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Tropical Isabella Grape Juices: Bioactive Compounds and Antioxidant Power Depends on Harvest Season

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Abstract: Grapes are important sources of antioxidants compounds and one of the most used varieties to elaborate juices is "Isabel", or "Isabella", as it is called in North-America. This study aimed to evaluate the antioxidant activity of Isabella grape juices from the tropical semi-arid climate produced in Brazil on March and September, 2010. Total phenolics and anthocyanins were determined, as well as the antioxidant capacity by DPPH, FRAP and hydrophilic ORAC methods. Test *T* was used to compare statistical difference at 5% of significance level. As expected, the results showed that the season can play a significant role on phenolics content and antioxidant power. Polyphenols content in juices varied from 82.9 ± 0.92 to 102.2 ± 1.59 mg GAE 100 mL^{-1} and anthocyanins content ranged from 44.3 ± 2.01 to 129.5 ± 2.82 mg cyanidin-3-glucoside 100 mL^{-1}. Regression analyses showed a high correlation of antioxidant capacity and anthocyanins content. Grapes from Sao Francisco River Valley demonstrated to be a good source of antioxidant for human diet.

Key words: Isabella grape, *Vitis labrusca*, grape juice, antioxidant activity, anthocyanins, phenolic content.

1. Introduction

Oxidative stress is commonly regarded as a key factor in several chronic diseases [1] and a possible way to attenuate oxidative stress is the consumption of antioxidant substances found in natural foods, such as phenolic compounds [2].

Polyphenols are a group of secondary metabolites present in fruits and vegetables and, in this group, flavonoids are the most important, with high antioxidant power [3]. The consumption of juice helps to fulfill the recommended fruit servings [4] and improves *in vivo* antioxidant status [5].

Fruits and juices have received special attention because of their high content of polyphenols [5, 6]. Indeed, the intestinal absorption of polyphenols from juices is reported to be better than for whole fruits, which could contribute for antioxidant status improvement [7, 8].

Grapes are important sources of antioxidant compounds that can act in prevention and control of some chronic diseases. The consumption of grape juice and grape products have been shown to increase serum antioxidant power [9, 10], and reduce bladder dysfunction in animal model [11, 12].

In Brazil, the most used grape variety to elaborate juices is Isabella. This cultivar has demonstrated good adaptation to tropical semi-arid climate, which is characteristic of the sub-middle Sao Francisco River Valley region. In this region, two crops of grapes variety can be harvested per year, due to its peculiar

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conditions, with high temperatures throughout the year, solar radiation and water availability for irrigation, which, along with other factors such as date of grape harvested and soil type, can influence the phenolic content of the fruit [13].

In the last years, the interest for foods which could improve health and quality of life has increased. The consumption of fruits and vegetables rich in antioxidants has increased due to their ability to prevent chronic diseases [14, 15]. This possible role could be related to antioxidant activity of natural juices, which have shown protective effects against several chronic diseases. Consumption of 480 mL day^{-1} of purple grape juice promoted reductions in DNA damage and in free radical levels in healthy Koreans [16].

In this way, the present study aimed to compare the major flavonoids profile in grape juices from Isabella produced during two different harvests in the same year in the sub-middle São Francisco River Valley region. Total phenolic and anthocyanins contents were determined, as well as the composition of flavonoids by HPLC. The total antioxidant capacity was investigated against DPPH radical, FRAP and scavenging activity of reactive oxygen species (ORAC).

2. Materials and Methods

2.1 Fruits and Juices

The grapes were harvested in March and September 2010, which corresponds to the first and second harvests of the year carried out by Embrapa Semi-arid Bebedouro, located in Petrolina, Pernambuco State, Brazil (09°09′S, 40°22′W, 365.5 m). The harvests of the Isabella grapes were done when the total acidity and soluble solids reached 6-7 g L^{-1} [17] and 23 ºBrix, respectively. The fruits were previous stored at 10 ºC until processing. Ten kilograms of fruits were processed by steam extraction method during 60 min and the temperature of extraction was maintained at 75-85 °C. The grapes were previously sanitized with hypochlorite solution (200 mg L^{-1}) for 15 min and then rinsed with water. The súlfur dioxide (80 mg L^{-1}) was added to inhibit oxidative reactions [18]. The juice was bottled in glass bottles previously washed and sterilized, threaded with polyethylene caps and stored at 16 °C until the moment of the analysis.

2.2 Total Polyphenolic and Anthocyanins Contents

Total phenolic content was determined by the Folin-Ciocalteu method, which was adapted from Swain and Hillis [19]. Fifty microliters of juice, 800 µL of ultrapure water, and 50 µL of Folin–Ciocalteu reagent were combined and mixed using a vortex. The mixture was allowed to react for 3 min, then 100 µL of 1 N Na_2CO_3 solution were added and mixed. The solution was incubated at room temperature (24 °C) in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer (Sinergy HT-Biotek, Winooski-USA) and the results were expressed as Gallic acid equivalents (GAE mg 100 mL^{-1} of juice).

Total anthocyanins were quantified according to the method described by Wrolstad [20] and adapted by Abe et al. [21]. An aliquot of the juice was diluted using 0.025 M potassium chloride buffer, pH = 1.0, according to the sample color. The absorbance was read at 510 and 700 nm using a microplate reader (Sinergy HT, Biotek, Winooski-USA). Another aliquot was diluted in the same proportions in 0.4 M sodium acetate buffer, pH 4.5, and the absorbance read at the same wavelengths. The absorbance was then calculated using Eq. 1 (according to the refereed method).

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

The anthocyanin content (mg $100g^{-1}$) was calculated as cyanidin 3-glucoside (PM = 449.2) using Eq. 2.

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

where, £ = molar absorptivity (26,900 mol L^{-1}), 1 = tray thickness (cm), MW= molecular weight and DF= dilution factor.

2.3 HPLC Analysis

The analyses were carried out in Waters Aliance e2695 HPLC system equipped with a photodiode array

detector and fluorescence detector. The separation of polyphenols was done on a C18 pre-column (4.0 × 3.0 mm, Gemini NX, Phenomenex) and column (150 × 4.6 mm, 3 µm Gemini NX, Phenomenex, Torrance, CA) at controlled temperature (40 °C). The mobile phase used was A: deionized water: phosphoric acid (pH = 2.05), B: methanol and C: acetonitrile. The gradient program was used 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. Flow rate and injection volume were 0.6 mL min^{-1} and 20 µL, respectively. The identification peak of each polyphenols was based on the comparison of relative retention time (RT), peak area percentage, spectra data (220, 320 and 360 nm) and fluorescence (360 nm emission) with polyphenols standards. The standard curves for all polyphenols were determined by using the same chromatographic conditions described above.

2.4 Antioxidant Activity

The DPPH (1,1-diphenyl-2-picrylhydrazil) assay was according to the method described by Brand-Williams, Cuvelier and Berset [22] with some modifications. The absorbance was measured in a Sinergy HT-Bioteck (Winooski-USA) spectrometer at 515 nm before addition of samples and after 30 min. The percentage scavenging DPPH: radical was calculated using the following equation $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

The FRAP assay was done according to Prior et al. [23] with some modifications. The stock solutions included 0.3 M acetate buffer pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM ferric chloride solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL of ferric chloride solution. The reaction was prepared with 90 µL juice, 270 µL deionized water and 2.7 mL FRAP solution. After homogenization, the sample was kept in water bath at 37 °C for 30 min in dark condition. Absorbance was taken at 595 nm. The standard curve was linear between 25 and 1,000 µM of Trolox. Results were expressed in µM TE 100 mL^{-1} juice.

For hydrophilic oxygen radical absorbance capacity (ORAC) assay, an automated plate reader (Sinergy HT, Biotek, Winooski-USA) with filters of emission 520 nm and excitation 485 nm was used [24]. Analyses were conducted in phosphate buffer pH 7.4 and the peroxyl radical was generated by 2,2'-azobis (2-amidino-propane) dihydrochloride solution, prepared just before the analysis and fluorescein was used as the substrate. Fluorescence was read at every 1 min, for 80 min. The standard curve was done with Trolox (1 to 70 µM TE) and the results were expressed in µM TE 100 mL^{-1} of juice.

2.5 Statistical Analysis

All the chemical analysis was conducted in triplicate. The data was performed by ANOVA and *t*-student means test for comparison of Isabella juices produced with grapes harvested in the two crops in the same year. All data were expressed in means ± SD. A difference was considered to be statistically significant when $P \leq 0.05$.

3. Results and Discussion

3.1 Total Phenolics Content and Anthocyanins

The results showed that Isabella grape juice have a similar content of polyphenols compared to commercial samples from Spain [25]. Fig. 1 shows that grapes harvested on September showed higher amounts of total polyphenols and anthocyanins contents. In this period of the year, on the South hemisphere, higher solar irradiation and higher temperatures could stimulate the production of secondary plant metabolites, resulting in increased flavonoids and pigments biosynthesis [26]. March 2010 (91 mm) was a very rainy season in Brazil compared to September

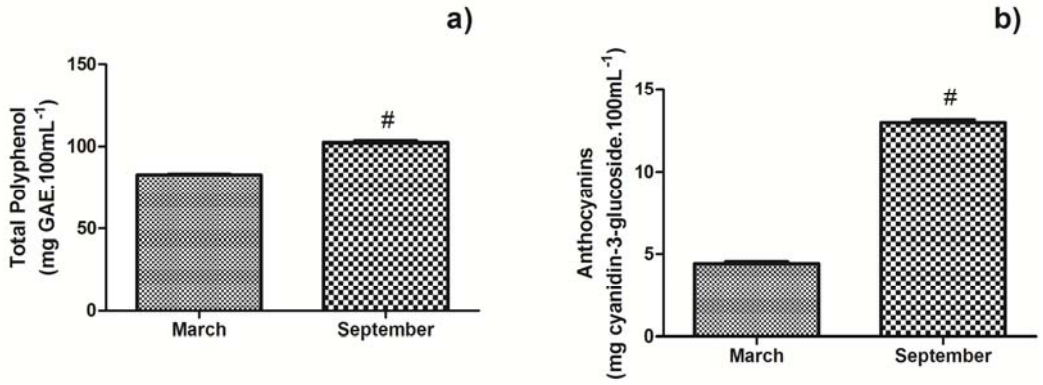

Fig. 1 Total phenolics (a) and anthocyanins (b) in Isabella grape juices produced in different times of the year. Results expressed in mean ± SD of a triplicate. Rows sharing different superscript are significantly different ($P < 0.05$). The results show a clear improvement of phenolics and anthocyanins contents on the samples harvested on September 2010 compared to March, 2010.

(2.7 mm) [27], which corroborates our hypothesis.

The content of total polyphenol in Isabella grape juices was higher than in pear juice [28], sour cherry juice [29], orange, grapefruit, apple and pineapple juices [30] and it could be related with the content of anthocyanins present in red and purple fruits.

A recent clinical trial showed that the consumption of 300 mL of apple juice at lunch and 300 mL of grape juice at dinner, for 2 weeks, increased plasma total antioxidant capacity and decreased the malondialdehyde levels. In the same way, the consumption of grape juice may contribute to the inhibition of lipid oxidation and it has a great potential to enhance *in vivo* antioxidant power and then protect the human health against oxidative stress [10]. Rats supplemented with 2% freeze-drying jaboticaba peel (*Myrciaria jaboticaba Vell Berg*, with 75.6 g 100 g^{-1} of cyanidin 3-glycoside) showed improved plasmatic antioxidant activity evaluated by ORAC (22.96 Trolox equiv μL^{-1}) [31].

3.2 Determination of Major Phenolic Compounds of Isabella Grape Juice by HPLC

Twenty polyphenolic compounds were determined in Isabella grape juices, among phenolic acids, flavonoids (flavan-3-ols and flavonols) and the trans-resveratrol (Table 1). There were significant differences ($P \leq 0.05$) in the content of all compounds among the juices from the grapes of the two seasons. Proanthocyanidin B1 is the most prominent polyphenol present in the juices (March 27.43 ± 0.024, September 41.65 ± 1.239 mg L^{-1}). The grapes harvested on September showed higher concentrations of 11 phenolic compounds (vanilic acid,

Table 1 Content of phenolic compounds (mg L^{-1}) in tropical Isabella grape juices from sub-middle Sao Francisco River Valley, Northeast region of Brazil, done in March and September of the year 2010.

	March 2010	September 2010
Caffeic acid	2.50 ± 0.000^{a}	2.30 ± 0.000^{b}
Vanilic acid	0.97 ± 0.024^{b}	1.83 ± 0.024^{a}
Chlorogenic acid	1.87 ± 0.024^{a}	1.68 ± 0.024^{b}
p-coumaric acid	1.45 ± 0.000^{a}	1.20 ± 0.000^{b}
Trans-Resveratrol	0.57 ± 0.024^{b}	0.73 ± 0.024^{a}
Isorhamnetin	1.10 ± 0.000^{b}	1.65 ± 0.000^{a}
Kaempferol	0.90 ± 0.000^{a}	0.85 ± 0.000^{b}
Quercetin	0.30 ± 0.000^{a}	0.05 ± 0.000^{b}
Rutin	0.85 ± 0.000^{b}	1.00 ± 0.000^{a}
Ferrulic acid	0.43 ± 0.024^{b}	0.98 ± 0.024^{a}
Catechin	2.57 ± 0.085^{b}	4.52 ± 0.062^{a}
Epicatechin	0.28 ± 0.024^{b}	0.35 ± 0.000^{a}
Proanthocyanidin A2	1.48 ± 0.024^{a}	1.27 ± 0.024^{b}
Proanthocyanidin B1	27.43 ± 0.024^{b}	41.65 ± 1.239^{a}
Proanthocyanidin B2	2.73 ± 0.118^{b}	4.40 ± 0.071^{a}
Epigallocatechin gallate	1.00 ± 0.000^{b}	1.83 ± 0.170^{a}
Gallic acid	1.33 ± 0.024^{b}	2.42 ± 0.024^{a}
Siringic acid	10.23 ± 0.062^{a}	7.13 ± 0.047^{b}
Total	55.50 ± 0.082^{b}	73.55 ± 1.219^{a}

Results expressed in mean ± SD of a triplicate. Rows sharing different superscript are significantly different ($P \leq 0.05$).

trans-resveratrol, isorhamnetin, rutin, ferrulic acid, catechin, epicatechin, proanthocyanidin B1, proanthocyanidin B2, epigallocatechin gallate, gallic acid) compared to the ones harvested on March. On the other hand, juices from the first harvest showed higher concentrations of seven phenolic compounds (caffeic acid, chlorogenic acid, p-coumaric acid, kaempferol, quercetin, proanthocyanidin A2, siringic acid).

Differently from cranberry, that is rich in proanthocyanidin A-type [32], Isabella grape juices are rich in proanthocyanidins B1 (epicatechin-(4β→8)-catechin). Proanthocyanidin from grape seed has showed cardioprotective properties by its antioxidant activity, as well anti-apoptotic, anti-necrotic and anti-endonucleolytic potentials [33, 34]. Proanthocyanidins intake in American adults (values adjusted for energy intake) was higher in women than in men, especially, between 31 and 70 years-old and the major food source of total proanthocyanidins was tea, legumes and wine [35].

There are other important polyphenols in juices, as resveratrol and epigallocatechin gallate. These compounds have shown important effects in osteoarthritis, by modulation of inflammation [36]. Daily consumption of a cup (250 mL) of Isabella juice can provide 0.14-0.18 mg of resveratrol and 0.25-0.46 mg of epigallocatechin gallate. Rats fed diet supplemented with resveratrol (consumption about 0.04 μg day^{-1}) and Brazilian Cabernet-Sauvignon wine (resveratrol 0.15 mg) improved vascular function and aerobic capacity with improvements in maximum O_2 consumption, tolerance to exercise and time to exhaustion [37].

3.3 Antioxidant Activity

DPPH assay is based on the reduction of the radical in the presence of a hydrogen donating antioxidant. In the present research, both tropical Isabella grape juices were able to reduce 100% the stable radical DPPH. Thus, scavenging capacity of DPPH radical in Isabella grape juices were superior than cherry juice (24.9% ± 0.7% to 54.2% ± 5.6%) [38]. ferric reduction potential by FRAP assay but not in antioxidant activity by ORAC (Table 2) between the two samples. In this study, regression analysis showed a strong correlation between antioxidant capacity and anthocyanins contents: ORAC (R^2 = 0.9906) showed a high dependence of anthocyanins concentration for the juice from the fruits harvested in September; and for FRAP (R^2 = 0.9826), a high dependence of anthocyanins content in juice from grapes harvested in March.

Table 2 Antioxidant activity of tropical Isabella grape juices from sub-middle Sao Francisco River Valley, Northeast region of Brazil, elaborated in March and September of the year 2010.

	March	September
DPPH (%)	100	100
FRAP (μM TE 100 mL^{-1})	89.2 ± 5.01[b]	118.6 ± 1.04[a]
ORAC-H (μM TE 100 mL^{-1})	201.9 ± 12.58[a]	206.3 ± 15.19[a]

Results are expressed in mean ± SD of a triplicate. Rows sharing different superscript are significantly different ($P \leq 0.05$).

Significant differences ($P \leq 0.05$) were observed in

Although, Isabella grape juices have shown lower levels of polyphenols than Clementine fruit juice (922.3 ± 4.6 to 999. 5 ± 25.3 mg L^{-1}) and sugarcane (402.3 ± 7.9 to 664.5 ± 3.9 mg L^{-1}) [39]. The antioxidant activity by ORAC assay was higher in grape juices of this study compared with the antioxidant activity of these juices (Clementine and sugar cane), especially for Isabella grape juice produced in September (Table 2).

4. Conclusions

In conclusion, the results of this research indicated that Isabella grape juices from the sub-middle Sao Francisco River Valley region, Northeast of Brazil have expressive amount of polyphenols and anthocyanins, important bioactive compounds, which may be considered as a good complementary source of natural antioxidants in the human diet.

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