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Physicochemical and microbiological profile of honey from *Melipona bicolor* (Lepeletier, 1836) from southern Brazil

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Abstract: Stingless bee honey has been attracting interest among consumers owing to its wide variety, sensorial characteristics, therapeutic properties, and high added value. Several studies have been carried out through native bee honey; however, studies around Melipona bicolor honey - a rare species remain scarce. Accordingly, this research aimed to evaluate the physical-chemical and microbiological profile of M. bicolor honey based on the current stingless bee honey legislation of Parana. Hence, three honey samples (200 ml each) from the M. bicolor bee were collected by suction and stored in sterile bottles. The collecting occurred in three different meliponaries in the Metropolitan region of Curitiba, state of Parana, directly from the bee boxes, it was collected from 4 to 6 boxes from each meliponarie, totalizing next to 2 kg from each farm. They were evaluated for moisture content, reducing sugars, total sugars, apparent sucrose, pH, water activity, hydroxymethylfurfural (HMF), aerobic mesophilic count, total and thermotolerant coliforms, yeast, and mold counts. The average result for moisture content was 24.8%, reducing sugars 62.96%, total sugars 65.6%, and pH 2.95. No significant amounts of HMF were detected in the honey from the three properties analyzed. The results of counting aerobic mesophilic bacteria (2.6 to 4.08 log CFU/ ml), total and thermotolerant coliforms (≤ 3.0 MPN/ml), and counting molds and yeasts (≤1.0 to 2.43 log CFU/ml), together with the data of analyzing physical-chemical parameters, indicated that the M. bicolor honey analyzed in this study complies with the Identity Standards and Quality imposed by Parana State Legislation for Meliponiculture; therefore, it is suitable for consumption and ready for sale.
Keywords: Stingless bees; meliponine; Quality; Food Safety.

1. Introduction

Belonging to the order Hymenoptera and the subfamily Meliponinae, stingless bees have a wide diversity of shapes and sizes and are present throughout the tropical and subtropical regions of the world, with more than 500 species (BRAGHINI et al., 2022; VILLAS BÔAS, 2012). They are characterized by an atrophied stinger, having other defense mechanisms developed throughout their evolutionary process. Meliponines are fundamental for pollination, acting in 80% of natural vegetation, being exclusive pollinators on 65% of them (CELLA et al., 2017; MICHENER, 2007).

Meliponiculture is a growing activity in tropical countries and, in addition to pollination services, beekeeping products, such as honey, propolis, resin, and pollen, are essential for human nutrition, health, and food security (ENGEL, 2023). As for honey, its particular characteristics and differentiated properties have made it an object of study of great interest among researchers, in addition to attracting consumers from different regions of the world, increasing its commercial value. Having countless varieties, these honeys differ not only by the species of bee that produced them but also by the local botanical composition and soil and climate conditions (NORDIN et al., 2018; EMBRAPA, 2023).

In Brazil, the diversity of biomes, differences in temperature and moisture, and the abundant composition of local flora influence the properties of honey in each region, preventing the standardization of legislation that represents reality in general (NORDIN et al., 2018). Accordingly, the impossibility of achieving the established parameters hinders the selling process of honey. Furthermore, it allows fraud to occur, putting consumer health at risk (SPINK et al., 2019). In this sense, it is essential to characterize honey from stingless bees, correlating it with the bee species and the characteristics of the place where the meliponary is located (BILUCA et al., 2016).

Among the stingless bee species, *Melipona bicolor*, popularly called Guaraipo, is known for its importance in plant pollination, honey production, and the preservation of biodiversity (ÁVILA et al., 2018). Furthermore, the honey produced by these bees is valued for its unique characteristics and unique flavor, having a more acidic flavor and more fluid texture, compared to honey from *Apis mellifera* (BILUCA et al., 2017; FALEIROS-QUEVEDO & FRANCOY, 2022). With an annual production of around 2 kg and selective floral preference, these bees are predominantly present in the southern and southeastern regions of Brazil. They are found more specifically in the Atlantic Forest biome, having nests built in tree cavities, generally closer to the ground or the base of the trunk, making it difficult to perceive (NOGUEIRA-NETO, 1997). They also present polygyny, which consists of the presence of more than one queen in the same nest, something rare among stingless bees (ALONSO et al., 1998). All these factors, combined

with the therapeutic potential attributed to stingless bee honey, make this a precious and relatively rare product that has good value in the national and international markets (PIMENTEL et al., 2021; BRAGHINI et al., 2022).

Conversely, it is important to note that there are few reports in the literature about the characterization of *M. bicolor* honey. The objective of this work was to evaluate the physicochemical and microbiological parameters of honey from the *M. bicolor* collected in three different regions in the metropolitan region of Curitiba, state of Parana (PR), southern Brazil. The data was compared with parameters recommended by the current state legislation to investigate the standardization and quality of the product, verifying whether this honey is suitable for consumption and ready for commercialization.

2. Materials and methods

Honey was harvested from *M. bicolor* (SisGen A0818FE) in three different meliponaries located on private properties on February 23, 2023. The first harvest was in Mandirituba, which produced a total of 2.053 kg of honey. The second harvest was carried in Fazenda Rio Grande (2.312 kg). The third harvesting was also located in Mandirituba (2.283 kg) (Table 1). The forests at the three sites are characterized as secondary formations in a medium and advanced stage. Honey was collected from, on average, 4 to 6 boxes from each place. There was no prior feeding of the bees, as it was a period at the end of summer.

All harvests were carried out through suction, using a honey-sucking machine that consists of a small tank with a pressure-based hose (Figure 1A), developed by producers Benedito Perin Uczai and Salete Perin Uczai. First, the external cleaning of the boxes was carried out, followed by the removal of the lid and canvas, and later by piercing the jars with the help of a knife. After drilling, a hose pipe was positioned inside the pots, suctioning the honey (Figure 1B). After harvesting, the honey was filtered using thread 40 fabric (92% Polyamide, 8% Elastane) (Figure 1C), followed by packaging the honey in previously sterilized glass containers. The material was transported under refrigeration to the laboratory and stored in the refrigerator at 4ºC until analysis (Figure 1D). The maximum storage time was 14 months, and analyses were carried out within this period. The samples were identified according to the collection location (A, B, C).

Identification	Harvest City	Coordinates
A	Mandirituba	-25.874460, -49.404861
B	Fazenda Rio Grande	-25.724054, -49.300099
C	Mandirituba	-25.803695, -49.303595

Table 1 – Identification and collection sites of honey samples from the stingless *M. bicolor bee* in the metropolitan region of Curitiba.

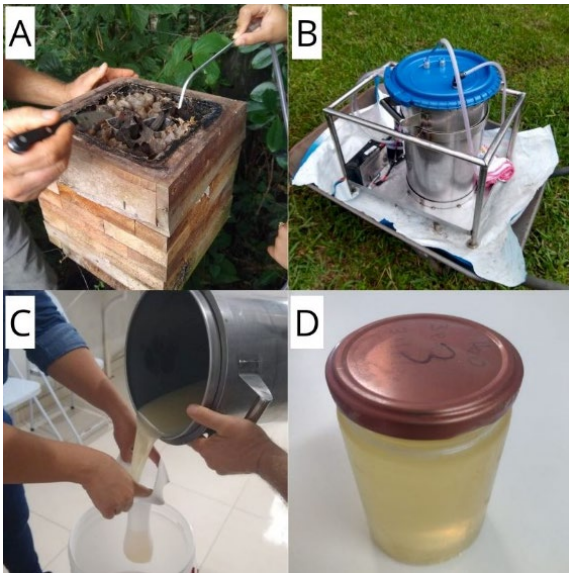

Figure 1 – Honey harvesting process (A) using a sucking machine (B) followed by filtration (C) and packaging in a previously sterilized glass container (D).

2.1. Physicochemical Parameters

The three (200 ml) samples were evaluated for moisture, determination of reducing sugars, total sugars and apparent sucrose, pH, water activity (aW), and determination of Hydroxymethylfurfural (HMF) content.

2.1.1. Moisture

The moisture index was determined in duplicate using an Abbé Refractometer (AOAC, 2000) from the Quality Control and Food Safety Laboratory of the Federal University of Parana, UFPR. The selected honey samples were removed from the refrigerator and kept at rest until they reached room temperature. Using a Pasteur pipette, a small amount of honey was placed on the reading prism. After the focus and calibration adjustments were made, the moisture index was obtained, determined by the refractive index (IR).

2.1.2. Sugars

The determination of total sugars, reducing sugars, and apparent sucrose in honey was carried out using the Lane- Enyon method (1923), also known as the titration method with Fehling solutions A and B (Instituto Adolfo Lutz, 2008). The objective is to determine the reduction of cupric ions to cuprous oxide under the action of heat in an alkaline medium, carried out by reducing sugars. To carry out the procedure, three samples were selected, one from each location. Sugar analyses were carried out at the Pharmaceutical Biotechnology Laboratory, UFPR.

Initially, 5 g of honey sample was weighed in a 100 ml volumetric flask, completing the volume with distilled water. This solution (50 ml) was pipetted into a 100 ml volumetric flask, followed by the addition of 2 ml of neutral lead acetate and an active charcoal spatula tip (Merck, Darmstadt, Germany), stirred, and left to rest for 10 minutes. The volume was completed with distilled water. The contents of the volumetric flask were filtered over 0.4 g of sodium monoacid phosphate per milliliter of lead acetate added in a 250 ml Erlenmeyer flask, followed by a second filtration without the use of sodium monoacid phosphate.

2.1.3. Blank titration

To perform the blank titration, in a 250 ml Erlenmeyer flask, 20 ml of Soxhlet solution (10 ml of Fehling A + 10 ml of Fehling B), 45 ml of distilled water, and 19 ml of the standard glucose solution were added to glass beads, heating the flask until it boils in a maximum time of 4 minutes. After boiling, three drops of methylene blue were added. One minute after the start of boiling, titration was carried out with glucose solution until the blue color disappeared. The procedure was repeated until the duplicate results were the same.

2.1.4. Reducing Sugars

To pre-titrate the sample, 45 ml of distilled water and 5 ml of honey sample were added to a 250 ml Erlenmeyer flask with 20 mL of Soxhlet solution. After heating and boiling, titration was carried out with glucose solution until the blue color disappeared. Two drops of methylene blue were added, continuing with the titration after one minute until the blue color completely disappeared. The volume of glucose solution used was considered as K. The sample titration follows the same principle as the previous titration, with the difference that the glucose solution added is considered to be K-2. The procedure was repeated until equal results were obtained in duplicate.

2.1.5. Total Sugars

To carry out the hydrolysis of the samples, 50 ml of the clarified sample was pipetted into a 100 ml volumetric flask, followed by the addition of 1 ml of hydrochloric acid, and kept in a water bath at 67°±3 for 15 minutes. Then, after cooling the contents of the volumetric flask to room temperature, neutralization was carried out by adding 2.8 ml of 5 N sodium hydroxide with the aid of litmus paper as an indicator. After filling the volume of the flask with distilled water, titrations were carried out following the principle of reducing sugars; however, the inverted sugar solution was used.

The results were expressed in g/100 g using the following formulas:

$$ARG = \frac{(b-a)}{10\,.\,v} \cdot 5 \cdot f1 \cdot f2$$

$$ANR = \left[\frac{(b-a)\cdot 5\cdot (f1\cdot 2)}{10}\cdot v - AR\right]\cdot f2$$

ARG is glucose-reducing sugars, ANR is non-reducing sugars, and AR is glucose-reducing sugars. f1 represents all dilutions and quantities of mass or volume used, f2 represents the conversion factor for expressing the results in glucose, a represents the number of ml of glucose solution used in titrating the sample, b is equivalent to the number of ml of the glucose solution used in the blank titration and v is the volume of the prepared sample used in the titration, in ml. To express total sugars, the sum of AR in glucose plus ANR in sucrose was performed. Sugars in sucrose were expressed using the formula: Total Sugars in Sucrose = (AR in glucose x 0.96) + ANR in sucrose. To express total sugars in glucose, the following formula was used: Total Sugars in Glucose = AR in glucose + (ANR in sucrose ÷ 0.96).

2.1.6. pH

The pH assessment was carried out by the analytical standards of the Instituto Adolfo Lutz (Instituto Adolfo Lutz, 2008). To measure the pH of the honey samples, a Luca-210 benchtop pH meter (São José do Rio Preto, SP) was used at the Quality Control and Food Safety Laboratory. After calibration with buffer solutions pH 4.0 and 7.0, its electrode was inserted directly into the tube containing honey, revealing its pH. The pH of each sample was measured in duplicate.

2.1.7. Water activity

To evaluate the water activity (Aw) of the samples, the portable HP 23aw ROTRONIC device, belonging to the Animal Nutrition Laboratory, UFPR, was used, placing approximately 2 ml of the sample in a stainless-steel sample holder connected to a measuring probe. Analyzes were performed in duplicate (AOAC, 2000).

2.1.8. Hydroxymethylfurfural

The quantitative analysis of hydroxymethylfurfural was carried out at the Center for Biopharmacy Studies, UFPR using the High-Performance Liquid Chromatography (HPLC) method using an Agilent 1100 Series chromatography (Waldbronn, Germany), composed of a G1311A quaternary pump, G1379A degasser, automatic injector G1320A and G1315B photodiode detector as described in Jeuring & Kuppers (1980). For that purpose, 10 g of honey was diluted to 50 ml with distilled water. The standard solution was prepared by diluting 100 mg of 5-(Hydroxymethyl) furfural to 1000 ml with distilled water. Then, 10 ml of this solution was diluted to 100 ml, resulting in a standard solution of 0.01 mg/ml^{-1}. The resulting sample and standard solutions were filtered using a 0.45 µm membrane filter. The chromatographic analysis occurred in an isocratic manner, using methanol and Milli-Q water as the mobile phase, in a 90:10 (v/v) ratio, with an injection volume of 20 µL; flow of 1.0 mL /min; XBridge column, C18, 5 µm, 4.6x150 mm; detection at 285 nm.

Quantification of the HMF concentrations of the samples was done by comparing the heights of the sample peaks with the heights of the peaks corresponding to the HMF standard, then the following formula was used to obtain the results:

$$HMF\left(\frac{mg}{kg}\right) = \frac{C \cdot PH \cdot x}{PH'} \cdot W$$

Where C is the concentration of the standard mg/L, PH is the height of the unknown peak, PH' is the height of the standard peak, W is the weight of the sample in grams and the dilution factor.

2.2. Microbiological Parameters

Standard plate counts of mesophilic bacteria, the most probable number of total and thermotolerant coliforms, and counts of molds and yeasts were performed. The analyses were carried out at the UFPR Quality Control and Food Safety Laboratory.

2.2.1. Sample preparation

A tube from each property (A, B, and C) was selected containing 25 ml of honey, which was added in vitro to 225 ml of saline (0.9%) buffered peptone solution (0.1%), thus obtaining the first dilution (10^{-1}). To carry out subsequent dilutions (10^{-2} to 10^{-5}), tubes containing 9 ml of the same diluent were used (ISO 4833-1: 2013; ISO 4833-2: 2014)

2.2.2. Standard plate count of aerobic mesophilic bacteria

To count aerobic mesophilic bacteria, the dilutions were distributed in Petri dishes with standard counting agar - PCA (Oxoid Ltd., Basingstoke – United Kingdom) and incubated at 35°C (±2) for 48 h. Values were expressed as Log CFU/ml^{-1} (Ryser & Schuman, 2015).

2.2.3. Most likely number of Total and Thermotolerant Coliforms

To analyze the presence of coliforms, tubes containing 10 ml of Lauryl Sulfate Tryptose Broth - LST (Oxoid Ltd. Basingstoke, United Kingdom) were used with the presence of an inverted Duhran tube inside each tube. 1.0 ml of each sample dilution was pipetted into the tubes, followed by homogenization and incubation at 35±2°C/48 h. The positive tubes were transferred to tubes with Brilliant Lactose Bile Green Broths (Kasvi, Roseto degli Abruzzi, Italy) and EC (HiMedia Laboratories Pvt. Ltd. - Mumbai, India) and incubated at 36 and 44.5°C, respectively, for 48 h. The result was expressed in MPN/ml^{-1} (Salfinger & Tortorello, 2015; Rice et al., 2012; Wehr & Frank, 2004).

2.2.4. Yeast and mold counts

To count yeasts and molds, 0.1 mL of each dilution was inoculated onto Potato Dextrose Agar (BDA) plates (HiMedia Laboratories Pvt. Ltd. - Mumbai, India) acidified with 10% tartaric acid (Neon – Suzano, São Paulo), spreading with a Drigalski loop. Then, the plates were incubated at 25ºC for five days. To count colonies and calculate the results, plates with 10 to 150 colonies were selected. The results were multiplied by their corresponding dilution factor and expressed in Log CFU/ml^{-1} (Salfinger & Tortorello, 2015; Pitt & Hocking, 2009; Wehr & Frank, 2004).

3. Results

Honey from the three locations had moisture equivalent to an average of 24.76%, reducing sugars 62.96%, and pH 2.95 (Table 2). No significant amounts of HMF were detected in the honey from the three locations analyzed (Figure 2).

Physicochemical parameters/Sample	A	B	C	Average
Moisture (%)	24.80	24.70	24.8	24.76
Reducing sugars (%)	63.36	61.52	64.0	62.96
Total sugars (%)	66.80	64.80	65.28	65.60
apparent sucrose (%)	3.40	3.30	1.30	2.60
pH	3.07	2.84	2.96	2.95
Water activity	0.644	0.689	0.677	0.67
HMF	Absent	Absent	Absent	–

Table 2 – Physicochemical parameters of *Melipona bicolor* honey samples collected in the metropolitan region of Curitiba-PR. A: 25.874460, -49.404861; B: -25.724054, -49.300099; and C: -25.803695, -49.303595.

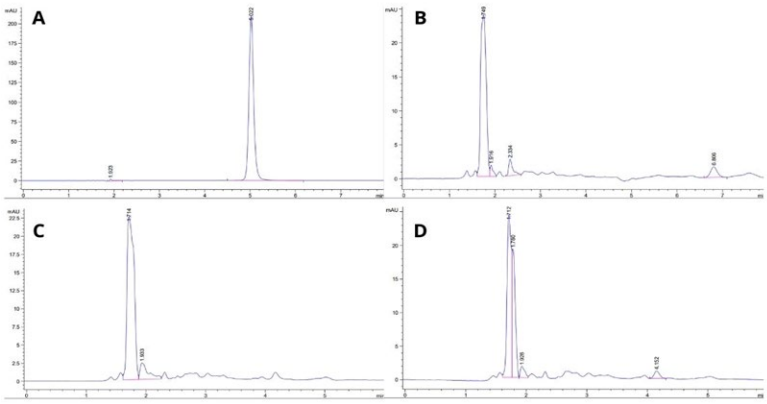

Figure 2 – Standard peak for HMF at concentration 0.01 mg/ml (A); HMF HPLC chromatography of *Melipona bicolor* honey collected in the metropolitan region of Curitiba-PR. A: -25.874460, -49.404861; B: -25.724054, -49.300099; and C: - 25.803695, -49.303595; and D: -25.803695, -49.303595.

As for the microbiological parameters of *M. bicolor* honey, the counting results in Standard values in plates of aerobic mesophilic bacteria were 3.29 (A), 2.46 (B), and 4.08 (C) log CFU/ml. The results for the most likely number of total and thermotolerant coliforms found were ≤ 3 MPN/ mL for all samples. The mold and yeast counts were 2.43 log CFU/ml for honey from property A and 1.78 log CFU/ml for honey from property B, with the absence of molds and yeasts being observed in honey from location C (Table 3).

Microbiological Parameters	A	B	C
Aerobic Mesophilic Bacteria (CFU/ml)	3.29 log	2.6 log	4.08 log
Total and Thermotolerant Coliforms (MPN/ml)	≤3	≤3	≤3
Molds and Yeasts (CFU/ml)	2.43 log CFU/ml	1.78 log CFU/ml	≤1 log CFU/ml

Table 3 – Microbiological parameters of *Melipona bicolor* honey samples collected in forests consisting of secondary formations in medium and advanced stages located in the metropolitan region of Curitiba, PR. A: -25.874460, -49.404861; B: -25.724054, -49.300099; and C: -25.803695, -49.303595.

4. Discussion

The reason for choosing the *M. bicolor* bee to carry out this research is related to the fact that this is a species of bee typical of the southern region of Brazil (NOGUEIRA-NETO, 1997), in addition to being a species threatened with extinction (ÁVILA et al., 2018). Guaraipo honey has a different flavor, having higher acidity and higher moisture content compared to European bee honey (BILUCA et al., 2017; FALEIROS-QUEVEDO & FRANCOY, 2022). Such characteristics prevent, among other factors, the application of the parameters established for *A. mellifera* honey described in Normative Instruction N° 11 of 2000 of the Ministry of Agriculture, Livestock and Supply (BRASIL, 2000).

Given the analysis of the physicochemical and microbiological parameters carried out, it was found that the results obtained were within the recommended standards for stingless bee honey by the Legislation and Technical Regulations for Stingless Bee Honey in Parana (PARANÁ, 2017). It is important to consider that the physical-chemical properties, chemical composition and biological activities of honey depend on the species of bee that produced it, the physiological state of the colony, the composition of the flora used, the level of maturity of the honey, geographic region, the conditions climate at the time of harvest, season, among other factors (ECHEVERRIGARAY et al., 2021; SOUSA et al., 2021; CHUTTONG et al., 2016). In this work, the harvest was carried out during the summer period, with temperatures varying between 18 and 25°C throughout the day. In summer, there is an increase in the level of honey production by bees. Temperature is a determining factor for bees to carry out their activities. As they are ectothermic beings, there is a significant exchange of heat between bee organisms and the environment. Low temperatures reduce your metabolism, even preventing you from flying. While very high temperatures stimulate the colony's ventilation behavior to reduce the temperature (CAMPOS et al., 2010; DE MOURA et al., 2022).

Determining the moisture content of honey is directly related to the maturity of the product and the possibility of the development of unwanted microorganisms (OLIVEIRA et al., 2023). According to the Physico-Chemical Characteristics of the main Meliponiculture Products and Legislation and Technical Regulations on Identity and Quality (RTIQ), the maximum moisture content established for refrigerated honey is 35 g/100 g. Therefore, the results obtained ($\bar{x}$ =24.76g/100g) are in line with the requirements recommended by the current legislation for honey from native stingless bees (PARANÁ, 2017). It is important to highlight that

stingless bee honey has a higher moisture content compared to *A. mellifera* honey, whose maximum allowable limit established by the Technical Regulation of Identity and Quality is 20 g/100 g (BRASIL, 2000). This occurs due to the production process carried out by each species of bee because, during honey production, *A. mellifera* fly their wings incessantly close to the combs to dehumidify the honey, which also contributes to preventing fermentation from occurring, preventing its deterioration (EYER et al., 2016; MITCHELL, 2019). Stingless bee honey has a different production process, being deposited in jars. Therefore, it has a greater tendency to ferment (BARBOSA et al., 2013; REINHARD-JESAJAS et al., 2023). Furthermore, the high temperature and moisture of tropical and subtropical regions, the composition of the nectar used, and the time in which the honey was collected also influence the moisture content of native bee honey (ÁVILA et al., 2018; BRASIL, 2000), as we can see in the research carried out by Ávila (2019), Nascimento (2015) and Borsato (2013).

As for reducing sugars, they are predominantly determined by glucose and fructose, the main monosaccharides present in honey. This characteristic is influenced by the flowering of the region where the meliponary is located and the time in which the honey was collected (ÁVILA et al., 2018; OLIVEIRA et al., 2023). IN. MAPA nº 11/2000 stipulates a minimum concentration of 65% for reducing sugars; however, this value applies to honey from Africanized bees (BRASIL, 2000). The RTIQ for stingless bee honey in Parana establishes a minimum reducing sugar value of 47% (PARANÁ, 2017). Therefore, the values obtained follow the minimum requirements established by legislation and are close to the values obtained by other authors (BORSATO, 2013; NASCIMENTO et al., 2015; BILUCA et al., 2016).

Around 60% of total sugars are made up of reducing sugars. There is no limit established in the RTIQ for stingless bee honey in Parana for total sugars. On the other hand, Echeverrigaray et al. (2021) presented results obtained from analyses of the total sugars in *Melipona* honey, with averages of 80.39%, a value significantly different from the results obtained in the present work ($\bar{x}$ =65.63%). However, it is important to highlight that, even if honey is produced by the same species of bee, numerous factors influence the composition of honey and its physical-chemical characteristics, which justifies the difficulty in establishing standards of identity and quality that represent reality in general (NORDIN et al., 2018). In this case, the different hypothesis may be related to the fact that the honey samples in the study conducted by Echeverrigaray et al. (2021) were collected in an area composed of native and exotic forests, and, in the present work, the honey samples were collected from regions composed of secondary formations in medium and advanced stages.

Apparent sucrose is defined as the main disaccharide present in honey (DEMETERCO, 2016). According to legislation and RTIQ recommendations, concentrations must have a limit of 5% (PARANÁ, 2023). Some honey present undetectable values of apparent sucrose (BATISTON et al., 2020; BILUCA et al., 2016), however, other studies indicated the presence of apparent sucrose at levels by the recommended limit (BORSATO, 2013; NASCIMENTO et al., 2015; ÁVILA et al., 2019). Concentrations above the limits indicate that the collection was carried out before the honey matured, that is, the sucrose has not yet been completely transformed into glucose and fructose by the action of invertase (JULIKA et al., 2020). Therefore, it is possible to verify that the results obtained ($\bar{x}$ =2.6%) are within the standards established by legislation (PARANÁ, 2023).

Honey from stingless bees has an acidic character, with low pH and high acidity. Such parameters are directly related to the quality of the product, as they can indicate its deterioration (NORDIN et al., 2018). Reduced pH values inhibit microbial growth; however, acidity values greater than 60 mEq kg^{-1}, which is the maximum level of free acidity allowed in honey (AOAC, 1998), together with low pH values, may suggest fermentation of sugars into organic acids, causing the formation of acetic acid (BERGAMO et al., 2019).

The RTIQ for stingless bee honey in Brazil establishes a maximum pH of 4.7 (PARANÁ, 2017), with the average pH of *M. bicolor* honey in Brazil equivalent to 3.42. With this, we can conclude that the pH of the honey analyzed in this work ($\bar{x}$ =2.95) meets the RTIQ requirements for *M. bicolor* honey in Brazil and is close to the pH values of *Melipona bicolor honey* identified in Brazil (ÁVILA et al., 2019, BATISTON et al., 2020, BILUCA et al., 2016, BORSATO, 2013; BRASIL, 2023).

Another parameter related to honey deterioration is water activity, consisting of a measure that evaluates the availability of water susceptible to chemical and enzymatic reactions. The greater the water activity, the greater the possibility of microorganisms multiplying (ECHEVERRIGARAY et al., 2021). The water activity necessary for the development of microorganisms is below 0.98, being around 0.7 for molds, 0.8 for yeasts, and 0.9 for bacteria (ÁVILA et al., 2018). The RTIQ for Native Stingless Bee Honey does not have a parameter for water activity in stingless bee honey in Parana; however, it establishes a scale of 0.52 to 0.8 for honey collected in São Paulo, values close to those water activity results obtained (PARANÁ, 2017; SAO PAULO, 2017).

Hydroxymethylfurfural (HMF) is a molecule resulting from the acid decomposition reactions of monosaccharides present in honey (glucose and fructose) and the condensation of carbohydrates with free amine groups, known as the Maillard reaction, a non-enzymatic browning reaction. HMF may or may not be present in fresh honey. The presence of this molecule in minimal quantities can occur naturally, as well as its gradual increase over time. Therefore, HMF is classified as a parameter of the freshness and quality of honey (GRAINGER et al., 2017; SHAPLA et al., 2018; NORDIN et al., 2018). Furthermore, exposing honey to high temperatures leads to an increase in the formation of HMF, which may be caused by inadequate heating carried out on crystallized honey to facilitate the processing process in warehouses or by high temperatures caused by the climate, including exposure to the sun. Therefore, high HMF content indicates heating, prolonged storage, and inadequate processing. Furthermore, it can be considered an indicator of honey adulteration due to the addition of sweeteners such as beet or cane sucrose, corn sugar, rice sugar, fructose, high-fructose corn syrups, maltose, and inverted sugar (OROIAN et al., 2023; ROBERTSON, 2019; ÁVILA et al., 2018). The HMF levels recorded for *M. bicolor honey* in Paraná vary from minimum levels of 0.21 (ÁVILA et al., 2019) and 5.88 (BORSATO, 2013)

to 31.58 (NASCIMENTO et al., 2015) with the maximum limit being considered to be 40 mg/kg (PARANÁ, 2017). No significant amounts of HMF were detected in the honey from the three properties analyzed, indicating the presence of HMF by the values recommended by the RTIQ. Therefore, it was found that there was no heating of the honey, nor were there any inappropriate processing processes during its handling that would increase the HMF content. Another justification for the results obtained can be attributed to the storage time since, from the moment of harvest to the moment of analysis, there was a short period, which could be considered insufficient for a significant accumulation of HMF to occur in the honey (NORDIN et al., 2018).

Regarding the microbiological parameters of *M. bicolor* honey, the results of the standard plate count of aerobic mesophilic bacteria showed 3.29 log10 CFU/mL^{-1} for location A, 2.46 log10 CFU/mL^{-1} for location B and 4.08 log10 CFU/mL^{-1} for location C. There are no recommended microbiological parameters for stingless bee honey in Brazil. The differences in characteristics between *M. honey and A. mellifera* prevent the use of the parameters recommended by IN N°11 of 2000 (BRASIL, 2000). The results for total and thermotolerant coliforms, ≤0.3/g MPN, were found for all samples. Parana Legislation recommends a limit of 10^2 for coliforms in *M. bicolor* honey (PARANÁ, 2017). Therefore, the findings obtained are within the parameters determined by the Legislation and consolidate previous results (SILVA et al., 2021; DELGADO et al., 2020; ÁVILA et al., 2019). The presence of coliforms in honey indicates contamination, which can be acquired through a source outside the nest being carried by the bee. Contamination can also occur due to the lack of adoption of Good Manufacturing Practices when harvesting the honey or during its transportation, among other factors (SILVA et al., 2021).

The Legislation of the State of Paraná allows counts of up to 4.00 log CFU/mL^{-1} of molds and yeasts (PARANÁ, 2017). The results obtained when counting molds and yeasts were 2.43 log CFU/mL^{-1} for samples from location A and 1.78 log CFU/mL^{-1} for location B, being absent in samples collected from location C. Therefore, they are aligned with the requirements established by State Legislation for honey from native stingless bees. The presence of molds and yeasts may be related to the honey production process since the presence of yeasts, together with high moisture, favors spontaneous fermentation after the crystallization process, as phase separation causes an increase in water activity, creating a favorable environment for the fermentation of honey and the consequent multiplication of yeasts (SILVA et al., 2022; CHIRIFE et al., 2006). However, it is important to highlight that some yeasts are related to the development and nutrition of bees (ECHEVERIGARAY, 2021).

Samples A and B presented counts of 2.43 and 1.78 CFU/ mL, respectively. The absence of molds and yeasts observed in sample C may be correlated to the fact that this sample is the only one that did not show crystallization, in addition to having a reduced apparent sucrose content compared to the other samples. The presence of molds and yeasts was observed in previous research (ECHEVERRIGARAY et al., 2021; SILVA et al., 2021; DA SILVA et al., 2022; DE SOUSA et al., 2022). However, its absence has also been previously reported (SILVA et al., 2016).

5. Conclusion

Physico-chemical and microbiological parameters of honey are fundamental to verify its quality and safety, enabling it to be sold by the standards required by legislation ensuring consumer health. The moisture content, sugars, pH, and HMF obtained in the present study indicate that the *M. bicolor* honey collected in three different properties located in the metropolitan region of Curitiba, PR, is aligned with the minimum requirements existing in the Legislation and Technical Regulations of Identity and Quality for Meliponiculture in Brazil. Therefore, they are suitable for consumption and commercialization. However, the lack of criteria for water activity and standard counts of mesophilic bacteria highlights the importance of determining parameters that represent the reality of stingless bee honey, both to help the producer market the product and to prevent fraud. Furthermore, the distinctions between the physical-chemical and microbiological parameters of honey from bees native to different regions, even if it is produced by the same species, make it difficult to develop legislation that applies generally to the entire Brazilian territory. Therefore, each State must establish its parameters, having scales that enable compliance with the required standards, regardless of the species of bee used, or even classify the parameters according to the respective genera.

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