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

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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

*Abbreviations:* ORAC, oxygen radical absorbance capacity; TE, Trolox equivalent; FRAP, ferric reducing ability; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; SD, standard deviation; JP, jaboticaba peel; JPT, jaboticaba peel tea; GAE, gallic acid equivalent; BHT, 2,6-di-*tert*-butyl-4-methylphenol; FLD- fluorescence detector, DAD -diode array detector; ESI, electrospray ionization interface; MS, mass spectrometry; HPLC, high performance liquid chromatography; LC, liquid chromatography.

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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