




# Development of isotonic beverage with functional attributes based on extract of *Myrciaria jaboticaba* (Vell) Berg

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## Abstract

Isotonic repositories are specially designed to promote rehydration during or after physical exercise. These beverages are processed for commercialization from synthetic flavorings and dyes with sensorial characteristics similar to those of fruits, in order to attenuate their natural acidity. The aim of the present study was to develop two formulations of isotonic drinks without synthetic dyes and with functional attributes based on concentrated hydroethanolic extracts of peel and pulp of *Myrciaria jaboticaba*. Determination of chemical (sodium, potassium and calcium, total phenolics, total anthocyanins, total flavonoids, total condensed tannins), physical (objective color analysis), and physical-chemical characteristics (pH, acidity, total soluble solids), as well as sensory evaluation through preference testing was carried out. In addition, the drinks presented dark red coloration, according to the chromaticity diagram. The sensory evaluation results revealed that the beverage formulated with 12% pulp extract stood out as the preferred beverage among the judges.

**Keywords:** bioactive phytochemicals; jaboticaba; sensory analysis color.

**Practical Application:** Processed beverage based on pulp and jaboticaba peel.

## 1 Introduction

During physical activity it is essential to maintain water and electrolytes for body homeostasis. Therefore, adequate fluid intake during exercise is recommended, which is essential for athletic performance and term regulation (Lewis et al., 2013).

Beverages are considered isotonic when they have an osmotic concentration similar to that found in body fluids. This feature allows quick absorption of the drink after ingestion, improving the performance of athletes and preventing muscle fatigue. According to RDC 18 of April 27, 2010 (Brasil, 2010), which regulates food intended for athletes, an isotonic drink should have a sodium concentration ranging from 460 to 1150 mg L<sup>-1</sup> and up to 8% carbohydrates.

During the processing of isotonic beverages, flavorings and synthetic dyes with sensory characteristics similar to fruit are used. However, experimental studies have found concerns among consumers about the chemical composition of these beverages. Accordingly, researchers including Kobylewski & Jacobson (2012), El-Wahab & Moram (2013), Carochi et al. (2014) are exploring the toxicity of synthetic dyes as well as their effects on health.

The food industries have been using natural colors instead of synthetic ones, especially anthocyanins, which represent one of the most colorful classes of substances in the vegetable kingdom (Wallace & Giusti, 2011).

Jaboticaba stands out as one of the richest Brazilian sources of anthocyanins, phytochemicals with antioxidant, antiviral, antimicrobial, anti-inflammatory and antitumor properties (Wu et al., 2013a). It is evident that during the processing of jaboticaba, to obtain the pulp, 43% of peel is generated, being discarded. However, this residue is rich in phenolic constituents, among which the anthocyanins, minerals and dietary fibers stand out (Silva et al., 2017; Souza et al., 2017).

Recent study by Barros et al. (2019) analyze the influence of different types of acids and pH on the recovery of bioactive compounds present in jaboticaba peel. They found that the hydroethanolic mixture (ethanol:water 50:50 v.v<sup>-1</sup>) at pH 1.0 acidified with formic acid stood out from the others. Thus, there is a growing application of jaboticaba peel extract in several products, such as Petit Suisse cheese (Pereira et al., 2016a, b) and mortadella (Baldin et al., 2018).

Received 28 May, 2019

Accepted 08 Oct., 2019

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In addition, in response to consumers' call for a healthy life and associated with the consumption of natural products, studies have been conducted exploring the production of fruit-based isotonic (Gironés-Vilaplana et al., 2013; Gironés-Vilaplana et al., 2016).

The present study puts forward an innovative proposal as a strategic alternative to current market options, considering that an isotonic beverage based on concentrated jaboticaba extract is not yet available. It is also worth noting the full use of this fruit is associated with its recognized functional properties. Developing an isotonic beverage with functional appeal from concentrated extracts of pulp and jaboticaba peel, absent from synthetic dyes and flavorings, represents a promising prospect for the beverage industry.

## 2 Materials and methods

### 2.1 Reagents and solutions

The compounds used were ethyl alcohol, 3,5-dinitrosalicylic acid (DNS), bovine serum albumin (BSA), hydrochloric acid (HCl), Folin-Ciocalteu, gallic acid, sodium borohydride/chloranil (BSC), catechin, and butanol were obtained from Sigma-Aldrich-Merck KGaA (Darmstadt, Germany). Potassium sorbate, sodium benzoate, sodium chlorides, citric acid and sucrose were purchased from Pryme Foods (São Paulo-Brazil). Ultra-pure water was obtained from Electro-Eletrônica Gehaka Ltda (São Paulo-Brazil).

### 2.2 Instrumentation

The equipment used was: spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan), spectrophotometer ColorQuest XE (HunterLab - Reston, Virginia, USA), cryoscope (MK 540, ITR, Esteio, Brazil), ICP OES (SPS-5), rotary evaporator (Fisatom-801, Brazil), and centrifuge (Hettich-Universal 320R, Tuttlingen, Germany).

### 2.3 Raw material

The hydroethanolic extracts (ethanol: water 70:30 v.v<sup>-1</sup>) of pulp and peel of jaboticaba fruits (*Myrciaria jaboticaba* (Vell.) Berg.) Were used as raw material for the processing of isotonic beverages. The fruits were purchased in the municipality of Itororó-Bahia from August 2016 to April 2017.

Hydroethanolic extracts (EHE) were obtained according to Fuleki & Francis (1968). The peels or pulps were ground in blender using a hydroethanol solution in the ratio 1: 2 peels or pulps: solvent extractor (m.v<sup>-1</sup>). The mixture was allowed to stand in the absence of light for 24 hours. Subsequently, the homogenate was filtered through fine nylon fabric, followed by centrifugation at 14,030 x g for 10 minutes. Finally, the supernatants were concentrated until complete removal of the solvent in a rotary evaporator at 40 °C.

### 2.4 Preparation of formulations of Isotonic Beverages (BI)

For the preparation of the BI formulations, preliminary tests (data not shown) were carried out, aiming to obtain a product with a total sugar content close to 8% and osmolarity concentration in accordance with the legislation of beverages for

**Table 1.** Preparation of formulations of isotonic beverages.

Ingredients	Formulation A	Formulation B
	(BI_A) %	(BI_B) %
Pulp Extract (v.v <sup>-1</sup> )	10.00	12.00
Peel extract (v.v <sup>-1</sup> )	4.00	4.00
Sucrose (m.v <sup>-1</sup> )	4.00	6.00
Citric acid (m.v <sup>-1</sup> )	0.07	0.07
Potassium sorbate (m.v <sup>-1</sup> )	0.01	0.01
Sodium benzoate (m.v <sup>-1</sup> )	0.05	0.05
Sodium Chloride (m.v <sup>-1</sup> )	0.04	0.04

athletes (Brasil, 2010). Two formulations of BI were performed as shown in Table 1. The isotonic beverages were prepared by dissolving the ingredients in water and subjecting the solution to a slow heat treatment (65 °C for 30 min).

### 2.5 Nutrition composition

For quantification, bovine serum albumin (BSA) analytical curves were constructed at concentrations ranging from 0.2 mg mL<sup>-1</sup> to 1.2 mg mL<sup>-1</sup> and the readings were made at 595 nm (Bradford, 1976). The results were expressed in g of BSA 100 mL<sup>-1</sup> of BI. Lipids were determined according to Association of Official Analytical Chemists (2010), and the results were expressed in g 100 mL<sup>-1</sup> of beverage. The energy value was calculated from the sum of the corresponding calories for proteins, lipids, and carbohydrates which provide 4.0, 9.0, and 4.0 kcal g<sup>-1</sup>, respectively (Brasil, 2003).

#### *Determination of sugars (reducing, non-reducing, and total)*

Reducing sugars (RS) were determined by the method of 3,5-dinitrosalicylic acid (DNS) proposed by Miller (1959). For the determination of total sugars (SCT) the method adapted by Matissek et al. (1998) was used: in this stage, non-reducing sugars (SNR) were hydrolyzed with concentrated HCl under heating. The SNRs were calculated by the difference between the SCT and RS contents. To quantify the sugars, analytical glucose curves were generated with the readings taken at 540 nm. The results were expressed as mg of glucose 100 mL<sup>-1</sup>.

### 2.6 Physical-chemical characterization

Measurements of pH, titratable total acidity (TTA), and soluble solids (TSS) content were determined according to the methodology described by Association of Official Analytical Chemists (2010).

After the previous digestion of samples in concentrated HNO<sub>3</sub>, minerals were analyzed by ICP OES. The control of the operating conditions of ICP OES was carried out with ICP-Expert Vista-Varian software, calibrated under specific conditions of wavelength and slit for each element. Argon was used as the entrainment gas with a flow of 2.0 mL min<sup>-1</sup>. All determinations were performed with three replicates and the results expressed in mg L<sup>-1</sup>.

### Determination of osmolarity

The osmolarity of the isotonic was determined by cryoscopy, according to Gomes & Oliveira (2011), by measuring the freezing point of the samples. Osmolarity was obtained according to Equation 1:

$$\text{Osmolarity} \left( \text{mOsm kg}^{-1} \right) = \frac{T_c}{K_c} * 1000 \quad (1)$$

where:  $K_c = 1.86 \text{ } ^\circ\text{C mol}^{-1} \text{ kg}^{-1}$  (cryoscopic water constant);  $T_c$  = freezing point temperature of the beverage samples in degrees Celsius.

### 2.7 Physical characterization

#### Objective color determination

For objective color determination, the CieLab color system was used, with the color components ( $L^*$ ,  $a^*$  and  $b^*$ ) being used. The values of  $C^*$  (color saturation) and  $h^*$  (hue angle) were calculated from the values of  $a^*$  and  $b^*$  according to Equations 2 and 3:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h^* = \arctan\left(b^* / a^*\right) \quad (3)$$

### 2.8 Chemical characterization

Total phenolic compounds (TPC) were determined according to ISO 14502-1: (International Organization for Standardization, 2005) using Folin-Ciocalteu reagent.

The test is based on the reduction of the phosphomolybdol-phosphotungstic acids by the phenolic hydroxylates, giving rise to blue tungsten and molybdenum oxides, where the coloring allows determination of the concentration of reducing substances. For quantification, an analytical curve of gallic acid was used at concentrations of 0.01, 0.03, 0.05, 0.07, and 0.09 mg mL<sup>-1</sup>. Absorbances were determined at 765 nm. The results were expressed as gallic acid equivalents (EAG) 100 g<sup>-1</sup> extract.

Total flavonoids (TF) were determined according to He et al. (2008) using sodium borohydride/chloroanil reagent (BSC). The method is based on the property of the aluminum cation in forming stable complexes with the flavonoids and avoiding the interference of other phenolic constituents. The absorbances were determined at 490 nm. For quantification, catechin analytical curves were generated at concentrations of 0.03 to 0.5 mg mL<sup>-1</sup>. The results were expressed as mg of catechin 100 mL<sup>-1</sup> sample.

Total anthocyanins (TA) were determined according to the methodology proposed by Lees & Francis (1972). A dilution was performed in order to obtain an absorbance value between 0.200 and 0.800. The anthocyanin content was obtained by Equation 4 and the results were expressed in equivalent of the main anthocyanin, cyanidin-3-glucoside.

$$TA = \left( \frac{Abs_{535} * MM_{Cianidina-3-glucosideo} * FD}{\epsilon} \right) * 100 \quad (4)$$

where: TA = total anthocyanins expressed as mg of the predominant anthocyanin in 100 g of sample;  $Abs_{535nm}$  = absorbance at 535 nm; MM = cyanidin-3-glucoside molar mass (449.2 g.mol<sup>-1</sup>);  $\epsilon$  = is the

molar extinction coefficient of cyanidin-3-glucoside in ethanolic solution acidified to 535nm, whose value is 26900L/cm.mg; FD = dilution factor.

### 2.9 Sensory analysis

Sensory evaluation was performed through the ordination test with 110 volunteer testers representing both sexes and ages between 17 and 60 years. Each taster received three isotonic beverage samples: the two isotonic formulations of jaboticaba and a commercial isotonic drink with grape flavor. Since there is no jotunaba-flavored isotonic beverage on the market, a drink whose coloration was similar to the elaborated beverage was chosen. The test was conducted in the Sensory Analysis laboratory in individual booths. The samples were presented to the tasters in disposable cups coded with three-digit numbers, at a temperature of  $4 \pm 2 \text{ } ^\circ\text{C}$ , and in a randomized manner. The tasters were asked to order the samples for color, aroma, taste, and overall impression preference, assigning the value 1 (one) for the least preferred sample and the value 3 (three) for the most preferred sample for each attribute analyzed. The study was approved by the Human Research Ethics Committee of the State University of Southwest of Bahia (CAAE: 64801417.5.0000.0056).

### 2.10 Statistical design

The experiment was conducted using the completely randomized design with three replicates, and the results were presented as mean  $\pm$  standard deviation. The results were analyzed using analysis of variance where  $\alpha = 0.05$ . The data obtained from the sensorial evaluation were evaluated by Friedman's non-parametric test at the 5% level of significance (Lawless & Heimann, 2010).

## 3 Results and discussion

### 3.1 Nutritional composition of BI

Results obtained from the nutritional composition of the elaborated BI were compared, with the labeling of the nutritional composition of four isotonic beverages commercially obtained (Table 2). The nutritional table of the commercial isotonic beverages is based on a 20 mL portion, however, the values were converted to a 100 mL portion, aiming to observe the equivalence of nutrients present with the beverages elaborated in this study.

As for the protein and lipid contents, it was verified that traditional isotonic beverages do not present these components. However, the amount of these constituents in the elaborated beverages from natural fruits was very low and did not interfere in the osmolality of the elaborated isotonic.

According to Erickson et al. (2017) fruits have considerable amounts of RS however, the values of non-reducing sugars found were higher. This result can be justified by the amount of sugar (sucrose) added to the formulations of BI prepared. Regarding the total carbohydrate concentrations for the compared isotonic beverages, equivalent contents were observed. These are in accordance with the current legislation, which establishes that electrolytic repositories for athletes must contain 4 to 8% total carbohydrates (Brasil, 2010). It should be noted that carbohydrate

**Table 2.** Nutritional composition of formulations A (BI\_A) and B (BI\_B) of elaborated isotonic drinks and commercial beverages.

Determinations	BH_A	BH_B	Gatorade	Bioleve	Powerade	Marathon
Proteins (g 100 mL <sup>-1</sup> )	0.04 <sup>a</sup> ± 0.01	0.05 <sup>a</sup> ± 0.02	0	-	-	0
Lipids (g 100 mL <sup>-1</sup> )	0.07 <sup>a</sup> ± 0.02	0.08 <sup>a</sup> ± 0.01	0	-	-	0
Reducing sugars-RS (g of glucose 100 mL <sup>-1</sup> )	1.62 <sup>a</sup> ± 1.36	1.78 <sup>a</sup> ± 1.34	-	-	-	-
Non-reducing sugars -SRN (g of glucose 100mL <sup>-1</sup> )	3.60 <sup>a</sup> ± 0.45	3.61 <sup>a</sup> ± 0.83	-	-	-	-
Total Carbohydrates -SCT (g of glucose 100 mL <sup>-1</sup> )	5.22 <sup>a</sup> ± 1.47	5.39 <sup>a</sup> ± 1.83	6.0	6.5	3.7	6.02
Sodium (mg 100 mL <sup>-1</sup> )	59.4 <sup>a</sup> ± 5.4	61.4 <sup>a</sup> ± 6.8	45.0	46.0	50.0	29.0
Potassium (mg 100 mL <sup>-1</sup> )	50.0 <sup>a</sup> ± 2.96	60.0 <sup>a</sup> ± 3.6	12.0	12.0	12.5	10.0
Calcium (mg 100 mL <sup>-1</sup> )	50.0 <sup>a</sup> ± 7.03	69.6 <sup>a</sup> ± 4.38	-	-	-	-
Calories (kcal 100 mL <sup>-1</sup> )	18.45 <sup>a</sup> ± 0.58	19.18 <sup>a</sup> ± 0.69	24.0	20.0	15.5	24.1

The averages followed by the same letter in each row do not differ statistically from each other by the F test ( $p \geq 0.05$ ). Mean values ± standard deviation.

concentration should not exceed 8% SCT, considering that carbohydrate-rich beverages can reduce gastric emptying (Pound & Blair, 2017).

As for the sodium content, it was found that they conformed to Brazilian legislation (Brasil, 2010) with an established concentration of sodium between 450 and 1150 mg L<sup>-1</sup>.

Considering body fluids, sodium (Na<sup>+</sup>) is the main extracellular cation, and it performs important functions including maintenance of cellular osmotic balance, regulation of the basic acid balance, and transmission of nerve impulses among others (Jackson et al., 2018). It is also the main electrolyte eliminated in the sweating process during long-term physical exercises.

Among the analyzed minerals, potassium was the most abundant element present in jaboticaba, mainly in the peel. The elaborated beverages presented higher values than those found in commercial hydroelectrolytic repositories, however, it was in compliance with the recommendations of Brazilian legislation. According to ANVISA, isotonic drinks may contain up to 700 mg potassium L<sup>-1</sup> (Brasil, 2010).

The calcium concentration obtained in formulations A and B of the beverage were 500 and 696 mg L<sup>-1</sup>. During physical exercises, calcium plays an essential role because it initiates muscle contractions (França & Martini, 2014). According to Mettler & Mannhart (2017), calcium, potassium, and magnesium are considered to be of little representativeness in said activity in comparison to sodium. Although intense physical activities cause loss of calcium in the sweat, this will not lead to hypocalcemia (low levels of blood calcium).

For the formulations BI\_A and BI\_B, energy values of 18.45 and 19.18 kcal 100 mL<sup>-1</sup>, respectively, were obtained. These values are in agreement with other commercial isotonic drinks (Table 2) used by athletes as electrolyte replacement that present values ranging from 15 to 24 kcal 100 mL<sup>-1</sup>.

### 3.2 Physicochemical characterization of BI

The results of the physical-chemical characterization of the isotonic beverages with 10% pulp (BI\_A) and 12% pulp (BI\_B) are presented in Table 3. The pH values obtained for the beverages were lower than 4.0. These values ensure the safety of the beverage by making it inhospitable to the proliferation of pathogenic bacteria, including *Clostridium botulinum*, since the minimum pH for the multiplication of strains varies between 4.6 and 4.8 (Santos et al., 2013).

Santos et al. (2013) evaluated three formulations of isotonic organic tangerine beverage and obtained pH values ranging from 3.1 to 3.3 and titratable total acidity (TTA) values between 0.13 and 0.21 mg 100 g<sup>-1</sup> citric acid. These studies demonstrated variation in the results obtained for the same type of beverage and corroborate the low pH and high acidity of isotonic drinks observed in this study.

In relation the mean of (TSS) for beverages BI\_A and BI\_B, we observed 7.83 °Brix for both. It should be noted that this determination was important for the formulation of isotonic formulations, since it influences the determination of the osmolarity of the formulated isotonic. Osmolarity for the isotonic should be within the osmotic value range of human blood plasma ranging from 285 to 295 mOsm kg<sup>-1</sup>. According to Brazilian legislation, this value can be up to 330 mOsm kg<sup>-1</sup> for isotonic beverages (Brasil, 2010). Therefore, the values found in this study are in accordance with the legislation's recommendations.

#### Analyses of BI instrumental color parameters

The results of the colorimetric characterization (Table 4) demonstrated that there was no statistical difference ( $p \geq 0.05$ ) between the two processed isotonic for any of the analyzed parameters.



**Table 3.** Physical-chemical determinations of BI\_A (10% pulp extract) and BI\_B (12% pulp extract).

Determinations	BI_A	BI_B	Means
pH	3.48 <sup>a</sup> ± 0.06	3.47 <sup>a</sup> ± 0.06	3.47 ± 0.06
TTA (mg 100g <sup>-1</sup> Citric acid)	0.67 <sup>a</sup> ± 0.36	0.78 <sup>a</sup> ± 0.42	0.72 ± 0.39
TSS (°Brix)	7.56 <sup>a</sup> ± 1.31	8.10 <sup>a</sup> ± 1.41	7.83 ± 1.36
Osmolarity (mOsm kg <sup>-1</sup> )	281.31 <sup>a</sup> ± 0.69	328.41 <sup>a</sup> ± 0.71	304.86 ± 0.70

The means followed by the same letter in each row do not differ statistically from each other by the F test ( $p \geq 0.05$ ). Mean values ± standard deviation.

**Table 4.** Mean values of instrumental color parameters of the isotonic formulations based on concentrated extracts of pulp and jaboticaba peel.

Isotonic	Coordinates				
	L*	a*	b*	C*	h*
BI_A	20.87 <sup>a</sup> ± 0.29	2.55 <sup>a</sup> ± 0.45	-0.21 <sup>a</sup> ± 0.01	2.56 <sup>a</sup> ± 0.45	-0.08 <sup>a</sup> ± 0.02
BI_B	20.43 <sup>a</sup> ± 0.11	2.87 <sup>a</sup> ± 0.39	-0.04 <sup>a</sup> ± 0.04	2.87 <sup>a</sup> ± 0.39	-0.03 <sup>a</sup> ± 0.02
Means	20.65 ± 0.20	2.71 ± 0.42	-0.125 ± 0.02	2.72 <sup>a</sup> ± 0.42	-0.05 <sup>a</sup> ± 0.02

The means followed by the same letter in each column do not differ statistically from each other by the F test ( $p \geq 0.05$ ). Mean values ± standard deviation. Where: L\*: luminosity; a\*: change from green to red; b\*: variation from yellow to blue; C\*: saturation; h\*: pitch angle.

**Table 5.** Chemical characterization of the produced beverages: total phenolic (TPC), total flavonoids (TF), total anthocyanins (TA).

Determinations	Control	BI_A	BI_B	Mean
TPC (mg EAG 100 mL <sup>-1</sup> )	1.44 <sup>b</sup> ± 0.00	44.64 <sup>a</sup> ± 0.15	52.40 <sup>a</sup> ± 0.16	48.52 ± 0.15
TF (mg of catechin 100 mL <sup>-1</sup> )	–	7.34 <sup>a</sup> ± 0.94	10.49 <sup>a</sup> ± 0.53	8.92 ± 0.73
TA (mg of cyanidin-3-glucoside 100 mL <sup>-1</sup> )	–	2.56 <sup>a</sup> ± 0.73	2.67 <sup>a</sup> ± 0.64	2.61 ± 0.68

The means followed by the same letter in each column do not differ statistically from each other by the F test ( $p \geq 0.05$ ). Mean values ± standard deviation.

The average results observed for the parameter (L\*) that represents the luminosity, determines when a producer is presented light or dark, these are compatible with the dark coloration, characteristic of the product. Therefore, there is no significant difference between the processed isotonic. Regarding the parameter a\* corresponds to the color range that varies from (green to red), the observed scores indicate by the chromaticity diagram that the isotonic ones are close to red. Regarding the values observed for the parameter b\* that represents the color range from (blue to yellow), they indicate that the isotonic ones are close to blue color, which is related to the purple coloration of the jaboticaba. Therefore, the analyzed isotonic ones present global, dark red appearance.

In relation to the parameters C\* and h\*, although they use the same diagram as the color spaces L\*, a\*, b\*, they differ because they use cylindrical coordinates. The C\* indicates the “chroma” and the h\* represents the hue angle. The chroma value C\* is 0 in the center and increases by the distance of the center. The values of h\* represent the color tone, the smaller the angle h\* the nearer the coordinate axis a\*. The values of C\* and h\* obtained, represent that the drinks are in the range of color between red and blue (purple).

Based on these parameters, it can be inferred that the beverages are translucent, and they are reddish as the presence of blue (purple) color, within what is expected for products based on jaboticaba fruit, since the fruit shows dark coloration, originating from the presence of anthocyanins.

### 3.3 Chemical characterization of BI

The mean TPC for the isotonic beverages produced in this study was 48.52 mg EAG 100 mL<sup>-1</sup> (Table 5). When compared with the control beverage (without addition of extracts), it was verified that the addition of the extracts added phenolic constituents to the elaborated isotonic drinks. According to Prior (2003), a food must contain at least 20 mg 100 g<sup>-1</sup> to be considered rich in anthocyanins. Elaborated beverages contained about 13.05% anthocyanin constituents, because the amount of extracts used was suitable to obtain a satisfactory coloration.

Values similar to those verified in this study were obtained by Burin et al. (2011) when evaluating the TA of the isotonic beverages formulated with grape powder extract; the obtained contents of 2.5 mg 100 mL<sup>-1</sup>. Mercali et al. (2015), when studying juice of natural jaboticaba obtained an average anthocyanin content of 14.8 mg 100 mL<sup>-1</sup>. While Gironés-Vilaplana et al. (2013), found lower values when studying isotonic beverages based on açai and lemon juice and açai, obtaining values of 0.74 mg.100 mL<sup>-1</sup> and 0.93 mg.100 mL<sup>-1</sup>, respectively.

The average levels of TF were 8.92 mg of catechin 100 mL<sup>-1</sup>, similar to the value verified by Silva et al. (2017) for jaboticaba peel tea (8.3 mg catechin 100 mL<sup>-1</sup>). According to the U.S. Department Agriculture (2014), the fruits of jaboticaba must contain at least 1.10 mg 100 g<sup>-1</sup> of flavonoids. Therefore, the drinks produced here have high relative levels of flavonoids from extracts of jaboticaba. It should be noted that the elaborated isotonic can be considered a functional beverage. It is a source of bioactive phytochemicals important in the inhibition of lipid

**Table 6.** Sum of the isotonic beverages order test.

Attributes	Beverages Isotonic		
	BI_A	BI_B	Commercial
Aroma	186 <sup>a</sup>	219 <sup>ab</sup>	242 <sup>b</sup>
Color	186 <sup>b</sup>	274 <sup>a</sup>	190 <sup>b</sup>
Flavor	219 <sup>b</sup>	271 <sup>a</sup>	158 <sup>c</sup>
Global Impression	196 <sup>b</sup>	274 <sup>a</sup>	178 <sup>b</sup>

Values with equal letters within a row do not differ statistically by the Friedman test ( $p \geq 0.05$ ). Minimum difference = 34.

peroxidation and contributing to homeostasis of physiological functions. These characteristics are innovative aspects that differ from commercial isotonic beverages.

### 3.4 Sensory analysis

The results of the sensory analysis on sample preference are presented in Table 6.

BI\_B, and the commercial beverages, were found to be preferred in the aroma category. Although the commercial product uses artificial flavorings (information described on the label), this was not sufficient to make it preferable to BI\_B. However, it was preferred in relation to BI\_A, likely because of the lower phenolic and flavonoid contents in BI\_A; both compounds are responsible for the aroma of the product. This may be related to the polyphenol content since these phytochemicals are associated with the flavor and color characteristics of the beverages (Soto-Vaca et al., 2012).

Color is an important sensory attribute and should be determinant for acceptance and preference in this type of product. Synthetic dyes are preferred to natural dyes precisely because of their color and stability during processing and storage. However, there are associations between the consumption of these dyes and diseases such as cancer and allergies (Gukowsky et al., 2018). The BI\_B beverage was significantly preferred over the commercial product, providing motivation for further work with natural dyes obtained from jaboticaba.

Although there were no significant differences in the color profile of the formulated beverages, the tasters preferred BI\_B.

With regard to taste, the commercial beverage was significantly less preferred. It is a nationally recognized brand and its product has good market share, so this means that isotonic jaboticaba beverages would be well accepted in the market for sensory aspects.

Regarding global impression, again the BI\_B isotonic beverage was preferred differing from both BI\_A and the commercial beverage. BI\_A did not differ from the commercial isotonic.

Consumer preferences are increasingly turning to products that are free of additives, especially artificial ones. Industries are looking for viable natural alternatives to substitute for artificial additives. The beverages developed, in addition to not containing dyes and artificial flavorings contain bioactive compounds that can improve athlete performance, besides reducing the oxidative stress damages caused by exercise (Takacs et al., 2015).

In spite of the significant tannin content that is responsible for jaboticaba's astringency, and consequently the astringency of the product, this was not a negative factor in the decision of the tasters. They preferred the beverages formulated with jaboticaba extract to the already commercialized beverage.

## 4 Conclusion

It is possible to elaborate an isotonic drink with functional appeal, without artificial colorants and flavorings, based on concentrated pulp extract and jaboticaba peel. The formulated beverages can be considered promising for the beverage market. They are distinguished by substantial contents of bioactive constituents coming especially from the shell of the jaboticaba. Additionally, the elaborated isotonic beverages were sensorially preferred to the successful commercial drink, thus highlighting their marketing potential.

## Acknowledgements

The authors thank the Coordination for the Improvement of Higher Education Personnel – CAPES, for the granting of the scholarship.

## References

- Association of Official Analytical Chemists – AOAC. (2010). *Official methods of analysis: international fruits and fruit products* (Chap. 37). Gaithersburg: AOAC.
- Baldin, J. C., Munekata, P. E. S., Michelin, E. C., Polizer, Y. J., Silva, P. M., Canan, T. M., Pires, M. A., Godoy, S. H. S., Fávoro-Trindade, C. S., Lima, C. G., Fernandes, A. M., & Trindade, M. A. (2018). Effect of microencapsulated Jaboticaba (*Myrciaria cauliflora*) extract on quality and storage stability of mortadella sausage. *Food Research International*, 108(5), 551-557. <http://dx.doi.org/10.1016/j.foodres.2018.03.076>. PMID:29735090.
- Barros, H. D. F. Q., Baseggio, A. M., Angolini, C. F. F., Pastore, G. M., Cazarin, C. B. B., & Marostica-Junior, M. R. (2019). Influence of different types of acids and pH in the recovery of bioactive compounds in Jaboticaba peel (*Plinia cauliflora*). *Food Research International*, 124(2), 16-26. PMID:31466635.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-254. [http://dx.doi.org/10.1016/0003-2697\(76\)90527-3](http://dx.doi.org/10.1016/0003-2697(76)90527-3). PMID:942051.
- Brasil, Agência Nacional de Vigilância Sanitária – ANVISA. (2003, December 23). Resolução RDC nº 360, de 23 de dezembro de 2003. *Diário Oficial [da] República Federativa do Brasil*.
- Brasil, Agência Nacional de Vigilância Sanitária – ANVISA. (2010, April 27). Resolução RDC nº 18, de 27 de abril de 2010. *Diário Oficial [da] República Federativa do Brasil*.
- Burin, V. M., Rossa, P. N., Ferreira-Lima, N. E., Hillmann, M. C. R., & Boirdignon-Luiz, M. T. (2011). Anthocyanins: optimisation of extraction from Cabernet Sauvignon grapes, microcapsulation and stability in soft drink. *International Journal of Food Science & Technology*, 46(1), 186-193. <http://dx.doi.org/10.1111/j.1365-2621.2010.02486.x>.
- Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. F. R. (2014). Adding molecules to food, pros and cons: a review on synthetic and natural food additives. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 377-399. <http://dx.doi.org/10.1111/1541-4337.12065>.
- El-Wahab, H. M. F. A., & Moram, G. S. (2013). Toxic effects of some synthetic food colorants and/or flavor additives on male rats.

- Toxicology and Industrial Health*, 29(2), 224-232. <http://dx.doi.org/10.1177/0748233711433935>. PMID:22317828.
- Erickson, J., Sadeghirad, B., Lytvyn, L., Slavina, J., & Johnston, B. C. (2017). The scientific of guideline recommendations on sugar intake: a systematic review. *Annals of Internal Medicine*, 166(4), 257. <http://dx.doi.org/10.7326/M16-2020>. PMID:27992898.
- França, N. A. G., & Martini, L. A. (2014). Funções plenamente reconhecidas de nutrientes – Cálculo. *ILSI: International Life Sciences Institute Brasil*, 1, 3-24.
- Fuleki, T., & Francis, F. J. (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science*, 33(1), 72-77. <http://dx.doi.org/10.1111/j.1365-2621.1968.tb00887.x>.
- Gironés-Vilaplana, A., Huertas, J. P., Moreno, D. A., Periago, P. M., & García-Viguera, C. (2016). Quality and microbial safety evaluation of new isotonic beverages upon thermal treatments. *Food Chemistry*, 194, 455-462. <http://dx.doi.org/10.1016/j.foodchem.2015.08.011>. PMID:26471579.
- Gironés-Vilaplana, A., Villaño, D., Moreno, D. A., & García-Viguera, C. (2013). New isotonic drinks with antioxidant and biological capacities from berries (maqui, açai, and blackthorn) and lemon juice. *International Journal of Food Sciences and Nutrition*, 64(7), 897-906. <http://dx.doi.org/10.3109/09637486.2013.809406>. PMID:23815554.
- Gomes, J. C., & Oliveira, G. F. (2011). *Análises físico-químicas de alimentos*. Viçosa: UFV.
- Gukowsky, J. C., Xie, T., Gao, S., Qu, Y., & He, L. (2018). Rapid identification of artificial and natural food colorants with surface enhanced Raman spectroscopy. *Food Control*, 92, 267-275. <http://dx.doi.org/10.1016/j.foodcont.2018.04.058>.
- He, X., Liu, D., & Liu, R. H. (2008). Sodium borohydride/chloranil-based assay for quantifying total flavonoids. *Journal of Agricultural and Food Chemistry*, 56(20), 9337-9344. <http://dx.doi.org/10.1021/jf070954+>. PMID:18798633.
- International Organization for Standardization – ISO. (2005). *ISO 14502-1:2005 (E): determination of substances characteristic of green and black tea*. Geneva: ISO.
- Jackson, S. L., Cogswell, M. E., Zhao, L., Terry, A. L., Wang, C. Y., Wright, J., Coleman King, S. M., Bowman, B., Chen, T. C., Merritt, R., & Loria, C. M. (2018). Association between urinary sodium and potassium excretion and blood pressure among adults in the United States. *Circulation*, 137(3), 237-246. <http://dx.doi.org/10.1161/CIRCULATIONAHA.117.029193>. PMID:29021321.
- Kobylewski, S., & Jacobson, M. F. (2012). Toxicology of food dyes. *International Journal of Occupational and Environmental Health*, 18(3), 220-246. <http://dx.doi.org/10.1179/1077352512Z.00000000034>. PMID:23026007.
- Lawless, H. T., & Heimann, H. (2010). *Sensory evaluation of food: principles and practices*. New York: Springer.
- Lees, D. H., & Francis, F. G. (1972). Standardization of pigment analysis in cranberries. *HortScience*, 7(1), 83-84.
- Lewis, E. J. H., Fraser, S. J., Thomas, S. C., & Wells, G. D. (2013). Changes in hydration status of elite Olympic class sailors in different climates and the effects of different fluid replacement beverages. *Journal of the International Society of Sports Nutrition*, 10(1), 11. <http://dx.doi.org/10.1186/1550-2783-10-11>. PMID:23432855.
- Matissek, R., Schnepel, F. M., & Steiner, G. (1998). *Análisis de los alimentos: fundamentos, métodos, aplicaciones*. Zaragoza: Acribia.
- Mercali, G. D., Gurak, P. D., Schmitz, F., & Marczak, L. D. (2015). Evaluation of non-thermal effects of electricity on anthocyanin degradation during ohmic heating of jaboticaba (*Myrciaria cauliflora*) juice. *Food Chemistry*, 171, 200-205. <http://dx.doi.org/10.1016/j.foodchem.2014.09.006>. PMID:25308660.
- Mettler, S., & Mannhart, C. (2017). Hydration, drinking and exercise performance. *Swiss Sports & Exercise Medicine*, 65(1), 16-21. Retrieved from [https://sgsm.ch/fileadmin/user\\_upload/Zeitschrift/65-2017-1/1-2017\\_3\\_Mettler.pdf](https://sgsm.ch/fileadmin/user_upload/Zeitschrift/65-2017-1/1-2017_3_Mettler.pdf)
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426-428. <http://dx.doi.org/10.1021/ac60147a030>.
- Pereira, E. P. R., Cavalcanti, R. N., Esmerino, E. A., Silva, R., Guerreiro, L. R. M., Cunha, R. L., Bolini, H. M. A., Meireles, M. A., Faria, J. A. F., & Cruz, A. G. (2016a). Effect of incorporation of antioxidants on the chemical, rheological, and sensory properties of probiotic petit suisse cheese. *Journal of Dairy Science*, 99(3), 1762. <http://dx.doi.org/10.3168/jds.2015-9701>. PMID:26805976.
- Pereira, E. P. R., Faria, J. A. F., Cavalcanti, R. N., Garcia, R. K. A., Silva, R., Esmerino, E. A., Cappato, L. P., Arellano, D. B., Raices, E. S. L., Silva, M. C., Padilha, M. C., Meireles, M. A., Bolini, H. M. A., & Cruz, A. G. (2016b). Oxidative stress in probiotic Petit Suisse: Is the jaboticaba skin extract a potential option? *Food Research International*, 81(2), 149-156. <http://dx.doi.org/10.1016/j.foodres.2015.12.034>.
- Pound, C. M., & Blair, B. (2017). Energy and sports drinks in children and adolescents. *Paediatrics & Child Health*, 22(7), 406-410. <http://dx.doi.org/10.1093/pch/pxx132>. PMID:29491725.
- Prior, R. L. (2003). Fruits and vegetables in the prevention of cellular oxidative damage. *The American Journal of Clinical Nutrition*, 78(3, Suppl.), 570S-578S. <http://dx.doi.org/10.1093/ajcn/78.3.570S>. PMID:12936951.
- Santos, E. S. M., Alves, R. M., & Lima, C. S. (2013). Elaboração tecnológica e aceitação sensorial de bebida isotônica orgânica de tangerina (*Citrus reticulata* Blanco). *Revista do Instituto Adolfo Lutz*, 72(1), 87-92. <http://dx.doi.org/10.18241/0073-98552013721547>.
- Silva, J. K., Batista, A. G., Cazarin, C. B. B., Dionisio, A. P., Brito, A. S., Marques, A. T. B., & Junior, M. R. M. (2017). Functional tea from a Brazilian berry: overview of the bioactives compounds. *Food Science and Technology (Campinas)*, 76B, 292-298. <http://dx.doi.org/10.1016/j.lwt.2016.06.016>.
- Soto-Vaca, A., Gutierrez, A., Losso, J. N., Xu, Z., & Finley, J. W. (2012). Evolution of phenolic compounds from color and flavor problems to health benefits. *Journal of Agricultural and Food Chemistry*, 60(27), 6658-6677. <http://dx.doi.org/10.1021/jf300861c>. PMID:22568556.
- Souza, C. G., Andrade, D. M. L., Jordão, J. B. R., Ávila, R. I., Borges, L. L., Vaz, B. G., Valadares, M. C., Gil, E. S., Conceição, E. C., & Rocha, M. L. (2017). Radical scavenger capacity of jaboticaba fruit (*Myrciaria cauliflora*) and its biological effects in hypertensive rats. *Oxidative Medicine and Cellular Longevity*, 2017, 1-10. <http://dx.doi.org/10.1155/2017/2383157>. PMID:29422986.
- Takacs, B. O., Nelli, C. M., Anjos, L. P., Souza, M. S. J., Carvalho, S. F., Xavier, T., & Alvarenga, M. L. (2015). Avaliação do consumo de alimentos antioxidantes em atletas de handebol. *Revista Brasileira de Nutrição Esportiva*, 9(53), 491-497. Retrieved from <http://www.rbne.com.br/index.php/rbne/article/view/579/505>
- U.S. Department Agriculture – USDA. (2014). *Database for the flavonoid content of selected foods, release 3.1*. Retrieved from <http://www.ars.usda.gov/nutrientdata>
- Wallace, T. C., & Giusti, M. M. (2011). Selective removal of the violet color produced by anthocyanins in procyanidin-rich unfermented cocoa extracts. *Journal of Food Science*, 76(7), 1010-1017. <http://dx.doi.org/10.1111/j.1750-3841.2011.02322.x>. PMID:22417537.
- Wu, S. B., Long, C., & Kennelly, E. J. (2013a). Phytochemistry and health benefits of jaboticaba, emerging fruit crop from Brazil. *Food Research International*, 54(1), 148-159. <http://dx.doi.org/10.1016/j.foodres.2013.06.021>.