementation with 480 mL of grape juice for 8 weeks showed protection against DNA damage in healthy adults of both genders.²⁰ All of these facts indicate that grape juice has the potential to maintain health and collaborate in the control of disease.

Grape juice is a representative product of grape processing and there is a constant need for improvement of its sensory quality and nutritional properties. Brazilian grape juice is mainly produced using Isabella cultivar, owing to its large production and the foxy aroma/flavor characteristic of *Vitis labrusca* cultivars. However, the color of Isabella grape juice generally needs to be improved, as the color is the first sensory attribute evaluated by consumers. The grape BRS-Cora, developed by the Brazilian Agricultural Research Agency (EMBRAPA) via the cross between 'Muscat Belly A' and 'H. 65.9.14',²¹ is frequently used to produce grape juice in Brazil, especially to produce blends with Isabella cultivar to improve the final color of the juice. Furthermore, the polyphenol content and antioxidant properties of BRS-Cora are equally important.

A quick search of the literature demonstrates that only a few reports have addressed the phenolic profile and antioxidant potential of Isabella cultivar grape juice, and most of them are of local relevance, which also applies to BRS-Cora grape juice. Brazil is very important as a grape juice producer, which makes the present work of international relevance for both researchers and industry. Thus the aim of the present work was to evaluate the phenolic profile and antioxidant properties of grape juices produced with Isabella and BRS-Cora cultivars.

MATERIAL AND METHODS

Sample and juice preparation

Grapes (cv. Isabella and BRS-Cora) were harvested and provided by Embrapa Tropical Semi-arid, Petrolina, Pernambuco State, Brazil (latitude 9° 8' 8.9" S, longitude 40° 18'33.6" W, altitude 373 m). Ripe fruits, which showed mean values of 0.7 g 100 mL⁻¹, 3.8 and 23 °Brix for total acidity, pH and sugar level, respectively, were harvested and stored at 10 °C for 12 h after processing. First, the grapes were sanitized with hypochlorite solution (100 mg L⁻¹) for 15 min and rinsed with water. Fruits were then processed by the steam extraction method for 60 min at 75–85 °C. Sterilized glass bottles were used for juice storage (16 °C) until analysis, within 30 days of juice bottling.

Total polyphenol content (TPC)

TPC was determined using the Folin–Ciocalteu method,²² with slight modifications. The extracts, distilled water and Folin–Ciocalteu's reagent were added to vials and mixed thoroughly. After 3 min, 2 mol L⁻¹ sodium carbonate solution were added and the mixture was kept in the dark at room temperature for 2 h. The absorbance was read at 725 nm using a spectrophotometer (Synergy HT, Biotek, Winooski, VT, USA). Gallic acid was used to prepare a standard curve and the results were expressed as gallic acid equivalents (mg GAE 100 mL⁻¹ of juice).

Total anthocyanins

Total anthocyanins were measured according to Wrolstad,²³ with some modifications, as described by Abe *et al.*²⁴ Appropriate dilutions from the samples were carried out using 0.025 mol L⁻¹ potassium chloride buffer (pH 1.0). The absorbance was read at 510 and 700 nm using a microplate reader (Synergy HT). A second dilution was made for each sample using 0.4 mol L⁻¹ sodium acetate buffer (pH 4.5) and the absorbance was read at the same wavelengths. The calculations were done as shown in the equation below:

$$A = [(A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH} = 1.0}] - [(A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH} = 4.5}] \quad (1)$$

The results were expressed as mg cyanidin 3-glucoside 100 mL⁻¹. The final results were calculated as follows:

$$C (\text{mg } 100 \text{ mL}^{-1}) = A.MW.DF.100 \div \xi.1 \quad (2)$$

where ξ = molar absorptivity (26900 mol L⁻¹), i = path length (cm), MW = molecular weight of cyanidin 3-glucoside and DF = dilution factor.

Analysis by high-performance liquid chromatography (HPLC)

Phenolic compounds were identified and quantified by HPLC technique using a Waters Alliance e2695 HPLC system equipped with a photodiode array detector (DAD) and fluorescence detectors (FLD). Separations were performed on a C18 column (150 × 4.6 mm, 3 μm Gemini NX, Phenomenex, Torrance, CA, USA); preceding the analytical column a C18 guard column was used (4.0 × 3.0 mm, Gemini NX) at 40 °C. The mobile phase consisted of a 25 mmol L⁻¹ solution of potassium dihydrogen phosphate in ultrapure water (PURELAB Option Q, Elga Systems, Woodridge, IL, USA) with pH adjusted to 2.05 using phosphoric acid (Fluka, Switzerland) as solvent A, methanol (HPLC grade, JT Baker, Phillipsburg, NJ, USA), as solvent B and acetonitrile (HPLC grade, JT Baker) as solvent C. The elution gradient used was as follows: 0 min 100% A; 18 min 87.5% A, 2.5% B, 10.0% C; 30 min 83.5% A, 3.2% B, 13.3% C; 36 min 75.0% A, 5.0% B, 20.0% C; 48.5 min 65.0% A, 8.3% B, 26.7% C; 50 min 65.0% A, 8.3% B, 26.7% C and 65 min 100% A. The flow rate and injection volume were 0.6 mL min⁻¹ and 10 μL, respectively. The juices were previously filtered through a 0.45 μm membrane before injection into the HPLC system. Phenolic compounds were identified by comparing their relative retention times (RT) and UV spectral data with those of identical standards at 520 nm (pelargonidin 3-O-glucoside, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside), 220 nm (gallic acid, procyanidin B1, (–)-epicatechin gallate and (–)-epigallocatechin gallate), 320 nm (chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid) and 360 nm (isorhamnetin-3-O-glucoside and rutin). The fluorescence detector was used for the detection of the vanillic acid, procyanidin B2 and A2, (–)-epicatechin, (+)-catechin and syringic acid at 280 nm of the excitation and 360 nm of the emission. The standards caffeic, vanillic, ferulic and gallic acids were purchased from Chem Service (West Chester, PA, USA). Chlorogenic, syringic and *p*-coumaric acids were purchased from Sigma (UK). Pelargonidin 3-O-glucoside, cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside, procyanidin B1, procyanidin B2, procyanidin A2, epicatechin, catechin, epicatechin gallate, epigallocatechin gallate, rutin and isorhamnetin-3-O-glucoside were obtained from Extrasynthese (Genay, France). Calibration curves were prepared

for these 21 standard phenolic compounds using the same chromatographic conditions. Table 1 shows the concentration range of each standard used for the calibration curves, standards purity (SP), equations of the calibration curves, coefficients of determination (R^2), detection limits (LD), quantification limits (LQ) and assay repeatability through coefficient of variation (CV). Data collection and analysis were carried out using Empore™ 2 software (Milford, CT, USA).

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazil) assay according to Brand-Williams *et al.*,²⁵ with some modifications. Briefly, 61 $\mu\text{mol L}^{-1}$ DPPH ethanol solution were added to the extracts, followed by shaking and incubation for 30 min in the dark. The absorbance was read at 515 nm using a Synergy HT spectrometer. The results were expressed as $\mu\text{mol Trolox equivalents (TE) 100 mL}^{-1}$ of juice.

Hydrophilic oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was determined according to Dávalos *et al.*²⁶ The extracts were prepared in phosphate buffer (PB) (75 mmol L^{-1} , pH 7.4). The peroxy radical was generated using 2,2'-azobis (2-amidinopropane) dihydrochloride solution (AAPH) and fluorescein was used as the substrate. The reaction mixture consisted of extracts, 0.0014 mmol L^{-1} fluorescein, and 0.4 mol L^{-1} AAPH. The reaction was carried out in a plate reader (Synergy HT) with filters of emission at 520 nm and excitation at 485 nm. The incubation was conducted at 37 °C. The fluorescence was recorded every minute for 80 min. The Trolox standard was used to prepare a standard curve and the results were expressed as $\mu\text{mol TE 100 mL}^{-1}$ juice.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to Rufino *et al.*,²⁷ with slight modifications. In the dark, FRAP reagent was prepared with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} FeCl_3 . Samples or standard solutions, ultrapure water and FRAP reagent were mixed and allowed to react in a water bath for 30 min at 37 °C. After cooling to room temperature, the absorbance of the samples and standards was read at 595 nm. Results were expressed in $\mu\text{mol TE 100 mL}^{-1}$ juice.

Statistical analysis

All analyses were carried out in triplicate and data were expressed as the mean value \pm standard error (SEM). The statistical analyses were carried out using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The significance of the data was determined using Student's *t*-test at $P < 0.05$.

RESULTS AND DISCUSSION

Total phenolic and anthocyanin content

Fruits and plant-derived beverages such as fruit juices are dietary sources of polyphenols. Polyphenols have received increasing interest from consumers and the food industry because of the direct association between their consumption and the prevention of several diseases. Plant polyphenols have antioxidant properties and therefore an important role against radical oxygen species

(ROS).^{28,29} Enhancements in the cognitive function of adults after the ingestion of 6–9 mL grape juice kg^{-1} body weight for 12 weeks have also been reported.³⁰ Other authors have found positive effects in the lipid profile,¹⁹ antioxidant status³¹ and antimutagenic activity²⁰ after grape juice consumption, which supports the potential health benefits of this product.

In the present study, BRS-Cora grape juice showed higher bioactive substance concentrations than that found in grape juice prepared with the Isabella cultivar. BRS-Cora grape juice showed three times higher TPC ($221.6 \pm 1.83 \text{ mg GAE } 100 \text{ mL}^{-1}$) than Isabella ($74.86 \pm 4.25 \text{ mg GAE } 100 \text{ mL}^{-1}$) (Fig. 1a). Furthermore, BRS-Cora grape juice also had an anthocyanin content that was five times higher ($73.88 \pm 1.30 \text{ mg cyanidin-3-glucoside } 100 \text{ mL}^{-1}$) than Isabella grape juice ($15.40 \pm 0.20 \text{ mg cyanidin-3-glucoside } 100 \text{ mL}^{-1}$) (Fig. 1b).

The results presented here are in good agreement with those reported for Campbell Early grape juice, for which TPC was found at a concentration of $308.2 \pm 11.0 \text{ mg GAE } 100 \text{ mL}^{-1}$ and content of $496.6 \pm 15.0 \text{ mg}$ expressed as malvidin-3-glucoside 100 mL^{-1} for total anthocyanins³². Minor differences are expected due to different cultivars, climate conditions, soil quality and losses during the juice-making process.^{13–15,33,34} Compared to other fruit juices, the analyzed grape juices are rich sources of polyphenol compounds. Pala and Toklucu³⁵ observed a lower anthocyanin content in pomegranate juice ($42.6 \pm 1.37 \text{ mg cyanidin 3-glucoside } 100 \text{ mL}^{-1}$).

HPLC analysis

Phenolic compounds are widely distributed in plants, and grapes are well recognized as good sources of polyphenols from several classes. In the present study, 21 phenolic compounds were positively identified and quantified by HPLC (Table 2).

Anthocyanins represent a significant portion of the total polyphenols in grapes.³² These compounds are among the most important groups of water-soluble pigments, concentrated mainly in the fruit skin, being responsible for the purplish color of the grape and the antioxidant properties.³⁶ Isabella grape juice showed a higher content of four monoglycosylated anthocyanins (Table 2), but delphinidin 3-*O*-glucoside was eight times more concentrated in BRS-Cora. Some works have shown prevalence of anthocyanins 3,5-diglucoside in non-vinifera grape cultivars and their hybrids.^{37–39} Diglycosidic anthocyanins were not investigated in the present study; however, as can be noted in Table 2, the phenolic total content (anthocyanins + phenolic acids + flavonoids/proanthocyanidins) was higher in BRS-Cora grape juice, supporting the TPC data. These compounds may be able to avoid certain diseases, thus providing a better life quality.⁴⁰

The major phenolic compounds of BRS-Cora grape juice were delphinidin 3-*O*-glucoside, proanthocyanidin B1, ferulic acid and caffeic acid. Proanthocyanidin B1, malvidin 3-*O*-glucoside, syringic acid and catechin were the major ones in Isabella grape juice. In general, the concentration of individual phenolic compounds was different ($P \leq 0.05$) between the samples. BRS-Cora grape juice rendered a higher concentration of most of the phenolics quantified, which is in agreement with the trend observed in TPC and anthocyanin analyses.

Among all the phenolic compounds identified, only isorhamnetin and malvidin 3-*O*-glucoside were not found in BRS-Cora grape juices, and *p*-coumaric acid and epicatechin gallate were not found in Isabella grape juices. *trans*-Resveratrol, kaempferol-3-*O*-glucoside, myricetin and quercetin were not identified in either juice.

Table 1. Concentration range (CR, mg L⁻¹) of the standards used to prepare the calibration curves, standards purity (SP, %), equations of the calibration curves, coefficients of determination (R²), detection limits (LD, mg L⁻¹), quantification limits (LQ, mg L⁻¹) and assay repeatability through coefficient of variation (CV, %)

Phenolic compound	CR (mg L ⁻¹)	SP (%)	Equation of calibration curves	R ²	LD (mg L ⁻¹)	LQ (mg L ⁻¹)	VC (%)
1. Pelargonidin 3-O-glucoside	0.625–20	≥98	Y = 4.26e + 004 X + 2.71e + 003	0.9996	0.02	0.05	1.21
2. Cyanidin 3-O-glucoside	0.625–20	≥98	Y = 6.42e + 004 X + 4.84e + 003	0.9997	0.04	0.12	2.88
3. Delphinidin 3-O-glucoside	0.625–20	≥95	Y = 5.56e + 004 X + 6.28e + 003	0.9995	0.09	0.30	3.39
4. Peonidin 3-O-glucoside	0.625–20	≥95	Y = 6.35e + 004 X + 4.12e + 003	0.9995	0.04	0.13	0.62
5. Malvidin 3-O-glucoside	0.625–20	≥95	Y = 3.47e + 004 X + 1.46e + 004	0.9997	0.27	0.89	1.26
6. Epicatechin gallate	1.25–20	≥98	Y = 8.93e + 004 X + 8.55e + 003	0.9997	0.13	0.42	1.97
7. Epigallocatechin gallate	1.25–20	≥98	Y = 9.64e + 004 X + 3.22e + 003	0.9999	0.09	0.31	2.32
8. Gallic acid	5.00–80	≥99	Y = 1.34e + 005 X + 5.31e + 004	0.9996	0.12	0.41	1.10
9. Procyanidin B1	1.25–20	≥80	Y = 1.17e + 005 X + 8.54e + 003	0.9996	0.09	0.31	1.45
10. <i>p</i> -Coumaric acid	2.5–40	≥98	Y = 1.09e + 005 X + 1.79e + 004	0.9997	0.08	0.27	2.79
11. Caffeic acid	2.5–40	≥99	Y = 9.99e + 004 X + 1.94e + 004	0.9996	0.08	0.26	2.79
12. Chlorogenic acid	2.5–40	≥99	Y = 5.42e + 004 X + 8.96e + 003	0.9996	0.09	0.30	1.43
13. Ferrulic acid	2.5–40	≥97	Y = 9.30e + 004 X + 1.80e + 004	0.9996	0.09	0.30	2.19
14. Isorhamnetin-3-O-glucoside	0.625–10	≥95	Y = 3.06e + 004 X + 2.48e + 003	0.9995	0.03	0.09	1.05
15. Rutin	0.625–10	≥98	Y = 2.77e + 004 X + 1.84e + 003	0.9994	0.06	0.20	2.93
16. Procyanidin A2	1.25–20	≥98	Y = 1.66e + 006 X + 5.87e + 005	0.9972	0.23	0.76	2.83
17. Catechin	2.50–40	≥98	Y = 4.01e + 006 X - 4.07e + 005	0.9994	0.06	0.19	1.79
18. Procyanidin B2	2.50–40	≥90	Y = 1.87e + 006 X + 4.44e + 004	0.9977	0.27	0.89	1.88
19. Syringic acid	2.50–40	≥95	Y = 1.91e + 006 X - 7.75e + 004	0.9998	0.28	0.93	2.30
20. Vanillic acid	2.50–40	≥97	Y = 1.23e + 007 X + 3.99e + 006	0.9990	0.30	1.00	1.95
21. Epicatechin	1.25–20	≥98	Y = 1.61e + 007 X + 2.94e + 006	0.9991	0.26	0.86	2.95

DAD: 220 nm (compounds 6–9); 320 nm (compounds 10–13); 360 nm (compounds 14–15); 520 nm (compounds 1–5) and fluorescent at 280 nm of the excitation and 360 nm of the emission (compounds 16–21).

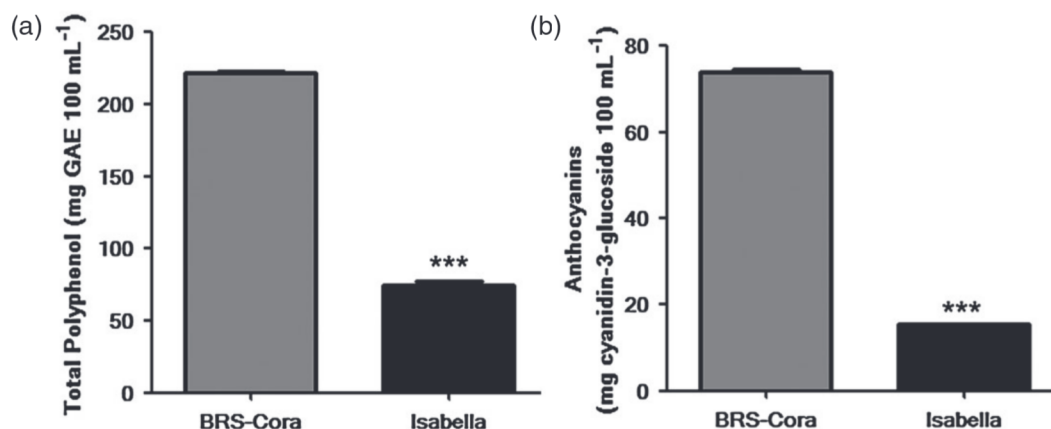


Figure 1. Total polyphenols (a) and anthocyanin contents (b). GAE, gallic acid equivalent. Data are expressed as means \pm SEM. Coefficient of variation (CV) for total polyphenols and total anthocyanins were 3.2% and 1.6%, respectively. Asterisks indicate a significant difference between BRS-Cora and Isabella grape juice, as analyzed by Student's *t*-test (***) $P \leq 0.001$.

Supporting the findings of the present study, Sensoy⁴¹ also found caffeic acid and syringic acids as the major compounds in varieties of red grapes. However, grape juices prepared with Sauvignon Blanc cultivar showed higher catechin and lower caffeic acid concentrations than those found in the present study, within the range of 4.5–7.7 and 0.41–3.7 mg L⁻¹, respectively.⁴² Such differences may be correlated with the different grape cultivars compared as the present study, which used red grapes, and these authors used Sauvignon Blanc, which is a white grape. However, Ghafoor *et al.*³² did not find caffeic acid in grape juice prepared with Campbell Early grape. According to Sun *et al.*,⁴³ ellagic acid

is the major compound observed in different polyphenol profiles of some grape varieties (Fernão Pires, Castelão, Vital, Vinhão, Espadeiro, Azal Tinto). The present study is in accordance with the literature in relation to the variation in polyphenol composition among different grape cultivars. BRS-Cora was developed by Embrapa, Brazil, with the objective of creating new varieties for grape juice as an alternative to emerging Brazilian regions for grape production, with high productivity, sugar content, color intensity and good sensory quality of grape juice, as well as the *Vitis labrusca* cultivars Isabella and Concord.⁴⁴ Therefore, it was expected that BRS Cora grape juice had a higher anthocyanins

Table 2. Phenolic compounds (mg L⁻¹) in BRS-Cora and Isabella grape juices as evaluated by HPLC-DAD-FLD

	BRS-CORA	Isabella
Anthocyanins		
Pelargonidin 3-O-glucoside	0.33 ± 0.03 ^b	3.28 ± 0.03 ^a
Cyanidin 3-O-glucoside	2.77 ± 0.03 ^b	3.27 ± 0.03 ^a
Delphinidin 3-O-glucoside	23.3 ± 0.10 ^a	2.80 ± 0.50 ^b
Peonidin 3-O-glucoside	0.55 ± 0.00 ^b	6.39 ± 0.05 ^a
Malvidin 3-O-glucoside	ND	23.2 ± 0.18
Σ Anthocyanins	26.98 ± 0.09 ^b	38.85 ± 0.15 ^a
Phenolic acids		
Gallic acid	3.52 ± 0.03 ^a	1.75 ± 0.00 ^b
Caffeic acid	7.42 ± 0.29 ^a	1.50 ± 0.00 ^b
Syringic acid	6.02 ± 0.13 ^b	11.38 ± 0.40 ^a
Vanillic acid	0.58 ± 0.03 ^a	0.25 ± 0.00 ^b
Ferulic acid	19.20 ± 1.13 ^a	0.62 ± 0.03 ^b
Chlorogenic acid	1.85 ± 0.45	1.85 ± 0.00
<i>p</i> -coumaric acid	1.32 ± 0.03	ND
Σ Phenolic acids	39.90 ± 0.51 ^a	17.35 ± 0.16 ^b
Flavonoids/proanthocyanidins		
Isorhamnetin 3-O-glucoside	ND	0.55 ± 0.00
Catechin	2.57 ± 0.03 ^b	2.68 ± 0.03 ^a
Epicatechin	1.83 ± 0.03 ^a	0.20 ± 0.00 ^b
Epicatechin gallate	1.95 ± 0.09	ND
Epigallocatechin gallate	4.65 ± 0.07 ^a	0.48 ± 0.02 ^b
Proanthocyanidin A2	1.15 ± 0.05 ^b	1.35 ± 0.03 ^a
Proanthocyanidin B1	20.78 ± 0.69 ^b	32.57 ± 0.20 ^a
Proanthocyanidin B2	3.33 ± 0.03 ^a	1.92 ± 0.39 ^b
Rutin	5.42 ± 0.03 ^a	1.63 ± 0.03 ^b
Σ	41.68 ± 0.14	41.38 ± 0.08
Flavonoids/proanthocyanidins		
Σ Phenolic compounds (anthocyanins + phenolic acids + flavonoids/proanthocyanidins)	108.86 ± 0.37^a	97.58 ± 0.17^b

Data are expressed as means ± SEM. Values followed by a different superscript in the same row are significantly different ($P \leq 0.05$) from Cora grape according to the Student's *t*-test. ND, not detected.

content, as shown in Fig. 1. Additionally, Table 2 shows that BRS Cora also presents a higher content of other phenolic compounds compared to Isabella grape juice, suggesting that the presence of this cultivar in the juice can contribute to improve benefits of grape juice consumption for human health. Furthermore, different techniques used for juice production may generate different polyphenol contents in the final product, but it is not the case for the juices evaluated.

As previously mentioned, phenolic compounds have shown health benefits against LDL oxidation as well as in the reduction of DNA damage and free radical level in blood plasma.^{45–47} Furthermore, proanthocyanidins, which are among the major phenolic compounds of BRS Cora and Isabella grape juice, have been associated with the prevention of urinary tract infections.⁴⁸ Grape seed extracts containing catechin, epicatechin and proanthocyanidins also demonstrated positive effects on prevention of Alzheimer's disease in animal models.⁴⁹ Sato *et al.*⁵⁰ demonstrated that caffeic acid has strong antioxidant potential *in vitro* and *in vivo*. Finally, syringic acid has shown a hepato-protective effect in mice subjected to induced liver injury.⁵¹ Thus phenolic compounds

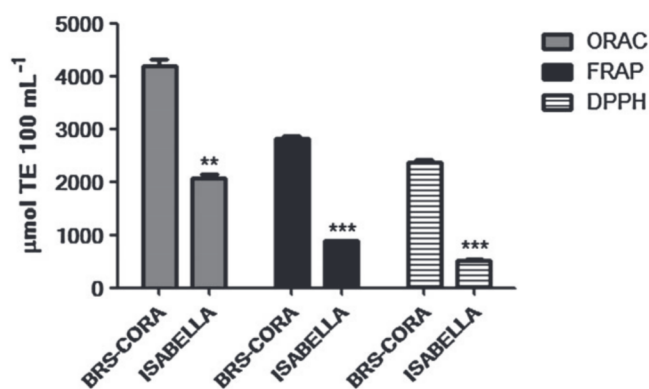


Figure 2. Antioxidant potential of grape juices as evaluated by ORAC, FRAP and DPPH methods (µmol Trolox equivalent (TE) 100 mL⁻¹ juice). Data are expressed as means ± SEM. Coefficients of variation (CV) for ORAC, FRAP and DPPH were 6.3%, 2.9% and 4.9%, respectively. Asterisks indicate a significant difference between BRS-Cora and Isabella grape juice as analyzed by Student's *t*-test (** $P \leq 0.01$; *** $P \leq 0.001$).

Table 3. Pearson's correlation coefficients (*r*) between bioactive compounds and antioxidant capacity of BRS-Cora and Isabella grape juices

Parameter	Polyphenols			Anthocyanins		
	ORAC	FRAP	DPPH	ORAC	FRAP	DPPH
<i>r</i>	0.9872	0.9966	0.9989	0.9916	0.9963	0.9992
<i>P</i>	0.0002	<0.0001	<0.0001	0.0001	<0.0001	<0.0001
<i>r</i> ²	0.9745	0.9931	0.9978	0.9833	0.9925	0.9983

r, correlation coefficient; *r*², determination coefficient.

identified in BRS-Cora and Isabella will likely provide health benefits for consumers.

Antioxidant activity

Oxidative stress may rise in the cell, affecting the integrity of biomolecules and increasing the risk of oxidative-related diseases. Polyphenols found in berry fruits possess antioxidant properties,⁵² which may be beneficial against oxidative stress. The *in vitro* evaluation of antioxidant capacity is helpful in anticipating the potential of food products to prevent oxidative damage *in vivo*. It is also important for consumers, helping them to make decisions and choose foods to achieve a better diet. Data about the bioavailability of polyphenols are still scarce due to the complexity of this kind of investigation. However, some studies showing the presence of these compounds in blood and tissues and, most recently, metabolomics databases with information on metabolites are becoming available, which may contribute to establishing relationships among different sources of polyphenols, their intake and human health improvement.^{53–55}

In the present study, the antioxidant properties of grape juices were evaluated by oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP) and DPPH radical scavenging activity. ORAC relies on chain-breaking antioxidant capacity against peroxy radicals,⁵⁶ FRAP involves the reduction of ferric to ferrous ions via electron transfer,²⁷ and DPPH involves hydrogen donation.²⁵ Peroxy radicals are reactive oxygen species (ROS), thus being detrimental to health due to the oxidative processes in which they are active. Because of their relatively long half-life,

their effect may occur at both a cellular level and in biological fluids. Ferric ions are also detrimental to biological systems, as they catalyze the oxidation of proteins and lipids. Finally, DPPH is a synthetic radical that has been widely applied to *in vitro* antioxidant evaluations as a preliminary screen of the antioxidant properties of several food products.⁵⁷

The results of the antioxidant evaluation are summarized in Fig. 2. The antioxidant activity of BRS-Cora grape juice was 2.3-, 3- and 4.5-fold higher than Isabella grape juice, as evaluated by ORAC, FRAP and DPPH, respectively. These results are supported by the higher TPC and anthocyanins found in BRS-Cora grape juice (Fig. 1). Furthermore, a strong positive correlation was found in TPC or anthocyanins and the remaining antioxidant assays (ORAC, FRAP and DPPH) (Table 3).

Antioxidants can stop free radical chain reactions via the donation of hydrogen from their phenolic hydroxyl groups or electrons, thus generating stable end-products that do not take part in further oxidation processes.⁵⁸ Therefore, in general, the antioxidant activity of food products reflects their polyphenol content, as demonstrated in the present study. Both grape juices had antioxidant properties in neutralizing peroxy radicals and in reducing ferric ions, as evaluated by ORAC and FRAP, respectively. Both methods possess biological relevance.^{59,60} Thus grape juice consumption will likely have health benefits. Finally, BRS-Cora, which is a relatively new cultivar (it was launched in 2004),⁴⁴ showed the highest antioxidant properties in all antioxidant assays when compared to Isabella. However, BRS-Cora is still underused. Thus the grape juice industry should consider the use of BRS-Cora to produce grape juice or use them to produce blends with Isabella, improving not only the color of the juice but also their health benefits.

CONCLUSION

BRS-Cora grape juice showed higher TPC, anthocyanin content and antioxidant potential than Isabella grape juice. Twenty-one phenolic compounds were positively identified and quantified using HPLC-DAD-FLD. Little difference was found in the identity of phenolics between the cultivars; however, their quantification demonstrated significant differences in the grape juices made with BRS-Cora and Isabella grapes. BRS-Cora grape juice had up to two and ten times higher concentrations of gallic acid and epigallocatechin gallate, respectively, than that found in Isabella grape juice. Furthermore, epicatechin gallate was identified and quantified only in BRS-Cora. Gallic acid and polyphenols bearing galloyl groups probably had an influence on the higher antioxidant potential of BRS-Cora grape juice. In this way, the use of BRS-Cora is a good alternative to produce grape juice or in blends with different cultivars. The new cultivar BRS-Cora has been used to improve the color of Brazilian grape juice; however, as new information, the present work demonstrated that the use of BRS-Cora may also offer improvements in the phenolic profile and antioxidant potential of the product, being a potential functional food.

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REFERENCES

- Kar P, Laight D, Rooprai HK, Shaw KM and Cummings M, Effects of grape seed extract in Type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. *Diabetic Med* **26**:526–531 (2009).
- Arimoto-Kobayashi S, Zhang X, Yuhara Y, Kamiya T, Negishi T and Okamoto G, Chemopreventive effects of the juice of *Vitis coignetiae* Pulliat on two-stage mouse skin carcinogenesis. *Nutr Cancer* **65**:440–450 (2013).
- Vislocky LM and Fernandez ML, Biomedical effects of grape products. *Nutr Rev* **68**:656–670 (2010).
- Bravo L, Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* **56**:317–333 (1998).
- Jacob JK, Hakimuddin F, Paliyath G and Fisher H, Antioxidant and antiproliferative activity of polyphenols in novel high-polyphenol grape lines. *Food Res Int* **41**:419–428 (2008).
- Joseph JA, Shukitt-Hale B and Willis LM, Grape juice, berries, and walnuts affect brain aging and behavior. *J Nutr* **139**:1813s–1817s (2009).
- Middleton E, Kandaswami C and Theoharides TC, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev* **52**:673–751 (2000).
- Monagas M, Gómez-Cordovés C, Bartolomé B, Laureano O and Ricardo-Da-Silva JM, Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J Agric Food Chem* **51**:6475–6481 (2003).
- Sandhu AK, Gray DJ, Lu J and Gu L, Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins. *Food Chem* **126**:982–988 (2011).
- De Nisco M, Manfra M, Bolognese A, Sofo A, Scopa A, Tenore GC *et al.*, Nutraceutical properties and polyphenolic profile of berry skin and wine of *Vitis vinifera* L. (cv. Aglianico). *Food Chem* **140**:623–629 (2013).
- Machado MM, Dos Santos Montagner GFF, Boligon A, Athayde ML, Da Rocha MIUM, Lera JPB *et al.*, Determination of polyphenol contents and antioxidant capacity of no-alcoholic red grape products (*Vitis labrusca*) from conventional and organic crops. *Quimica Nova* **34**:798–803 (2011).
- Treutter D, Managing phenol contents in crop plants by phytochemical farming and breeding: visions and constraints. *Int J Mol Sci* **11**:807–857 (2010).
- Cazarin CBB, Correa LC, Da Silva JK, Batista AG, Furlan CP, Biasoto ACT *et al.*, Tropical Isabella grape juices: bioactive compounds and antioxidant power depends on harvest season. *J Food Sci Eng* **3**:64–70 (2013).
- Ky I, Lorrain B, Kolbas N, Crozier A and Teissedre PL, Wine by-products: phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different french grape varieties. *Molecules* **19**:482–506 (2014).
- Ky I and Teissedre PL, Characterisation of mediterranean grape pomace seed and skin extracts: polyphenolic content and antioxidant activity. *Molecules* **20**:2190–2207 (2015).
- Sabir A, Comparison of green grafting techniques for success and vegetative development of grafted grape cultivars (*Vitis* spp.). *Int J Agric Biol* **13**:628–630 (2011).
- Verma SK, Singh SK, Krishna H and Patel VB, Comparative performance of different grafting techniques in grape cv. Pusa Urvashi. *Indian J Horticult* **69**:13–19 (2012).
- Stein JH, Keevil JG, Wiebe DA, Aeschlimann S and Folts JD, Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* **100**:1050–1055 (1999).
- Castilla P, Echarri R, Davalos A, Cerrato F, Ortega H, Teruel JL *et al.*, Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am J Clin Nutr* **84**:252–262 (2006).
- Park YK, Park E, Kim JS and Kang MH, Daily grape juice consumption reduces oxidative DNA damage and plasma free radical levels in healthy Koreans. *Mutat Res – Fund Mol M* **529**:77–86 (2003).
- Camargo UA, Maia JDG and Ritschel PS, *Embrapa Uva e Vinho – novas cultivares brasileiras de uva*. Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Bento Gonçalves, Brazil (2010).

- 22 Swain T and Hillis WE, The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *J Sci Food Agric* **10**:63–68 (1959).
- 23 Wrolstad RE, *Color and Pigment Analyses in Fruit Products*. Oregon Agricultural Experimental Station, Corvallis, OR (1993).
- 24 Abe LT, Mota RVD, Lajolo FM and Genovese MI, Compostos fenólicos e capacidade antioxidante de cultivares de uvas *Vitis labrusca* L. e *Vitis vinifera* L. *Cienc Tecnol Aliment* **27**:394–400 (2007).
- 25 Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity. *LWT – Food Sci Technol* **28**:25–30 (1995).
- 26 Dávalos A, Bartolomé B and Gómez-Cordovés C, Antioxidant properties of commercial grape juices and vinegars. *Food Chem* **93**:325–330 (2005).
- 27 Rufino MDM, Alves RE, de Brito ES, Perez-Jimenez J, Saura-Calixto F and Mancini J, Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem* **121**:996–1002 (2010).
- 28 Scalbert A and Williamson G, Dietary intake and bioavailability of polyphenols. *J Nutr* **130**:2073S–2085S (2000).
- 29 Scalbert A, Johnson IT and Saltmarsh M, Polyphenols: antioxidants and beyond. *Am J Clin Nutr* **81**:215S–217S (2005).
- 30 Krikorian R, Nash TA, Shidler MD, Shukitt-Hale B and Joseph JA, Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment. *Br J Nutr* **103**:730–734 (2010).
- 31 Yuan LH, Meng LP, Ma WW, Xiao ZX, Zhu X, Feng JF et al., Impact of apple and grape juice consumption on the antioxidant status in healthy subjects. *Int J Food Sci Nutr* **62**:844–850 (2011).
- 32 Ghafoor K, Al-Juhaimi F and Choi YH, Effects of grape (*Vitis labrusca* B.) peel and seed extracts on phenolics, antioxidants and anthocyanins in grape juice. *Pak J Bot* **43**:1581–1586 (2011).
- 33 Tanrioven D and Eksi A, Phenolic compounds in pear juice from different cultivars. *Food Chem* **93**:89–93 (2005).
- 34 Burin VM, Ferreira-Lima NE, Panceri CP and Bordignon-Luiz MT, Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: evaluation of different extraction methods. *Microchem J* **114**:155–163 (2014).
- 35 Pala ÇU and Toklucu AK, Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. *J Food Compos Anal* **24**:790–795 (2011).
- 36 Santos TD, Cavalcante RN, Rostagno MA, Queiroga CL, Eberlin MN and Meireles MAA, Extraction of polyphenols and anthocyanins from the jambul (*Syzygium cumini*) fruit peels. *Food Public Health* **3**:12–20 (2013).
- 37 Zhu L, Zhang YL and Lu J, Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *Int J Mol Sci* **13**:3492–3510 (2012).
- 38 Wang HB, Race EJ and Shrikhande AJ, Characterization of anthocyanins in grape juices by ion trap liquid chromatography-mass spectrometry. *J Agric Food Chem* **51**:1839–1844 (2003).
- 39 Rebello LPG, Lago-Vanzela ES, Barcia MT, Ramos AM, Stringheta PC, Da-Silva R et al., Phenolic composition of the berry parts of hybrid grape cultivar BRS Violeta (BRS Rubra × IAC 1398–21) using HPLC-DAD-ESI-MS/MS. *Food Res Int* **54**:354–366 (2013).
- 40 Pojer E, Mattivi F, Johnson D and Stockley CS, The case for anthocyanin consumption to promote human health: a review. *Compr Rev Food Sci Food Saf* **12**:483–508 (2013).
- 41 Sensoy RIG, Determination of phenolic substances and antioxidant activities in some grape cultivars by HPLC. *J Anim Plant Sci* **22**:448–451 (2012).
- 42 Fracassetti D, Lawrence N, Tredoux AGJ, Tirelli A, Nieuwoudt HH and Du Toit WJ, Quantification of glutathione, catechin and caffeic acid in grape juice and wine by a novel ultra-performance liquid chromatography method. *Food Chem* **128**:1136–1142 (2011).
- 43 Sun B, Ricardo-da-Silva JM and Spranger MI, Quantification of catechins and proanthocyanidins in several portuguese grapevine varieties and red wines. *Cienc Tecnol Viticola* **16**:23–34 (2001).
- 44 Camargo UA and Maia JDG, BRS CORA: nova cultivar de uva para suco, adaptada a climas tropicais. Comunicado Técnico – Embrapa Uva e Vinho (2004).
- 45 Frankel EN, Waterhouse AL and Teissedre PL, Principal phenolic phytochemicals in selected california wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J Agric Food Chem* **43**:890–894 (1995).
- 46 Teissedre PL, Frankel EN, Waterhouse AL, Peleg H and German JB, Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J Sci Food Agric* **70**:55–61 (1996).
- 47 de Camargo AC, Regitano-d’Arce MAB, Biasoto ACT and Shahidi F, Low molecular weight phenolics of grape juice and winemaking byproducts: antioxidant activities and inhibition of oxidation of human low-density lipoprotein cholesterol and DNA strand breakage. *J Agric Food Chem* **62**:12159–12171 (2014).
- 48 Howell AB, Botto H, Combesure C, Blanc-Potard AB, Gausa L, Matsumoto T et al., Dosage effect on uropathogenic *Escherichia coli* anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. *BMC Infect Dis* **10**:94 (2010).
- 49 Wang J, Ho L, Zhao W, Ono K, Rosensweig C, Chen L et al., Grape-derived polyphenols prevent Aβ oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer’s disease. *J Neurosci* **28**:6388–6392 (2008).
- 50 Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T et al., In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *Int J Pharm* **403**:136–138 (2011).
- 51 Itoh A, Isoda K, Kondoh M, Kawase M, Watari A, Kobayashi M et al., Hepatoprotective effect of syringic acid and vanillic acid on CCl₄-induced liver injury. *Biol Pharm Bull* **33**:983–987 (2010).
- 52 Côté J, Caillet S, Doyon G, Dussault D, Salmieri S, Lorenzo G et al., Effects of juice processing on cranberry antioxidant properties. *Food Res Int* **44**:2907–2914 (2011).
- 53 Manach C, Williamson G, Morand C, Scalbert A and Remesy C, Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**:230S–242S (2005).
- 54 Seeram NP, Lee R and Heber D, Bioavailability of ellagic acid in human plasma after consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice. *Clin Chim Acta* **348**:63–68 (2004).
- 55 Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L et al., Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. *Arch Toxicol* **88**:1803–1853 (2014).
- 56 Dávalos A, Gomez-Cordoves C and Bartolome B, Extending applicability of the oxygen radical absorbance capacity (ORAC-fluorescein) assay. *J Agric Food Chem* **52**:48–54 (2004).
- 57 de Camargo AC, Vieira TMFS, Rasera GB, Caniniatti-Brazaca SG, Alencar SM and Regitano-Darce MAB, Lower solvent concentration and time for extraction of peanut skin antioxidants at optimized conditions, in *Peanuts: Production, Nutritional Content and Health Implications*, ed. by Cook RW. Hauppauge, NY, pp. 31–50 (2014).
- 58 Göktürk Baydar N, Özkan G and Yaşar S, Evaluation of the anti-radical and antioxidant potential of grape extracts. *Food Control* **18**:1131–1136 (2007).
- 59 Benzie IFF and Strain JJ, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal Biochem* **239**:70–76 (1996).
- 60 Prior RL, Hoang H, Gu LW, Wu XL, Bacchiocca M, Howard L et al., Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *J Agric Food Chem* **51**:3273–3279 (2003).