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Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region

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ABSTRACT

Monofloral honeys produced by the stingless bee *M. Subnida* Duke and *Melipona scutellaris* Latrelle in a Brazilian semi-arid region were analyzed regarding their physicochemical (moisture, protein, proline, hydroxymethyl-furfural, color, electrical conductivity, pH, free acidity, and sugar profile) and sensory aspects (color, viscosity and flavor). The floral source influenced the color, acidity, sugar profile, ash and proline content in the honeys. The intensity of the acidic flavor, sweet taste and color in the honeys varied according to the floral source. No differences were perceived for honeys from the same floral source produced by different bee species. Principal component analysis revealed that most of the variability was defined by the water content, total acidity, glucose, sucrose and color, as well as by acid taste, acid flavor, honey flavor and sweet taste. These findings reveal that Brazilian monofloral honeys produced by stingless bees possess well-defined characteristics that are influenced by the floral source.

1. Introduction

Honey is a natural food consumed without any processing and is characterized by its complex composition, which varies according to the bee species, geographical region, available floral source and storage conditions (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). The major components of honey are sugars mostly fructose and glucose although other minor components, such as enzymes, proteins, organic acids, minerals, pollen grains, waxes and phytochemicals, are also present (Manzanares, García, Galdón, Rodríguez, & Romero, 2014). Some of these constituents are naturally found in nectar (or pollen), while others are inserted by bees during the honey maturation process, thereby defining the differences in composition and functional properties of the honeys (Sant'Ana, Sousa, Salgueiro, Lorenzon, & Castro, 2012).

Traditionally, the combined studies of melissopalinological, physicochemical and sensory parameters are used to determine the botanical origin and quality of monofloral honeys. Still, the mineral content and the sugar profile have been suggested as criteria for the characterization of monofloral honeys (Manzanares et al., 2014). The quantitative descriptive analysis (QDA) is usually applied as a tool to access specific sensory characteristics in honey (Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009). However, the descriptive method Ranking Descriptive Analysis (RDA) has been cited as useful to study sensory properties of foods because it is less time consuming (in comparison to QDA) and allows, in addition to the descriptions of the attributes, the ranking among the samples for each assessed sensory attribute (Richter, Avancini, Prudencio, & Benassi, 2010). Researchers have stated that application of multivariate statistical methods is a highly effective tool for characterizing honeys of different geographical, botanical and entomological origins (Ferreira, Aires, Barreira, &

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Estevinho, 2009; Habib, Al Meqbali, Kamal, Souka, & Ibrahim, 2014; Silvano, Varela, Palacio, Ruffinengo, & Yamul, 2014).

The semiarid northeastern region of Brazil has a unique biodiversity of stingless bees and native flora, enabling the production of honey with particular characteristics. In this region, a biome (caatinga) composed by botanical species exists that is adapted to the typical climatic conditions of one rainy and one dry regular season during the year. Some of the botanical species are commonly visited by native bee species, known as indigenous bees, stingless bees, or meliponini bees (Silva et al., 2013, 2014). Among the miliponini species already identified in the north and northeastern regions of Brazil, special attention has been given to *Melipona subnitida* Ducke (jandaíra) and *Melipona scutellaris* Latrelle (uruçu); these bee species typically visit botanical species available only in a specific period of the year (rainy or dry season), thus producing different types of honeys during the year.

Considering these aspects, studies on the characterization of monofloral honeys produced by native stingless bees in the Brazilian northeastern region could be important incentives for the maintenance of the stingless bee population and to help conserve the flora and indigenous bees in this region. In this study, honeys produced by jandaíra and uruçu bees from specific botanical sources, which are typically found in the northeastern region of Brazil during the rainy and dry seasons, were characterized in terms of their sugar content, physicochemical parameters and sensory attributes.

2. Material and methods

2.1. Experimental design and honey samples

The experimental design included four different monofloral honeys produced by two different stingless bee species in the semiarid region of Brazilian northeastern (Seridó region, state of Rio Grande do Norte and Agreste region of state of Paraíba) collected in three different occasions (4 × 2 × 3). Each of the 24 samples analyzed was composed by a mixture of honeys collected in four different meliponaries. The samples of honey from *Ziziphus joazeiro* Mart. (juazeiro) and *Mimosa quadrivalvis* L. (malícia) produced by the stingless bees *M. subnitida* Ducke (jandaíra) and *M. scutellaris* Latrelle (uruçu) were collected during the 2012 dry season, while the honey samples from *Mimosa arenosa* willd Poir (jurema branca) and *Croton heliotropiifolius* Kunth (velame branco) produced by the both bee species (jandaíra and uruçu) were collected during the 2013 rainy season. Samples were stored in sterilized amber glass containers, shipped to the laboratory, and then maintained at 6–8 °C in the dark until analysis. To ensure that the honeys were monofloral, the melissopalynological procedure was applied to all samples (Silva et al., 2013). For this procedure, 10 g of each honey sample was diluted in 20 mL of distilled water and then centrifuged at 4000 *g* for 20 min. The sediment was dried at 40 °C and then mounted with Entellan Rapid (Merck, 1.07961.0500). The honeydew elements and pollen grains ($n = 500$) were counted and identified in 20 distinct optical areas using an optical microscope (Nikon Optiphot II microscópio; 400× and 1000×). The pollen grains were compared to reference images of University of São Paulo, São Paulo, Brazil. All samples contained more than 80% pollen grains of the same botanical origin.

2.2. Physicochemical analysis

All of the physicochemical parameters were determined in triplicate according to the Harmonized Methods of the International Honey Commission – IHC (2002) and the Association of Official Analytical Chemists – AOAC (1990). The water content was determined via refractometry using an Abbé-type refractometer at 20 °C (Q767-B, Tokyo, Japan); the corresponding values were obtained according to the table of Chatway. The brix values in honeys were determined via refractometry following the AOAC Official method 31.119. The total acidity was analyzed using the sum of free acidity and lactonic acidity determined by titration of 10 g of honey dissolved in 75 mL of distilled water with 0.05 mol/L NaOH to pH 8.3, with the results expressed as mmol H^+/L. The pH was measured in a solution of 10 g of honey in 75 mL ultrapure water using a digital potentiometer (QUIMIS, Q488AS). The protein content was measured using the Kjeldahl method, based on the conversion of the organic nitrogen present in the sample to $(NH_4)_2SO_4$, using 6.25 as the conversion factor. The proline content was determined via the measurement of the absorbance at 510 nm of the resulting product between proline and ninhydrin in an acidic medium. The electrical conductivity was measured at 20 °C in a 20 g/100 mL (w/v) solution of honey in ultrapure water using a radiometer (Analyser, model 600), with the results expressed as mS/cm. The ash content was measured after burning the sample at 550 °C. The color was determined using the Pfund method; the absorbance of the honeys [diluted to 50 g/100 mL (w/v) in ultrapure water] was determined at 636 nm and then converted to the Pfund scale ($\text{mmPfund} = -38.7 + 371.39 \times \text{Abs}$). Hydroxymethyl furfural (HMF) was measured using the method of White (Silvano et al. 2014).

2.3. Sugar profile

Approximately 0.5 g of honey was weighed directly in polypropylene tubes and then mixed with 10 mL of water. Next, a milliliter of this dissolution was passed through a 0.20-μm cellulose acetate membrane prior to HPLC analysis. The determination of the sugars (fructose, glucose, sucrose, maltose and arabinose) was performed using a 1100 series Hewlett–Packard chromatograph equipped with a refractive index detector operated at 50 °C and a 300 × 7.8 mm CARBOsep CHO 682 column (Transgenomic, Glasgow, U.K.) operating at 80 °C. Distilled water was used as the mobile phase (flow rate 0.4 mL/min). The HPLC sample peaks were identified by comparing the retention times obtained from standards. The honey samples were also spiked with standards (Sigma®) to verify the identity of the chromatographic peaks. Duplicate injections were performed, and the average peak areas were used for the quantification.

2.4. Sensory analysis

This work was submitted to the National Committee in Research Ethics (Brazil) and approved under number CAAE: 06371012.8.0000.5188. Sensory analyses were performed using Ranking Descriptive Analysis (RDA) according to the procedures described by Richter et al. (2010). The panel was composed of 19 assessors, all members (students, technicians and teachers) of the Federal University of Paraíba (João Pessoa, Brazil), with ages ranging from 16 to 62 years and with previous experience in sensory analysis. The sessions were performed in a tasting room, separated from the area where the samples were prepared and maintained at room temperature (25 °C) during the evaluation. The coded samples were presented in random order to each assessor in 40-mL glass vials sealed with a twist-off cap and at room temperature. The order of presentation was balanced and randomized to eliminate contrast effect and positional bias. Mineral water was provided for the assessors to perform mouth-rinsing between samples. The sessions were performed between 10:00 and 12:00, and the panelists declared that they were not hungry at the commencement of the tests. During the initial sessions, the assessors underwent training in descriptive analysis, during which they generated descriptors over the course of seven sessions. Due to the high number of honey samples, the panel opted to evaluate four samples per session (honeys of juazeiro and malícia produced by jandaíra and uruçu bee) and (honeys of jurema branca and velame branco produced by jandaíra and uruçu bee). The common descriptors chosen by the panel were compiled, together with significant descriptors selected for the formulation of a glossary (amber color, viscosity, honey flavor, acid flavor sweet taste, bitter taste, and acid taste). The honey samples were evaluated by RDA in two distinct sessions, where each sample was individually evaluated using the glossary and then ordered according to increasing intensity for each of the attributes.

2.5. *Data analysis*

All analyses were performed in triplicate in three different occasions. The mean values obtained for the physicochemical variables studied in honey samples were compared using the Two-Way ANOVA followed by Tukey test. The sensory data were analyzed by the Friedman test, considering four samples, 19 judges and the critical value: 21. Principal Component Analysis (PCA) was performed to integrate significant data. For this, the physicochemical and sensory data that differed among honeys from distinct floral sources, as well as those considered characteristic of honeys produced by stingless bee, such as water content and acidity, were normalized and submitted to PCA multivariate analysis. All statistical analyses were performed using Statistica Software 5.1. A p-value ≤ 0.05 was considered to be significant.

3. Results and discussion

3.1. *Water content (g/100 g)*

The water content of the honey samples ranged from 23.9 to 28.9 g/100 g, and no differences were observed ($p \geq 0.05$) among honeys from the same floral source produced by different bee species (Table 1), with the exception of honey from jurema branca. In honey, water content has been linked to the floral source, although climatic conditions, soil, collection period and processing aspects may strongly affect this parameter (Silvano et al., 2014). The water content values found in the assessed honeys are similar to those reported in previous studies (22–35 g/100 g) involving characterization of honeys produced by jandaíra and uruçu bees in different states of northeastern Brazil (Silva et al., 2013; Sousa et al., 2013; Souza et al., 2006), which indicate that the analyzed samples presented satisfactory quality.

3.2. °*Brix*

The °Brix values for the honey samples ranged from 71.1 to 74.7. No differences ($p \geq 0.05$) were observed regarding the floral source among the assessed honey samples (Table 1). However, honeys from malícia differed ($p \leq 0.05$) according to the bee species. The °Brix values are directly related to the levels of sugars present in honey, making them an important indicator of possible adulteration (Guarrini et al., 2009). Generally, honeys produced by stingless bees exhibit lower °Brix values when compared to honeys produced by *A. mellifera* (≥ 75), as honeys from stingless bees possess higher water content and a lower percentage of total sugars (Guarrini et al., 2009; Habib et al., 2014). Previous studies found °Brix values similar to those detected in this study for honeys produced by the uruçu bee in the state of Paraíba - Brazil (average of 72 °Brix) (Campos, Gois, & Carneiro, 2010), and for honeys produced by stingless bees in South American countries (57.5–75.0 °Brix) (Souza et al., 2006).

3.3. *pH and acidity*

The pH values found in honeys ranged from 3.1 to 5.3, differing ($p \leq 0.05$) among the honeys from different floral sources and/or bee species. Previous studies describe pH values less than and greater than (2.9–6.5) those found in this study for multifloral

Table 1
Physicochemical parameters (n:3, mean values ± standard deviation) and the sugar profiles of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources.

Physicochemical parameters	Stingless bee/monofloral honeys							
	jandaíra (*M. Subnida* Duke)				uruçu (*M. scutellaris* Latrelle)			
	juazeiro	malícia	velame branco	jurema branca	juazeiro	malícia	velame branco	jurema branca
Water (g/100 g)	$23.9^{Ad} \pm 0.4$	$27.2^{Ab} \pm 0.2$	$25.6^{Ac} \pm 0.4$	$28.9^{Aa} \pm 0.2$	$24.3^{Ab} \pm 0.3$	$26.5^{Aa} \pm 0.8$	$25.8^{Aa} \pm 0.4$	$25.4^{Bab} \pm 0.6$
°Brix	$74.7^{Aa} \pm 0.2$	$71.1^{Bb} \pm 0.2$	$72.4^{Aab} \pm 1.2$	$72.0^{Aab} \pm 1.0$	$74.3^{Aa} \pm 0.5$	$73.0^{Aa} \pm 0.6$	$72.5^{Aa} \pm 0.5$	$72.8^{Aa} \pm 1.2$
pH	$5.3^{Aa} \pm 0.4$	$3.1^{Bc} \pm 0.2$	$3.8^{Ab} \pm 0.01$	$3.6^{Ab} \pm 0.02$	$4.2^{Ba} \pm 0.05$	$4.0^{Ab} \pm 0.02$	$3.5^{Bc} \pm 0.4$	$3.6^{Ac} \pm 0.1$
TA (mmol H^+/L)	$28.2^{Ac} \pm 0.9$	$86.8^{Aa} \pm 0.4$	$17.8^{Bd} \pm 1.0$	$37.8^{Bb} \pm 0.8$	$30.4^{Ac} \pm 1.2$	$66.1^{Ba} \pm 2.4$	$32.1^{Ac} \pm 0.4$	$42.4^{Ab} \pm 0.7$
Protein (g/100 g)	$0.5^{Aa} \pm 0.1$	$0.4^{Aab} \pm 0.1$	$0.3^{Aa} \pm 0.0$	$0.2^{Aa} \pm 0.1$	$0.5^{Aa} \pm 0.0$	$0.4^{Aab} \pm 0.0$	$0.3^{Ab} \pm 0.0$	$0.3^{Ab} \pm 0.0$
Proline (mg/kg)	$20.5^{Aa} \pm 3.7$	$11.9^{Ab} \pm 2.1$	$5.9^{Ab} \pm 0.8$	$10.8^{Ab} \pm 1.1$	$17.4^{Aa} \pm 0.9$	$7.5^{Bb} \pm 0.5$	$4.6^{Ac} \pm 1.0$	$8.9^{Ab} \pm 0.7$
EC (μS/cm)	$598^{Bb} \pm 2.6$	$636^{Aa} \pm 1.0$	$300^{Bd} \pm 3.1$	$520^{Bc} \pm 0.2$	$670^{Aa} \pm 5.1$	$514^{Bc} \pm 2.3$	$340^{Ad} \pm 1.5$	$571^{Ab} \pm 3.4$
Ash (g/100 g)	$0.52^{Aa} \pm 0.0$	$0.04^{Ab} \pm 0.0$	$0.11^{Ab} \pm 0.0$	$0.12^{Ab} \pm 0.0$	$0.41^{Aa} \pm 0.0$	$0.03^{Bb} \pm 0.0$	$0.12^{Ab} \pm 0.0$	$0.1^{Ab} \pm 0.0$
Color (nm Pfund)	$95.4^{Ba} \pm 0.2$	$55.6^{Bb} \pm 0.02$	$35.8^{Bd} \pm 0.3$	$54.1^{Bc} \pm 0.1$	$103.4^{Aa} \pm 0.6$	$82.8^{Ab} \pm 0.4$	$55.9^{Ac} \pm 0.4$	$57.2^{Ac} \pm 1.1$
HMF	nd	nd	nd	nd	nd	nd	nd	nd
Sugars (g/100 g)								
Glucose (g/100 g)	$37.7^{Ac} \pm 0.4$	$45.4^{Aa} \pm 0.3$	$42.1^{Bb} \pm 0.6$	$45.7^{Aa} \pm 0.4$	$38.1^{Ac} \pm 0.3$	$42.6^{Ba} \pm 0.2$	$43.3^{Aa} \pm 0.1$	$41.4^{Bb} \pm 0.5$
Fructose (g/100 g)	$59.2^{Aa} \pm 1.1$	$50.0^{Bd} \pm 0.9$	$55.7^{Ab} \pm 1.9$	$52.6^{Ac} \pm 2.5$	$57.6^{Aa} \pm 1.5$	$55.5^{Aab} \pm 1.0$	$53.8^{Bb} \pm 0.9$	$53.6^{Ab} \pm 0.7$
Sucrose (g/100 g)	$1.6^{Ab} \pm 0.2$	$3.9^{Aa} \pm 0.2$	$0.7^{Bc} \pm 0.0$	$1.2^{Bbc} \pm 0.2$	$2.6^{Ba} \pm 0.5$	$1.9^{Ba} \pm 0.3$	$2.0^{Aa} \pm 0.3$	$3.0^{Aa} \pm 0.5$
Maltose (g/100 g)	nd	nd	nd	nd	nd	nd	nd	nd
Arabinose (g/100 g)	nd	$0.5^{A} \pm 0.0$	$1.0^{A} \pm 0.1$	$0.4^{A} \pm 0.1$	$0.3^{b} \pm 0.0$	$0.4^{Ab} \pm 0.1$	$0.7^{Ba} \pm 0.1$	$0.2^{Ac} \pm 0.0$
TS	$68.2^{Aab} \pm 0.7$	$63.2^{c} \pm 1.0$	$67.9^{Abc} \pm 1.9$	$71.2^{Aa} \pm 1.4$	$67.6^{Aab} \pm 1.6$	$62.7^{Ab} \pm 1.4$	$68.0^{Aab} \pm 2.3$	$71.2^{Aa} \pm 1.5$
F/G	$1.5^{Aa} \pm 0.2$	$1.1^{Bc} \pm 0.0$	$1.3^{Ac} \pm 0.0$	$1.1^{Bc} \pm 0.0$	$1.5^{Aa} \pm 0.0$	$1.3^{Ab} \pm 0.0$	$1.2^{Bb} \pm 0.0$	$1.2^{Ab} \pm 0.0$

TA = Total acidity; EC = Electrical conductivity; TS = Total Sugars; nd = not detected.: (*Ziziphus joazeiro*); malícia: (*Mimosa quadrivalvis*); Velame branco: (*Croton heliotropiifolius*); Jurema branca: (*Mimosa arenosa*). Different capital letters in the same row indicates significant differences ($p \leq 0.05$) between honeys from the same botanical source produced by distinct bee species according to the Tukey test. Different lowercase letters in the same row indicates significant differences ($p \leq 0.05$) between honeys from different floral source produced by the same bee species according to the Tukey test.

honeys produced by stingless bees (Sousa et al., 2013). In general, honeys from a floral source or honeydew honeys exhibit acidic pH values, without a direct relationship with the botanical source. However, the pH values in honeys have been linked with other parameters that are influenced by geographical and botanical origin, such as mineral content (Vanhanen, Emmertz & Savage, 2011).

The acidity values ranged from 17.88 to 86.85 mmol H^+/L in the samples evaluated. Differences were observed ($p \leq 0.05$) among the honeys from different floral sources and/or bee species (Table 1). Previous studies that characterized wild honey produced by stingless bees in north and northeastern Brazil reported lower and higher acidity values (18.5–95.2 mmol H^+/L) when compared to those observed in the present study (Alves, Carvalho, Souza, Sodré, & Marchini, 2005; Sousa et al., 2013). The acidity value corresponds to the balance of organic acids present in honey, (Vit, 2008), which vary according to the floral composition and the bee species. This variation may explain the differences observed.

3.4. *Protein and proline*

The mean protein values in honeys ranged from 0.2 to 0.5 g/100 g (Table 1), and no difference ($p > 0.05$) in this parameter was observed among the honeys from the same floral source, despite being produced by bees from different species. These results reinforce the findings of Habib et al. (2014), who revealed that the protein content in honeys is dependent on the floral source and that different values may be associated with the presence of proteins derived from nectar of flowers and enzymes introduced by bees in honeys.

The proline levels in honey ranged from 20.5 to 4.6 mg/kg (Table 2). The honeys from juazeiro produced by both bee species also exhibited higher proline contents ($p \leq 0.05$) when compared to the other honeys analyzed; however, honeys from the same floral source exhibited similar proline contents ($p \geq 0.05$). Proline is the main free amino acid present in honeys produced by stingless bees, making it useful for the characterization of the botanical source because is related to the floral source and the amount of pollen present in the honeys (Truzzi, Annibaldi, Illuminati, Finale, & Scarponi, 2014).

3.5. *Sugar quantitative profile*

The type of sugar present in the greatest amounts in the honeys was fructose, followed by glucose and sucrose (Table 1). With the exception of honeys from juazeiro, the honeys of the same floral source produced by different bee species differed ($p \geq 0.05$) in the amounts of each detected sugar; this difference was also found for different floral sources. In the floral honeys, the glucose content corresponds to the characteristics of the nectar of the predominant flowers and varies among the plant species (Escuredo, Dobre, Fernández-González, & Seijo, 2014). The glucose and fructose levels of the honeys studied were higher than those reported in previous studies, with multifloral honeys produced by stingless bees in Ecuador (25.5 g/100 g of total sugars for glucose and 25.2 g/100 g of total sugars for fructose) (Guerrini et al., 2009) and in southern Brazil (8.2–35.39 g/100 g of total sugars for glucose and 31.88–45.46 g/100 g of total sugars for fructose) (Rizélio et al., 2011).

The fructose/glucose (F/G) ratio in the honeys evaluated ranged from 1.1 to 1.5 (Table 1), which is similar to the ratio found by Oddo et al. (2008) (1.4) in honey samples produced by stingless bees in Australia. The F/G ratio directly influences the sweet taste of honey because fructose is sweeter than glucose. The sucrose contents found are lower than those described in previous studies for multifloral honeys produced by the jandaíra bee (3.7 g/100 g of total sugars) and the uruçu bee (5.3–8.8 g/100 g of total sugars) (Campos et al., 2010; Souza et al., 2013). These results demonstrate the lack of adulteration and honey collection at the ideal maturation time because high sucrose content may result from the addition of commercial sugar or may be attributed to the early honey collection.

3.6. *Electrical conductivity*

The electrical conductivity (EC) of the honeys studied ranged from 300 to 670 μS/cm(Table 1), classifying them as floral honeys (≤800 μS/cm) (Codex Alimentarius, 2001). Differences were found in the EC ($p \geq 0.05$), regardless of the floral source or bee species.

Table 2

Intensity values of the sensory attributes (assessed by RDA) of monofloral honeys produced by different stingless bees (jandaíra and uruçu) in the semiarid region of Northeastern Brazil from different floral sources.

Sensory attributes	Stingless bee/monofloral honeys			
	Jandaíra (*M. subnida* Duke)		Uruçu (*M. scutellaris* Latrelle)	
	juazeiro	malícia	juazeiro	malícia
Amber color	62^{a}	22^{b}	71^{a}	38^{b}
Viscosity	71^{a}	20^{b}	61^{a}	38^{b}
Honey flavor	68^{a}	29^{b}	58^{a}	35^{b}
Acid flavor	44^{b}	72^{a}	21^{c}	53^{ab}
Sweet taste	62^{a}	16^{c}	69^{a}	43^{b}
Acid taste	40^{b}	74^{a}	19^{c}	54^{ab}
Bitter taste	52^{a}	60^{a}	56^{a}	20^{b}
	velame branco	jurema branca	velame branco	jurema branca
Amber color	26^{c}	56^{ab}	37^{cb}	71^{a}
Viscosity	55^{a}	44^{ab}	64^{a}	27^{b}
Honey flavor	29^{c}	56^{ab}	$39^{\ bc}$	66^{a}
Acid flavor	58^{ab}	28^{c}	65^{a}	39^{bc}
Sweet taste	49^{a}	66^{a}	25^{b}	50^{a}
Acid taste	52^{ab}	43^{bc}	71^{a}	21^{c}
Bitter taste	59^{a}	36^{b}	$44^{\ ab}$	51^{ab}

Rank sum values of answers of 19 assessors for the ordination of intensity of assessed sensory attributes in honey samples, considering four samples, 19 judges and the critical value: 21. Different letters in the same row indicate significant differences ($p \leq 0.05$) according to Newell and MacFarlane (1987); juazeiro: (*Ziziphus joazeiro*); malicia: (*Mimosa quadrivalvis);* velame branco: (*Croton heliotropiifolius*); jurema branca: (*Mimosa arenosa*).

Campos et al. (2010) observed values from 539.6 to 586 μS/cm for multifloral honeys produced by uruçu bees in a semiarid region of northeastern Brazil. Results similar to those of the present study were previously reported for monofloral honeys produced by *A. mellifera* in a semiarid region of the United Arab Emirates (254–605 $\mu S\ cm^{-1}$) (Habib et al. 2014). The electrical conductivity intensity of honeys has been described as a measure of the amount of mineral elements possessing good electrical conductivity property contained in the honey (Yadata, 2014). However, higher electrical conductivity values do not necessarily correspond to higher amounts of ash in honeys (Escuredo et al., 2014), as evidenced in the present study. Other factors, such as floral source, the amount of organic acids and proteins, and storage time can also influence the electric conductivity of honey samples (Karabagias et al., 2014).

3.7. *Ash*

Honeys of the same botanical origin did not differ ($p \geq 0.05$) for different producer bee species (Table 1). The highest ash values ($p \leq 0.05$) were observed for honeys from juazeiro. Ash content, representing the total minerals present in honey, is directly related to the environmental, geographical and botanical aspects of the region where it is produced (Finola, Lasagno, & Marioli, 2007). The ash content observed for honeys from malícia, velame branco and jurema branca are similar to the levels reported for honeys from angico (*Anadenanthera* sp.) (0.01–0.02 g/100 g) produced by jandaíra bees (Silva et al., 2014) and for wild honeys (0.1–0.302 g/100 g) produced by uruçu bees (Campos et al., 2010) in a Brazilian semiarid region, reinforcing the influence of geographic factors on this parameter (Santos, Moreira, & Maria, 2015). The high ash content in some honey may be due to the nectar characteristics of some botanical species, such as juazeiro in the present study.

3.8. *Color and HMF*

The color of honeys on the Pfund scale exhibited values from 35.8 to 103.4 (Table 2). Honeys from juazeiro produced by both bee species exhibited the highest values ($p < 0.05$) on the Pfund scale, being classified as amber. Interestingly, these honeys also exhibited the highest ash and pH values ($p < 0.05$) when compared to all other honey samples. Previous studies have reported that the color intensity of honeys varies according to pH and mineral content, exhibiting a positive correlation with these two parameters (Guerrini et al., 2009). However, factors such as exposure to light, heat and storage time, as well as enzymatic reactions, may also affect this parameter.

HMF was not detected in honey samples characterizing the evaluated monofloral honeys from stingless bees as fresh and high quality honeys (Habib et al., 2014). HMF is widely known as a parameter of freshness in honeys because it is absent in fresh honeys and tends to increase during processing and/or aging of the product.

3.9. *Sensory analysis*

The sensory characteristics of aroma and acidic flavor, sweet taste and intense amber color (caramel) described for monofloral honeys are reported in the sensory evaluation of honeys produced by stingless bees (Ferreira et al., 2009; Souza et al., 2013); however, there are no previous studies on the ordering of these attributes according to the perceived intensity. In general, honeys from the same floral source were observed to have similar sensory attributes and did not differ with respect to the bee species that produced them. The physicochemical and sensory characteristics of honeys vary according to botanical origin, maturation time, weather and storage conditions (Ferreira et al., 2009).

Considering the ordering of the intensity of the attributes of amber color, viscosity, honey aroma and sweet taste, the judges determined that the honeys from juazeiro produced by both species of stingless bees were more intense ($p \leq 0.05$) compared with the honeys from malícia (Table 2). Previous studies have reported a direct correlation of these sensory attributes in honeys with physicochemical parameters, such as °Brix, pH, ash content, proteins, color and total sugars (Habib et al., 2014). This correlation may explain the difference in intensity of the attributes of honeys from juazeiro and malícia perceived by the judges in the evaluation because the °Brix values, protein, ash, total sugar and instrumental color were higher ($p \leq 0.05$) in the honeys from juazeiro compared to those in the honeys from malícia for both bee species (Table 2). This result is also corroborated by the fact that the judges determined the honeys from malícia as being more acidic (aroma and acid taste) than those from juazeiro ($p \leq 0.05$); in addition, the judges determined that the honeys from malícia did not differ ($p \geq 0.05$) in aroma and acid taste when considering the bee species (Table 2). The greater intensity of aroma and acid taste described in the sensory evaluation for honeys from malícia compared to those from juazeiro appears to be associated with their higher total acidity, lower pH index, and the lowest total sugar, which could mask the acidity (Table 1). These features are possibly derived from the nectar offered by the malícia flower to the bee species, considering that acidity is a parameter strongly related to the botanical origin (Beay, Solomon, Bultossa, Adgaba, & Melaku, 2015).

Similar to the observed intensity ordering of the attributes of honeys from juazeiro and malícia, honeys from jurema branca were perceived as being more intense ($p \leq 0.05$) regarding the attributes of color, viscosity, honey aroma and sweet taste when compared to honey from velame branco (Table 2), but were not considered to be different ($p > 0.05$) when considering the bee species. In the physicochemical analysis, honeys from velame branco exhibited high values for °Brix, pH, ash, protein, instrumental color and total sugars (Table 1), partially explaining the sensory characterization. In contrast, higher intensities for the attributes of acid aroma, acid taste and bitter taste were described for honeys from velame branco (Table 2), when compared to honeys from jurema branca. Although these attributes are directly correlated with the total acidity levels, the ash content, and the pH and type of organic acids present in monofloral honeys (Manzanares et al., 2014), high acidity is typical physicochemical and sensory characteristic of honeys produced by stingless bees (Vit, 2008). In the present study, honeys from malícia and velame branco exhibited higher intensity for the attributes of aroma and acid taste and lower intensity for amber color when compared to honeys from juazeiro and jurema branca. This result is in agreement with the previous findings that honeys produced by stingless bees that exhibit less intense amber color have more intense aroma and acid taste (Ferreira et al., 2009).

Once the individual behavior of each honey was studied, a PCA was used to assess the overall effect of the variables (floral source and bee specie) on the principal components that define the honey samples. The physicochemical parameters of the honey samples that provide a major contribution to PC1 were water content, total acidity, glucose and color; sensory parameters that contribute in this PC were acid taste and acid flavor together with honey flavor and sweet taste, which were in contrast to the aforementioned result (Fig. 1A). PC1, which explains 48.15% of the variance, clearly separates acid taste and acid flavor from sweet taste, water content, glucose and total acidity. However, PC2, which explains 17.14% of the variance, was only defined by sucrose level; the remaining variability not taken into account by PC1 was explained by this variable. For PC1, the water content, total acidity and glucose were found to be the most important variables that explain the

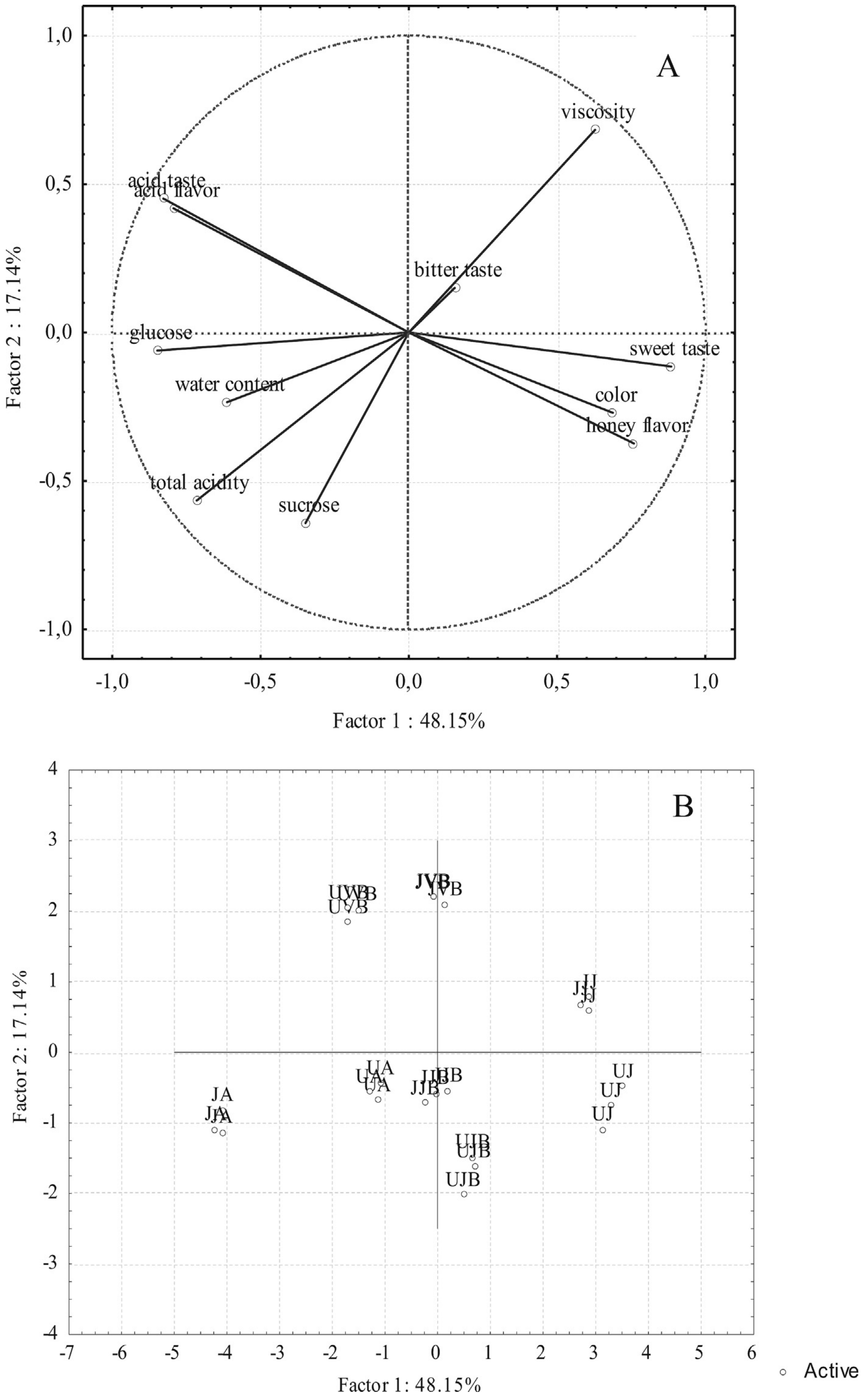

Fig. 1. (A) Principal Component Analysis (PCA) of the physicochemical (water content, total acidity, glucose, sucrose) and sensory (acid flavor, acid taste, viscosity, sweet taste, bitter taste, honey flavor, color) aspects of monofloral honey; (B) Distribution of samples according PCA: JJ (juazeiro/jandaíra); UJ (juazeiro/uruçu); JA (malícia/jandaíra); UA (malícia/uruçu); JJB (jurema branca/jandaíra); UJB (jurema branca/uruçu); JVB (velame branco/jandaíra); UVB (velame branco/uruçu).

separation of the honey of malícia produced by both bee species studied from the other tested honeys (Fig. 1B).

Previous studies also described the characteristics of acid flavor and acid taste for the honey of malícia produced in a semiarid region of Brazil (Campos et al., 2010; Sousa et al., 2013), which are strongly associated with the nectar of the floral source collected by bees (Belay et al., 2015; Silvano et al., 2014). PC1 also explains the separation of the honeys of juazeiro produced by uruçu bees from the other honeys assessed (Fig. 1B), which could be related to the higher values of color, honey flavor and sweet taste when compared with the honeys of the other floral sources studied (Table 2). The honey of juazeiro produced by jandaíra bees also exhibited higher values for these same parameters (color, honey flavor and sweet taste); note that this honey was ranked by the sensory panel as being more intense regarding acid taste and acid flavor, which could influence the distribution of the samples according to the PCA (Table 2; Fig. 1B). However, the honey of juazeiro exhibited no differences ($p \geq 0.05$) when produced by different bee species regarding the physicochemical parameter total acidity.

According to PC2, the similarity observed between the honey of jurema branca and velame branco produced by jandaíra was defined by the levels of sucrose (Fig. 1B). These samples exhibited the lowest sucrose amounts when compared to the other assessed honey samples (Table 1). This result suggests that this variable strongly contributes, with the same weight, despite the floral source, for the unique characteristics in these two honeys, which appears in the y axis (Fig. 1B); this result is similar to results found for the honeys of jurema branca and velame branco produced by jandaíra in the sensory evaluation by RDA (Table 2). For the honey samples studied, the water content exhibited a positive correlation with glucose ($r = 0.88$) and with total acidity ($r = 0.5$), whereas a negative correlation was observed with viscosity ($r = -0.54$) and color ($r = -0.50$). As expected, honeys with lower water content (juazeiro and velame branco) were ranked as being more intense for the viscosity attribute when compared to the other honeys (malícia and jurema branca), regardless of the bee species involved. A strong positive correlation ($r = 0.89$) was also observed for acid flavor and acid taste, revealing the accuracy of the panel regarding the sensory evaluation performed. In contrast, a negative correlation was observed between total acidity and sweet taste ($r = -0.63$). The highest total acidity values were observed for malícia honey produced by both bee species assessed; this honey exhibited lower values for the intensity of sweet taste in the sensory evaluation by RDA.

4. Conclusions

Monofloral honeys produced by distinct stingless bee species in a Brazilian northeastern region presented unique characteristics related to the floral source and a good correlation between the physicochemical and sensory parameters. These findings revealed that RDA is a feasible technique for establishing differences between honey samples. In particular, honeys from juazeiro differed from other honeys regarding their intense color and high proline, glucose, and ash contents, while honeys from malícia exhibited the highest acidity.

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References

Alves, R. M. O., Carvalho, C. A. L., Souza, B. A., Sodré, G. S., & Marchini, L. C. (2005). Physico-chemical characteristics of honey samples of stingless bee *Melipona* mandaçaia Smith (Hymenoptera:Apidae). *Ciência e Tecnologia de Alimentos, 25*, 644–650.

AOAC (Association of Official Analytical Chemists). (1990). *Official methods of the analysis of official analytical chemists* (15th ed., vol. II) USA: Virginia.

Belay, A., Solomon, W. K., Bultossa, G., Adgaba, N., & Melaku, S. (2015). Botanical origin, colour, granulation, and sensory properties of the Harenna forest honey, Bale, Ethiopia. *Food Chemistry, 167*, 213–219.

Campos, F. S., Gois, G. C., & Carneiro, G. G. (2010). Physico-chemical parameters of the honey of stingless bee *Melipona scutellaris* produced in the Paraíba. *Zootecnia, 7*, 186–190.

Castro-Vázquez, L., Díaz-Maroto, M. C., González-Viñas, M. A., & Pérez-Coello, M. S. (2009). Differentiation of monofloral citrus, rosemary, eucalyptus, lavender, thyme and heather honeys based on volatile composition and sensory descriptive analysis. *Food Chemistry, 12*, 1022–1030.

Codex Alimentarius. (2001). *Commission Standards. Official methods of analysis*. CODEX STAN. 12–1981, Rev. 1 1987, Rev. 2.

Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry, 149*, 84–90.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*, 1438–1443.

Finola, M. S., Lasagno, M. C., & Marioli, J. M. (2007). Microbiological and chemical characterization of honeys from central Argentina. *Food Chemistry, 100*, 1649–1653.

Guerrini, A., Bruni, R., Maietti, S., Poli, F., Rossi, D., Paganetto, G., et al. (2009). Ecuadorian stingless bee (Meliponinae) honey: a chemical and functional profile of an ancient health product. *Food Chemistry, 114*, 1413–1420.

Habib, H. M., Al Meqbali, F. T., Kamal, H., Souka, U. D., & Ibrahim, W. H. (2014). Physicochemical and biochemical properties of honeys from arid regions. *Food Chemistry, 153*, 35–43.

International Honey Commission – IHC. (2002). Swiss Bee Research Centre. FAM, Liebefeld, CH-3003 Bern, Switzerland.

Karabagias, I. K., Badeka, A. V., Kontakos, S., Karabournioti, S., & Kontominas, M. G. (2014). Botanical discrimination of Greek unifloral honeys with physico-chemical and chemometric analyses. *Food Chemistry, 165*, 181–190.

Manzanares, A. B., García, Z. H. B., Galdón, R., Rodríguez, E. R., & Romero, C. D. (2014). Physicochemical characteristics of minor monofloral honeys from Tenerife, Spain. *LWT Food Science and Technology, 55*, 572–578.

Newell, G. J., & Macfarlane, J. D. (1987). Expanded tables for multiple comparison procedures in the analysis of ranked data. *Journal of Food Science, 52*, 1721–1725.

Oddo, P. L., Heard, T. A., Rodríguez-Malaver, A., Pérez, R. A., Fernández-Muiño, M. A., Sancho, M. T., et al. (2008). Composition and antioxidant activity of trigona carbonaria honey from Australia. *Journal of Medicinal Food, 11*, 789–794.

Richter, V. B., Avancini, T. C., Prudencio, S. H., & Benassi, M. T. (2010). Proposing a ranking descriptive sensory method. *Food Quality and Preference, 21*, 611–620.

Rizélio, V. M., Tenfen, L., Silveira, R., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2011). Development of a fast capillary electrophoresis method for determination of carbohydrates in honey samples. *Talanta, 93*, 62–66.

Santos, A., Moreira, R. F. A., & Maria, C. A. B. (2015). Study of the principal constituents of tropical angico (*Anadenanthera* sp.) honey from the atlantic forest. *Food Chemistry, 171*, 421–425.

Sant'Ana, L. D., Sousa, J. P. L. M., Salgueiro, F. B., Lorenzon, M. C. A., & Castro, R. M. (2012). Characterization of monofloral honeys with multivariate analysis of their chemical profile and antioxidant activity. *Journal of Food Science, 77*, 135–140.

Silvano, M. F., Varela, M. S., Palacio, M. A., Ruffinengo, S., & Yamul, D. K. (2014). Physicochemical parameters and sensory properties of honeys from Buenos Aires region. *Food Chemistry, 152*, 500–507.

Silva, T. M. S., Santos, F. P., Evangelista-Rodrigues, A., Silva, E. M. S., Silva, G. S., Novais, J. S., et al. (2013). Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis, 29*, 10–18.

Silva, T. M. G., Silva, P. R., Camara, C. A., Silva, G. S., Santos, F. A. R., & Silva, T. M. S. (2014). Chemical analysis and antioxidant potential of angico honey collected by stingless bee Jandaíra. *Journal of Chemistry, 5*, 1370–1379.

Sousa, J. M. B., Aquino, I. S., Magnani, M., Albuquerque, J. R., Santos, G. G., & Souza, L. S. (2013). Physicochemical aspects and sensory profile of stingless bee honeys from Seridó region, State of Rio Grande do Norte, Brazil. *Semina: Ciências Agrárias, 34*, 1763–1772.

Souza, B., Roubik, D., Barth, O., Heard, T., Enríquez, E., Carvalho, C., et al. (2006). Composition of stingless bee honey: setting quality standards. *Interciência, 31*, 867–875.

Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., & Scarponi, G. (2014). Determination of proline in honey: comparison between official methods, optimization and validation of the analytical methodology. *Food Chemistry, 150*, 477–481.

Vanhanen, L. P., Emmertz, A., & Savage, G. P. (2011). Mineral analysis of mono-floral New Zealand honey. *Food Chemistry, 128*, 236–240.

Vit, P. (2008). Review: valorization honey of stingless bees (Meliponini). *Brazilian Journal of Pharmaceutical Science, 50*, 20–28.

Yadata, D. (2014). Detection of the electrical conductivity and acidity of honey from different areas of Tepi. *Food Science and Technology, 2*, 59–63.