



## Physical-Chemical Parameters of Honey of Stingless Bee (Hymenoptera: Apidae)

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors ASN, LCM and CALC designed the study and interpreted the data. Authors RAO performed statistical analysis. Authors ASN, CALC, DFDA and TAS participated in study conduction and data interpretation. Author ASN drafted the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

The objective of this study was to evaluate the physico-chemical quality of honeys samples of stingless bee (Meliponinae). Physico-chemical analyses were: moisture, color, pH, acidity, electrical conductivity, hydroxymethylfurfural, ashes, diastasic activity, reducing sugars, and sucrose. For the parameters hydroxymethylfurfural, ashes, reducing sugars and sucrose, the samples complied with the requirements of the current Brazilian legislation for honey. The diastasic activity and moisture parameters did not meet thresholds in the legislation, pointing to the need to create a specific legislation for honey of stingless bees, given the large number of species and the different characteristics of the honey that they produce.

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## 1. INTRODUCTION

The floral honey of stingless bee (Meliponinae) is a food produced from the nectar that bees collect, transform, combine with specific substances of their own, store and let it mature inside pots in the colonies [1]. The amount of honey produced by stingless bee is smaller than that produced by *Apis mellifera* bees; however, its differentiated sensory characteristics make it attractive and aggregate higher market values to it [2-4].

Honey contains a complex mixture of carbohydrates, mainly glucose and fructose, in addition to enzymes, amino acids, organic acids, minerals, aromatic substances, vitamins, pigments, beeswax, and pollen that contribute to its color, smell and flavor [5-6]. Water is the second largest component in honey composition and its content can be influenced by the botanical origin of the nectar, climatic conditions and the handling during the harvesting of the honey. The water content is considered one of the most important features as it affects several characteristics of honey, such as viscosity, specific weight, maturity, flavor and crystallization [7].

The purpose in determining the physical-chemical parameters of honeys is to compare the results obtained with the standards prescribed by international and national institutions to ensure product quality, both for domestic consumption and exports, protecting the consumer from purchasing an adulterated product [2,8].

Obtaining physical-chemical parameters of honey is important not only for its characterization [9], but it is also essential to ensure product quality on the market. Because of the diversity of Meliponinae species and little information regarding the characteristics of the honey produced by this bee species, determining the physical-chemical parameters of the honey is crucial for the process of creating specific legislation for stingless bee honey in Brazil. Therefore, efforts to characterize the physical-chemical parameters of stingless bee honey are important to generate information on the quality standard of this product. In this study, we analyzed samples of Meliponinae honey regarding its physical-chemical parameters.

## 2. MATERIALS AND METHODS

The study was carried out in the municipality of Guaraqueçaba, Paraná State, Brazil, (25°17'15"S; 48°19'1"W, altitude 20 m) (Fig. 1) where there were already structured apiaries of Meliponinae bee. The stingless bee culture in Paraná State is performed by small producers with great potential for this type of honey activity.

The samples, consisting of 250 mL of honey, were obtained directly from local beekeepers according to the peak of honey production in the region. We collected 30 samples the species *Cephalotrigona capitata* (Smith, 1854), *Melipona bicolor* (Lepelletier, 1836), *Melipona marginata* (Lepelletier, 1836), *Melipona mondury* (Smith, 1863), *Melipona quadrifasciata* (Lepelletier, 1936), *Melipona scutellaris* (Latreille, 1811), *Melipona seminigra* (Friese, 1903), *Scaptotrigona xanthotricha* (Moure, 1950) and *Tetragonisca angustula* (Latreille, 1811).

The samples were collected with disposable syringes, afterwards, they were placed in plastic containers properly identified, packed in thermal boxes with ice and forwarded to the laboratory of useful insects of the Department of Entomology and Acarology at the "Luiz de Queiroz" College of Agriculture, University of São Paulo, Piracicaba, São Paulo State, Brazil, where they were stored under refrigeration ( $\approx 8^{\circ}\text{C}$ ). The physico-chemical analyzes were performed logos after collection of samples not exceeding the period of 30 days. We assessed the following physical-chemical parameters:

### 2.1 Moisture

Sample moisture was recorded using a portable digital refractometer (Atago PAL-Moisture 4573) with the range expressed in percentage (%) [10].

### 2.2 Color

To determine the color of the honey samples, we used a spectrophotometer operating within the absorbance range of 560 nm, in a 1 cm quartz cell and the white was provided by pure glycerin. The colors were classified in the Pfund scale [11] according to the values recorded.

### 2.3 pH and Acidity

The pH and acidity were determined in accordance with the methodology adopted by

Moraes and Teixeira [12]. The pH value was determined using a solution containing 10 g of honey dissolved in 75 mL of distilled water, homogenized and subjected to reading in a pH meter.

Acidity was obtained by performing the neutralization of acidic solution of honey (10 g of honey dissolved in 75mL of distilled water) using a sodium hydroxide solution 0.1 N and 1% of phenolphthalein indicator solution until a pink color was obtained for 10 seconds. The reading of the sodium hydroxide volume 0.1 N required in the titration was recorded. The result is expressed in meq kg<sup>-1</sup> using the equation:

Acidity = V (NAOH) x PA, where:

V (NAOH) = NAOH volume (mL)

PA = sample weight (g)

## 2.4 Electrical Conductivity

The electrical conductivity was measured using an HI8820N conductivity meter. A solution containing 10 g of honey dissolved in 50 mL of distilled water was used for the readings ( $\mu\text{S cm}^{-1}$ ) of each sample [13].

## 2.5 Hydroxymethylfurfural (HMF)

The determination of hydroxymethylfurfural (HMF) was based on the readings in different absorbance scales (284 and 336 nm wavelengths) in a spectrophotometer [14]. The HMF is expressed in mg kg<sup>-1</sup> in the equation:

HMF = (A284 – A336) x 149.7 x 5 x D/W, where:

A284 = absorbance at 284 nm

A336 = absorbance at 336 nm

D = dilution factor, if necessary

W = weight of honey sample (g)

## 2.6 Ashes

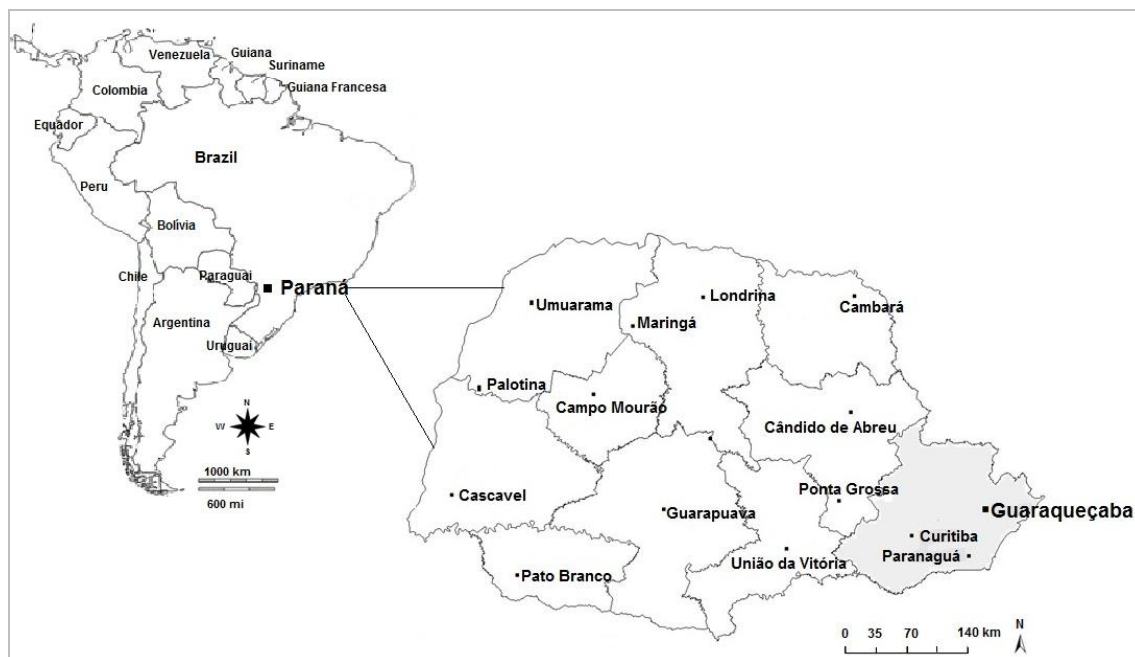
The ash content in the samples was determined by performing the incineration of 1 g of honey (crucible) in a muffle at 550°C for 3 hours [15]. The result is expressed in percentage (%) according to the equation:

Ashes = [(m1-m2)/m3] x 100, where:

m1 = weight of the crucible with the ash (g).

m2 = weight of the crucible (g)

m3 = weight of honey sample (g)



**Fig. 1. Location of the municipality of Guaraqueçaba, Paraná State, Brazil (25°17'15"S; 48°19'1"W, altitude 20 m) where the honey samples were obtained**

## 2.7 Diastasic Activity

The diastasic activity was determined with the A.O.A.C. [14] methodology. A solution of buffered honey of starch-honey was kept in water bath at 40°C for a period necessary to obtain the specific endpoint (in a spectrophotometer operating within an absorbance range of 660 nm). The diastasic activity corresponds to the number in the Gothe scale that is obtained by dividing 300 by the time in minutes to obtain an absorbance lower than 0.235 nm (specific endpoint).

## 2.8 Reducing Sugars and Sucrose

Reducing sugars and sucrose were determined according to the method used by Lane and Eynon [16] and Copersucar [17] modified by Marchini et al. [8].

## 2.9 Analysis of Variance (ANOVA)

The assumptions of the analysis of variance were verified by means of optimal processing family of Box-Cox [18] and the Hartley test [19] was applied to check the homogeneity of the variances. The delineation was completely randomized with treatments consisting of honey samples (with three repetitions) of the species *C. capitata*, *M. bicolor*, *M. marginata*, *M. mondury*, *M. quadrifasciata*, *M. scutellaris*, *M. seminigra*, *S. xanthotricha* and *T. angustula* that were analyzed separately, that is, only one factor.

After verifying the ANOVA assumptions, the F test ( $p < 0.05$ ) was applied to check possible differences between treatments. The Tukey test was applied ( $p < 0.05$ ) for the variables that showed a difference. The data were analyzed in the computer program Sas/Stat [20].

## 3. RESULTS AND DISCUSSION

### 3.1 Moisture

We observed a statistical difference in the average values of moisture between the honey samples according to bee species (Table 1). Among the samples analyzed, the average moisture ranged from 25.99% (*M. bicolor*) to 36.89% (*M. quadrifasciata*). The threshold for stingless bee honey is 35% [21].

The moisture of the honey samples was higher than the threshold for Brazilian and international standards [22] that allows a maximum of 20% for

honey of *Apis mellifera*. The honey from stingless bees shows higher moisture when compared to *Apis mellifera* honey, because high hygroscopicity is a basic characteristic of Meliponinae honey. This high hygroscopic behavior is preserved even if the environment where these bees live have low humidity [23].

In samples of stingless bee honey from different states in Brazil, Pereira [24] observed no statistical difference in comparison with different species of honeybees and local species, and the moisture value exceeded the threshold established by the current Brazilian legislation. This fact, according to the author, shows that the water content in honey is an intrinsic characteristic of bee species with no significant influence of the producing region of origin. Studies on Meliponinae [4,23,25-27] show a variation of moisture in honey according to bee species, and this variation is above the threshold allowed by current legislation, however, within the limits established by Villas-Bôas and Malaspina [21].

The higher moisture content characteristic of honey from stingless bees can be influenced by the air relative humidity and, possibly, by the different containers that beekeepers used for the storage of stingless bee honey (made of wax and resin) and those used for *Apis mellifera* honey (made of pure wax) [25]. Moisture is one of the most relevant characteristics of honey, because it influences viscosity, specific weight, maturation, crystallization, taste and conservation of this food. In addition, after extraction, moisture changes according to storage due to water transfer [28]. Moisture levels within the reference standards enhance the shelf life of the product, since it provides an unfavorable condition for microbial development [29].

A high water content in honey facilitates the proliferation of yeasts, causing a fermentation process, which makes the product unfit for human consumption and hinders its marketing [30]. Despite containing various bacteriostatic and bactericidal properties, honey is not considered sterile and is, therefore, susceptible to contamination [31] by filamentous fungi, yeasts and bacteria. The high moisture in stingless bee honey and the aforementioned factors reinforce the need to store this product in refrigerated chambers to avoid degradation or even modification of the physical-chemical properties of honey, thereby ensuring a product with quality to the consumer.

**Table 1. Average values of the physical parameters-chemicals of the honeys of Meliponinae, from Guaraqueçaba, Paraná, of State, Brazil, being**

Species	Physical-chemical parameters										
	n	Moi. (%)	Color	pH	Acidity (meq kg <sup>-1</sup> )	Cond. (µS cm <sup>-1</sup> )	HMF (mg kg <sup>-1</sup> )	Ashes (%)	DA (Gothe)	RA (%)	Suc (%)
C.ca	2	32.10 ab	0.925 a	3.04 ed	34.33 ab	728.34 a	35.40 ab	0.195 a	0.18 ab	75.21 a	0.36 a
M.bi	4	36.18 a	0.705 ab	3.32 bcde	48.58 a	535.67 a	31.58 b	0.185 a	0.12 a	68.43 b	0.57 a
M.ma	3	32.44 ab	0.700 ab	2.93 e	22.55 b	624.00 a	48.09 ab	0.140 a	0.19 ab	67.39 b	0.85 a
M.mo	3	29.97 ab	0.790 ab	3.50 bcd	37.89 ab	514.78 a	51.38 ab	0.246 a	0.20 ab	67.77 b	0.85 a
M.qd	4	36.89 a	0.640 ab	3.18 cde	35.00 ab	576.33 a	42.63 ab	0.160 a	0.13 a	71.63 ab	0.85 a
M.sc	4	33.98 ab	0.562 ab	3.48 cde	27.25 b	540.40 a	40.86 ab	0.157 a	0.11 a	66.41 b	0.70 a
M.se	3	27.85 ab	0.723 ab	3.72 ab	30.44 b	548.22 a	29.50 b	0.216 a	0.20 ab	69.12 ab	1.61 a
S.xa	3	29.84 ab	0.600 ab	3.58 abc	28.78 b	621.89 ab	58.27 a	0.210 a	0.62 b	66.32 b	1.22 a
T.an	4	25.99 b	0.302 b	4.08 a	27.00 b	722.42 a	27.99 b	0.327 a	22.43 c	66.75 b	0.82 a

Means followed by the same letter in the column do not differ statistically ( $p>0.05$ ); C.ca (*Cephalotrigona capitata*); M.bi (*Melipona bicolor*); M.ma (*Melipona marginata*); M.mo (*Melipona mondury*); M.qd (*Melipona quadrifasciata*); M.sc (*Melipona scutellaris*); M. se (*Melipona seminigra*); S.xa (*Scaptotrigona xanthotricha*); T.an (*Tetragonisca angustula*); n (number of samples); Moi (moisture); cond. (electrical conductivity); HMF (hydroxymethylfurfural); DA (diastásica activity); RA (reducing sugars) and Suc (sucrose)

### 3.2 Color

The color of the honey samples ranged from light amber (0.302 nm) to dark amber (1.225 nm). There was significant difference in the honey color of *Tetragonisca angustula* when compared with the other species (Table 1). The average values for color observed for the honey samples analyzed are in compliance with the current regulations that may vary from the white-water to dark amber [22]. The honey color depends almost exclusively on floral origin. Generally, dark-colored honey has more minerals than light-colored honey. Studies show that darker honeys may have four to six times more minerals than light-colored honeys, especially manganese, potassium, sodium and iron [32].

### 3.3 pH and Acidity

The pH and acidity parameters showed statistical differences among the species. For pH, there was a variation in the average value from 2.93 (*M. marginata*) to 4.08 (*T. angustula*). Acidity had an average value ranging from 22.55 to 48.58 meq kg<sup>-1</sup> (Table 1). Brasil [22] establishes a maximum of 50 meq kg<sup>-1</sup>; Villas-Bôas and Malaspina [25] suggested the maximum limit for stingless bee honey of 85 meq kg<sup>-1</sup>, therefore, the average values of acidity of the honey samples are within the limits established. However, the pH values are not standardized by national or international legislations.

The pH is a physical-chemical parameter associated with the microbial development in any food. In the specific case of honey, the pH values recorded by several authors [4,23,24,33] range, in general, between 3.3 and 4.7. These values prevent the development of microorganisms that require neutral or basic pH values, significantly limiting the spectrum of potentially contaminating microorganisms [34]. The pH value of honey may also be influenced by the nectar pH, in addition to differences in the soil composition or the use of plant species for the final composition of honey [35]. For Evangelista-Rodrigues et al. [36], the difference of pH values among the honeys from different species of stingless bees, even when produced in the same region, could be explained by the mandibular substances that are added to nectar during transport to the colony.

Honey samples analyzed by Oliveira et al. [37] showed acidity values of 69.06 meq kg<sup>-1</sup> for *T. angustula* and a range from 92.09 to 102.10 meq kg<sup>-1</sup> for *S. depilis* confronting the values required

in legislation. According to Vit et al. [38], honeys of *T. angustula* showed higher acidity when compared to *Melipona* honey. In this study, comparing *Melipona* with *T. angustula* honey, we observed that *Melipona* honey showed higher acidity (Table 1).

The broad range of acidity values found for the stingless bee honey samples studied (Table 1) can also be verified in the study conducted by Cortopassi-Laurino and Gelli [39], who observed a variation in the average value for acidity from 30.0 to 90.0 meq kg<sup>-1</sup> in analysis of honeys from different Meliponinae species. Souza et al. [27] compiled results of 152 samples of honeys of various Meliponinae species from eight countries in the Americas and found values ranging from 5.9 to 109.0 meq kg<sup>-1</sup>. Stingless bee honey usually features high acidity in comparison to *A. mellifera* honey. This feature can be detected by the taste, constituting one of the parameters that defines consumers' preference for stingless bee honey. However, acidity can be directly related to the maturation state of honey, and it increases with fermentation [40].

### 3.4 Electrical Conductivity

The average values of electrical conductivity observed in the honey samples ranged from 514.78 to 728.34 μS cm<sup>-1</sup>. Only the honey from *S. xanthotricha* differed from the other honey samples in this parameter (Table 1). The electrical conductivity can be used to identify the botanical origin of honey [41]. There are no values for conductivity established by the current Brazilian legislation. Electrical conductivity is closely related to the concentration of minerals, organic acids and proteins, and it is a parameter that shows great variability depending on the floral source of honey. As electrical conductivity varies with the botanical origin [42], floral honey must have conductivity values below 800 μS cm<sup>-1</sup>, while values for honeydew honey must be greater than 800 μS cm<sup>-1</sup> [43].

### 3.5 Hydroxymethylfurfural (HMF)

For the studied species, we observed a statistical difference for the HMF parameter, and the highest average values were verified in the honey samples of *S. xanthotricha* (58.27 mg kg<sup>-1</sup>) and *M. mondury* (51.38 mg kg<sup>-1</sup>) (Table 1). This parameter shows values within the range established by the current legislation [22] that allows a maximum of 60 mg kg<sup>-1</sup>.

The hydroxymethylfurfural is a chemical compound formed by the reaction of certain sugars with acids and it is used as an indicator of honey quality [8] regarding product adulteration or storage in inadequate conditions. When honey is subjected to high temperatures, inadequate storage conditions or addition of invert sugar, the HMF content increases and it is one of the most common degrading products in honey, indicating its aging [31]. In honey of *M. fasciculata*, Holanda et al. [44] found that the HMF content in the samples ranged from 5.44 to 70.79 mg kg<sup>-1</sup>. The authors observed that 92.86% of the samples analyzed had HMF values below the maximum values established by national (60 mg kg<sup>-1</sup>) and international legislation (80 mg kg<sup>-1</sup>, tropical countries) for honey. The HMF content found in honey samples of other *Melipona* species showed low values, as in *M. asilvai* with 0.52-7.93 mg kg<sup>-1</sup> [26] and *M. mandacaia* with 5.79 mg kg<sup>-1</sup> [23], different from results found in this study that showed higher levels.

There is a wide variation in the range of HMF content in stingless bee honey; however, there is no definition whether human exposure to HMF poses a potential risk to health. According to Louise et al. [45], the following points are relevant to discussion on the subject: high HMF concentrations, its cytotoxicity, irritation to the eyes, upper respiratory tract, skin, and mucous membranes.

### 3.6 Ashes

The ash content indicates the amount of minerals found in honey, which is influenced by the botanical origin of the nectar. The honey samples assessed showed no statistical difference among the species (Table 1). The honeys classified as light amber showed lower ash content ranging from 0.1 to 0.2%, while the dark amber honeys have contents equal to or greater than 0.3%.

The ash content is related to honey color, that is, the darker the honey color, the higher its ash content [46]. For Chaves et al. [25], the ash content in honey denotes the amount of minerals in the product, while the mineral content is related to the soil type. The ash content in honey is usually reduced and depends on the nectar composition of plants used in its production [47].

The stingless bee honey must have a lower ash content since it shows a light color as a differentiating feature [25]. The light color allows the stingless bee honey to reach high prices on

the market, since, in most cases, the consumer chooses the product just by appearance [27]. Honey stingless bee samples collected in Rio Grande do Norte State, Brazil, showed ash contents ranging from 0.1 to 1.1% [48], as follows: *Frieseomellita flavicornis* (0.30%), *M. compressipes fasciculata* (0.10%), *M. scutellaris* (0.10%), *M. subnitida* (0.20%), *M. quadrifasciata* (0.58%), *M. quinquefasciata* (0.10%), *Nanotrigona testaceicornis* (0.60%) and *Scaptotrigona* sp. (0.30%). These results, as well as the results in this study, show the low ash content in honey samples. For this parameter, all samples are within the range permitted by the legislation.

### 3.7 Diastasic Activity

The diastasic activity of the honey samples studied ranged between 0.11 and 22.43 (Gothe scale) and showed a statistical difference among species (Table 1). We observed that 88.88% of the samples are below the minimum threshold (8 in the Gothe scale) established by the Brazilian legislation [22].

Honey samples of stingless bees analyzed by Vit and Pulcini [49], also displayed most values for the diastasic activity below the minimum required: *T. angustula*, 16.50-35.60; *M. favosa favosa*, 2.60-3.50; *Nanotrigona* sp., 8.70; *M. compressipes*, 2.60-3.00; *M. lateralis*, 2.60-3.00; *M. paraensis*, 2.60-3.00; *Frieseomellita* sp., 6.60-13.70; *M. eburnean*, 3.40; *M. crinita*, 3.00 and *Scaptotrigona* sp., 2.60 (Goth scale).

The diastasic activity tends to decrease along the time in honeys of *Apis* [50]. Stramm [51] observed that the honey samples of *M. subnitida* showed moisture and diastasic activity outside the limits prescribed by the Brazilian legislation. Furthermore, parameters more sensitive to changes due to storage were the HMF content, free acidity, electrical conductivity for honey of *M. subnitida*. For honey of *M. fasciculata*, Holanda et al. [44] also found that the values of diastasic activity and HMF contents were below the minimum limits established by the Brazilian legislation.

### 3.8 Reducing Sugars and Sucrose

The averages of reducing sugars are within the standards established by Villas-Bôas and Malaspina [21], which indicate an acceptable minimum of 50%, as well as by the current legislation [22] that establishes a minimum of

65%. Among the samples analyzed, the average of sugar contents ranged from 66.12 to 75.21%. The sucrose content showed a maximum value of 1.61%, and the maximum acceptable is 6% for sucrose. The honey samples differed statistically according to the bee species for reducing sugars and no difference was observed for sucrose (Table 1).

Stingless honey has lower sugar content and normally fructose is prevalent, which is one of the factors responsible for sweetness of honey and its hygroscopicity [52]. The taste of stingless bee honey is influenced by the low sugar content and the acidic pH, which shows a clear preference of consumers for this type of honey. Taking into account the composition of stingless bee honey, mainly in terms of the parameters of reducing sugars that are lower and high moisture compared to honey of *Apis*, this product can undergo fermentation quickly if not properly stored after the harvesting.

According to Azeredo et al. [53], the high sucrose content means, in most cases, a premature harvesting of honey, that is, a product in which sucrose was not thoroughly transformed into glucose and fructose by the action of the invertase enzyme. In General, the sucrose content does not exceed 8%. Silva et al. [54] evaluated the concentration of sugars in honey samples stored in different packaging in terms of length of storage period. The authors found an increase of reducing sugars in honey along the storage period. This increase is probably attributed to the transformation of sucrose into glucose and fructose caused by the activity of the enzyme invertase, since the inactivation of this enzyme occurs by heating the honey. The sucrose content is also important to know whether the bees fed on sugar or if the honey was adulterated by direct addition of sucrose [55].

#### 4. CONCLUSION

The parameters reducing sugars, sucrose, hydroxymethylfurfural and ash in the honey samples of stingless bees comply with the requirements of the current Brazilian legislation. The parameters of the diastasic activity and moisture do not comply with the limits. This fact points to the necessity of creating a specific legislation for stingless bee honey, given the large number of bee species and the different characteristics of the honey that they produce.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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