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# Microbiological characteristics of meliponine honey marketed in the State of Paraná – Brazil

**Características microbiológicas do mel meliponineos comercializados no Estado do Paraná – Brasil**

**Características microbiológicas de la miel de meliponina comercializada en el Estado de Paraná – Brasil**

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**Abstract**
Meliponine is a honey with particular characteristics; it has a high percentage of humidity, which can favor the development of microorganisms, thereby causing changes in the quality of the product. The objective of this study was to evaluate the microbiological characteristics of meliponine honey produced by different species and marketed in the State of Paraná-Brazil. The sample unit was composed of honey of *Tetragonisca angustula* (n = 15), *Scaptotrigona bipunctata* (n = 05), *Melipona quadrifasciata quadrifasciata* (n = 05) and *Melipona bicolor schencki* (n = 01). The following microbiological parameters were evaluated: counts of total and thermotolerant coliforms, fungi count and detection of *Salmonella* spp. Principal component analysis (PCA) was used to evaluate the possible relationships among species, locality and type of microorganism. For microorganisms of the total coliform group, 15.38% of the analyzed samples had values >3 MPN/g. The presence of thermotolerant coliforms was observed in 7.69% of the samples, all of which were *T. angustula* samples. For fungi counts, 100.00% of the analyzed samples had values within the established range created by the Agricultural Defense Agency of Paraná. The presence of *Salmonella* spp. was not observed in any of the samples evaluated. The amount of water in the honey is related to the location of the apiary and edaphoclimatic factors. Adoption of good handling practices by beekeepers is indispensable for the safety of the final product.
**Keywords:** Microbiological quality; Coliforms; Fungi; *Salmonella*; PCA.

**Resumo**
Os meliponíneos elaboram um mel diferenciado com características particulares, apresenta elevado percentual de umidade o que pode favorecer o desenvolvimento de microrganismo ocasionando alterações na qualidade do produto. O objetivo deste estudo foi avaliar as características microbiológicas do mel de meliponíneos produzido por diferentes espécies e comercializado no Estado do Paraná-Brasil. A unidade amostral foi composta de mel de *Tetragonisca angustula* (n=15), *Scaptotrigona bipunctata* (n=05), *Melipona quadrifasciata quadrifasciata* (n=05) e *Melipona bicolor schencki* (n=01). Parâmetros microbiológicos foram avaliados: contagem de coliformes totais e

termotolerantes, contagem de fungos e detecção de *Salmonella* spp. A análise de componentes principais - ACP foi utilizada para avaliar as possíveis relações entre espécie, localidade e tipo de microrganismo. Para microrganismos do grupo coliformes totais, 15,38% das amostras analisadas apresentaram valores >3 NMP/g. Em 7,69% das amostras foi observado a presença de coliformes termotolerantes, sendo todas amostras de *T. angustula*. Para contagem de fungos, 100,00% das amostras analisadas apresentaram valores dentro do estabelecido pela Agência de Defesa Agropecuária do Paraná. Não foi observado a presença de *Salmonella* spp em nenhuma das amostras avaliadas. Algumas amostras se assemelharam segundo a espécie produtora e a quantidade de água no mel é relacionada com a localização do meliponário, devido a fatores edafoclimáticos. Adoção de boas práticas de manipulação pelos meliponicultores se tornam indispensáveis para conferir segurança alimentar ao produto final.
**Palavras-chave:** Qualidade microbiológica; Coliformes; Fungos; *Salmonella*; ACP.

**Resumen**
La meliponíne produce una miel diferenciada con características particulares, presenta un alto porcentaje de humedad, lo que puede favorecer el desarrollo de microorganismos provocando cambios en la calidad del producto. El objetivo de este estudio fue evaluar las características microbiológicas de la miel de meliponíne producida por diferentes especies y comercializada en el Estado de Paraná-Brazil. La unidad de muestra estuvo compuesta por miel de *Tetragonisca angustula* (n = 15), *Scaptotrigona bipunctata* (n = 05), *Melipona quadrifasciata quadrifasciata* (n = 05) y *Melipona bicolor schencki* (n = 01). Se evaluaron parámetros microbiológicos: recuento de coliformes totales y termotolerantes, recuento de hongos y detección de *Salmonella* spp. Análisis de componentes principales: se utilizó ACP para evaluar las posibles relaciones entre especies, localidad y tipo de microorganismo. Para los microorganismos del grupo de coliformes totales, el 15,38% de las muestras analizadas presentaron valores >3 NMP/g. En el 7.69% de las muestras se observó la presencia de coliformes termotolerantes, siendo todas muestras de *T. angustula*. Para contar hongos, el 100,00% de las muestras analizadas presentaron valores dentro de lo establecido por la Agencia de Defensa Agropecuaria de Paraná. No se observó presencia de *Salmonella* spp en ninguna de las muestras evaluadas. Algunas muestras fueron similares según la especie productora y la cantidad de agua en la miel está relacionada con la ubicación del meliponario, debido a factores edafoclimáticos. La adopción de buenas prácticas de manipulación por parte de los productores de miel es fundamental para garantizar la seguridad alimentaria del producto final.
**Palabras clave:** Calidad microbiológica; Coliformes; Hongos; *Salmonella*; ACP.

## 1. Introduction

Honey is a natural, healthy and clean product that naturally contains microorganisms at minimum levels that provide subsidies for its control in industry and its natural characteristics (Bogdanov, 2009). Containing a distinct and unique microflora, honey has a high antimicrobial activity that is generally related to physical factors such as osmolarity, chemicals, and the presence of volatile compounds and hydrogen peroxide. The enzyme glucose oxidase and phenolic compounds are a natural barrier to the development of microorganisms in honey due to their oxidizing characteristics (Eteraf-Oskouei & Najafi, 2013).

The microbiota present in honey can be divided into two microorganisms' groups. The microorganisms inherent to honey, which are the primary source of contamination, are bacterial spores, molds and yeasts that, under normal conditions of humidity, do not interfere with honey quality. There is secondary contamination directly related to the extraction, manipulation and processing of the product. This microbiota is one of the main criteria for evaluating product quality, along with other physical, chemical and sensory characteristics (Silva et al., 2017).

The total coliforms group is composed of non-sporulating, gram-negative bacteria that are, lactose fermenters, with acid and gas production at temperatures ranging between 32° and 37°C. The thermotolerant coliforms, whose natural habitat is the intestinal tract of warm-blooded animals, may be present in other environments such as water and vegetables, and they demonstrate whether the food has had contact with fecal material (Sereia et al., 2017).

The presence of microorganisms in honey may be variable and depend on floral origin, moisture, harvesting techniques and storage temperature (Al-Waili, Salom, Al-Ghamdi, & Ansari, 2012). The quality of the honey is related to the hygiene practices of the producer, but it is also related to the hygiene habits of the bees. Many species land in fecal matter

(Nogueira-Neto, 1997), which can cause a change in the quality of the final product, reducing shelf-life or rendering the product unfit for human consumption.

The conservation techniques (refrigeration, pasteurization, dehydration and fermentation) adopted by beekeepers are empirical. Brazilian and international regulations do not include microbiological quality parameters for meliponine honey (Brasil, 2000; FAO, 2001). The absence of specific legislation and the diversity of meliponine species in Brazil reinforces the need to obtain more specific information on the physicochemical and microbiological characteristics of honey for the establishment of quality and identity standards (Carvalho et al., 2013).

The Technical Regulation of Identity and Quality of Honey of Stingless bees for the state of Parana was established by the Agency of Agricultural and Livestock Defense of Parana, Ordinance N° 63 of March 10, 2017, based on results from studies conducted in the region (Adapar, 2017). This standard determines which parameters should be considered to assess the quality of honey produced by stingless bees. The objective of this study was to evaluate the microbiological characteristics of meliponine honey produced by different species and marketed in the State of Paraná.

## 2. Materials and Methods

### *2.1 Collection of samples*

The samples were obtained from six distinct regions (Figure 1) of the State of Paraná-Brazil, comprising 14 different cities (Table 1 and Figure 1). We analyzed 26 samples of meliponine honey from the following species: *T. angustula* (n = 15), *S. bipunctata* (n = 5), *M. q. quadrifasciata* (n = 5) and *M. b. schencki* (n = 1).

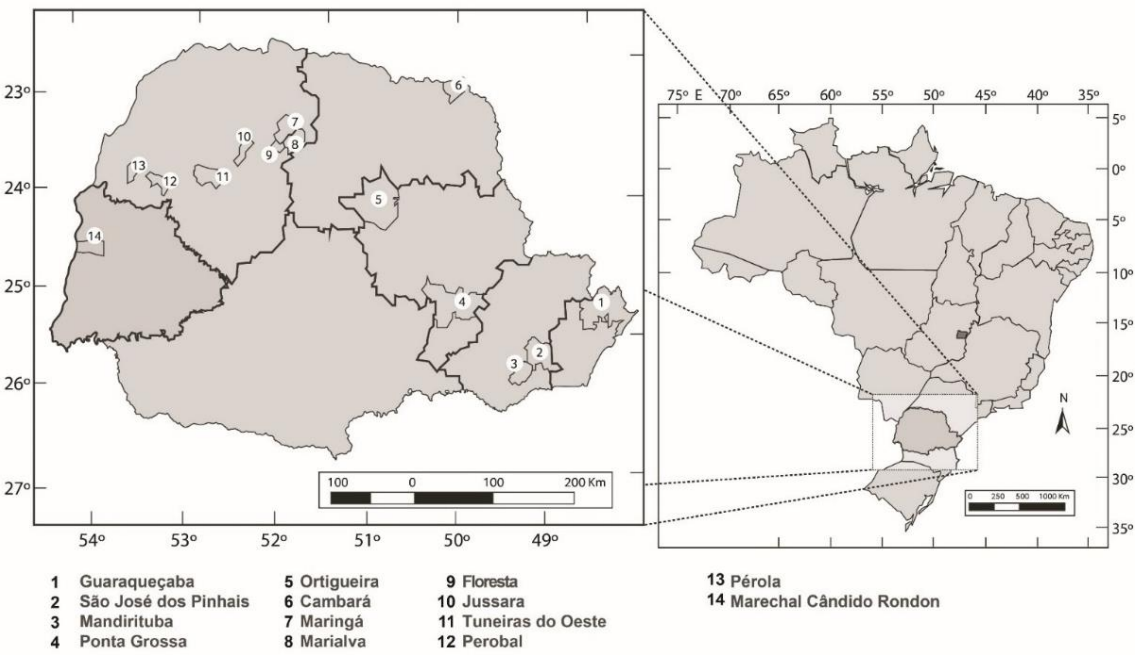
The samples were collected from their colonies according to the practical methods of harvesting of each producer. After harvesting, the honey was packed in properly sterilized polypropylene or glass jars and stored under refrigeration at a temperature of 5°C until the start of the analysis.

**Table 1.** Geographical coordinates of the cities in which the honey samples were collected.

| Cities of Paraná - Brazil | Geographical coordinates | State Region |
|---|---|---|
| Guaraqueçaba | 25° 18' 25" S 48° 19' 44" W | Coast from Paraná |
| São José dos Pinhais | 25° 32' 06" S 49° 12' 21" W | Curitiba/metropolitan area |
| Mandirituba | 25° 46' 44" S 49° 19' 33" W | Curitiba/metropolitan area |
| Ponta Grossa | 25° 05' 42" S 50° 09' 43" W | Campos gerais |
| Ortigueira | 24° 12' 28" S 50° 56' 56" W | Campos gerais |
| Cambará | 23° 02' 45" S 50° 04' 26" W | North |
| Maringá | 23° 25' 30" S 51° 56' 20" W | Northwest |
| Marialva | 23° 29' 06" S 51° 47' 31" W | Northwest |
| Floresta | 23° 35' 56" S 52° 04' 51" W | Northwest |
| Jussara | 23° 37' 15" S 52° 28' 08" W | Northwest |
| Tuneiras do Oeste | 23° 52' 15" S 52° 52' 33" W | Northwest |
| Perobal | 23° 53' 45" S 53° 24' 36" W | Northwest |
| Pérola | 23° 48' 18" S 53° 41' 02" W | Northwest |
| Marechal C. Rondon | 24° 33' 21" S 54° 03' 25" W | West/Itaipu |

Source: Authors.

**Figure 1.** Geographical distribution of cities where honey samples were collected.




Source: Authors.

### *2.2 Microbiological analysis*

Following methods described by APHA's international standards, the APHA technical committee on microbiological methods for food (Salfinger & Tortorello, 2015) and the data collection techniques described by Pereira, Shitsuka, Parreira, & Shitsuka, (2018), the following microbiological analysis were performed: counting of total and thermotolerant coliforms, fungi counting and *Salmonella* spp. counts (Brasil, 2003). Aseptically, 25 g of honey were added to 225 mL of peptone water (Himedia, Mumbai, India) (0.1%) and homogenized. Subsequent decimal dilutions were prepared in sterile peptone water and analyzed by counting plates or by The Most Probable Number technique (MPN), depending on the type of microorganism.

#### *2.2.1 Total coliforms*

The Most Probable Number technique (MPN/g), obtained using Hoskins table analysis (APHA, AWWA, WEF, 2012), was used. The presumptive test was initially carried out using a series of the three dilutions. One mL was inoculated into a series of three tubes using lauryl sulphate tryptose broth - LST (Acumedia, Lansing, EUA) to determine total coliforms. The tubes were incubated at 35°C for 48 h. The confirmatory test included inoculating three loops of each LST-positive tube into tubes containing brilliant green bile broth - BGBB (Biomark, Pune, India) at 35°C for 48 h. The presence of gas and turbidity in the environment defines this analysis as positive.

#### *2.2.2 Thermotolerant coliforms*

Escherichia coli broth - EC (Acumedia, Lansing, EUA) was used for the detection of thermotolerant coliforms. Three loops of each LST-positive tube were transferred to EC broth and incubated at 45°C for 48 h. The presence of gas and turbidity in the environment defines this analysis as positive.

#### *2.2.3 Fungi counts*

For fungi counts, 1.0 mL of the dilutions were plated using potato dextrose agar - PDA (Acumedia, Lansing, USA) acidified with tartaric acid (Synth, São Paulo, Brazil) at 10.00% to pH 3.5. The incubation was carried out in a bacteriological oven (TE-392/2, Tecnal, Brazil) for seven days. After this time, the plates were removed from the oven, and the number of colony forming units (CFU/g) was counted.

#### *2.2.4 Analysis of Salmonella spp.*

The sample was prepared according to section 2.3.1 and incubated for 20 h at 36°C in a bacteriological oven. Aliquots of 0.1 mL of the pre-enriched samples were transferred to separate tubes containing 10.0 mL of rappaport vassiliadis soy broth – RVS (Acumedia, Lansing, USA) and 10.0 mL of selenite cystine broth – SC (Biomark, Pune, India) and incubated at 41°C for 24 h in a water bath (Q226M2, Quimis, Brasil). Isolation of typical colonies of Salmonella spp. was performed on solid medium: brilliant green phenol red lactose sucrose agar - BLPS (Acumedia, Lansing, USA) and hektoen enteric agar - HE (Acumedia, Lansing, USA).

### *2.3 Moisture and water activity*

The moisture was determined using a bench refractometer (WY1A; ABBE, USA) with the aid of a Chataway table (AOAC, 1990; Bogdanov, 2009). A digital meter (LabSwift; Novasina, Switzerland) using a dew point fixation technique in an encapsulated mirror = measured the water activity.

### *2.4 Statistical analysis*

The multivariate statistical method of principal component analysis using Matrix Laboratory Matlab® software, version R2013b, was used to verify the similarities and differences among the data obtained from the samples (Pestana & Gageiro, 2014). To make a general evaluation of the data, it was necessary to use the main components analysis method for determining which samples were similar (scores) and which motives or charges characterized separation (loadings). Eight major components were used to explain 97.43% of the total variance of the data. This technique aims to reduce the number of variables analyzed. All determinations were performed in triplicate.

## 3. Results and Discussion

### *3.1 Total and thermotolerant coliforms*

It was observed in Table 2, that 15.38% and 7.69% of the analyzed samples had > 3 MPN/g values of total and thermotolerant coliforms, respectively. All of these samples originated from *T. angustula*. This species of bee is considered one of the most hygienic among the meliponineos, but this does not exclude the possibility that this bee landed on undesirable materials (Nogueira-Neto, 1997). Results > 3 MPN/g can be attributed to failures in good practices of manipulation by the beekeeper in relation to the samples. Some species of meliponine, such as *M. subnitida* and *T. spinipes*, exhibit anti-hygienic habits, with direct records of the collection of fecal material (Nogueira-Neto, 1997). There are reports of visits of *S. bipunctata* and *M. q. quadrifasciata* to vertebrate excrement (Nogueira-Neto, 1997). However, all honey samples from these species had values < 3 MPN/g for thermotolerant coliforms.

**Table 2.** Microbiological quality of honey samples grouped by meliponine species from regions of the State of Paraná, Brazil.

| | | Coliforms (MPN/g) | | | | | |
|---|---|---|---|---|---|---|---|
| **Species** | **Sample** | **35°C** | **45°C** | **Fungi (CFU/g)** | ***Salmonella* (25 g)** | **Moist (%)** | **Aw** |
| | 1 | <3.00 | 0.00 | 3.60x10⁴ | Away | 24.07 | 0.69 |
| *T. angustula* | 2 | 215.40 | 3.00 | 3.50x10⁴ | Away | 24.87 | 0.72 |
| | 3 | <3.00 | 0.00 | 8.10x10⁴ | Away | 24.60 | 0.71 |
| | 4 | <3.00 | 0.00 | 5.80x10⁴ | Away | 24.73 | 0.71 |
| | 5 | <3.00 | 0.00 | 5.40x10⁴ | Away | 26.87 | 0.73 |
| | 10 | <3.00 | 0.00 | 2.00x10⁴ | Away | 24.07 | 0.71 |
| | 11 | <3.00 | 0.00 | 7.60x10⁴ | Away | 24.60 | 0.71 |
| | 12 | <3.00 | <3.00 | 3.40x10⁴ | Away | 25.53 | 0.72 |
| | 13 | <3.00 | 0.00 | 1.30x10⁴ | Away | 23.27 | 0.70 |
| | 15 | 10.67 | <3.00 | 1.10x10⁴ | Away | 24.47 | 0.73 |
| | 16 | 3.00 | <3.00 | 2.30x10⁴ | Away | 24.33 | 0.70 |
| | 18 | <3.00 | <3.00 | 2.20x10⁴ | Away | 25.53 | 0.71 |
| | 20 | <3.00 | <3.00 | 2.60x10⁴ | Away | 25.13 | 0.72 |
| | 24 | 125.30 | 23.00 | 3.80x10⁴ | Away | 24.33 | 0.72 |
| | 25 | <3.00 | 0.00 | 3.60x10⁴ | Away | 23.93 | 0.72 |
| Mean | | 24.36 | 2.13 | 3.75x10⁴ | 0.00 | 24.69 | 0.71 |
| Median | | 1.00 | 0.00 | 3.50 | 0.00 | 24.60 | 0.71 |
| Standard deviation | | 61.72 | 5.84 | 2.12 | 0.00 | 0.85 | 0.01 |
| *S. bipunctata* | 7 | <3 | 0.00 | 4.70 x10⁴ | Away | 31.13 | 0.76 |
| | 17 | <3 | <3 | 2.50 x10⁴ | Away | 27.40 | 0.73 |
| | 19 | <3 | <3 | 2.70 x10⁴ | Away | 25.67 | 0.71 |
| | 23 | <3 | <3 | 1.00 x10⁴ | Away | 25.80 | 0.71 |
| | 26 | <3 | 0.00 | 3.50 x10⁴ | Away | 26.73 | 0.73 |
| Mean | | 1.20 | 0.60 | 2.90 x10⁴ | 0.00 | 27.35 | 0.73 |
| Median | | 1.00 | 1.00 | 2.70 | 0.00 | 26.73 | 0.73 |
| Standard deviation | | 0.45 | 0.55 | 1.33 | 0.00 | 2.23 | 0.02 |
| *M. q. quadrifasciata* | 6 | <3 | 0.00 | 4.40 x10⁴ | Away | 34.07 | 0.79 |
| | 8 | <3 | 0.00 | 2.50 x10⁴ | Away | 25.40 | 0.70 |
| | 14 | <3 | 0.00 | 8.40 x10⁴ | Away | 27.67 | 0.72 |
| | 21 | <3 | 0.00 | 3.10 x10⁴ | Away | 38.73 | 0.84 |
| | 22 | <3 | 0.00 | 1.30 x10⁴ | Away | 41.80 | 0.85 |
| Mean | | 1.00 | 0.00 | 3.94 x10⁴ | 0.00 | 33.53 | 0.78 |
| Median | | 1.00 | 0.00 | 3.10 | 0.00 | 34.07 | 0.79 |
| Standard deviation | | 0.00 | 0.00 | 2.73 | 0.00 | 7.00 | 0.07 |
| *M. b. schencki* | 9 | <3.00 | 0.00 | 4.20 x10⁴ | Away | 33.13 | 0.78 |

Moist (%): moisture; Aw: water activity. Source: Authors.

Honey samples from *M. fasciculata* from the state of Maranhão, Brazil, had low counts of total and thermotolerant coliforms that were within the limits established by the Mercosul countries and the State Legislation ($10^2$ MPN/g). This

suggested good practices in relation to the manipulation of honey (Adapar, 2017; Fernandes, Rosa, & Conti-Silva, 2018; Mercosul, 1994). Good practices may be associated with the absence of microorganisms from the coliform group in trigonine honey samples in the State of Bahia, Brazil (Souza, Marchini, Oda-Souza, Carvalho, & Alves, 2009) and honey samples from the *M. compressipes*, *M. subnitida* and *M. scutellaris* species from the State of Piauí, Brazil (Monte et al., 2013; Souza, Marchini, Oda-Souza, et al., 2009). In samples of honey from *Melipona* spp. in the state of Amazonas, 33.33% had positive results for coliforms at 35°C and 45°C. The foraging flight areas of bees may influence the microbiological quality of honey (Matos, Nunes, Mota & Laureano, 2011).

The positive results verified in this study for the coliform group may be related to the fact that the quantification of these microorganisms in honey represents accurate data in the samples but not in the environment (Souza, Marchini, Oda-Souza, et al., 2009). The samples that are positive for thermotolerant coliforms may suggest the presence of other microorganisms that accompany *Escherichia coli* in feces. The presence of microorganisms in food is an indicator of the possible presence of pathogenic microorganisms that are difficult to identify (Gerba, 2015).

### *3.2 Fungi counts*

All samples of honey analyzed had values above those allowed in the Mercosur countries (Mercosul, 1994) (Table 2), in which honey can contain a maximum of 100 CFU/g, but the values were within the limit established by Paraná legislation ($10^4$ CFU/g) (Adapar, 2017). The maximum and minimum values were verified in sample 14 (*M. q. quadrifasciata*) and 23 (*S. bipunctata*).

The origin of fungi in honey is often naturally occurring and probably comes from primary sources when nectar is being harvested, stored and matured. Fungi may also be incorporated during processing, as spores can be found in the air (Al-Waili et al., 2012). Usually, this flora is found in concentrations below 100 CFU/g, because they are controlled by industrial practices that prevent fermentation (Mercosul, 1994).

It is recommended that meliponine honey should be collected aseptically to prevent possible external contamination (Schlabitz, Silva, & Souza, 2010). Apiaries in unhealthy environments and environmental variables may be responsible for counts of microorganisms above the standards in analyzed samples.

When evaluating the microbiological quality of 14 samples of honey produced by five species of stingless bees in the State of Bahia (*Tetragonisca*, *Frieseomelitta*, *Nannotrigona*, *Partamona* and *Scaptotrigona*), Souza, Marchini, Oda-Souza, et al., (2009) reported that 50% of the samples had results above the maximum established by the Mercosul Resolution. Fernandes et al., (2018), analyzing 40 samples of *M. fasciculata* honey from the State of Maranhão, verified that 5% of the samples had presented results above the maximum established by the Mercosur Resolution. However, under current state legislation (Adapar, 2017), 100% of the analyzed samples were within the established limit and were suitable for human consumption.

Honey samples from *T. angustula* in the city of Misiones, Argentina, collected aseptically had differences in microbiological quality, with mean values for molds and yeasts of $1.05x10^3$ CFU/g (Pucciarelli et al., 2014). Osmophilic yeasts are the microorganisms that influence the quality of honey. Yeasts are present due to acidic conditions of the medium and because they are not inhibited by high concentrations of sugar. This can lead to fermentation of the product, promoting the production of ethanol and carbon dioxide and generating unpleasant flavors (Tornuk et al., 2013).

### *3.3 Salmonella spp.*

*Salmonella* is the most common cause of gastrointestinal disease, leading to serious consequences in children under 5

years of age, the elderly over 65 years of age, and people with weakened immune systems. Outbreaks of salmonellosis detected in several parts of the world, and it is estimated that in the United States, 1 million foodborne diseases are caused by this microorganism (Mba-Jonas et al., 2018). In Canada, 87.500 domestic cases of food infection caused by *Salmonella* occur annually (Vrbova et al., 2018).

We did not identify *Salmonella* spp. in any of the analyzed samples (Table 2). In honey from *M. compressipes*, *M. subnitida* and *M. scutellaris* from the State of Piauí, Brazil, and in honey from *T. angustula* from the city of Misiones, Argentina, we did not detect *Salmonella*, suggesting the adoption of good management practices by the beekeeper, adequate facilities, and the use of disposable materials or stainless steel for the extraction, packaging and storage of honey (Monte et al., 2013).

***3.4 Moisture and water activity (Aw)***

Water is the main component of many foods and has influence on the food biochemical stability. The water excess found in meliponine honey is due to the low rate of dehydration of the nectar during the transformation process of honey (Monte et al., 2013). Meliponine honey may contain a moisture content above 20.00% (Anacleto, Souza, Marchini, & Moreti, 2009; Souza, Marchini, Oda-Souza, et al., 2009). Due to the moisture content the amount of free water (water activity) in this honey is also high. Water activity is one of the main factors that prevents or limits microbial development. Just as moisture is responsible for product stability, it modulates the microbial response and determines what kind of microorganism will develop in the product. Adverse conditions of Aw may cause osmotic stress and the sporulation of microorganisms, and under optimal water activity, conditions induce germination and growth. The production of secondary metabolites (toxins) is also affected by the Aw value of the medium.

An average of 29.65% moisture was observed, ranging from 23.27 to 41.80%, in *T. angustula* sample 13 and *M. quadrifasciata* sample 22, respectively (Table 2). Samples of honey from *T. angustula* from the city of Misiones, Argentina, had moisture contents of 24.00% (Pucciarelli et al., 2014). In the Northeast region of Brazil, honey samples from *M. subnitida* had a humidity of 24.80% (de Almeida-Muradian et al., 2013). In another study carried out in the Southern region of Brazil, more specifically in Santa Catarina State, the honey samples of *M. bicolor*, *M. quadrifasciata*, *S. bipunctata* and *T. angustula* had a humidity of 30.08%, 31.23%, 29.77% and 26.98%, respectively (Batiston, Frigo, Stefani, Silva, & Araujo, 2020).

The mean water activity observed in the samples was 0.75, with a variation of 0.69 to 0.85 for *T. angustula* sample 1 and *M. quadrifasciata* sample 22, respectively (Table 2). Variations in water activity values were found between 0.65 and 0.69 for samples of meliponas in the state of Piauí (Monte et al., 2013) and from 0.66 to 0.85 for samples of meliponas from different localities in the state of Bahia, northeastern Brazil (Souza, Marchini, Dias, et al., 2009). Values varying from 0.59 to 0.82, with an average of 0.66, were identified in *T. angustula* honey samples collected in São Paulo, Brazil (Anacleto et al., 2009).

Despite the known influence of moisture and water activity on the growth of microorganisms in a medium, the moisture and water activity of the honey samples did not explain the positive results found for the coliform group, mainly the thermotolerant coliforms. These bacteria require a water activity greater than 0.91 for growth (Bogdanov, 2014; Damodaran, Parkin, & Fennema, 2018), and the maximum Aw found in these analysis was 0.85. This is insufficient for the development of enteric pathogens, considering contamination post-processing of the product by unhygienic habits of the manipulator, which does not exclude the survival of these bacteria in the medium.

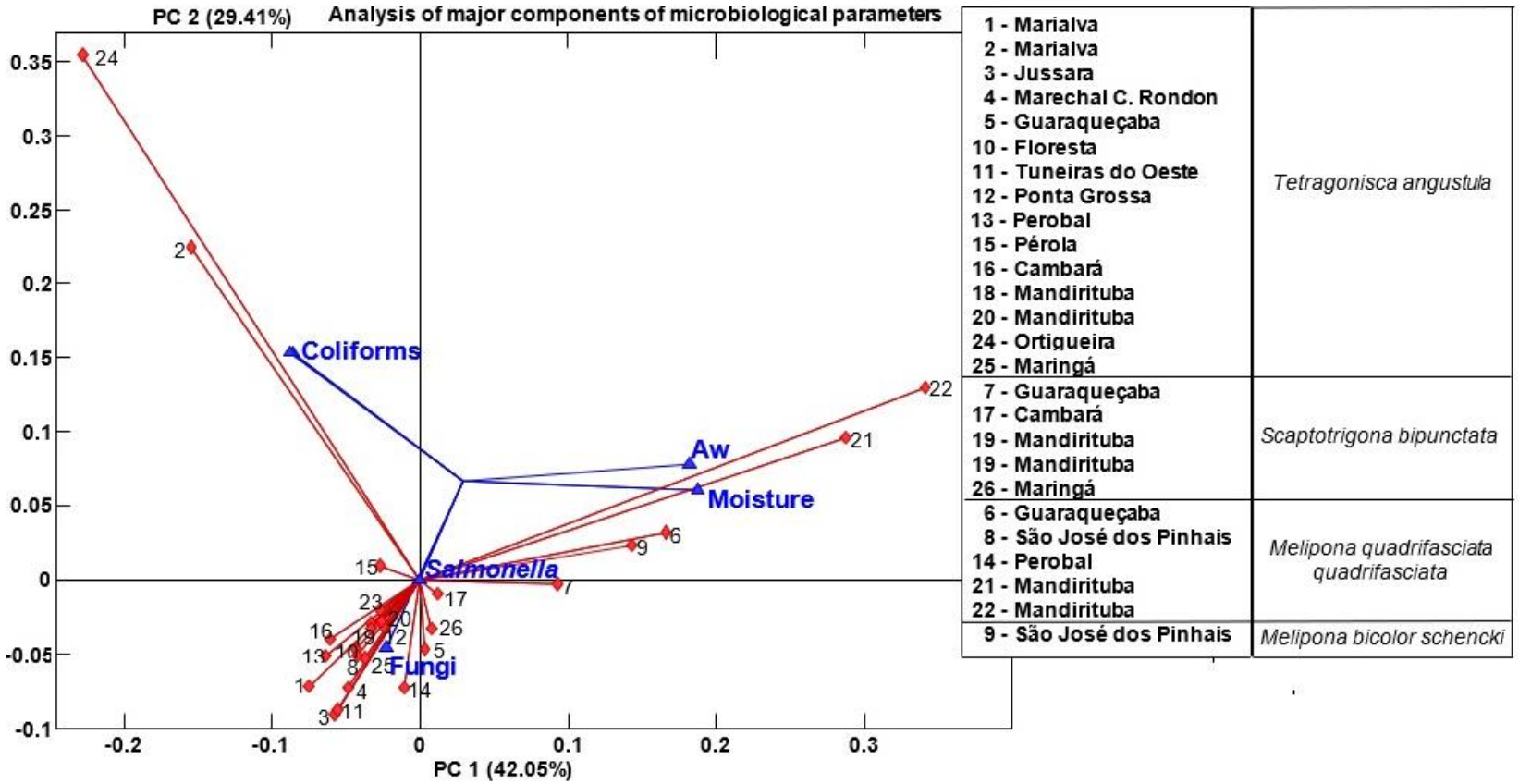
Due to the high water content of meliponine honey, the application of dehumidification techniques may be an option for conservation (Ramli et al., 2018). Without causing changes in quality and acceptability by consumers, this technique may help

to prolong the shelf-life of the product (Carvalho et al., 2009).

### *3.5 Principal component analysis*

Figure 2 presents the results of the principal component analysis by projection of principal component 1, which accounts for 42.05% of the explained variance, against principal component 2, which accounts for 29.41% of the explained variance. This analysis separates samples with higher counts of coliforms (negative quadrant of component 1 and positive quadrant of component 2) that may be influenced by water and moisture activity (positive quadrant of component 1 and 2), and it also separates samples influenced by significant levels of fungi (negative quadrant of component 1 and 2).

**Figure 2.** Correlations between microbiological characteristics and the description of the meliponine honey through principal component analysis. PC: principal component.




Source: Authors.

Figure 2 also presents the projection of samples with the highest counts of coliforms (negative quadrant of component 1 and positive quadrant of component 2), represented by samples two, fifteen and twenty-four, both produced by *T. angustula* bees, from the cities of Marialva, Perobal and Ortigueira, respectively.

The positive quadrant of component 1 and component 2 included most of the samples from the genus Melipona due to the moisture content and water activity. We observed that samples six, twenty-one and twenty-two, all from honey of bees of the species *M. q. quadrifasciata*, and sample nine, from the species *M. b. schencki*, had high values of moisture content and water activity. We observed that samples twenty-one and twenty-two, both from Mandirituba, were the samples that had the highest moisture content and water activity, probably due to the location of the apiary near the coast. Sample six from Guaraqueçaba and sample nine from São José dos Pinhais were less affected than the others.

Samples influenced by significant fungi content were grouped in the negative quadrant of component 1 and component 2. Most of the samples showed significant fungi counts, and samples three, four, five, eleven and fourteen were the samples that had the highest counts (from *T. angustula* bees).

## 4. Conclusions

Most meliponine honey samples from different species and localities in the state of Paraná, Brazil, were considered suitable for commercialization. The location of an apiary can influence the type of microorganism present in the honey, and the adoption of good practices of manipulation in relation to the product, from the management of the hive to consumption, guarantees the quality of the product and allows supervision by the authorities.

Future investigations on physical and chemical analysis of stingless honey, in conjunction with scientific articles that address the same theme, can contribute to improving the productivity and quality of the honey produced, resulting in the advancement of food and nutritional security.

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