Functional tea from a Brazilian berry: Overview of the bioactives compounds

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ARTICLE INFO

ABSTRACT

The consumption of berries has become a trend with confirmed health properties; however, fruit peels have been wasted despite their high concentrations of bioactive compounds. Tea made from jaboticaba (*Myrciaria jaboticaba*) peel (JP) could be an alternative to make use of this byproduct and contribute to increased intake of polyphenols. For this reason, this study investigated the JP chemical composition and bioactive profile, as well as the antioxidant capacity of its aqueous extract (JPT − 25 g L^{-1}). The peel corresponded to approximately 35% of fruit weight. Total fiber content was approximately 30 g 100 g^{-1}, and the soluble portion represented 8.50 ± 0.21 g 100 g^{-1}. JPT showed strong antioxidant capacity according to all methods tested (FRAP, DPPH, and ORAC). Phenolic, flavonoid, and anthocyanin content were 54.55 mg GAE, 8.33 mg catechin, and 4.34 mg cyanidin 3-glucoside in 100 mL, respectively. Phenolic content in a cup of JPT (250 mL) was approximately 150 mg GAE, which could be considered an important source of bioactives. Cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, and ellagic acid were identified in JPT by LC-DAD-ESI/MS. Other polyphenols were investigated by HPLC-DAD-FLD in the hydrolyzed extract, and gallic acid and rutin were the prevalent compounds after cyanidin-3-*O*-glucoside. The hydrolysis increased total phenolic, but not affected the antioxidant capacity according to DPPH e FRAP. The JPT color, anthocyanins and antioxidant capacity showed stability in refrigerated conditions, even without additives, for up to three days. These findings suggest that aqueous extract could be an adequate way to utilize the functional properties of jaboticaba peel.

1. Introduction

A number of studies have highlighted the health benefits of red-black fruits, such as berries, which are considered a source of bioactive compounds (especially anthocyanins) with antioxidant and anti-inflammatory potential. The literature has shown the important effects of berries against several conditions, such as obesity, urinary tract infection, cardiovascular diseases, insulin resistance and inflammatory bowel disease (Batista et al., 2014; Blumberg et al., 2013; Leite-Legatti et al., 2012; Xiao et al., 2015).

Jaboticaba, as well as the worldwide well-known acai (*Euterpe oleracea*), can be called Brazilian berry. The jaboticaba plant belongs to the *Myrtaceae* family and is largely distributed in the South and Southeast regions of Brazil. The main species is *Myrciaria jaboticaba*, popularly known as jaboticaba "sabará". The jaboticaba fruit has a dark purple peel and a pleasant flavor. The fruit consumption is mainly in its fresh form and its use is still mostly domestic (Sato & Cunhad, 2007), which shows potential for bigger investments in production and marketing of jaboticaba crops and products (Balerdi, Rafie, & Crane, 2006).

In general, jaboticaba fruit peels are not widely consumed, but

recently some new food formulations have sought to include these fruit byproducts in order to aggregate nutritional and commercial value (Dessimoni-Pinto, Moreira, Cardoso, & Pantoja, 2011). Jaboticaba peels can also be dried in order to extend their shelf-life, and provide an alternative to improve the consumption of this source of bioactive compounds (Leite-Legatti et al., 2012; Wu, Long, & Kennelly, 2013).

Few investigations report the polyphenol content of *M. jaboticaba*; however, some bioactive compounds have been described: anthocyanins, mainly cyanidin 3-glucoside and delphinidin 3-glucoside; ellagitannins; ellagic acid; gallic acid; quercetin; vitamin C; limonene; terpenes; dietary fibers; and others (Batista et al., 2014; Plaza et al., 2016; Reynertson et al., 2006). These compounds could be directly related to antioxidant, antiproliferative, antimutagenic, and anti-inflammatory *in vitro* and *in vivo* activity exhibited by *M. jaboticaba* peel, and reported by recent studies (Batista et al., 2014; Dragano et al., 2012; Lenquiste, Batista, Marineli, Dragano, & Maróstica Júnior, 2012; Plaza et al., 2016).

Extracts of vegetable parts have been reported in the literature as excellent sources of bioactive compounds. Aqueous extraction is a well-known method for beverage preparation; furthermore, infusions are among the most frequently consumed beverages worldwide (Piljac-Žegarac, Valek, Stipčević, & Martinez, 2010). Their consumption is increasing, including in Western countries. For example, tea sales reached $6.8 billion in 2005, increasing by 10% in one year in the United States (de Mejía, Song, Heck, & Ramírez-Mares, 2010).

Studies have confirmed the medicinal properties of age-old aqueous infusions such as green tea, black tea, and yerba mate, among others (Cabrera, Artacho, & Gimenez, 2006; de Mejía et al., 2010). The health-promoting properties of these beverages are mainly due to the presence of bioactive compounds with strong antioxidant properties (Socha et al., 2013). Tea made from jaboticaba peels could be an efficient way to use this portion of the fruit and take advantage of its functional properties. In this sense, this study reports the chemical and functional characterization of jaboticaba peel as well as its aqueous extract (tea).

2. Material and methods

2.1. Jaboticaba peel powder (JP)

Jaboticaba (*Myrciaria jaboticaba* Berg) fruits were kindly donated by the company Indústria e Comércio Lagoa Branca Ltda, located at Boa Vista II farm in Casa Branca city (Sao Paulo, Brazil). Once sanitized, the fruits were peeled and the peels were separated and oven-dried with air circulation at 40 °C (Marconi, Piracicaba, SP, Brazil) for 72 h. Dried peels were ground into a fine powder (Marconi, model MA 630/1, Piracicaba, SP, Brazil), sifted (20 mesh), and stored in an amber flask at −20 °C until further analyses.

2.1.1. JP − chemical characterization

The proximate composition of JP peel was determined by analyses of protein (Dumas nitrogen analyzer − Velp Scientifica, Usmate, MB, Italy), lipids (Bligh & Dyer, 1959), ash, and moisture (AOAC, 2002). Carbohydrates were calculated by difference. Total dietary fiber and insoluble fractions were determined in the peel powder using the enzymatic-gravimetric method (AOAC, 2002), and soluble fraction was calculated by difference. Pectin (total and soluble) was determined according to Bitter and Muir (1962), and insoluble pectin was calculated by difference. The percentage of the peel weight in the whole fruit was calculated by weighing 10 whole fruits with the peel.

In order to determine reducing sugars, a 70% ethanol solution was added to the samples and allowed to reaction during 60 min at 100 °C. After evaporation of the ethanol, the extract was filled with water and then analyzed according to the Somogyi-Nelson method, using a Synergy HT, Biotek microplate reader (Winooski, USA), with readings set at 520 nm (Nelson, 1944).

2.2. Jaboticaba peel tea (JPT)

In order to obtain the JPT, 25 g of JP were immersed in 1000 mL of boiling water (90−100 °C), manually shaken at 0, 15, and 35 min at room temperature (22−23 °C), after the extract was filtered through a paper filter (18 mm) coupled to a vacuum system. The extraction was performed in triplicate for each analysis.

2.2.1. Chemical characterization

A BioTek Synergy HT Microplate Reader (Winooski, USA) coupled to the data software program Gen5™ 2.0 was used for colorimetric and fluorometric analyses in 96-well microplates (transparent or dark for ORAC).

2.2.1.1. Bioactive compounds. **Total phenolic compounds:** The reaction of the water-diluted JPT with Folin-Ciocalteu's reagent and the saturated solution of sodium carbonate (allowed 2 h to react in the dark at room temperature − 22−23 °C) were used to determine the total phenolic content (Swain & Hillis, 1959), with some adaptations to microplate assay. The absorbance was read at 725 nm. Results were expressed in terms of the used standard− gallic acid equivalents (mg GAE mL^{-1}).

Total yellow flavonoids: As described by Zhishen, Mengcheng, and Jianming (1999), sodium nitrite, aluminum chloride, and sodium hydroxide were added to diluted JPT. Catechin was used to build a standard curve and the results were expressed as catechin equivalents (mg CE mL^{-1}).

Monomeric anthocyanins method: JPT was diluted in potassium chloride buffer (pH = 1.0) in order to reach absorbance of 0.4−0.6, and the same dilution factor was used in acetate buffer (pH = 4.5). The absorbance readings were done at 520 and 700 nm (Wrolstad, 1976).

The absorbance (A) was then calculated using Equation (1):

$$A = [(A_{520\ nm} - A_{700\ nm})\ pH = 1.0] - [(A_{520\ nm} - A_{700\ nm})\ pH = 4.5] \quad (1)$$

The anthocyanin concentration (C) was calculated as cyanidin-3-O-glucoside (PM = 449.2) using Equation (2), and expressed as mg cyaniding-3-O-glucoside equivalents mL^{-1}:

$$C = A.\ MW.\ DF\ /\xi.\ L \quad (2)$$

Where ξ = molar absorptivity (26,900 mol L^{-1}), L = pathlength (cm), MW = molecular weight, and DF = dilution factor.

Condensed tannins: JPT were mixed with working solution − 1% vanillin (w/v) in methanol and 8% HCl in methanol (1:1). After 20 min at 30 °C, the absorbance was read at 500 nm. Catequin was used as standard and results were expressed as μg catequin equivalent (CE) mL^{-1} (de Camargo, de Souza Vieira, Regitano-D'Arce, Calori-Domingues, & Canniatti-Brazaca, 2012; Price, Hagerman, & Butler, 1980).

2.2.1.2. JPT − antioxidant capacity. **Ferric reducing antioxidant power (FRAP):** FRAP reagent (acetate buffer, TPTZ in HCl, FeCl$_3$) was mixed with water-diluted JPT or standard solutions (Trolox). After 30 min at 37 °C, the samples were cooled to room temperature and the absorbance was read at 595 nm. The results were expressed as μmol Trolox equivalent (TE) mL^{-1} (Benzie & Strain, 1996).

DPPH assay: A DPPH-methanol solution was added to diluted

JPT and kinetic readings were made at 515 nm until the stabilization of absorbance (A), which occurred at 2 h. Five concentrations of JPT were used to calculate IC_{50}, using water as a reaction control (Rufino et al., 2010). Calculations were made as follows (Equation (3)):

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (3)$$

The samples concentration required to reduce 50% of the DPPH radical (IC_{50}) was calculated by linear regression, and the result showed in g L^{-1}. As well as in the other antioxidant measurements, DPPH scavenging capacity was calculated using trolox as standard and the results expressed as μmol TE mL^{-1}.

Oxygen radical absorbance capacity (ORAC): Diluted extract (JPT) or standard solution, fluorescein solution, and AAPH were prepared in phosphate buffer (pH 7.4) and added to 96-wells black microplates in the dark. The readings were made in the following fluorescent filters: excitation wavelength at 485 nm and emission wavelength at 520 nm. Trolox was used as standard and results were expressed as μmol TE mL^{-1}. The linearity between the net area under the curve and the concentration was checked (Davalos, Gomez-Cordoves, & Bartolome, 2004).

2.2.2. Chromatographic characterization of phenolic compounds

For the chromatography analysis of anthocyanins and ellagic acid, the aqueous extract was prepared as described in the Section 2.2. The samples were filtered through a 0.45 μm syringe filter. To identify specific anthocyanin components in the extract, the general procedure for screening of phenolic compounds in plant materials was employed (Lin & Harnly, 2007) with some modifications. The LC-DAD-ESI/MS instrument consisted of a Varian 250 (Varian, CA, USA) coupled with a diode array detector (DAD) and a 500-MS IT mass spectrometer (Varian, CA). A Symetry C18 (Varian Inc., Lake Forest, CA) column (3 μm, 250 × 2 mm) was used at a flow rate of 0.4 mL min^{-1}. The oven temperature of the column was set at 30 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was varied linearly from 10% to 26% B (v/v) at 40 min, to 65% B at 70 min, and finally to 100% B at 71 min, and then held at 100% B for 75 min. The DAD was set at 270 and 512 nm for real-time read-out and UV/VIS spectra were recorded in the range of 190—650 nm. Mass spectra was simultaneously acquired using electrospray ionization in the positive and negative ionization modes (PI and NI) at a fragmentation voltage of 80 V for the mass range of 100—1000 amu. We used a drying gas pressure of 35 psi, a nebulizer gas pressure of 40 psi, a drying gas temperature of 370 °C, capillary voltages of 3500 V for PI and 3500 V for NI, and spray shield voltages of 600 V. The LC system was coupled to the MSD with a splitting of 50%.

In addition, other polyphenols in the JPT were performed by HPLC-DAD-FLD as described previously, including equipment and running conditions (Natividade, Corrêa, Souza, Pereira, & Lima, 2013). A solution of 1.2 M HCl and 0.26 g L^{-1} BHT in 50% methanol was added to the extract (Section 2.2). Later, the extract was kept at 90 °C for 30 min in water bath coupled with refrigerated reflux condenser (Batista et al., 2014). The DAD was set at the following wavelengths: 280 nm for identification of gallic acid and (−)-epicatechin gallate; 320 nm for phenolics acids; 360 nm for flavonols and 520 nm for anthocyanins. Fluorescence detector was used at 320 nm emission for identification of (+)-catechin, procyanidin A2 and (−)-epicatechin.

In order to compare the hydrolyzed to non-hydrolyzed JPT, it was determined total phenolics by colorimetric assay, FRAP and DPPH assays according aforementioned to JPT (Sections 2.2.1.1 and 2.2.1.2).

2.2.3. JTP − color measurements: stability evaluation

Measures of color were taken in JPT at room temperature using a spectrophotometer (Color Quest II − Hunter Lab, Reston, USA). CIELAB* system (D65, 10° observer, transmittance) was used and parameters a*, b*, and L* were registered. The extract was divided into two amber bottles, one was stored at refrigerated conditions (at 4—10 °C) and the other was kept at room temperature (22—23 °C). Color measurements of each bottle were taken and compared to D0, considered standard, by three days (72 h). Measurements were taken after the bottles reached room temperature. Color difference was calculated (ΔE) according to Equation (4):

$$\Delta E = \sqrt{(L^*_{dayX} - L^*_{day0})^2 + (a^*_{dayX} - a^*_{day0})^2 + (b^*_{dayX} - b^*_{day0})^2}(4)$$

Where L*, a*, and b* are color parameters; day 0 = standard; day X = 24 h, 48 or 72 h after storage.

In addition, it was carried out the following assays: total phenolic, monomeric anthocyanins and FRAP, as described in the Sections 2.2.1.1 and 2.2.1.2, in order to evaluate also the stability of bioactive compounds.

2.3. Statistical analyses

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

3. Results and discussion

Jaboticaba fruit has a thicker peel than other fruits like grapes and blueberries. We have found that this portion corresponds to approximately 35% of the fruit weight (4.97 ± 1.47 g, 1.77 ± 0.19 g, whole fruit and peel, respectively). The proximate composition of jaboticaba peel powder (JP) is shown in Table 1. The JP showed great level of total dietary fiber (33.86 g 100 g^{-1} ± 1.38), in agreement to a recent work that found a similar concentration of total fiber in JP (38.4 g ± 1.2) (Inada et al., 2015). Fiber has been associated with beneficial health properties and protection against certain diseases, and can also help intestinal transit, modulating the gut microbiota and control of serum lipids and glucose (Yapo & Koffi, 2008). JP showed a high level of soluble fibers, with pectin representing a large fraction of dietary fiber in JP, since we have found 9.00 ± 0.16 and 3.03 ± 0.05 g 100 g^{-1} JP of total and soluble pectin, respectively. Gurak, De Bona, Tessaro, and Marczak (2014) have found a similar soluble fiber level in jaboticaba peel (about 10%). The amounts of soluble fiber is the most important fraction in this case, since it could also be present in JPT, and the fraction is also strongly correlated with gut fermentation and its potential health effects

Table 1
Proximate Composition of jaboticaba peel powder.

	g 100 g^{-1}
Carbohydrate	72.06 ± 0.25
Reducing sugar	13.75 ± 0.47
Total dietary fiber	33.86 ± 1.38
Insoluble fiber	25.38 ± 0.24
Soluble fiber	8.50 ± 0.21
Lipids	2.94 ± 0.02
Proteins	6.85 ± 0.05
Moisture	14.63 ± 0.42
Ashes	2.98 ± 0.03

Soluble dietary fiber and carbohydrates were calculated by difference. Results are expressed as mean ± SD (n = 3).

(Eswaran, Muir, & Chey, 2013).

A recent study has shown that food byproducts correspond to more than one third of the waste generated by the food industry in developed countries (Mirabella, Castellani, & Sala, 2014). Seeds and peels represent the largest portion of byproducts and could be put to better use, especially as they are rich in fiber and phenolic compounds (Gorinstein et al., 2011).

Chaovanalikit and Wrolstad (2004) found the highest amount of total phenolic and anthocyanins concentrated in peels of four cultivars of cherries. Previous studies have also demonstrated larger amounts of phenolics in jaboticaba peel (Leite-Legatti et al., 2012), and also that JP added to a diet positively affected disorders *in vivo*, such as obesity, dyslipidemia, and insulin resistance (Batista et al., 2014; Lenquiste et al., 2012; Plaza et al., 2016). Since the tea is the second most-consumed beverage around the world (El-Beshbishy, 2005), the use of JP aqueous extract could be an alternative to spread the use and consumption of phenolic compounds from jaboticaba peel. The bioactive compounds profile of JPT is shown in Table 2.

Distinct extraction methods have been used to evaluate the bioactive compounds profile and antioxidant potential of JP. The phenolic level found in JP (Table 2) was up to 76 times greater than the level observed in acai (*Euterpe oleracea*) (0.31 mg GAE g^{-1}), and higher than in blackberry extracts and other exotic tropical red-black berries (Dai, Gupte, Gates, & Mumper, 2009; Kang et al., 2012). This is an indicator of the high bioactive concentration in JP aqueous extract. Although this study found lower levels of phenolic compounds in JP extract than reported by other authors (Abe, Lajolo, & Genovese, 2012; Leite-Legatti et al., 2012), still JPT proved to be an excellent source of bioactive compounds.

There is a number of variables that can influence polyphenol identification and quantification. The amount of polyphenols in fruits and vegetables can be modified by environmental conditions, post-harvest treatments, and processing (Chen, Xin, Yuan, Su, & Liu, 2014). Thus, it is possible to identify differences in polyphenol content in the same fruit grown in different seasons (da Silva et al., 2015). In addition, analytical choices can influence the polyphenol extraction because of their chemical characteristics. Organic solvents have been widely used to extract polyphenols due to their efficiency and wide applicability (Dai & Mumper, 2010). Matrix properties of the plant part, temperature, pressure, time and solvent are also factors that can affect the extraction processes (Azmir et al., 2013). In this way, this large range of solvents and methods used to extract phenolic compounds makes difficult the comparison of studies. Moreover, the focus of our study was to obtain a viable alternative for the JP consumption and not reaching the best method or solvent to polyphenol extraction.

A usual portion of JPT (cup 250 mL) provides polyphenols at the level of 148 mg GAE (Table 2), which is half the amount found in black tea and mate tea (Rechner et al., 2002; de Mejía et al., 2010)

and about one quarter of total polyphenols in green tea, whether decaffeinated or not (Yang & Liu, 2013). The antioxidant capacity of JPT (Table 2) was similar to that found in black tea according to FRAP assay (Luximon-Ramma et al., 2005; Rechner et al., 2002). This is a good result, since among teas, black tea is considered a major source of polyphenols. Furthermore, studies found higher antioxidant capacity (FRAP) and total phenolic levels in aqueous extract than in other organic extract of black teas (Luximon-Ramma et al., 2005, 2006). Dudonné, Vitrac, Coutière, Woillez, and Mérillon (2009) determined the radical scavenging capacity of 30 aqueous plant extracts and some of their results by ORAC assay were close to those shown in Table 2.

The DPPH IC_{50} value found in this study (Table 2) was lower than several other aqueous extracts of berries (5—30 g L^{-1}) (Gorinstein et al., 2013) and several medicinal plant extracts (Krishnaiah, Sarbatly, & Nithyanandam, 2011). A lower IC_{50} value is directly linked to high anti-radical capacity, observed in JPT. In addition, the antioxidant capacity of JPT measured by DPPH assay (Table 2) was similar to that reported for different commercial brands of white, green, and black teas. Whereas the *Camellia sinensis* infusions (white, green, and black tea) are the most studied and marketed until today, with health benefits that have been scientifically attributed to anti-radical action (Socha et al., 2013).

Berries are known to be rich in anthocyanins, compounds responsible for the red-purple colors observed in foods that belong to the flavonoids family. The glycosylated form of these flavonoids is more prevalent in nature, and glycosylation can influence certain types of biological benefits, such as antioxidant and anti-inflammatory action (Chen et al., 2014; Prior & Wu, 2006; Xiao, 2015). Lima, Correa, Saczk, Martins, and Castilho (2011) have shown that jaboticaba peel concentrates the largest amount of anthocyanins in the fruit and our results showed that even infusion was able to extract anthocyanins from jaboticaba peel (1.73 ± 0.03 mg of cyanidin-3-*O*-glucoside g^{-1} dry weight of JPT) by colorimetric method (Table 2). In addition, yellow flavonoid levels were in accordance with the results shown by another study with this fruit (Rufino et al., 2010) and higher than other fruit byproducts (da Silva et al., 2015) (Table 2). The higher flavonoid content could contribute to the antioxidant activity of JP. Other bioactive compounds with antioxidant properties are tannins, which are present in berries and nuts (Alezandro, Dubé, Desjardins, Lajolo, & Genovese, 2013). Condensed tannins in JPT was similar to result in peanut skin (de Camargo et al., 2012).
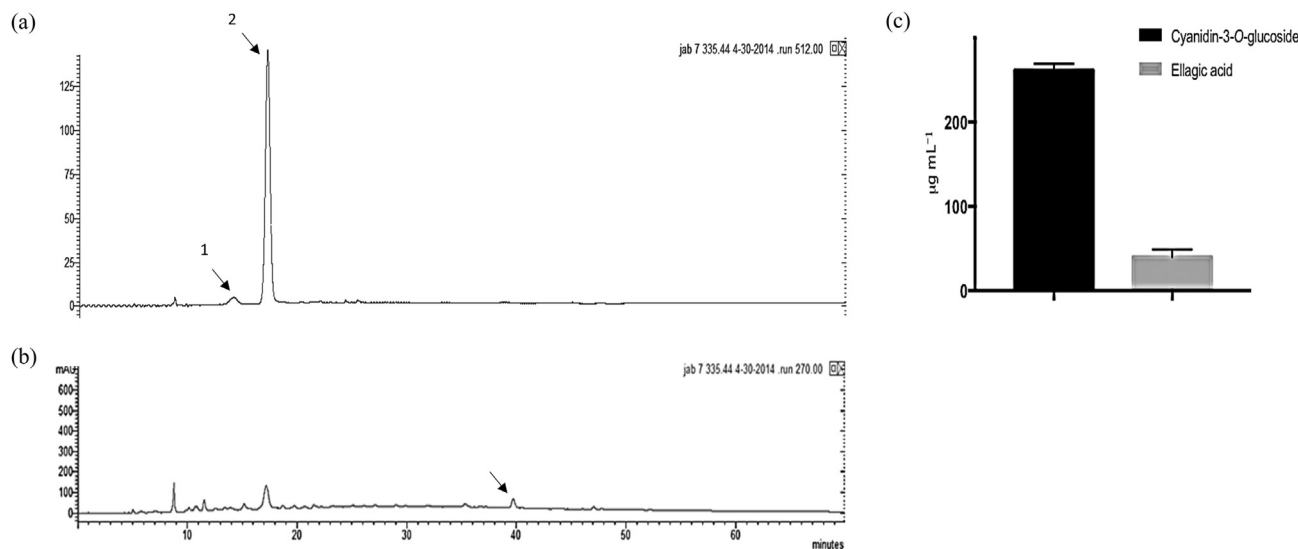
Delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside were identified in JPT and the results were confirmed by mass spectrometry (LC-DAD-ESI/MS) (Fig. 1). Similar findings have been reported in the literature (Inada et al., 2015); however, other authors have found higher amounts of these anthocyanins in JP (Leite et al., 2011), probably due to distinct methods of extraction, plant species the conditions of growing and harvest of the crop, since that factors

Table 2
Bioactive compounds and antioxidant capacity of JPT.

Parameters	mL^{-1} JPT	g^{-1} JP
Total phenolics	0.593 ± 0.041 mg gallic acid equivalents	23.73 ± 1.65 mg gallic acid equivalents
Total yellow flavonoids	0.083 ± 0.004 mg catechin equivalents	3.33 ± 0.170 mg catechin equivalents
Total monomeric anthocyanins	0.043 ± 0.001 mg cyanidin-3-*O*-glucoside equivalents	1.73 ± 0.030 mg cyanidin-3-*O*-glucoside equivalents
Condensed tannins	4.75 ± 0.20 mg catechin equivalents	189.88 ± 8.06 mg catechin equivalents
FRAP	8.8 ± 0.01 μmol TE	353.6 ± 14.8 μmol TE
ORAC	3.8 ± 0.37 μmol TE	153.8 ± 14.9 μmol TE
DPPH	5.15 ± 0.30 μmol TE	206.3 ± 12.1 μmol TE
IC50	3.54 g L^{-1}	

TE = Trolox equivalent. ORAC linearity test: concentration range = 0.25—3.0 g JP L^{-1}, slope = 9.2676, intercept = 11.021, R2 = 0.9951. Results are expressed as mean ± SD (n = 3).

Fig. 1. Chromatogram of anthocyanins at 512 nm (a): 1) delphinidin-3- O-glucoside (t_R = 15.7 min, $[M+H]^+$ = 465 m/z, MS/MS = 303 m/z, λ max = 253–520 nm); 2) cyanidin-3-O-glucoside (t_R = 17.2 min, $[M+H]^+$ = 449 m/z, MS/MS = 287 m/z, λ max = 280–514 nm); and ellagic acid at 270 nm (b) (t_R = 39 min, $[M+H]^+$ = 301 m/z, MS/MS = 257/229 m/z, λ max = 253–367 nm). Anthocyanins: 261.5 ± 7.6 μg cyanidin-3-O-glucoside mL^{-1} or 10,459.1 ± 303.96 μg cyanidin-3-O-glucoside g^{-1} and ellagic acid: 39.9 ± 8.9 μg ellagic acid mL^{-1} or 1595.9 ± 355.9 μg ellagic acid g^{-1} (c).

could influence the polyphenolic profile and consequently the antioxidant capacity found in berries.

In agreement with previous results, delphinidin-3-O-glucoside and cyanidin-3-O-glucoside are the major anthocyanins quantified in jaboticaba fruit (Inada et al., 2015; Leite et al., 2011). Cyanidin-3-O-galactoside was not found in jaboticaba peel, also confirmed by a previous study (Leite et al., 2011).

In an earlier study, Abe et al. (2012) found ellagic acid in 20 botanical families, and jaboticaba fruit was considered a promising source of ellagic acid derivatives in the diet. We also have investigated ellagic acid concentrations in JPT (Fig. 1). However, this finding could be underestimated, since ellagic acid is mainly found as polymeric ellagitannins in nature, requiring basic or acid hydrolysis for their quantification (Abe et al., 2012).

The HPLC-DAD-FLD analysis was carried out in the hydrolyzed JPT (Table 3). Cyanidin-3-O-glucoside and delphinidin-3-O-glucoside were shown as major phenolic compounds, despite the fact

that they were less concentrated in the hydrolyzed JPT extract. The release of aglycone after acid hydrolysis can explain this finding, even that anthocyanidins quantification was not performed. Significative level of gallic acid was also determined, since this compound can be, as well as ellagic acid, part of tannins structure.

Most of polyphenols in plants occurs in the conjugate form and acid hydrolysis could release their monomers, but some phenolic compounds can be degraded by factors such as high temperature (Goldman, Kader, & Heintz, 1999). Our findings suggest that the temperature used for the hydrolysis did not cause damage to phenolic compounds, since there was not any loss. The total phenolics were higher in hydrolyzed JPT (49.5 ± 1.25 mg GAE g^{-1}) than in the crude extract (23.73 ± 1.65 mg GAE g^{-1}). In the other hand, DPPH-IC50 did not show difference between the extracts (3.14 and 3.54 g L^{-1}, respectively); and the DPPH-trolox equivalent was lower in hydrolyzed JPT (223.3 ± 16.6 and 353.6 ± 14.85 μmol g^{-1}, hydrolyzed and crude, respectively). Therefore, hydrolysis can release

Table 3
Polyphenols in the aqueous extract of jaboticaba peel by HPLC-DAD/FLD.

Polyphenols		RT (min)[a]	Concentration (μg mL^{-1})[a]
Phenolic acid			
1	p-Coumaric acid	37.29 ± 0.043	0.15 ± 0.01
2	Benzoic acid	26.51 ± 0.019	0.15 ± 0.03
3	Gallic acid	11.40 ± 0.008	4.34 ± 0.11
Flavanols and procyanidins			
4	(−)-Epicatechin gallate	42.94 ± 0.005	0.08 ± 0.00
5	(+)-Catechin	24.20 ± 0.009	0.13 ± 0.01
6	(−)-Epicatechin	29.41 ± 0.035	0.06 ± 0.00
7	Procyanidin A2	43.80 ± 0.012	0.12 ± 0.05
Flavonols			
8	Isorhamnetin-3-O-glucoside	46.57 ± 0.004	0.10 ± 0.00
9	Kaempferol-3-O-glucoside	46.05 ± 0.005	0.62 ± 0.04
10	Quercetin	51.05 ± 0.015	0.09 ± 0.02
11	Myricetin	48.01 ± 0.004	0.06 ± 0.00
12	Rutin	43.19 ± 0.004	3.36 ± 0.10
Anthocyanins			
13	Delphinidin-3-O-glucoside	25.11 ± 0.015	0.465 ± 0.02
14	Cyanidin-3-O-glucoside	28.22 ± 0.023	8.96 ± 0.60
Total			**18.83**

[a] Data expressed in mean ± standard deviation.
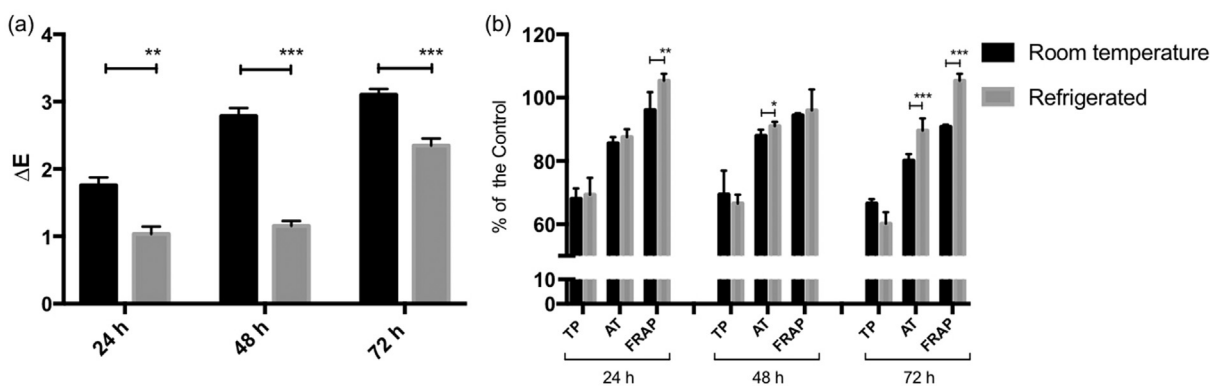
Fig. 2. JPT color difference (ΔE) (a) and percentage of the control (analyses in day0) of TP = total phenolics, AT = monomeric anthocyanins and FRAP (b); after storage of 24 h, 48 h, and 72 h in room temperature (25 °C) or refrigerated (4–10 °C). Results are expressed as mean ± SD (n = 3). $*P < 0.05$, $**P < 0.005$ and $***P < 0.001$ according Student's t-test.

the monomerics/aglycones compounds, becoming them more accessible to total phenolics assay. However, hydrolysis did not affect the antioxidant capacity of extract, indicating that the complex form of polyphenols has an antioxidant action as well as monomerics in JPT. Batista et al. (2014) has showed similar findings, since there was no difference in antioxidant capacity between hydrolyzed and non-hydrolyzed extracts of jaboticaba peel. The BHT used in the acid hydrolysis could also reduce Folin-Ciocalteau reagent and overestimate the total phenolic result, although it demonstrated minimum effects (Batista et al., 2016).

The color measurement can be linked to certain levels of bioactive compounds, in which degradation or transformation of such pigments can indicate phenolic compound alterations (Zhang et al., 2008). In order to verify the stability of the color and bioactive compounds in the aqueous extract (JPT) and to provide a direction for future studies, we have evaluated the color of the extract within 72 h under two different conditions of storage: RT (room temperature 22–23 °C) and RF (4–10 °C).

There were lower color changes under refrigerated conditions over the storage period of 24–72 h compared to the sample storage at room temperature (Fig. 2a). Temperature could be a factor in polyphenol degradation, since the ΔE indicative of color difference compared to control (Day 0) was 1.7, 2.4, and 1.3 times higher in RT than in RF after 24 h, 48 h, and 72 h storage, respectively. The lowest difference was observed after 72 h after storage, but after 24 h the difference was already 70% higher in RT. FRAP assays has also showed greater reduction in antioxidant capacity in RT after 24 h and 72 h (Fig. 2a). Total phenolics quantification was not affected by storage conditions (Fig. 2a). However, monomeric anthocyanins level (major pigments in JPT) was lower in RT than in RF after 48 and 72 h of storage (Fig. 2a). Therefore, we recommend that fresh extract should be used quickly within 24 h or kept refrigerated for up to 72 h.

4. Conclusion

Jaboticaba peel is already recognized as a source of bioactive compounds, in particular anthocyanins. Our findings indicate innovatively that functional substances can be extracted by aqueous infusion (tea), with high anti-radical capacity. Storage of the JPT at refrigerated conditions (4–10 °C) for up to 72 h did not promote major alterations. Therefore, the consumption of jaboticaba peel tea beverage could be considered an effective and simple way to take advantage of the antioxidants found in the byproduct of this fruit.

Acknowledgements

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