



RESEARCH ARTICLE - BEES

Quality of Honey Produced by Four Species Of Stingless Bees in the Central Region of the State of Tocantins

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Abstract

Meliponiculture, the rational breeding of native stingless bees, is considered an excellent sustainable alternative to assist in the pollination process and is an economically viable activity. In the *cerrado* of Tocantins, the meliponine species that stand out most due to their wide distribution are: *Scaptotrigona tubiba*, *Melipona fasciculata*, *M. rufiventris* and *Tetragonisca angustula*. The bibliographic collection about these species is still little explored, hence there is a need for research to deepen the existing knowledge in the area. For this reason, the aim of this study was: a) to quantify the honey production of four meliponine species: *T. angustula*, *M. fasciculata*, *M. rufiventris*, and *S. tubiba*; b) to determine the physicochemical characteristics of the product; c) measure the biological parameters of the colony and d) evaluate the profile and sensory acceptance of honey in the municipalities, Palmas and Miracema, in the Tocantins. The study evaluated the biological parameters of the colony, honey production, and physicochemical analysis. The highest honey production came from the species *T. angustula* in the two collections for the municipality of Palmas. For Miracema, the species *S. tubiba* and *M. fasciculata* were evaluated, respectively. The physicochemical parameters evaluated fit the norms assigned to honey quality control. Results showed that honey from *M. fasciculata* was the sensory profile that obtained the best average among the characteristics observed in the study. There was a positive and negative correlation between the biological parameters, with a significant difference only between the characters' height and diameter of the honey pot.

Introduction

Stingless bees play a key role in various ecosystems, and they have been standing out as the primary agent for pollinating many native and cultivated plant species. They also ensure the maintenance of genetic variability, productivity, and the quality of many fruits (Bartelli & Nogueira-Ferreira, 2014; Roubik, 2018a).

These bees can be found in urban environments as long as there are favorable conditions for their survival. However, their ability to succeed in urban environments will depend on several factors, such as food availability (e.g., nectar, pollen, and water) and nesting resources (e.g., mud, oil, resin), as

well as other intrinsic factors related to the geographical distribution of each species (Souza et al., 2005; Antonini et al., 2012; Singh, 2016).

Although almost three hundred species of native bees are known in Brazil, only a few are properly studied. Stingless bees of the genus *Meliponini* stand out for their honey production (Pereira et al., 2017). However, some species, for example, *Meliponini rufiventris*, despite being endemic to the *Cerrado* biome, are in danger due to devastating anthropic practices (Silveira et al., 2018).

Stingless bees have a limited honey production compared to *Apis mellifera* (Zuccato et al., 2017; Shadan et al., 2018). The limitation is due to various factors, such as the



absence of standards of quality control, limited knowledge in the field, and low levels of industrial production. However, despite this limitation in the industry, stingless bee honey is available in traditional markets, and it has a significantly higher price than the honey of *A. mellifera* (Sekuan Wei & Ghoshalb et al., 2018).

The composition of bee honey depends on the nectar and the physiology of the species that produces it, providing its physical, chemical, biochemical, and sensory characteristics. In addition to climatic and soil conditions, handling practices in processing also interfere with physical-chemical properties, product quality, and consumer preference (Araújo et al., 2017).

Therefore, achieving the honey physicochemical parameters becomes essential not only for the characterization of the diversity of meliponini species but also to ensure the quality of the product on the market. (Zielinski et al., 2014; Nascimento et al., 2015).

In addition, to the knowledge we already have on the types of honey, it is also necessary to understand the relevant characteristics of each species of bee to meet their needs. For example, characteristics of harvesting, food storage by bees, the nest structure, and the sanity of colonies (Alves & Imperatriz-Fonseca, 2010; Pereira et al., 2011).

Therefore, the objective of this study was: a) to quantify the honey production of four species of meliponines: *T. angustula*, *M. fasciculata*, *M. rufiventris*, and *S. tubiba*; b) to determine the physical and chemical characteristics of the product; c) to assess the biological parameters of the colony, and d) to evaluate the profile and sensory acceptance of the honey from stingless bees in two municipalities in the Tocantins, Palmas and Miracema, as previously described.

Material and Methods

Study Area and Stingless Bee Species

The hives used in this study were situated in two municipalities of Tocantins: Palmas and Miracema, 90.5 km away from each other. Thus, we performed the procedures for honey harvesting in the respective cities. The physical-chemical and entomological evaluations were conducted at the Laboratory of Production, Food Technology, and Entomology, in the Agrarian Sciences Complex of the State University of Tocantins, in the municipality of Palmas.

We used four species of stingless bees in the study: *Melipona rufiventris*, *Scaptotrigona tubiba*, *Melipona fasciculata* and *Tetragonisca angustula*, popularly known as: *uruçu amarela*, *tubi*, *tiúba* and *jatai*, these are the species found most frequently in Tocantins *Cerrado* (a savanna like vegetation). These species were populated by processes of divisions and transfers of colonies.

Monitoring of the Bee Colonies

For the monitoring of the colonies, four in each municipality – in the same locality, referring to the species mentioned above a review form was used with the following observation parameters: water supply, hive productivity, Batum, nest development, and general needs. During the study, the colonies of both municipalities were monitored weekly. We made the water supply using disposable cups (50 ml) with stones (previously cleaned) placed in the bottom to prevent the melyphs from drowning. We observed the productivity of the hives by removing the lid from the boxes and visually diagnosing any products left by the melyphs. In addition, the Batum was monitored by its presence.

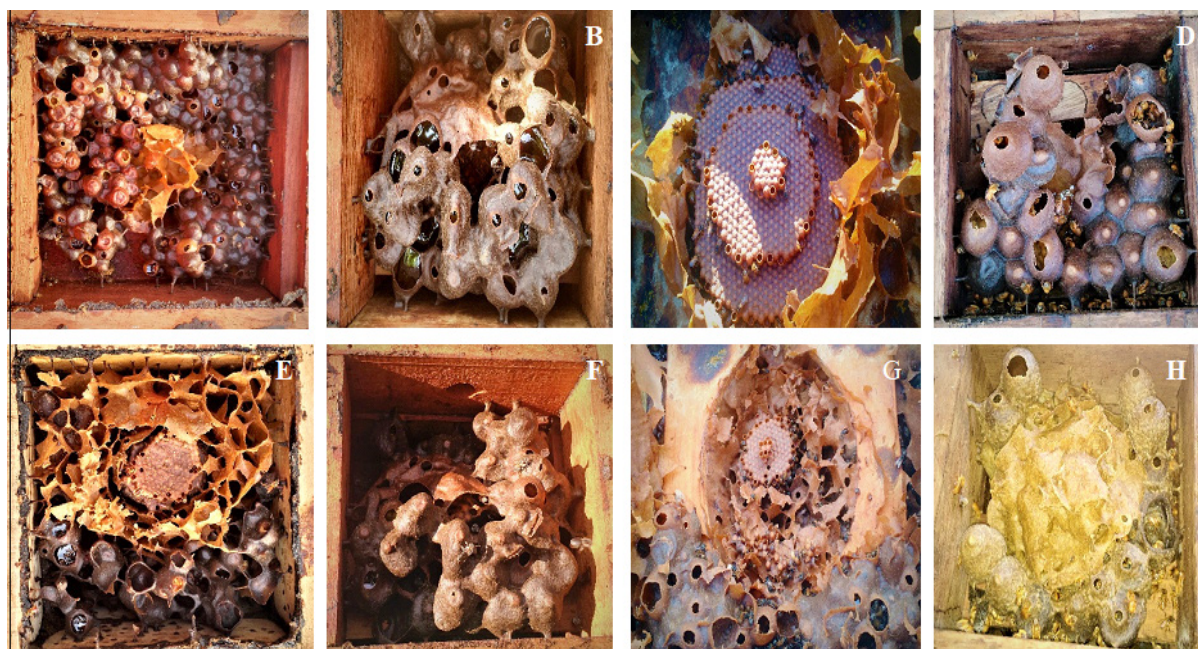


Fig 1. Bee Colonies of the species: *Tetragonisca angustula* (A and E), *Melipona fasciculata* (B and F), *Scaptotrigona tubiba* (C and G) and *Melipona rufiventris* (D and H) in the municipalities of Palmas and Miracema, respectively.

The development of the nest was carried out visually by observing the following characteristics: the presence of the queen and its sanity; the space occupied by the chicks in the tenements; the number of worker bees and the presence of drones in the hive. Finally, we allocated space for observations not previously accounted for, which could interfere with each species' honey production. Images of the colonies of each species were taken in the same monitoring week in both locations, Palmas and Miracema (Fig 1).

Experimental Design

We used a completely randomized factorial (2x2x4) experimental design. The first factor corresponded to the municipalities: Palmas and Miracema, the second to harvests (first and second), and the third corresponded to stingless bees: *M. rufiventris*, *M. fasciculata*, *T. angustula*, and *S. tubiba*. Each treatment had three boxes with nest and over nest considering a repetition.

The harvest interval was thirty-six days with the first one in September. The cities were chosen for their proximity and environment similarity, and the modular boxes were selected for the ease of handling and observation of the sanity parameters of the colonies exemplified in the study.

Evaluation of Honey Production

The period defined for this study was around 7a.m. and 5p.m. These times were chosen so that the honey was first and second harvested with less interference regarding avoiding wear of the colonies. Thus, avoiding the hottest times of the day. The boxes were handled as little as possible, using the syringe method with a capacity of 20ml.

The honey was collected following hygiene protocols, avoiding possible contaminants, and using appropriate materials at the time of harvest and storage. After this process, the samples were kept at a temperature of 10°C to maintain the physicochemical characteristics, until the moment they were taken to the Food Technology Laboratory of the Agricultural Sciences Complex of the State University of Tocantins, where the evaluations recommended in this study.

Evaluation of Biometric Parameters

The colony biological parameters evaluated in the four species were: diameter and height of brood cells, height and diameter of honey pots, the weight of pollen mass deposited in closed pots, and height of pollen pots. Both assessments were carried out in the same weekly period and in the morning, according to Brito et al. (2013), adapted.

For the evaluations of space occupied by the honey pots, height and diameter, and height of the pollen pot, the measurement was carried out directly using a manual caliper, with a measurement range of 0.01mm – 150mm and an error of 0.03mm. As for the other parameters, a number 60 sewing thread was used so that these cells would not be damaged. Finally, to quantify the mass of the pollen pots, an analytical balance was used.

Evaluation of Physical-Chemical Parameters

We determined the physicochemical parameters following the Brazilian legislation, which includes the physicochemical parameters of honey from *Apis mellifera* (Azevedo & Beser, 2000)

All physicochemical parameters were determined in triplicate according to the standards of the International Honey Commission (IHC, 1997) and the Official Analysis Methods of the Association of Official Analytical Chemists (with some modifications).

The determination of moisture and total soluble solids was performed by the refractometric method, using a portable manual refractometer, model RT90 ATC. The equipment was calibrated with distilled water. The samples had a mathematical correction, an addition of 0.00023, for each degree Celsius higher than the established, 20°C ambient, according to Brazilian legislation (Brasil, 2000).

We determined the pH according to the methodology adopted by Moraes and Teixeira (1998). The method is based on simple titration, made by measuring the potential difference between two suitable electrodes, immersed in the solution analyzed and using a benchtop pH metro, model SP 3611, with 15 ml of honey in Becker, to monitor the pH measurement.

The Hach DR model spectrophotometer equipment measured the parameter “honey color” following the methodology by Larana (1981). The wavelength worked at was 540 nanometers and the reagent chosen for equipment calibration was pure glycerin. The values obtained were in absorbance, and after the conversion of the wavelength into honey color, using the formula $\text{mm Pfund} = -38.70 + 371.39 \times \text{Abs}$, the honey color classification proceeded (Ferreira et al., 2009)

We performed a sensory analysis with the honey of the four stingless bee species in this study. The parameters used for the analysis were: appearance, color, odor, flavor, texture, and overall evaluation, through a free profile spreadsheet, with scoring amplitude from 1 (disliked very much) to 9 (I liked it very much).

For this process, five tasters who were already consumers of this product, but had no experience with free profile sensory analysis, were invited. The samples were coded so that the compositions of notes were in accordance only with the preferences of each one. Palate cleaning was also performed between the samples (Grosso, 2006).

Statistical Analyses

Statistical analysis of variance (ANOVA) was used to evaluate the honey production of the four species of stingless bees in the two municipalities. When applicable, Tukey multiple comparison tests at 5% probability were used to identify differences between treatments.

The correlation between biological parameters and honey production of the four species was performed through

the construction of graphs that provided this interaction. According to the first and second collections, the analyses were carried out for the physicochemical characters, individualizing them by town.

Variance analyses and Tukey tests were performed using the software Sisvar (Sisvar for Windows v. 5.6, UFPA, Lavras, MG, BR). We calculated the means and standard errors with the software MS Excel (Excel 2010, Microsoft, Redmond, WA, USA). Then we produced the graphs with the Minitab Statistical Software and MiniTab Workspace. All analyses were performed using a significance level of 0.05.

Results and Discussion

Not all melipona species produced honey during the harvest period in their respective municipalities. For example, there was no production in the first collection, in Miracema, for the species *M. rufiventris* and *S. tubiba*. However, *T. angustula* produced 115.95 ml of honey in the same period. Also, the species *S. tubiba* (91.56 ml) stood out, in the first collection, in Palmas, followed by *M. rufiventris* (68.4 ml) and *M. fasciculata* (47.53 ml), according to Fig 2.

In the second collection, in Miracema, the highest honey production was verified with the melipona species

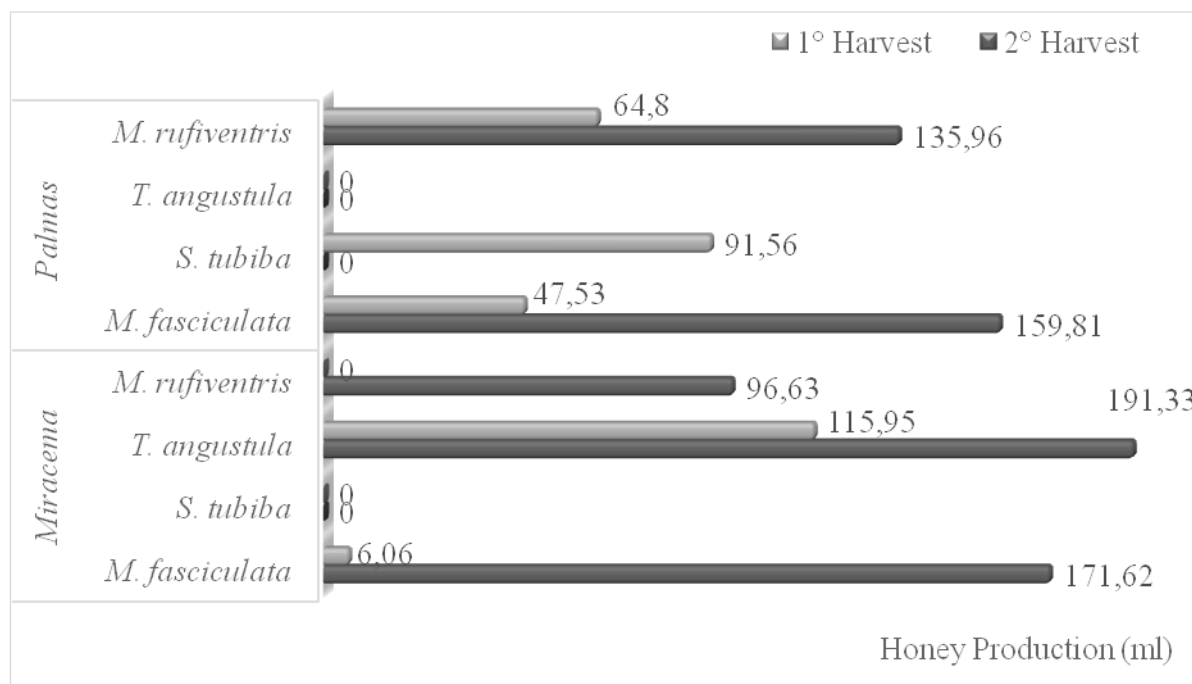


Fig 2. Honey production from the first and second harvest in the municipalities of Miracema and Palmas (TO), Brazil.

T. angustula with the summation of 191.33 ml, followed by *M. fasciculata* and *rufiventris* (171.62 and 96.63 ml), respectively. *S. tubiba* did not produce honey during this period. In the municipality of Palmas, honey production was not observed for *S. tubiba* and *T. angustula* bees, only for *M. fasciculata* and *rufiventris*, with averages of 159.81 and 135.96 ml, respectively (Fig 2).

Honeybee production is represented by the number of pots and the volume of honey contained within (Evangelista-Rodrigues et al., 2008). Thus, colonies with higher values of these variables have higher production. Furthermore, variations in the number of honey pots can easily occur, given the flowering time in each region.

Table 1 shows the significant differences in honey production, performed by the average of the three repetitions of each species of the four species of meliponines in the two municipalities, individually, in each harvest. It is possible to notice the range of quantification of production, both among bees and in the cities of the study. The municipality of Miracema had higher rates of honey production except for *T. angustula*, which did not show any production.

Table 1 - Average production (ml) of honey from four species of stingless bees in the municipalities of Palmas and Miracema (TO), Brazil, in two harvest seasons.

Species	1st Harvest	
	Municipality	
	Miracema	Palmas
<i>Melipona fasciculata</i>	57.2 Aa	2.02 Bb
<i>Scaptotrigona tubiba</i>	63.77 Aa	-
<i>Tetragonisca angustula</i>	-	59.97Aa
<i>Melipona rufiventris</i>	32.21 Aa	-
Species	2nd Harvest	
	Municipality	
	Miracema	Palmas
<i>Melipona fasciculata</i>	53.27 Aa	15.84 Aa
<i>Scaptotrigona tubiba</i>	-	30.52 A
<i>Tetragonisca angustula</i>	-	-
<i>Melipona rufiventris</i>	45.32 Aa	21.6 Aa

*Means followed by the same lowercase letter in the row and uppercase in the column do not differ statistically from each other by the Tukey test at the 5% probability level. Data were transformed into Log (x+1).

The current apicultural bloom in the city of Palmas, visually observed was mainly weeds (undergrowth), for example, *Vernonia difusa* Less., *Sida rhombifolia* L., and *Sida spinosa* L., with potential for the production of pollen and nectar. These plant species have flowering times ranging from September to December in the region, given the favorable environmental conditions. In addition to these, the tree species *Syzygium cumini* (L.) Skeels is also easily found in the landscape composition of the studied municipality, with its flowering season extended from April to September (Migliato, 2005).

The plant composition in the municipality of Miracema was slightly different from Palmas, as there were a higher number of native trees, visually observed. The species most frequently found were: *Caryocar brasiliense* Camb, *Anacardium occidentale* L., *Parkia pendula* Willd., *Cabralea canjerana* (Vell) and *Curatella americana* L. According to Migliato (2005), these species have distinct flowering seasons ranging from the second half of August to the end of November, given the appropriate edaphoclimatic conditions.

The state of the colonies is a factor that maintains a close relationship with honey production. Generally, when weak, they are more susceptible to temperature variations. They have activity shifted to later hours of the day compared to other colonies of the same species, drastically reducing their honey production (Hilário et al., 2001). According to

Venturieri (2008) knowledge of the biometric parameters is necessary to understand the productive indexes, identify similarities or differences between individuals, and evaluate each species' potential.

Another relevant point is the behavior and characteristics of production and reproduction in the boxes used in meliponaries. For Ribeiro et al. (2014), the construction of brood discs can be influenced by several factors. For instance, the number of bees in the colony, food (mainly pollen), and a queen's presence. Concerning honey pots, the larger the store, the greater volume of honey. Consequently, there will be more outstanding production and less waste of wax. However, population size can directly interfere with honey production at a proportional rate (Alves et al., 2007; Alves, 2010). The importance of the number of individuals refers to the fact that, typically, in populous colonies, many foragers collect more resources during flowering times, enabling defense against enemies, and maintaining adequate temperature for the development of the offspring (Faquinello et al., 2013).

There was a significant difference in the correlation between honey production and the diameter and height of honey pots (-0.28 and -0.33), see Table 2. The other measured variables had a positive correlation, however, with no significant difference.

Table 2 - Correlation of production and biometric variables of colonies of four species of stingless bees in the municipalities of Palmas-TO, Brazil.

Variables	PM	APP	MP	AC	DC	DPM
APP	-0,31 ^{ns}	-	-	-	-	-
MP	-0.3 ^{ns}	0.366 ^{ns}	-	-	-	-
AC	-0.309 ^{ns}	0.917 ^{ns}	0.522 ^{ns}	-	-	-
DC	-0.264 ^{ns}	0.94 ^{ns}	0.489 ^{ns}	0.983 ^{ns}	-	-
DPM	-0.287**	0.804 ^{ns}	0.241 ^{ns}	0.732 ^{ns}	0.749 ^{ns}	-
APM	-0.334**	0.85 ^{ns}	0.249 ^{ns}	0.773 ^{ns}	0.791 ^{ns}	0.989 ^{ns}

APP = Height of pollen pots; PM = Honey production; MP = Pollen pot mass; AC = Height of brood cells; DC = Diameter of brood cells; DPM = Diameter of honey pots and; APM = Height of honey pots, of the meliponous species: *M. fasciculata*, *M. rufiventris*, *S. tubiba*, and *T. angustula*. **Significant at 1%; *significant at 5%; ns = not significant.

The formation of honey pots with greater diameters and depths reduces the space occupied. It reduces honey consumption by workers bee in wax production, which

leads to a more significant amount of honey produced, revealing the statistical difference shown in Table 3 (Alves et al., 2012).

Table 3 - Correlation of production and biometric variables of colonies of four species of stingless bees in the municipality of Miracema-TO, Brazil.

Variables	PM	APP	MP	AC	DC	DPM
APP	0.025 ^{ns}	-	-	-	-	-
MP	-0.266 ^{ns}	0.221 ^{ns}	-	-	-	-
AC	0.202 ^{ns}	0.662 ^{ns}	0.657 ^{ns}	-	-	-
DC	0.064 ^{ns}	0.613 ^{ns}	0.662 ^{ns}	0.954 ^{ns}	-	-
DPM	0.176**	0.528 ^{ns}	0.741 ^{ns}	0.907 ^{ns}	0.87 ^{ns}	-
APM	0.279**	0.645 ^{ns}	0.57 ^{ns}	0.962 ^{ns}	0.9 ^{ns}	0.892 ^{ns}

APP = height of pollen pots; PM = Honey production; MP = Pollen pot mass; AC = Height of brood cells; DC = Diameter of brood cells; DPM = Diameter of honey pots and; APM = Height of honey pots, of the meliponous species: *M. fasciculata*, *M. rufiventris*, *S. tubiba*, and *T. angustula*. ** Significant at 1%; * significant at 5%; ns = not significant.

These bees separate the pollen and honey. They are stored in two types of pots, differentiated by size. The pollen pots are cylindrical or conical with about 3 cm high, and the ovoid honey pots, with approximately 1.5 cm in height, vary according to the meliponous species (Carvalho-Zilse et al., 2012; Pereira et al., 2012).

However, Rodrigues et al., (2018) state that empty pots, regardless of the final product, derives from the preparation of food stored in wax pots. Thus, they identified that the biometric characteristics of pollen and honey pots are related to the colony's size and vary according to their

purpose. The table below presents the results of humidity, pH, color, and Brix of the kinds of honey produced by the stingless bee species, *S. tubiba*, *M. rufiventris*, *M. fasciculata*, and *T. angustula*, in both harvests, first and second, in the municipalities of Miracema and Palmas.

The moisture contents found ranged from 24% - 31% (m/m) and 23% - 32% (m/m) for *Melipona fasciculata* and *Melipona rufiventris*, in the first and second collections, respectively. It was possible to observe that the species differ from each other and harvest periods (Table 4). For example, the species *T. Angustula* obtained 24.02% (m/m) in the first collection and 28% (m/m) in the second.

Table 4 - Analysis of moisture, pH, color, and total soluble solids (°Brix) for honey collected by the four melipona species in the municipalities of Palmas and Miracema during the two collections periods.

Samples	Humidity (%)	pH	Color	°Brix
<i>1M. fasciculata</i> ^(a)	25.2	4.18	Clear Amber	73.48
<i>1M. fasciculata</i> ^(a)	27.3	3.79	Amber extra clear	70.48
<i>1M. fasciculata</i> ^(a)	25.5	4.00	Amber extra clear	73.48
<i>1 S. tubiba</i> ^(a)	28.2	4.19	White	70.48
<i>1 T. angustula</i> ^(a)	26.3	4.48	Âmbar	72.48
<i>1 T. angustula</i> ^(a)	28.1	4.56	Âmbar	70.48
<i>1M. rufiventris</i> ^(a)	31	3.99	Âmbar	67.48
<i>1M. rufiventris</i> ^(a)	30.3	3.87	Amber extra clear	68.48
<i>1M. fasciculata</i> ^(b)	24	nd	Clear Amber	74.48
<i>1M. fasciculata</i> ^(b)	29.4	nd	Clear Amber	71.48
<i>1 T. angustula</i> ^(b)	24.2	4.39	Amber extra clear	74.48
<i>2 S. tubiba</i> ^(b)	31.3	4.0	White	67.98
<i>2 S. tubiba</i> ^(b)	29	4.03	White	69.48
<i>2 M. rufiventris</i> ^(b)	32.1	3.9	White	66.48
<i>2 M. fasciculata</i> ^(b)	23	4.09	White	75.48
<i>2 M. rufiventris</i> ^(a)	31.2	4.22	Amber extra clear	67.98
<i>2 M. rufiventris</i> ^(b)	30	3.97	White	68.48
<i>2 M. rufiventris</i> ^(b)	31.5	3.82	Clear Amber	67.48
<i>2 M. fasciculata</i> ^(b)	28.9	4.06	Amber extra clear	70.28
<i>2 M. fasciculata</i> ^(b)	32	3.58	White	66.48
<i>2 M. fasciculata</i> ^(b)	33	3.58	White	65.48

Note: Nd = Undetected Values; 1 and 2 = Order of Collection; (a) and (b) = Miracema and Palmas.

Current Brazilian and international legislation specific to honey from *Apis mellifera* maintains that honey must contain a maximum of 20% moisture (Brasil, 2000; Codex Stan 12, 2001). However, several studies that characterize honey from stingless bees show that higher humidity levels are a characteristic of this type of honey, such as the study by Almeida-Muradian (2013), Silva et al. (2013), and Sousa et al. (2016), when analyzing honey from *T. angustula*, *M. fasciculata*, and *M. scutellaris*, they found values ranging from 23.4 to 25.6% (m/m), from 22.2 to 24.4% (m/m) from 23.9 and 28.9% (m/m), respectively.

High levels of moisture can come from the low dehydration of the nectar during the natural transformation

process into honey. And it may also be related to several factors such as the humid tropical environment; the collection of nectar from lower flowers, and ripe fruits with higher water content; harvest time; degree of maturity reached in the hive; edaphoclimatic conditions; or even from the different species of bees (Guerrini et al., 2009; Vit et al., 2013).

High levels of moisture can also accelerate the fermentation process, resulting in the formation of undesirable organic compounds, which can give a bitter taste, and unwanted color, interfering with the product's shelf life and, consequently, with its commercialization (Da Silva et al., 2016).

For the soluble solids' concentration (°Brix), the values found ranged from 67.98 - 72.98° Brix and 65.48 - 75.48°

Brix for *M. asciculate* and *M. rufiventris* and *M. asciculate* in both, in the first and second collection, respectively. It is possible to observe that the species differ from each other and harvest seasons (see Table 4). For example, the species *M. asciculate* had a greater range of variation (10.00 °Brix) in the same collection between cities.

Honey from *Apis ascicula* bees generally has higher values of °Brix. Thus, lower values, obtained for honey from stingless bees, may be related to the high-water content found in this product and lower sugar content (De Sousa et al., 2016).

The pH values found ranged from 3.87 (*Melipona rufiventris*) to 4.56 (*Tetragonisca angustula*) and 3.58 (*Melipona asciculate*) to 4.22 (*Melipona rufiventris*), in the first and second collection, respectively (Table 04), defining an acidic character to the samples, similar to the pH observed for *Apis ascicula* kinds of honey (3.2 – 4.5), according to Brazilian and international legislation (Brasil, 2000; Codex Stan 12, 2001).

According to Crane (1985), pH values can be influenced by the pH of the nectar, soil, or association of nectars for

its composition. In addition, even if honey is produced in the same town, there are mandibular substances added to the nectar during its transport to the hive (Vit et al., 2013). Currently, pH is not a mandatory parameter in the quality control of Brazilian honey, nor is it recommended by international honey legislation. However, its evaluation is of great importance in defining the form of handling and storage of honey. It considers that pH and acidity are important antimicrobial factors, capable of providing greater stability and shelf life to the product, as well as influencing the speed of formation of 5-HMF (Guerrini et al., 2009).

The color of honey is considered a parameter of quality, preference, and acceptance among consumers. In our study, the color of the kinds of honey ranged from white to amber (Fig 3). These colors fit the current regulations, Brasil (2000) and Codex Alimentarius (2001), ranging from colorless to dark amber, for honey quality control that is based on the characteristics of honey produced specifically by *A. Mellifera* bees.

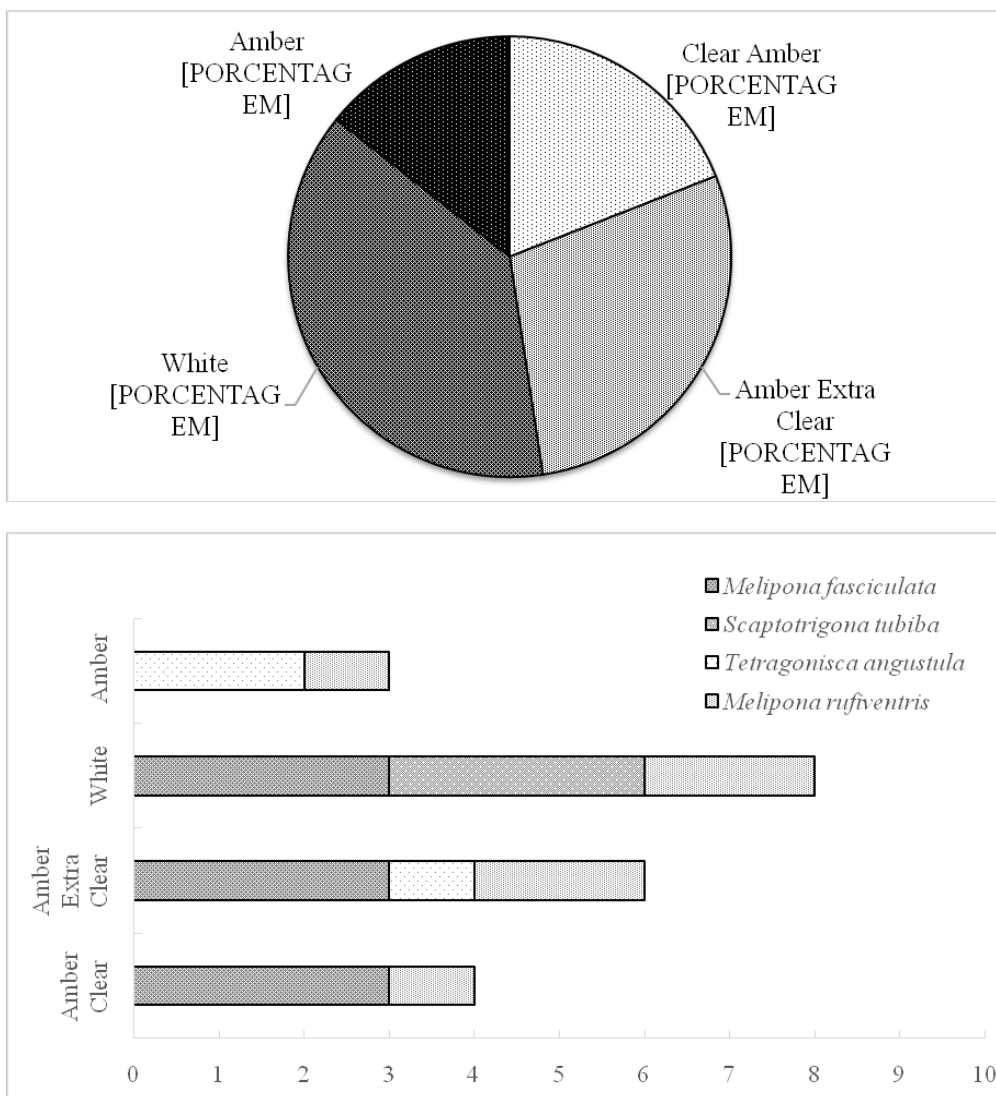


Fig 3. Color parameter of honey produced by stingless bees in the municipalities of Palmas and Miracema in two harvest seasons.

In our study, the predominance of the white color, with honey from the stingless bee species, agrees with the results obtained by Souza et al. (2006) and Anacleto et al. (2009). On the other hand, Lacerda et al. (2010) found lighter hues for stingless bees, adhering to the variations in landscape composition, temporal, and intrinsic characteristics of each species.

It is also noteworthy that several other factors namely, influence the color of honey: floral origin, honey maturation, climatic variations along the period of nectar flow, time, and temperature at which the honey remains stored in hives, presence of chemical elements, vitamins B and C, caramelization process, Maillard reaction products, and the content of flavonoids and phenolic acids. The greater the concentration of these components, the more intense the color of the honey (Lacerda et al., 2010; Lira et al., 2014; Braghini, 2016; Da Silva et al., 2016).

The development and approval of regulation for stingless bee honey, which meets standards regarding identity and consumer safety, is important and establishes a reference basis for regulatory agencies, thus allowing official marketing. But, unfortunately, the Brazilian legislation for honey does not behold the stingless bees.

Figure 4 shows the sensory profile of each species, performed by the average between the two municipalities. The average value attributed by the tasters to each attribute (appearance, color, texture, odor, flavor, and global evaluation) is marked on the corresponding axis. The center of the figure represents the zero point of the scale used in the assessment, while the intensity increases from the center to the periphery. The sensory profile is revealed when the points are connected (Spider chart).

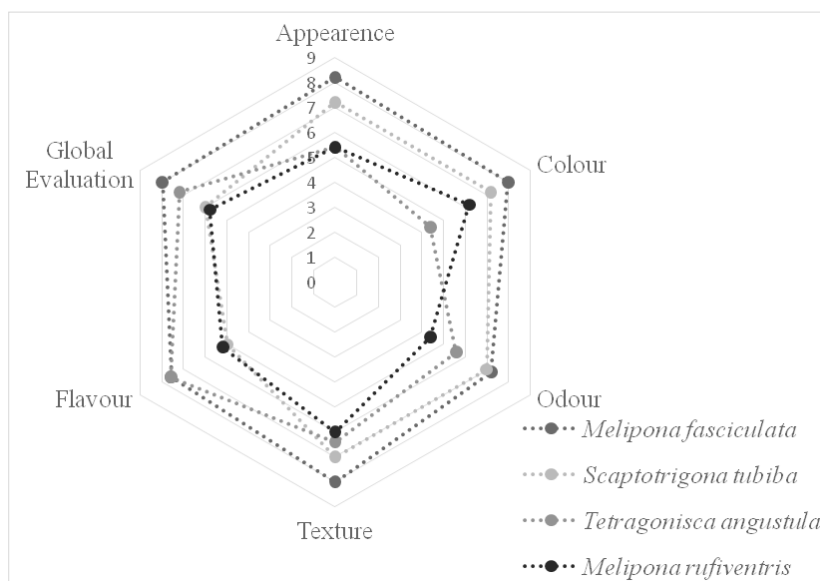


Fig 4. Sensory analysis of honey produced by stingless bees in the municipalities of Palmas and Miracema in two harvest seasons.

Sensory analysis is a technique used for evaluating the attributes perceived by the sense organs (organoleptic attributes) applied in many fields. It allows for establishing the organoleptic profile of different products, indicating consumer preference (Piana et al., 2004). Sensory characteristics stimulate the senses and provoke various degrees of reactions of desire

or rejection, in which the consumer chooses a food based on its level of sensory quality (Carvalho, et al., 2006).

Sensory analysis is essential for assessing consumer preferences according to honey processing, storage, and its impacts on botanical, geographic, and volatile identity (Sodré et al., 2008; Ribeiro et al., 2014; Costa et al., 2017).

Table 5 - Sensory analysis of honey produced by stingless bees in the municipalities of Palmas and Miracema in the two harvest seasons.

Parameters	Species of Meliponines			
	<i>M. fasciculata</i>	<i>S. tubiba</i>	<i>T. angustula</i>	<i>M. rufiventris</i>
Appearance	8.2 a	7.2 b	5.4 c	5.4 c
Colour	8 a	7.2 b	4.4 d	6.2 c
Odour	7.2 a	7 b	5.6 c	4.4 d
Texture	8 a	7 b	6.4 c	6 d
Flavour	7.6 a	5 c	7.6 a	5.2 b
Global Evaluation	8 a	6 c	7.2 b	5.8 d

*Means followed by the same lowercase letter on the line do not differ statistically from each other by Tukey's test at the 5% probability level.

Table 5 shows the marks given by the tasters concerning the sensory attributes related to the species *M. asciculate*, *S. Tubiba*, *T. Angustula*, and *M. Rufiventris*, where the first one presented higher marks for all the evaluated characters, differing statistically from each other at 5% probability by Tukey test.

Conclusion

The results of this study demonstrate that the highest production of honey (in the first and second collections), came from the species *T. Angustula* in the municipality of Palmas. As for Miracema, the highest production came from the species *S. tubiba* and *M. fasciculata*.

Considering the physicochemical analyses carried out, we verified that all the evaluated parameters fit the norms designated to the quality control of honey, even if specifically, of *A. mellifera*. The specie *M. fasciculata* obtained the best honey sensory profile by average among the characteristics observed in this study.

As indicated by the data analyses there was a positive and negative correlation between the biological parameters evaluated namely, height and diameter of honey pots and brood, and height and mass of pollen pots, with a significant difference only between the height and diameter of the character of the honey pot.

In light of these results and beyond the scientific purpose, this study supports the creation of a standard identity and specific quality control for the honey of stingless bees, contributing to the production and marketing of this product, like that, already used for *Apis mellifera* honey.

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Authors' Contributions

MR: conceptualization, methodology, formal analysis, investigation, resources, and writing.

RS: conceptualization, methodology, investigation, resources, project administration, review & editing, and project administration.

RD: methodology and formal analysis.

References

Almeida-Muradian, L.B., Stramm, K.M., Horita, A., Barth, O.M., Freitas, A.S., Estevinho, L.M. (2013). Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. *International Journal Food Science Technology*, 48: 1698-1706. doi: 10.1111/ijfs.12140.

Alves, R.M.O., Souza, B.A. & Carvalho, C.A.L. (2007). Notas sobre a bionomia de *Melipona mandaçaia* (Apidae: Meliponini). *Magistra*, 19: 177-264. doi: 10.1590/1676-06032015009714.

Alves, R.M.O., Carvalho, C.A.L. & Faquinello, P. (2012). Parâmetros biométricos e produtivos de colônias de *Melipona scutellaris* Latreille, 1811 (Hymenoptera: Apidae) em diferentes gerações. *Magistra*, 24: 05-111. doi:10.13140/RG.2.1.2447.9768.

Anacleto, D.D.A., Souza, B.D.A., Marchini, L.C. & Moreti, A.C.D.C.C. (2009). Composição de amostras de mel de abelha Jataí (*Tetragonisca angustula* Latreille, 1811). *Ciência e Tecnologia de Alimentos*, 29: 535-541. doi: 10.1590/S0101-20612009000300013.

Antonini, Y., Martins, R.P., Aguiar, L.M. & Loyola, R.D. (2012). Richness, composition and trophic niche of stingless bee assemblage in urban forest remnants. *Urban Ecosystems*, 16: 527-541. doi: 10.1007/s11252-012-0281-0.

Araújo, J.S., Chambó, E.D., Costa, M.A.P.C., Cavalcante, D.A., Silva, S.M.P., Carvalho, C.A.L. & Estevinho, L.M. (2017). Chemical composition and biological activities of monoand heterofloral bee pollen of different geographical origins. *International Journal of Molecular Science*, 18: e921. doi: 10.3390/ijms18050921.

Azeredo, L.C., Azeredo, M.A.A. & Beser, L.B.O. (2000). Características físico-químicas de amostras de méis de melíponas coletadas no Estado de Tocantins. In: Congresso Brasileiro de Apicultura. Florianópolis. doi: 10.1590/S0101-20612005000400004.

Bartelli, B.F. & Nogueira-Ferreira, F.H. (2014). Pollination services provided by *Melipona quadrfasciata* Lepeletier (Hymenoptera: Meliponini) in greenhouses with *Solanum lycopersicum* L. (Solanaceae). *Sociobiology*, 61: 510-516. doi: 10.13102/sociobiology.v61i4.510-516.

Braghini, F. (2016). Estabilidade de méis de abelhas sem ferrão (Meliponinae spp.) submetidos a diferentes condições térmicas. 156f. Dissertação (Mestrado) Programa de Pós-Graduação em Ciências dos Alimentos do Centro de Ciências Agrárias – Universidade Federal de Santa Catarina, Florianópolis-SC.

Brasil. Instrução Normativa 11 de 20 de outubro de 2000. Regulamento Técnico de Identidade e Qualidade do Mel. Diário Oficial da União. Legislação de Produtos Apícolas Derivados. Abastecimento, p. 23.

Camargo, R.C.R., Oliveira, K.L. & Berto, M.I. (2017). Mel de abelhas sem ferrão: proposta de regulamentação. *Brazilian Journal of Food Technology*, 20: e2016157. doi: 10.1590/1981-6723.15716.

Carvalho, C.A.L., Alves, R.M.O., Souza, B.A., Vêras, S.O., Alves, E.M. & Sodrê, G.S. (2013). Proposta de regulamento

- técnico de qualidade físico-química do mel floral processado produzido por abelhas do gênero *Melipona*. In: Stingless bees process honey and pollen in cerumen pots. Eds: Vit, P. & Roubik, D.W., p. 1-9. doi: 10.1590/1981-6723.15716.
- Carvalho-Zilse, G.A. (2012). Meliponicultura na Amazônia. Manaus: [s.n.], 50p.
- Codex Alimentarius Commission (2001). Codex Alimentarius Commission Standards. Codex Stan 12-1981, p. 1-8.
- Costa, A.C.V., Sousa, J.M.B., Silva, M.A.A.P., Garruti, D.S. & Madruga, M.S. (2017). Sensory and volatile profiles of monofloral honeys produced by native stingless bees of the Brazilian semiarid region. *Food Research International*, 105: 110-120. doi: 10.1016/j.foodres.2017.10.043
- Crane, E. (1985). O livro do mel, Livraria Nobel, São Paulo.
- DaSilva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O. & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, 196: 309-323. doi: 10.1016/j.foodchem.2015.09.051.
- Evangelista-Rodrigues, A., Góis, G.C., Silva, C.M. Souza, D.L., Souza, D.N., Silva, P.C.C., Alves, E.I. & Rodrigues, M. L. (2008). Desenvolvimento produtivo de colméias de abelhas *Melipona scutellaris*. *Revista Biotemas*, 21: 59-64. doi: 10.5007/2175-7925.2008v21n1p59
- Faquinello, P., Brito, B.B.P., Carvalho, C.A.L., Paula-Leite, M.C. & Alves, R.M.O. (2013). Correlação entre parâmetros biométricos e produtivos em colônias de *Melipona quadrifasciata anthidioides* Lapeletier (Hymenoptera: Apidae). *Ciência Animal Brasileira*, 14: 312-317. doi: 10.5216/cab.v14i3.18603.
- Ferreira, E.L., Lencioni, C., Benassi, M.T., Barth, M.O. & Bastos, D.H.M. (2009). Descriptive sensory analysis and acceptance of stingless bee honey. *Food Science and Technology International*, 15: 251-258. doi: 10.1177/1082013209341136.
- Ferrufino, U. & Vit, P. (2013). Pot-honey of six Meliponines from Amoro National Park, Bolivia. In: Vit, P.; Pedro, S.R.M. & Roubik, D. Pot-honey: a legacy of stingless bees. New York: Springer. p. 409-416. doi: 10.1007/978-1-4614-4960-7_29.
- Guerrini, A., Bruni, R., Maietti, S., Poli, F., Rossi, D., Paganetto, G., Muzzoli, M., Scalvenzi, L. & Sacchetti, G. (2009). Ecuadorian stingless bee (Meliponinae) honey: A chemical and functional profile of an ancient health product. *Food Chemistry*, 114: 1413-1420. doi: 10.1016/j.foodchem.2008.11.023.
- Hilário, S.D., Imperatriz-Fonseca, V.L. & Kleinert, A.M.P. (2001). Responses to climatic factors by foragers of *Plebeia pugnax* Moure (in litt.) (Apidae, Meliponinae). *Revista Brasileira de Biologia*, 61: 191-196. doi: 10.1590/S0034-71082001000200003.
- IHC (1997). Harmonized methods of the International Honey Commission. IHC responsible for the methods: Stefan. *Anal.* p. 10-16.
- Lacerda, J.J.J., Santos, J.S., Santos, A.S., Rodrigues, G.B. & Santos, M.L.P. (2010). Influência das características físico-químicas e composição elementar nas cores de méis produzidos por *Apis mellifera* no sudoeste da Bahia utilizando análise multivariada. *Química Nova*, 33: 1022-1026. doi: 10.1590/S0100-40422010000500003.
- Larana (1981). Laboratório Nacional de Referência Animal. Métodos analíticos oficiais para controle de produção, controle de produtos de origem animal e seus ingredientes. II - Métodos físicos e químicos. Mel. Brasília: Ministério da Agricultura, v.2, cap. 25, p. 1-15.
- Lira, A.F., Sousa, J.P.L.M., Lorenzon, M.C.A., Vianna, C.A.F.J. & Castro, R.N. (2014). Estudo comparativo do mel de *Apis mellifera* com méis de meliponíneos. *Acta Veterinaria Brasilica*, 8: 169-178. doi: 10.21708/AVB.2014.8.3.3560.
- Michener, C.D. (2000). The bees of the world. Johns Hopkins Univ. Press, p. 872.
- Migliato, K.F. (2005). *Syzygium cumini* (L.) Skeels - Jambolão: estudo farmacognóstico, otimização do processo extrativo, determinação da atividade antimicrobiana do extrato e avaliação da atividade antisséptica de um sabonete líquido contendo o referido extrato. Dissertação de Mestrado em Ciências Farmacêuticas. Araraquara, 179 p.
- Moraes, R.M. de & Texeira, E.W. (1998). Análise de mel. Pindamonhangaba: Instituto de Zootecnia. Manual Técnico, 41 p.
- Nascimento, A.S., Marchini, L.C., Carvalho, C.A.L., Araújo, D.F.D., Olinda, R.A. & Silveira, T.A. (2015). Physical-Chemical Parameters of Honey of Stingless Bee (Hymenoptera: Apidae). *Chemical Science International Journal*, 7: 139-149. doi: 10.9734/ACSJ/2015/17547.
- Pereira, F.M. et al. (2012). Manejo de colônias de abelhas-sem-ferrão. Teresina: Embrapa Meio-Norte. 31p.
- Pereira, F.M., Souza, B.A. & Lopes, M.T.R. (2021). Criação de abelhas sem ferrão. <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/166288/1/CriacaoAbelhaSemFerrao.pdf>. Accessed: January 2021.
- Ramón-Sierra, J.M., Ruiz-Ruiz, J.C. & Ortiz-Vázquez, E.D.L.L. (2015). Electrophoresis characterization of protein as a method to establish the entomological origin of stingless bee honeys. *Food Chemistry*, 183: 43-48. doi: 10.1016/j.foodchem.2015.03.015.
- Ribeiro, M.F., Santos-Filho, P.S. & Imperatriz-Fonseca, V.L. (2006). Size variation and egg laying performance in *Plebeia remota* queens (Hymenoptera, Apidae, Meliponini). *Apidologie*, 37: 191-206. doi: 10.1051/apido:2006046.

- Ribeiro, R.O.R., Mársico, E.T., Carneiro, C.S., Monteiro, M.L.G., Júnior, C.A.C., Mano, S. & Jesus, E.F.O. (2014). Classification of Brazilian honeys by physical and chemical analytical methods and low field nuclear magnetic resonance. *LWT – Food Science and Technology*, 55: 90-95. doi: 10.1016/j.lwt.2013.08.004.
- Rodrigues, C.S., Ferasso, D.C., Prestes, O.D., Zanella, R., Grando, R.C., Treichel, H., Coelho, G.C. & Mossi, A.J. (2018). Quality of Meliponinae honey: Pesticides residues, pollen identity, and microbiological profiles. *Environmental Quality Management*, 27: 39-45. doi: 10.1002/tqem.21547.
- Roubik, D.W. (2018a). The pollination of cultivated plants. A compendium for practitioners. Vols. 1 & 2. (Ed.) Rome: FAO, 324 p.
- Se, K.W., Ibrahim, R.K.R., Wahab, R.A. & Ghoshal, S.K. (2018). Accurate evaluation of sugar contents in stingless bee (*Heterotrigona itama*) honey using a swift scheme. *Journal of Food Composition and Analysis*, 66: 46-54. doi: 10.1016/j.jfca.2017.12.002.
- Shadan, A.F., Mahat, N.A., Wan Ibrahim, W.A., Ariffin, Z. & Ismail, D. (2018). Provenance establishment of stingless bee honey using multi-element analysis in combination with chemometrics techniques. *Journal of Forensic Sciences*, 63: 80-85. doi: 10.1111/1556-4029.13512.
- Silva, A.S., Alves, C.N., Fernandes, K.G. & Müller, R.C.S. (2013). Classification of honeys from Pará State (Amazon region, Brazil) produced by three different species of bees. *Journal Brazilian Chemical Society*, 24: 1135-1145. doi: 10.5935/0103-5053.20130147.
- Silveira, F.A., Melo, G.A.R., Campos, L.A.O., Marini Filho, O.J., Menezes-Pedro, S.R. (2018). *Melipona rufiventris* Lepelletier, 1836. In: ICMBio (Org), Livro vermelho da fauna ameaçada de extinção. V. 7. Invertebrados.
- Singh, A.K. (2016). Traditional Meliponiculture by Nagha tribes in Nagaland, India. *Indian Journal of Traditional Knowledge*, 154: 693-699.
- Sodré, G.S., Carvalho, C.A.L., Fonseca, A.A.O., Alves, R.M.O. & Souza, B.A. (2008). Perfil sensorial e aceitabilidade de méis de abelhas sem ferrão submetidos a processos de conservação. *Ciência e Tecnologia dos Alimentos*, 28: 72-77. doi: 10.1590/S0101-20612008000500012
- Sousa, J.M.B., Souza, I.A., Magnani, M. & Albuquerque, J.R. (2013). Physicochemical aspects and sensory profile of stingless bee honeys from Seridó region, State of Rio Grande do Norte, Brazil. *Semina: Ciências Agrárias*, 34: 1765-1774. doi: 10.54 33/1679-0359.2013v34n4p1765
- Sousa, J.M.B., Souza, E.L., Marques, G., Benassi, M.T., Gullón, B., Pintado, M.M. & Magnani, M. (2016). Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. *LWT-Food Science and Technology*, 65: 645-651. doi: 10.1016/j.lwt.2015.08.058.
- Souza, B., Roubik, D., Barth, O., Heard, T., Enríquez, E., Carvalho, C., Villas-Bôas, J. Marchini, L.C., Locatelli, J., Persano-Oddo, L., Almeida-Muradian, L. Bogdanov, S. & Vit, P. (2006). Composition of stingless bee honey: Setting quality standards. *Interciência*, 31: 867-875.
- Souza, S.G.X., Teixeira, A.F.R., Neves, E.L. & Melo, A.M.C. (2005). As abelhas sem ferrão (Apidae: Meliponina) residentes no campus Federação/Ondina da Universidade Federal da Bahia, Salvador, Bahia, Brasil. *Candombá. Revista Virtual*, 1: 57-69.
- Venturieri, G.C. (2008b). Criação de abelhas indígenas sem ferrão. Belém: Embrapa Amazônia Oriental, 60 p.
- Villas-Bôas, J.K. & Malaspina, O. (2005). Parâmetros físico-químicos propostos para o controle de qualidade do mel de abelhas indígenas sem ferrão no Brasil. *Mensagem Doce*, 82: 6-16.
- Vit, P. (2013). *Melipona favosa* Pot-Honey from Venezuela. In: Vit, P., Pedro, S.R.M. & Roubik, D.W. (Eds.); Pot-Honey A legacy of stingless bees. 1st ed., p. 363-373.
- Vit, P., Pedro, S.R.M. & Roubik, D.W. (2013). PotHoney: A Legacy of Stingless Bees. Springer, N. Y. 655 p.
- Zielinski, A.A.F., Ávila, S., Ito, V., Nogueira, A., Wosiacki, G., Windson, C. & Haminiuk, I. (2014). The association between chromaticity, phenolics, carotenoids, and in vitro antioxidant activity of frozen fruit pulp in Brazil: An application of chemometrics. *Journal of Food Science*, 79: C510-C516. doi: 10.1111/1750-3841.12389
- Zuccato, V., Finotello, C., Menegazzo, I., Peccolo, G. & Schievano, E. (2017). Entomological authentication of stingless bee honey by ¹H NMR-based metabolomics approach. *Food Control*, 82: 145-153. doi: 10.1016/j.foodcont.2017.06.024

