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Characterization of honey of stingless bees from the Brazilian semi-arid region

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ABSTRACT

The physicochemical characteristics of honey vary according to bee species, climate, region, period of collection, processing and storage. In this context, this work aimed to perform a comparative study of the physicochemical characteristics of *Melipona subnitida* and *M. fasciculata* honey collected at different periods and regions of the State of Piauí, Brazil. Twenty-nine honey samples were collected and evaluated by principal component analysis from physicochemical analysis data. Twenty-two percent of the parameters analyzed differed between species. Evaluating the collection period, the honey of *M. subnitida* and *M. fasciculata* presented differences among themselves. The study revealed a similarity between the physicochemical parameters of the honey of the two species of bees, in addition, the time was one of the determining factors in the formation of clusters.

1. Introduction

Brazil has a diversity of stingless bee species that represent economic and socioenvironmental importance in the different regions where they are distributed. In the Brazilian territory, there is a predominance of diverse climate and vegetation that makes possible the rational stingless bees beekeeping, as well as the production and commercialization of honey with different characteristics (Jaffé et al., 2015).

The Northeastern region of Brazil has the potential for honey production, mainly due to the diversified flora that guarantees the supply of nectar and pollen the whole year (Oliveira, Dias, Costa, Filgueira, & Sobrinho, 2012). In addition, in this region has been found of several species of bees belonging to the genus *Melipona* known to be the most productive and created by small farmers (Biluca, Braghini, Gonzaga, Costa, & Fett, 2016).

Meliponine honey is a concentrated solution of sugars, with a predominance of fructose and glucose (De Sousa et al., 2016). In composition is also found a range of minor constituents such as polyphenols, proteins, minerals, organic acids, aromatic compounds and pollen, which may vary with the origin of nectar, the entomological species, the edapho climatic conditions and the region of production (Shamsudin et al., 2019).

Considering the influence of different factors on honey composition and the scarcity of studies, there is a need to obtain information about the physicochemical characteristics of honeys produced by different species of bees. Due to the particularities of this product is necessary to know characteristics to evaluate the quality and identity of stingless bees honey. Mainly because Brazil does not have specific legislation (Carvalho et al., 2013).

Among the species of *Melipona* stand out the *Melipona subnitida* Duke (jandaíra), often created in the Northeast region (Carvalho, Koedam, & Imperatriz-Fonseca, 2014) and *M. fasciculata* Smith (tiúba) one of the most important species in honey production in the Brazilian Northeast (Holanda et al., 2015). These species present commercial value, mainly by the honey production, appreciated by the differentiated taste, conferred by their physicochemical characteristics and by the therapeutic properties (De Sousa et al., 2016).

In this context, the objective of this research was to perform a comparative study of the physicochemical aspects of the honeys produced by two species of stingless bees, *Melipona subnitida* and *M. fasciculata*, collected in the seasons and regions of Piauí State, northeastern Brazil, using statistical tools.

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2. Material and methods

2.1. Reagents and solvents

Methanol (Merck, Darmstadt, Germany) of HPLC grade. Water was supplied by a Milli-Q water purifier system from Millipore (Bedford, MA, USA), and used after filtration through a 0.45-μm pore sized membrane filter. Carboxylic acids standards were obtained from Sigma (St. Louis, MO, USA). The stock solution of ascorbic, tartaric, acetic, succinic, maleic, fumaric and citric acids (100 mg L^{-1}) was prepared in methanol–water (1:1). The stock solution was diluted to give different standard solutions. The determination of the carboxylic acids was performed as described by Santos, Santos, and Azevedo (2014). The solutions of the honey were prepared in a ratio of 1: 5 (honey: water), later filtered with PES syringe filters, model K18-230 ® and analyzed in the HPLC DAD.

2.2. Liquid chromatographic analysis

Chromatographic separation was performed on a high performance liquid chromatograph Agilent 1260 Infinity, coupled to a diode array detector (DAD), with a C18 reverse phase column (150 mm × 4.6 mm, 5 μm) at 30 The mobile phase composed of a solution of methanol and Mili-Q water (5:95, v / v), pH adjusted to 2.1 using hydrochloric acid, isocratic elution at 1.0 mL min^{-1} for 10 min. and injection volume of 20 μL. Samples and standards injections were performed in triplicate and the chromatograms obtained were recorded using Open Lab Software. The ascorbic acid, acetic acid. maleic acid, succinic acid, fumaric acid, citric acid, tartaric acid were monitored and quantified at wavelength of 215 nm.

2.3. Honey samples

Twenty-nine honey samples of *Melipona subnitida* and *M. fasciculata*, collected directly from the stingless bees beekeepers, in the municipalities of Murici dos Portelas, Cajueiro da Praia, Guadalupe and Parnaíba, located in the transition between cerrado and coastal vegetation, State of Piauí, Brazil. Aseptically the honey collected and stored in sterile plastic containers, transported in a refrigerated thermal box and sent of the Insects Studies Laboratory (INSECTA) of the Federal University of Recôncavo da Bahia, at the Center for Science, Agrarian, Environmental and Biological Sciences (CCAAB).

The samples of honey of both species (*M. fasciculata* and *M. subnitida*) were obtained in three distinct periods: E1 = season 1 (July 2015); E2 = season 2 (October 2015); and E3 = season 3 (March 2016). The normal climatic recorded for the capital of the State of Piauí varied as follows for the months of collection of honey samples: average temperature − 26.3 °C (March), 26.6 °C (July), 29.6 °C (October) and 27.4 °C (annual average); Relative humidity average − 85.1% (March), 69.4% (July), 56.2% (October) and 72.5% (annual average); average rainfall − 286.9 mm (March), 10.6 mm (July), 19.5 mm (October) and 1325.0 mm (annual average) (INMET, 2019).

2.4. Physicochemical analysis

The physicochemical characterization of the honey performed by determination of the parameters: moisture, reducing sugars and apparent sucrose, total soluble solids, free acidity and pH, hydroxymethylfurfural (HMF), diastase activity, and color, besides the determination of total phenolic compounds, total flavonoids and carboxylic acids. The analyses performed in triplicates.

2.4.1. Moisture

The moisture of the honey was recorded from a direct reading of the sample in a portable digital refractometer (Atago PAL-22S model), under ambient temperature and expressed as a percentage (ATAGO Co, 1988).

2.4.2. Reducing sugars and apparent sucrose

Reducing sugars and apparent sucrose were determined as the method described by Lane and Eynon (1934), with modifications of Marchini, Sodré, and Moreti (2004). For this, 2.5 g of the honey sample diluted in 50 mL of distilled water, 10 mL of this solution taken and diluted in 200 mL of distilled water, and the solution was titrated in 10 mL of the Fehling's reagent. Determination of the reducing sugar involved the reduction of Fehling's solution (alkaline copper) with a solution of reducing sugars of the honey. For the determination of sucrose, 10 mL of the initial solution (2.5 g of honey/50 mL distilled water) transferred to a 200 mL volumetric flask containing 20 mL of hydrochloric acid (0.75 mol L^{-1}) and placed in a 65° water bath. The sample then neutralized with sodium hydroxide (0.75 mol L^{-1}) and the flask volume filled with distilled water. The neutralized sample was subjected to Erlenmeyer titration containing 10 mL of Fehling's reagent.

2.4.3. Free acidity and pH

The free acidity quantified with titration of the honey sample with 0.1 mol L^{-1} sodium hydroxide. The pH was determined based on Association of Official Analysis Chemists (AOAC, 1990) using a digital potentiometer in a solution prepared with 10 g of honey sample diluted in 75 mL of distilled water.

2.4.4. Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural was determined according to the method AOAC (1990) based on absorbance reading in a spectrophotometer. An aliquot of 5.0 g of honey dissolved in 25 mL of distilled water and added to Carrez I solutions (0.5 mL) and Carrez II (0.5 mL), the solution filtered and the first 10 mL discarded. From the filtrate, the absorbance at 284 and 336 nm read with an aliquot of the solution filtered with 0.2% sodium bisulfite as blank. The HMF determined by the equation: HMF/100 g of honey = $(Abs_{284} - Abs_{336}) \times 14.97 \times 5$ g of the sample. The results expressed in milligram of the substance per kilogram of honey (mg kg^{-1}).

2.4.5. Diastase activity

Diastase activity was assessed by spectrophotometry according to the method of AOAC (2005), using a buffered solution of starch and honey, maintained in a water bath at 40 °C until an absorbance of less than 0.235 at a wavelength of 660 nm was obtained. The diastase activity was calculated using the time required for absorbance to reach the reading of 0.235 nm and expressed in Göthe degrees as the amount (mL) of 1% enzyme hydrolyzed starch, 1 g of honey in 1 h.

2.4.6. Total soluble solids

The total soluble solids were determined according to AOAC (2005) based on the reading of the honey sample in refractometer, model Abbe, at room temperature, expressed in ° Brix.

2.4.7. Color

The color classification of the honey performed by direct reading in a spectrophotometer in the length of 560 nm, using the glycerin as white and converted through the Pfund scale (Vidal & Fregosi, 1984).

2.4.8. Determination of total phenolic compounds

The content of total phenolic compounds determined by spectrophotometry using the Folin-Ciocalteu method described by Singleton, Orthofer, and Lamuela-Raventos (1999). Aliquots of 0.5 mL of honey solution (100 mg L^{-1}, prepared with distilled water) transferred into test tubes, adding 2.5 mL of Folin-Ciocalteu solution (10%) and 2 mL of sodium carbonate (75 g L^{-1}). The mixture was stirred and allowed to stand for 30 min at 40 °C. The absorbance measured in 760 nm using the spectrophotometer. To obtain the content of total phenolic compounds the calibration curve was defined using gallic acid as standard

with concentrations ranging from 10 to 80 mg L^{-1}. The results expressed in milligram equivalent of gallic acid per kilogram of the honey sample (mg EAG kg^{-1}).

2.4.9. Determination of total flavonoids

The content of total flavonoids determined by spectrophotometry, according to the colorimetric method with aluminum chloride (Woisky & Salatino, 1998). A solution of honey was prepared with distilled water, from which a 0.5 mL aliquot (1:10) was transferred to test tube (triplicate), adding 2.0 mL of distilled water and 0.15 mL of sodium nitrite (5%). After six minutes, 0.15 mL of aluminum chloride (10%) added, letting it stand for six minutes, followed by the addition of 2.0 mL of sodium hydroxide solution (4%) and distilled water to the final volume of 5.0 mL. The absorbance measured in 510 nm using the spectrophotometer. The results expressed in milligram equivalent of quercetin per kilogram of the honey sample (mg EQ Kg^{-1}).

2.5. Statistical analyses

Descriptive Statistics tools were used. The Mann-Whitney (*U* test) nonparametric test was used to compare the variables between species.

The results were also analyzed by the Principal component analysis (PCA), Biplot (Gabriel, 1971) and Ward's method cluster analysis (Ward, 1963) considering the general physicochemical characteristics of honey samples, by species, place and time of collection. Due to the large number of variables measured in different units, the analyses were performed based on mean-centered standardization.

A widely used criterion for determining the number of principal components is the Kaiser's criterion (Kaiser, 1960) which states that components with $\lambda i > 1$ represent sufficient portion of the total variation of the data. Another method that can be used to reinforce the decision based on the Kaiser's method is the plot scree or Cattell test (Cattell, 1966) which consists of observing the graph of the eigenvalues by the number of dimensions where the goal is to locate the point where the eigenvalues have a decreasing linear trend. The analyses were performed by STATISTICA 13.5 (TIBCO Software Inc., 2018).

3. Results and discussion

The results in relation to the physicochemical parameters evaluated in the honey of *M. fasciculata* and *M. subnitida* are shown in Table 1. It was found that 77.77% of the analysis performed did not differ significantly among the bee species studied. They differed only significantly in 22.22%, is highly significant at 1% for reducing sugars concentration and significant at 5% for the content of hydroxymethylfurfural, acetic acid, and color. The acetic acid associated with reducing sugars may influence the Maillard reaction, which consequently influences HMF and color. These parameters are associated with honey quality and can be influenced by external factors (temperature, humidity – collection and storage period) or microbial growth, with the two bee species being subjected to similar environmental conditions.

Reducing sugars influence the energy value of honey, characterizing it as an immediate source of energy (Peralta, Uetanabaro, & Lucchese, 2015). A high energy value can provide a medium with high osmotic pressure, reducing water activity (Aw), which may hinder the development of microorganisms and, consequently, favors longer shelf life of honey.

Svecová, Bordovská, Kalvachová, and Hajek (2015) reported that HMF content in honey varies according to the geographic origin as well as storage conditions. As for acetic acid, this may vary according to the composition of the nectar and the presence of microorganisms that use the sugars as an energy source, influencing the flavor and aroma (Sroka & Satora, 2017).

In general, the color ranged from white to amber (Table 1) and is certainly associated with the floral species and maturation level, according to Lacerda, Santos, Santos, Rodrigues, and Santos (2010). Evaluating different species of the genus *Melipona*, Silva, Alves, Fernandes, and Müllerd (2013) verified that they are statistically similar with respect to the color of the honey, whose characteristic is associated with the climatic conditions of the region and composition of the nectar.

Table 1
Median values of the physico-chemical variables of honey samples from *Melipona fasciculata* and *M. subnitida* from the semi-arid region of Brazil.

Variables	*M. fasciculata*	*M. subnitida*	U-test[1]	P value[2]
Moisture (%)	27.2 (26.4–27.9)	27 (27–27)	92	0.77^{NS}
pH	3.5 (3.4–3.7)	3.6 (3.3–3.9)	81	0.43 ^{NS}
Acidity (meq Kg^{-1})	23 (16–33)	28 (23–37)	70.5	0.21 ^{NS}
HMF (mg Kg^{-1})	2.0 (1.1–2.4)	4.4 (2.0–8.4)	52.5	0.04*
DA (Gothe)	0.04 (0.04–0.06)	0.05 (0.04–0.06)	98.5	1.00 ^{NS}
BRIX	72.1 (71.2–77.7)	71 (70–74)	72.5	0.24 ^{NS}
RS (%)	69 (67–71)	72 (71–76)	41	< 0.01**
SUC (%)	1.74 (0.74–2.78)	1 (1–3)	99	1.00 ^{NS}
TF (mg Kg^{-1})	202 (175–227)	122 (78–136)	72.5	0.24 ^{NS}
TFLV (mg Kg^{-1})	121.8 (78.3–135.7)	87 (61–105)	59.5	0.08 ^{NS}
ASC (mg Kg^{-1})	51.8 (32.9–75.9)	75 (34–117)	84	0.52 ^{NS}
ACE (mg Kg^{-1})	536 (230–1006)	3584 (1006–8079)	48.5	0.02*
MAL (mg Kg^{-1})	0.6 (0.0–0.6)	0.0 (0.0–0.9)	82	0.41 ^{NS}
SCA (mg Kg^{-1})	0.2 (0.1–0.6)	0.45 (0.14–0.50)	92.5	0.78 ^{NS}
FUM (mg Kg^{-1})	0.6 (0.5–2.7)	0.8 (0.0– 6.8)	98	0.97 ^{NS}
CTR (mg Kg^{-1})	0.0 (0.0–10.3)	0.0 (0.0–13)	99	1.00 ^{NS}
TR (mg Kg^{-1})	745 (688–10721)	9865 (760–16470)	62	0.10 ^{NS}
Color (Pfund)	0.5 (0.4–0.6)	0.33 (0.15–0.52)	52	0.04*

[1] Mann-Whitney U-test. Median and 25th and 75th percentiles in parentheses.
[2]NS – significant. * significant at 5%. ** significant at 1%;
(HMF: hydroxymethylsulfural. DA: diastase activity. BRIX: total soluble solids. RS: reducing sugars. SUC: apparent sucrose. TF: total phenols. TFLV: total flavonoids. ASC: ascorbic acid. ACE: acetic acid. MAL: maleic acid SCA succinic acid. FUM fumaric acid. CTR: citric acid. TR: tartaric acid).

Belay, Solomon, Bultossa, Adgaba, and Melaku (2013) reported significant variations of HMF content, among honey collected in different beehives, with no distinction of collection regions. De Sousa et al. (2016) observed a significant difference between *Melipona subnitida* and *M. scutellaris* honey in relation to the amount of reducing sugars and color, varying according to the entomological species and botanical origin. This observation was also reported by other authors when studding honey samples of different stingless bees (Biluca et al., 2016; Shamsudin et al., 2019).

Using the analysis of major components of the honey produced by *M. scutellaris* and *M. quadrifasciata* from the State of Bahia, Sodré, Carvalho, Fonseca, Oliveira, and Souza (2008) found that the separation of the samples occurred according to the type of thermal treatment applied (dehumidification process), not exclusively according to the entomological species.

The principal component analysis was found that the first component (PC1) explains 22.17% of the variation between the samples. This PC1 is formed by a positive combination of acidity, total phenolic compounds and fumaric acid. PC2, which explains 16.83% of the variation, is represented positively by pH and HMF and negatively by color (S1, Supplementary material). In order to explain satisfactorily the variability presented between the samples (Mardia, Keni, & Bibby, 1979) the need for a percentage generally higher than 70%.

In a study by Flanjak, Kenjerić, Bubalo, and Primorac (2015) with honey collected in different seasons of the year, the first two main components represented 83.3%, explained by the variation in color intensity, whose characteristic showed a relation with the botanical origin.

Based on the spatial distribution of the samples (Fig. 1), the Biplots,

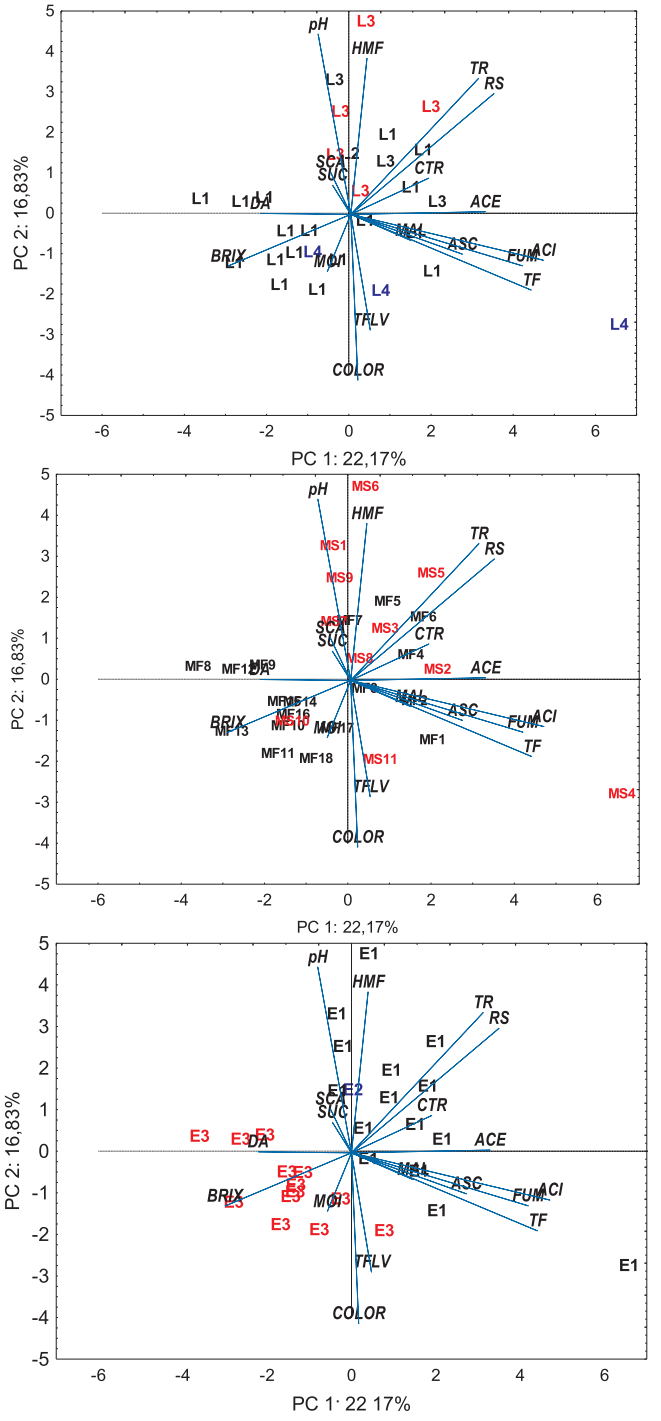

Fig. 1. Biplot of the 29 honey samples from stingless bees from the semi-arid region of Brazil, State of Piauí, based on 18 characteristics evaluated according to: (A) Location: L1 – Murici dos Portelas, L2 – Guadalupe, L3 – Parnaíba, L4 – Cajueiro da Praia; (B) Species: MF – Melipona fasciculata and MS – Melipona subnitida; and (C) Periods: E1 – July / 2015; E2 – October / 2015, E3 – March / 2016.

presented by local (Fig. 1A), bee species (Fig. 1B) and collection season (Fig. 1C) are observed. Comparing the three graphs (Fig. 1), a clear separation between the collection seasons was observed, influencing the physicochemical characteristics of the honey samples analyzed. Among the species of bees evaluated and the region of distribution, no distinction was make between these characteristics.

In general, honey samples collected at E1 and a high association of acidity, HMF, reducing sugars, total phenolic compounds, total flavonoids, ascorbic acid, acetic, maleic, fumaric, citric, tartaric and color, characterized E2. In contrast, E3 samples are negatively associated with moisture, pH, diastase activity; total soluble solids (° Brix), sucrose and succinic acid.

In Fig. 2 shows the dendrogram obtained from the Cluster analysis (Squared euclidean distance), considering the physical–chemical parameters of the honey. The formation of the three groups is observed. The first one is composed exclusively of samples that were collected in season 3 (E3) in the month of March, characterized by the rainy season. The second group corresponds to a sample from season 1 (month of July, dry season), belonging to the *M. subnitida* species, collected in Cajueiro da Praia municipality (near the coastal region). While the third group consists almost exclusively of E1 samples, produced by *M. subnitida* and *M. fasciculata* collected in July, in the municipality of Murici dos Portelas (located in the cerrado domain) and Parnaíba (near the coastal region), in addition to a sample of season 2, produced by *M. subnitida*, collected in the month of October in the municipality of Guadalupe (cerrado).

The distance between the groups was possibly due to the statistical differences observed between the samples for the HMF, reducing sugars, acetic acid and color parameters, observed in Table 1. In season 1, the honey produced by *M. subntida* and *M. fasciculata* presented higher concentrations of hydroxymethylfurfural, reducing sugars and acetic acid, with a predominance of lighter colors. In relation to these parameters, period 3 corresponds to samples with lower values and intensity of color tending to darker. The proximity of seasons 1 and 2 in the same group is due to the similarity in coloration, acidity and hydroxymethylfurfural values among honey samples.

The distancing of group 2 in relation to the others, confirms the results represented in the biplot (Fig. 1) and in S1 (Supplementary material) through which it was observed that the *M. subnitida* honey sample of season 1 presented a differentiated behavior due to the variables (acidity, fumaric acid, and total phenolic compounds).

The result of the present study corroborates with the observation of Carvalho et al. (2006) on the influence of the collection season, among other factors, on honey characteristics. During the year, changes in climatic conditions (temperature, relative humidity, insolation, etc.) characterize the seasons, which in turn influence the behavior and eating habits of bees (Oliveira et al., 2012). It is worth mentioning that the transition between the seasons is characterized by a change in the floristic landscape, which may be associated with the seasonal reduction of food for the bees, besides influencing the composition of the nectar and, consequently, the physical–chemical properties of honey (Carvalho et al., 2016).

Using cluster analysis, Moreti, Sodré, Marchini, and Otsuk (2009) observed that samples of honey collected in different periods were grouped in relation to the physical–chemical parameters evaluated, indicating the influence of the floral origin on the characteristics of the honey.

4. Conclusion

The analysis of samples of *Melipona subnitida* and *M. fasciculata* honey from the Brazilian semi-arid region showed similarities between the physicochemical parameters evaluated. The season of honey production was one of the determining factors in the formation of clusters. This data on honey of these species will complement information necessary for the future establishment of quality regulations for these products.

5. Compliance with ethical standards

The author Melissa ODA Souza declares that she has no conflict of interest

The author Rosane da S. Sant'ana declares that she has no conflict of interest.

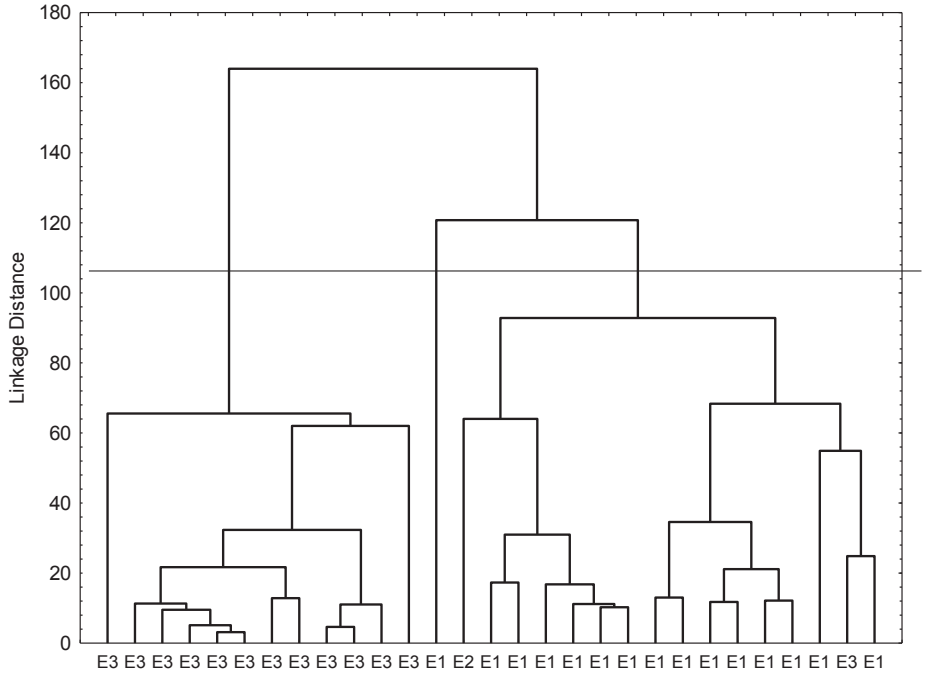

Fig. 2. Dendrogram obtained by cluster analysis, using the mean Euclidean distance and the Ward method for three collection times (E1, E2, E3) of honeys produced by two species of *Melipona* (*Melipona subnitida* and *Melipona fasciculata*) from the semi-arid region of Brazil, considering 18 characteristics evaluated.

The author Carlos Alfredo L. de Carvalho that he has no conflict of interest

The author Bruno de A. Souza declares that she has no conflict of interest

The author Fabio de Souza Dias declares that he has no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.127041.

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