Development of isotonic beverage with functional attributes based on extract of Myrciaria jabuticaba (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 Myricaria jabuticaba. 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 jabuticaba 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⁻¹ 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), Carocho 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).

Jabuticaba 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 jabuticaba, 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 jabuticaba peel. They found that the hydroethanolic mixture (ethanol:water 50:50 v/v) at pH 1.0 acidified with formic acid stood out from the others. Hus, there is a growing application of jabuticaba peel extract in several products, such as Petit Suisse cheese (Pereira et al., 2016a, b) and mortadella (Baldin et al., 2018).
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 isotonics (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 butanolic 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, Schimadzu, 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.) 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 hydroethanolic solution in the ratio 1:2 peels or pulps: solvent extractor (m.v−1). 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 14,000 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 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−1 to 1.2 mg mL−1 and the readings were made at 595 nm (Bradford, 1976). The results were expressed in g of BSA 100 mL−1 of BI. Lipids were determined according to Association of Official Analytical Chemists (2010), and the results were expressed in g 100 mL−1 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−1, 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−1.

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 HNO3, 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−1. All determinations were performed with three replicates and the results expressed in mg L−1.
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:

\[
\text{Osmolarity (mOsm \text{ kg}^{-1})} = \frac{T_f}{K_c} \times 1000
\]

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

2.7 Physical characterization

Objective color determination

For objective color determination, the CieLab color system was used, with the color components \((L^*, a^* \text{ and } b^*)\) 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:

\[
C^* = \sqrt{a^*^2 + b^*^2}
\]

\[
h^* = \arctan\left(\frac{b^*}{a^*}\right)
\]

2.8 Chemical characterization

Total phenolic compounds (TPC) 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, a calibration curve of gallic acid was used at concentrations of 0.01, 0.03, 0.05, 0.07, and 0.09 mg mL\(^{-1}\). Absorbances were determined at 765 nm. The results were expressed as mg of gallic acid equivalents (EAG) 100 g\(^{-1}\) extract.

Total flavonoids (TF) were determined according to Heimann, 2010) using Folin–Ciocalteu reagent. The test is based on the reduction of the phosphomolybdophosphate-solubilizing 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\(^{-1}\). Absorbances were determined at 535 nm. The results were expressed as mg of catechin 100 mL\(^{-1}\) 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.

\[
TA = \left(\frac{\text{Abs}_{S355} \times \text{MM}_{\text{Cyanidin-3-glucoside}} \times \text{FD}}{\varepsilon}\right) \times 100
\]

where: \(TA = \text{total anthocyanins expressed as mg of the predominant anthocyanin in 100 g of sample; Abs}_{535nm} = \text{absorbance at 535 nm; MM} = \text{cyanidin-3-glucoside molar mass (449.2 g mol}^{-1}\); \(\varepsilon = \text{is the molar extinction coefficient of cyanidin-3-glucoside in ethanolic solution acidified to 535 nm, whose value is 26900 L cm}^{-1}\text{mg}^{-1}\).
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⁻¹.

Considering body fluids, sodium (Na⁺) 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⁻¹ (Brasil, 2010).

The calcium concentration obtained in formulations A and B of the beverage were 500 and 696 mg L⁻¹. During physical exercises, calcium plays an essential role because it initiates muscle contraction (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⁻¹, 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⁻¹.

### 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⁻¹ 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⁻¹. According to Brazilian legislation, this value can be up to 330 mOsm kg⁻¹ 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 ≥ 0.05) between the two processed isotonics for any of the analyzed parameters.
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.

### Table 3. Physical-chemical determinations of BI_A (10% pulp extract) and BI_B (12% pulp extract).

| Determinations            | BI_A           | BI_B           | Means          |
|---------------------------|----------------|----------------|----------------|
| pH                        | $3.48 \pm 0.06$| $3.47 \pm 0.06$| $3.47 \pm 0.06$|
| TTA (mg 100g⁻¹ Citric acid)| $0.67 \pm 0.36$| $0.78 \pm 0.42$| $0.72 \pm 0.39$|
| TSS (°Brix)               | $7.56 \pm 1.31$| $8.10 \pm 1.41$| $7.83 \pm 1.36$|
| Osmolarity (mOsm kg⁻¹)    | $281.31 \pm 0.69$| $328.41 \pm 0.71$| $304.86 \pm 0.70$|

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     | $20.87 \pm 0.29$| $2.55 \pm 0.45$| $-0.21 \pm 0.01$| $2.56 \pm 0.45$| $-0.08 \pm 0.02$|
| BI_B     | $20.43 \pm 0.11$| $2.87 \pm 0.39$| $-0.04 \pm 0.04$| $2.87 \pm 0.39$| $-0.03 \pm 0.02$|
| Means    | $20.65 \pm 0.20$| $2.71 \pm 0.42$| $-0.125 \pm 0.02$| $2.72 \pm 0.42$| $-0.05 \pm 0.02$|

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⁻¹)    | $1.44 \pm 0.00$| $44.64 \pm 0.15$| $52.40 \pm 0.16$| $48.52 \pm 0.15$|
| TF (mg of catechin 100 mL⁻¹)| _             | $7.34 \pm 0.94$| $10.49 \pm 0.53$| $8.92 \pm 0.73$|
| TA (mg of cyanidin-3-glucoside 100 mL⁻¹)| _             | $2.56 \pm 0.73$| $2.67 \pm 0.64$| $2.61 \pm 0.68$|

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.

#### 3.3 Chemical characterization of BI

The mean TPC for the isotonic beverages produced in this study was $48.52 \text{ mg EAG 100 mL}^{-1}$ (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 $20 \text{ mg 100 g}^{-1}$ 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 $2.5 \text{ mg 100 mL}^{-1}$. Mercali et al. (2015), when studying juice of natural jaboticaba obtained an average anthocyanin content of $14.8 \text{ mg 100 mL}^{-1}$. While Gironés-Vilaplana et al. (2013), found lower values when studying isotonic beverages based on açai and lemon juice and açai, obtaining values of $0.74 \text{ mg,100 mL}^{-1}$ and $0.93 \text{ mg,100 mL}^{-1}$, respectively.

The average levels of TF were $8.92 \text{ mg of catechin 100 mL}^{-1}$, similar to the value verified by Silva et al. (2017) for jaboticaba peel tea (8.3 mg catechin 100 mL⁻¹). According to the U.S. Department Agriculture (2014), the fruits of jaboticaba must contain at least $1.10 \text{ mg 100 g}^{-1}$ 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
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.

| Attributes          | Beverages Isotonic |            |            |
|---------------------|--------------------|------------|------------|
|                     | BL_A               | BL_B      | Commercial |
| Aroma               | 186\textsuperscript{a} | 219\textsuperscript{b} | 242\textsuperscript{b} |
| Color               | 186\textsuperscript{a} | 274\textsuperscript{c} | 190\textsuperscript{b} |
| Flavor              | 219\textsuperscript{b} | 271\textsuperscript{a} | 158\textsuperscript{c} |
| Global Impression   | 196\textsuperscript{a} | 274\textsuperscript{c} | 178\textsuperscript{b} |

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

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.

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