



Physicochemical and microbiological analyses of clandestine honey commercialized in Rio Branco, Acre

Análises físico-químicas e microbiológicas de mel clandestino comercializado em Rio Branco, Acre

Giovana de Lira Teixeira^{1doi}, Rudney da Silva Maia Júnior^{1*doi}, Naiá Carla Marchi de Rezende Lago^{2doi}, André Buzutti de Siqueira^{3doi}, Patrícia Gelli Feres de Marchi^{4doi}, Cassio Toledo Messias^{1doi}

ABSTRACT: The commercialization of clandestine honey poses risks of adulteration, collection failure, and problems in production, packaging, storage, handling, and transportation, making it unsafe for consumption. The objective of this study was to analyze the physicochemical and microbiological properties of honey marketed in Rio Branco, Acre, Brazil. Ten duplicate samples were collected from street and municipal markets. Labeling standards and physicochemical parameters (moisture, ash and minerals, soluble solids, acidity, and pH) were evaluated using the Fiehe, Lund, and Lugol tests. Microbiological tests included detection of total and thermotolerant coliforms at 35°C and 45°C and mold and yeast counts (CFU/g). Only 50% of the samples had a moisture content within the established limit of 20%. All samples complied with the current standards for ash and mineral contents. The soluble solids had an average value of 76.5°Brix. In contrast, 70% of the samples exceeded the allowed limits of acidity, with an average pH of 2.43. Fiehe, Lund, and Lugol tests detected adulteration in 100% of the samples. Total coliforms were present in 30% of the samples, whereas thermotolerant coliforms were absent. Mold and yeast were observed in 60% of the samples. These honeys do not meet the Brazilian quality standards for stingless bees and, in the absence of standards for stinging bees, pose health risks to consumers.

Keywords: fraud; legislation; food quality; health.

RESUMO: A comercialização clandestina de mel apresenta riscos de adulteração, falhas na coleta e problemas na produção, embalagem, armazenamento, manuseio e transporte, tornando-o inseguro para o consumo. O objetivo deste estudo foi analisar as propriedades físico-químicas e microbiológicas do mel comercializado em Rio Branco, Acre, Brasil. Dez amostras duplicadas foram coletadas em feiras livres e mercados municipais. Os padrões de rotulagem e os parâmetros físico-químicos (umidade, cinzas e minerais, sólidos solúveis, acidez e pH) foram avaliados pelos testes de Fiehe, Lund e Lugol. Os testes microbiológicos incluíram a detecção de coliformes totais e termotolerantes a 35 °C e 45 °C e a contagem de bolores e leveduras (UFC/g). Apenas 50% das amostras apresentaram teor de umidade dentro do limite estabelecido de 20%. Todas as amostras atenderam aos padrões vigentes para teores de cinzas e minerais. Os sólidos solúveis apresentaram valor médio de 76,5 °Brix. Em contrapartida, 70% das amostras excederam os limites permitidos de acidez, com pH médio de 2,43. Os testes de Fiehe, Lund e Lugol detectaram adulteração em 100% das amostras. Coliformes totais estavam presentes em 30% das amostras, enquanto coliformes termotolerantes estavam ausentes. Bolores e leveduras foram observados em 60% das amostras. Esses méis não atendem aos padrões brasileiros de qualidade para abelhas sem ferrão e, devido à ausência de padrões para abelhas com ferrão, representam riscos à saúde dos consumidores.

Palavras-chave: fraude; legislação; qualidade dos alimentos; saúde.

INTRODUCTION

Beekeeping honey is present in Brazilian homes as either food or a natural remedy. According to Brazilian legislation (Brazil, 2000), honey is a food product produced from the nectar of flowers, secretions from the living parts of plants, or excretions of plant-sucking insects that remain in the living parts of plants, which bees extract and combine with their own specific substances for storage and maturation (Codex Alimentarius, 1981).

In Brazil, beekeeping is a significant source of additional income for small- and medium-sized rural producers (Aguiar *et al.*, 2016). According to data released by the Brazilian Institute of Geography and Statistics (IBGE), 64,188,949 tons of Brazilian honey

were produced in 2023 (IBGE, 2024), of which 28,555 tons were exported (IBGE, 2024).

Honey is composed of a concentrated sugar solution (75%), with a predominance of monosaccharides such as glucose and fructose, reduced amounts of disaccharides such as sucrose and maltose, water, vitamins, and minerals, such as calcium, copper, magnesium, and potassium (Damasia-gomes *et al.*, 2015; Bonagura *et al.*, 2024). The characteristics are influenced by the origin of the nectar, climatic and geographical conditions, raw materials used by the bees, and type of bee (Silva *et al.*, 2016; Conceição *et al.*, 2022; Ferreira *et al.*, 2023).

The increase in honey consumption, limited availability, and relatively high value stimulate the informal market, where some traders seek financial

¹ Federal University of Acre, Rio Branco, AC, Brazil.

² Moura Lacerda University Center, Ribeirão Preto, SP, Brazil.

³ Federal University of Roraima, Boa Vista, RR, Brazil.

⁴ Federal University of Mato Grosso, Barra do Garças, MT, Brazil.

*Corresponding author: jrmaiasr@gmail.com.

Received: 06/19/2025.

Accepted: 08/15/2025.

benefits to the detriment of the health and well-being of the consumer, leading them to adulterate the composition of food (Souza, 2018). The most common fraud observed in honey is the addition of commercial sugars, dextrins, and glucose; dilution by the addition of water or solvent; and mixing with stingless bee honey (Marquele-Oliveira *et al.*, 2017; Raypah *et al.*, 2022).

The most commonly used tests to characterize and ensure the quality of food include the physicochemical analysis of honey; assessments of the degrees Brix (°Bx), pH, and acidity; and the Fiehe's, Lund, and Lugol tests. Microbiological analyses assess risks to consumer health by detecting coliforms, molds, and yeasts (Pascual-Mate *et al.*, 2018). The objective of this study was to perform physicochemical and microbiological analyses of the types of honey commercialized in Rio Branco, Acre.

MATERIAL AND METHODS

Physicochemical and microbiological analyses were performed at the Food Technology Unit (UTAL) of the Federal University of Acre (UFAC), between October and November 2022. Ten duplicate honey samples were used, totaling 20 aliquots from open-air fairs and municipal markets that were not registered with the Official Inspection Services. The sellers identified five (A, C, D, H, and I) samples as coming from the Italian bee *Apis mellifera* Linnaeus 1758, and five (B, E, F, G, and J) from the urucu bee *Melipona scutellaris* Latreille, 1811.

The samples were classified by letters from A to J, followed by numbering 1 or 2 according to repetition. For comparison, all analyses included a honey sample with a federal inspection seal (SIF), identified as COM. Honey labels were observed and compared with the labeling standards recommended by Normative Instruction No. 11 of October 20, 2000 and the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA), instituted by Decree No. 9,013 enacted March 29, 2017, and updated in 2020 (Brazil, 2020).

Physicochemical analysis

The physicochemical analyses performed included assessments of the moisture content, ash and mineral contents, soluble solid content, and acidity and pH and the Fiehe, Lund, and Lugol reactions. Analyses were performed using two samples from the same batch and three replicates for each honey.

Moisture

The moisture content of the honey was determined using the Chataway refractometric method, 173/IV, from the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008), and the official methodology No. 969.38, described in the *Official Methods of Analysis* of the Association of Official Analytical Chemists (AOAC), according to the guidelines of the Manual of Official Methods for the Analysis of Foods of Animal Origin issued by the Ministry of Agriculture, Livestock and Supply (MAPA) (Brazil, 2019), using a benchtop digital refractometer (Abbe NOVA). The results were obtained from the relationship between the refractive index and percentage of water in the honey samples, using the Chataway Table for comparison. The readings were taken at temperatures between 25°C and 27°C and

corrected for a standard temperature of 20°C by adding 0.00023 to the refractive index for each degree above 20°C.

Ash and minerals

The ash content was measured using method 4.8 of the Adolfo Lutz Institute (IAL, 1985) and the ABNT NBR 15714-3 standard from the Manual of Official Methods for Analysis of Food of Animal Origin issued by MAPA (Brazil, 2019). The crucible was placed for 1 h in the muffle furnace (model q318S, Quimis) at 550°C and then immediately cooled in the desiccator at room temperature and weighed on a precision scale (model ATY224, Shimadzu). The sample (5 g) was added and incinerated on a heating plate in a fume hood (LUCA-15, Lucadema) until the smoke ceased. The crucible was then placed in the muffle furnace at 550°C for 3 h, cooled, and reweighed. The ash content was calculated as follows:

$$((\text{Final weight} - \text{Initial weight}) \times 100) / \text{Sample weight} = \% \text{ Ash}$$

Soluble solids

The amount of soluble solids was defined using a benchtop digital refractometer (Abbe). The °Bx was measured by adding a drop of honey to the prism and pressing the "READ" button, according to the method described by the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008).

Acidity

The acidity was detected using the 174/IV methodology from the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008) and AOAC method 962.19, which was guided by the Manual of Official Methods for the Analysis of Foods of Animal Origin issued by the MAPA (Brazil, 2019). The sample (10 g) was weighed and diluted in 75 mL of distilled water. Three drops of phenolphthalein were added, and samples were titrated with 0.1 N sodium hydroxide (NaOH) until the color changed from amber to pink, which indicated pH 8.5. The volume of NaOH that was used was recorded. The free acidity was calculated using the following formula:

$$\text{Volume} \times 50 \times \text{Solution factor} / \text{Weight} = \text{Acidity (mEq/kg)}$$

pH

The pH was measured using the potentiometric method and a pH meter (model LUCA-210, Lucadema), according to method 174/IV of the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008), the Brazilian Standard NBR 15714-6 of the Brazilian Association of Technical Standards (ABNT), and the guidelines of the Manual of Official Methods for the Analysis of Foods of Animal Origin, issued by MAPA (Brazil, 2019). The sample (10 g) was weighed in a beaker and diluted with 75 mL of water. The electrode was immersed in the solution, and the pH was recorded using a pH meter.

Fiehe's test

Fiehe's test was used to detect the addition of commercial glucose and overheating. Hydroxymethylfurfural was reacted with resorcinol in

an acidic environment, which produced a red color in the condensation compound. The analysis involved test tubes, a tube shaker (model AP-56, Phoenix), and the reagents resorcinol, hydrochloric acid P.A., and ethyl ether.

The procedure, which was adapted from that of the UTAL, involved combining 10 mL of 50% honey with 5 mL of ethyl ether; the solution rested until the etheric layer appeared. Using a 5 mL pipette, the etheric layer was transferred to a new test tube, and 5 drops of resorcinol hydrochloric solution were added. The solution turned red in the presence of commercial glucose or superheated honey (Brazil, 1981; Brazil, 2019). The results were compared to those of the inspected honey (COM) sample.

Lund test

The presence of albuminoids in honey was analyzed using the Lund test, according to methodology 182/IV of the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008). A 50 mL cup, 25 mL cup, 5 mL volumetric pipette, glass rod, and 0.5% tannic acid solution were used.

The sample (2 g) was diluted in 20 mL of distilled water and transferred to a 50 mL glass. A 0.5% tannic acid solution (5 mL) was added, and the volume was brought to 40 mL using distilled water, with homogenization. The solution was then allowed to stand for 24 h. In the presence of pure honey, a 0.6–3 mL deposit formed, whereas in adulterated honey, the deposit formation was nonexistent or negligible.

Lugol test

The presence of starch and dextrins in honey was investigated using the Lugol reaction and the procedure described by the Adolfo Lutz Institute (Torres; Bertolini; Franco, 2008). The sample (10 g) was weighed, diluted in 10 mL of distilled water, and stirred, followed by the addition of 1 mL of Lugol's solution. In the presence of commercial glucose, the solution was colored red-violet to blue. The results were compared to those of the inspected honey (COM) sample.

Microbiological Analysis

Microbiological analyses were performed to assess mold and yeast counts (colony-forming unit [CFU]/g), using the surface plating method, and the presence of total and thermotolerant coliforms at 35°C and 45°C, using the multiple tube technique. The sealed samples were taken to the UTAL microbiology sector and opened inside the vertical laminar flow bench (model 050, Pachane), to avoid the risk of external contamination.

Total and thermotolerant coliforms

Total and thermotolerant coliforms were analyzed using the multiple tube technique; nine test tubes were used, including three for each dilution containing Lactose Broth medium and an inverted Durhan tube. In a vertical laminar flow bench, a 10^{-1} dilution of each sample was prepared by collecting 25 g of each sample, adding 225 mL of 0.1% peptone saline solution, and homogenizing the mixture; then, 1 mL of the solution was added to three tubes containing lactose

and to another tube with 9 mL of peptone solution, forming a 10^{-2} dilution. A 10^{-3} dilution was prepared by adding 1 mL of the 10^{-2} dilution to three tubes and another with 9 mL of peptone solution; 1 mL of this dilution was then added to three other tubes containing lactose.

The tubes containing the dilutions were incubated in a culture furnace (model 002CB, Fanem LTDA) at approximately 36°C for 48 h. Subsequently, the presence or absence of gas formation was observed using inverted Durhan tubes. The results were presented as the most likely number (NMP).

The confirmatory phase was performed using positive samples containing gas with turbidities inside inverted Durhan tubes; the solutions were inoculated into test tubes containing 5 mL of *Escherichia coli* broth and inverted Durhan tubes. The samples were placed in a water bath (TE-0551, Tecnal) at 45°C for 24 h. Durhan tubes containing gas and light turbidity were considered positive.

Molds and yeasts

Molds and yeasts were quantified by counting the CFUs in Petri dishes containing Dicloran Rose Bengal Chloramphenicol (DRBC) Agar (Neogen). The analyses were performed in duplicate, with dilutions from 10^{-1} to 10^{-3} ; the plates were prepared in a vertical laminar flow bench, with approximately 20 mL of DRBC, and 0.1 mL of each dilution was inoculated on the surface of the plates and spread using a Drigalski loop. The plates were incubated in the biological oxygen demand (BOD) oven (model TE-371, Tecnal) at 22°C for 5 days, to detect the growth of molds and yeasts. The colonies were quantified using the following formula:

$$CFU/g = \text{Number of colonies} \times 10 \times \text{dilution factor}$$

Statistical analysis

The averages of the replicates were calculated using Excel (version 2019; Microsoft) and used for statistical analyses. Analysis of variance (ANOVA) and Tukey's test at a 5% probability were performed using the RStudio software, to compare the means.

RESULTS

Among the samples observed, 70% did not have labels, and 30% did. The honey with labels had little information on the origin, their contents, the species of bees involved, the nutritional table, warning about the restriction of honey consumption by children under one year of age, and identification of registration with the Official Inspection Services. Therefore, none of the honey samples met the requirements established by Normative Instruction No. 11 of October 20, 2000, and RIISPOA 2020 (Brazil, 2000; Brazil, 2020).

The honey samples had moisture values ranging from 17.4% to 24.6% (Table 1). Of the honey samples of the bee species *A. mellifera*, only samples A and I had a moisture content within the standards required by the Brazilian legislation, which established a maximum limit of 20%. Samples C, D, and H presented values greater than those allowed by law. Among the five honey samples identified as originating from *M. scutellaris*, only samples E, G, and J presented humidity levels within the limits allowed by legislation.

Table 1 – Physicochemical analysis of ten honey samples and one commercially inspected honey sample for comparison.

Samples	Humidity (%)	Ash (%)	Soluble solids °Bx	Free acidity (mEq/kg)	pH
A	19.4g	0.06bc ±0.055	78.95a	27.29d ±1.30	2.99c ±0.05
B	20.6d	0.03bcd ±0.010	77.6cde	107.61b ±1.22	2.55e ±0.02
C	23.8b	0.24bcd ±0.005	71.3f	116.71a ±3.49	1.82i ±0.07
D	23.6bc	0.02cd ±0.011	74.0ef	121.06a ±1.50	1.87i ±0.02
E	19.9F	0.16d ±0.005	78.4abc	15.42e ±1.98	2.93d ±0.04
F	24.6a	0.06b ±0.011	73.8ef	20.57de ±1.22	3.15b ±0.007
G	20.2e	0.38bcd ±0.042	78.1bcd	89.41c ±1.22	2.49f ±0.0
H	22.8c	0.18cd ±0.007	75.6def	93.76c ±12.62	2.44f ±0.01
I	19.6g	0.01d ±0.008	78.65ab	92.57c ±1.50	2.12g ±0.03
J	19.6g	0.21bcd ±0.008	78.65ab	120.6a ±0.96	2.02h ±0.01
COM	17.4h	0.35a ±0.013	80.9a	16.61e ±0.01	4.14a ±0.0
F Calculated	1780	60.244	78.938	609.99	3033.1

Means followed by the same letter in a column do not differ from each other according to the Tukey's test with a 5% probability. Data are presented as mean ± standard deviation.

The ash content ranged from 0.01% to 0.38% (Table 1). According to Normative Instruction No. 11, of October 20, 2000, the maximum allowable ash content for honey is 0.6 g/100 g. All honey samples meet the standard established by Brazilian legislation and qualified as fit for consumption (Brazil, 2000).

Although legislation does not recommend average values for total soluble solids, this parameter is used in scientific studies to determine the amount of dissolved substances in honey, especially sugar. Based on the values found in the ten samples evaluated, the possibility that the honey was extracted green was ruled out.

The acidity of *A. mellifera* honey samples ranged from 27.29 to 121.06 mEq/kg. Only sample A had a value within the limit allowed by legislation, which stipulated a maximum total acidity of 50 mEq/kg. Samples C, D, H, and I had values higher than allowed. For meliponine honey, the mean values ranged from 15.42 to 120.6 mEq/kg. Samples E and F complied with national standards, whereas samples B, G, and J showed acidities >50 mEq/kg.

The pH values were 1.82–3.15. According to MAPA, the pH values of honey can vary between 3.3 and 4.6. Therefore, except for the inspected commercial honey (COM), all samples showed values below the expectations.

In Fiehe's test, 100% of the samples showed an intense red coloration, indicating the possibility of additional commercial glucose or overheating (Table 2).

The analyzed samples neither showed deposits at the bottom of the beaker in the Lund reaction nor demonstrated negligible deposit values, indicating artificial honey or fraud by artificial substances. Only the inspected commercial honey (COM) produced a deposit >0.6 after 24 h.

In the Lugol reaction, all samples showed a color change from reddish-brown to dark blue, demonstrating the presence of starch or dextrin, which is indicative of adulteration. Only the commercial honey (COM) sample did not change color and was considered a negative honey (Table 2).

Table 2 – Results of the Fiehe, Lund and Lugol tests.

Samples	Fiehe	Lund	Lugol
A	+	SD	+
B	+	SD	+
C	+	SD	+
D	+	SD	+
E	+	SD	+
F	+	SD	+
G	+	SD	+
H	+	SD	+
I	+	SD	+
J	+	SD	+
COM	+	>0.6	-

Honey samples that produced a colorimetric reaction with resorcinol (Fiehe's reaction) and the Lugol reaction were considered "+" honeys, and those without any alterations were considered "-" honeys. SD = no deposits at the bottom of the cylinder.

The most probable number of total and thermotolerant coliforms 45°C (MPN/g) and standard mold and yeast counts (CFU/g) are shown in Table 3.

Microorganisms from the total coliform group were identified in samples E, I, and G, accounting for 30% of all samples. Thermotolerant coliforms (45°C) were not detected in any of the samples.

Samples E, F, H, and J did not show mold or yeast growth, similar to the control sample (COM). The other honey samples demonstrated mold and yeast counts ranging from 2.5×10^1 to 13×10^4 CFU/g. Normative Instruction No. 60 (Brazil, 2019) does not establish microbiological standards for honey; however, molasses and syrup categories have a maximum limit of 100 CFU/g for molds and yeasts. Therefore, samples A, B, C, and D were outside the standard established by the Brazilian legislation.

Table 3 – Microbiological analyses of honey samples and commercially inspected (control) honey sample.

Samples	Total coliforms (MPN/g)	Thermotolerant coliforms (MPN/g)	Molds and yeasts (CFU/g)
A	<3.0	<3.0	13 x 10 ⁴
B	<3.0	<3.0	7.7 x 10 ²
C	<3.0	<3.0	2.0 x 10 ³
D	<3.0	<3.0	1.2 x 10 ²
E	3.0	<3.0	Absent
F	<3.0	<3.0	Absent
G	4.85	<3.0	2.5 x 10 ¹
H	<3.0	<3.0	Absent
I	3.0	<3.0	0.5 x 10 ²
J	<3.0	<3.0	Absent
COM	<3.0	<3.0	Absent

DISCUSSION

Samples B, F, and G, which came from *M. scutellaris*, presented moisture levels above those allowed by legislation. For *A. mellifera*, samples C, D, and H also exceeded the limits established by the Brazilian legislation (Brazil, 2000). Although the maximum limit established by legislation is 20%, according to Carvalho *et al.* (2013), the maximum humidity value for meliponine honey is 30%. Raweh *et al.* (2023) found that the moisture content of honey is influenced by various factors such as the quality, stability, resistance to bacterial fermentation, and shelf life. Thus, the higher the moisture content, the shorter the useful life of the product.

The ash and mineral contents obtained were within legislative standards. According to Venturini, Sarcinelli and Silva (2007), determining the amount of ash and minerals helps in the detection of irregularities, such as a lack of good hygiene practices, impurities, and lack of decantation and/or filtration after the honey has been removed by the beekeeper.

The analysis of soluble solids yielded values ranging from 71.3 to 78.95 °Bx (Table 1). Flagini (2016) analyzed *Apis mellifera* honey collected from local beekeepers marketed in the same state and found values ranging from 77.1 to 79.4 °Bx, which is similar to values observed in samples A, D, I, and COM, also from the same species. In contrast, Aguiar *et al.* (2016) analyzed honey from stingless bees and found total soluble solids between 77.60 and 73.51 °Bx. These values are similar to those of samples B, E, G, and J from the stingless bees of *M. scutellaris*.

The degree of ripeness of honey can also be measured using this index, where more mature honey has higher °Bx values, and greener honey has lower values (Flagini, 2016; Silva, 2017). Therefore, based on the values found in the ten samples evaluated, the possibility that the honey was extracted green was ruled out.

Variations in the acidity content are related to the floral origin. The floral nectar collected by bees contains different organic acids and ions, and other factors, such as the bacterial behavior during the

maturation of the honey, can modify the acidity. Aguiar *et al.* (2016) demonstrated that the acidity of honey is associated with the control and development of microorganisms. The high acidity values can also result from the fermentation caused by microorganisms, which is favored by high humidity and temperature. Therefore, microorganisms convert sugars into alcohols via the oxidation of carboxylic acids (Parpinelli *et al.*, 2021).

Although pH is not a mandatory legislative parameter, pH analysis helps in the evaluation of the honey quality, as a low pH hinders the growth of microorganisms, favors the maintenance of shelf life, and promotes enzymatic breakdown (Alves *et al.*, 2015). Altered pH levels may be related to adulteration or fermentation of the honey (Capuci; Honorato, 2012).

All samples produced an intense red color in Fiehe's reaction, which indicated the addition of commercial glucose. However, inadequate storage, high moisture levels, high acidity, and aging of honey may also accelerate the formation of hydroxymethylfurfural (Finco; Moura; Silva, 2010; Alves *et al.*, 2015; Garcia *et al.*, 2018). The intense red color in the commercial honey sample (COM) may be related to honey overheating. All other samples failed the adulteration analyses, indicating possible fraud. In a study conducted by Aguiar *et al.* (2016) in the same state, Fiehe's test showed negative results only in honey samples from stingless bees.

In the Lund reaction, none of the uninspected honey samples showed a deposit at the bottom of the cylinder or a negligible deposit value, indicating artificial honey or fraud by artificial substances. Only the inspected commercial honey (COM) produced a deposit >0.6 after 24 h. Brazilian legislation stipulates a minimum deposit value of 0.6 mL and a maximum of 3.0 mL (Brazil, 2000). Deposits of <0.6 mL demonstrate that the honey is fake or contains added substances, while deposits >3.0 mL are related to feeding the bees protein hydrolysates, adding protein substances, or pressing the combs to obtain honey (Schlabitz; Silva; Souza, 2010).

In the Lugol reaction, starch and dextrin were observed in all samples except the control (COM), which indicates possible fraud in the samples sold in fairs and popular markets in the region. According to Araújo *et al.* (2024), the blue or purple color in the Lugol test is formed by the interaction between iodine and starch, which increases the viscosity of the product. Rambo *et al.* (2023) found that the presence of starch in honey indicates the addition of corn syrup or other sugars.

Total coliforms were identified in 30% of the samples, and enterobacteria are frequently found in the intestinal tracts of humans and animals. Contamination with these bacteria can cause diarrhea and urinary infections (Santos; Rangel; Azeredo, 2010). Thermotolerant coliforms were not detected in any samples. The absence of these microorganisms indicates that good hygiene practices were used in the harvesting, processing, and handling of honey. In addition, the high concentration of sugars in honey discourage the growth of microorganisms, and pH values <4.0 indicate very acidic honey, contributing to the preservation of honey and preventing the development of microorganisms (Moraes *et al.*, 2014).

The moisture content is directly related to the growth of microorganisms in honey; the higher the humidity, the greater the number of microorganisms, resulting in more fermentation (Araújo *et al.*, 2024).

Among the nonstandardized samples for molds and yeasts, samples C and D had moisture levels above the allowed level, with values of 23.8% and 23.6%, respectively.

Snowdon and Cliver (1996) found that molds and yeasts are common in honey, as they survive the high acidity, high sugar concentrations, and antimicrobial environment. Almeida *et al.* (2023), Rambo *et al.* (2023), and Araujo *et al.* (2024) observed that the occurrence of microorganisms in honey may be related to the methods used during collection, cleaning of the materials, temperature, storage, and hygiene of the handler.

CONCLUSIONS

The ten honey samples analyzed did not comply with legislation. This demonstrates that the lack of legislation that regulates all parameters of honey quality, from both *A. mellifera* and meliponines, enables the trade and sale of clandestine, adulterated, or counterfeit products, deceiving the consumer, and causing risks to their health. The results indicate the need for increased inspection of honey production and the marketing chain, especially in open fairs and municipal markets, as well as an enhanced public awareness of the risks of consuming clandestine foods of unknown origin.

ACKNOWLEDGMENTS

The authors thank the Federal University of Acre (UFAC) and Food Technology Unit (UTAL/UFAC) for providing the structures. This project did not receive any third-party funding or institutional support.

REFERENCES

- AGUIAR, L. *et al.* Physicochemical parameters of honey from stingless bees in the state of Acre. **Encyclopedia Biosphere**, v. 13, n. 23, p. 908-919, 2016.
- ALMEIDA, D. J. *et al.* Physicochemical analyses of honeys from native bees in urban areas. **Uniciências**, v. 27, n. 2, p. 150-153, 2023.
- ALVES, T. P. *et al.* Quality of honey bees *Apis mellifera* sold in the state of Alagoas, Brazil. **African Journal of Microbiology Research**, v. 9, n. 27, p. 1692-1698, 2015.
- ARAÚJO, J. M. *et al.* Evaluation of the quality of Amazonian honey sold in street markets. **Research, Society and Development**, v. 13, n. 8, p. 1-12, 2024.
- BONAGURA, T. *et al.* Quality Parameters of Brazilian Native Bee Honeys. **Brazilian Journal of Natural Sciences**, v. 6, n. 1, p. 1-13, 2024.
- BRAZIL. Ministry of Agriculture, Livestock and Supply – MAPA. **Ordinance No. 1, of October 7, 1981**. Analytical Methods for the Control of Products of Animal Origin and their Ingredients, consisting of Microbiological Methods and Physical and Chemical Methods. Available at: https://www.gov.br/agricultura/pt-br/assuntos/lfda/legislacao-metodos-da-rede-lfda/poa/metodos_oficiais_para_analise_de_produtos_de_origem_animal_1a_ed-2022assinado.pdf. Accessed on: 07 oct. 2024.
- BRAZIL. Ministry of Agriculture, Livestock and Supply – MAPA. **Normative Instruction No. 11, of October 20, 2000**. Technical Regulation of Honey Identity and Quality. Available at: <https://www.gov.br/agricultura/pt-br/assuntos/defesa-agropecuaria/suasa/regulamentos-tecnicos-de-identidade-e-qualidade-de-produtos-de-origem-animal-1/rtiq-mel-e-produtos-apicolas>. Accessed on: 07 oct. 2024.
- BRAZIL. Ministry of Agriculture, Livestock and Supply – MAPA. **Manual of official methods for the analysis of foods of animal origin**, 2019. Available at: <https://wp.ufpel.edu.br/inspleite/files/2019/04/Manual-de-Metodos-An%C3%A1lises-POA.pdf>. Accessed on: 07 oct. 2024.
- BRAZIL. Ministry of Agriculture, Livestock and Supply – MAPA. **Regulation of Industrial and Sanitary Inspection of Products of Animal Origin**, 2020. Available at: https://www.planalto.gov.br/ccivil_03/_ato2019-2022/2020/decreto/d10468.htm. Accessed on: 07 oct. 2024.
- CAPUCI, K.; HONORATO, C. Effect of storage temperature on honey quality. Centro Universitário da Grande Dourados-UNIGRAN. **Revista Ciência Exatas Terra**, v. 1, p. 54-61, 2012.
- CARVALHO, C. A. L. *et al.* **Proposal for a technical regulation of physicochemical quality of processed floral honey produced by bees of the genus Melipona**. Stingless bees process honey and pollen in cerumen pots. Mérida, Venezuela: Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, p. 1-9, 2013.
- CODEX ALIMENTARIUS. **Codex standard for honey: CXS 12-1981**, 1981. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252Fstandards%252FCXS%2B12-1981%252FCXS_012e.pdf. Accessed on: 25 jul. 2025.
- CONCEIÇÃO, V. S. *et al.* Geographic indication of bee honey from Alagoinhas-Bahia: A possibility. **Ingi Magazine – Geographical Indication and Innovation**, v. 6, n. 3, p. 1787-1800, 2022.
- DAMASIA-GOMES, L. *et al.* Physical-chemical characteristics of honey on Brazil. **EnciBio**, Centro Científico Conhecer - Goiânia, v. 11, n. 22, p. 670-682, 2015.
- FERREIRA, A. M. *et al.* Evaluation of the environmental quality of bee honey commercialized in the Municipality of João Pessoa-PB: a physical and chemical approach. **Brazilian Journal of Environmental Management and Sustainability**, v. 10, n. 25, p. 633-642, 2023.
- FINCO, A. F. D. B.; MOURA, L. L.; SILVA, I. G. Physical and chemical properties of *Apis mellifera* L. honey. **Food Science and Technology**, v. 30, p. 706-712, 2010.
- FLAGINI, B. **Bromatologia de mel produzido por *Apis mellifera* comercializado na cidade de Rio Branco – Acre**. 2016. 50f. Dissertação (Mestrado em Ciência, Inovação e Tecnologia) – Universidade Federal do Acre, Rio Branco, AC.

- GARCIA, L. N. H. C. *et al.* Physicochemical quality of bee honey from different blooms. **Revista Brasileira de Higiene e Sanidade Animal**, v. 12, n. 1, p. 11-20, 2018.
- IBGE. Brazilian Institute of Geography and Statistics. **Production of animal origin, by type of product – Bee honey**, 2024. Available at: <https://www.ibge.gov.br/explica/producao-agropecuaria/mel-de-abelha/br>. Accessed on: 19 Apr. 2025.
- IAL. INSTITUTO ADOLFO LUTZ. **Normas analíticas do Instituto Adolfo Lutz: métodos químicos e físicos para análise de alimentos**. 3. ed. São Paulo: IMESP, 1985.
- MARQUELE-OLIVEIRA, F. *et al.* Fundamentals of Brazilian honey analysis: an overview. **Honey analysis**, p. 139-170, 2017.
- Microsoft Corporation. **Microsoft Excel 2019**. Redmond, 2019.
- MORAES, F. J. *et al.* Physicochemical characterization of Africanized bee honey samples from the municipalities of Santa Helena and Terra Roxa (PR). **Brazilian Archive of Veterinary Medicine and Animal Science**, v. 66, n. 4, p. 1269-1275, 2014.
- PARPINELLI, R. S. *et al.* Microbiological characteristics of meliponine honey marketed in the State of Paraná-Brazil. **Research, Society and Development**, v. 10, n. 1, p. 1-13, 2021.
- PASCUAL-MATE, A. *et al.* Methods of analysis of honey. **Journal of Apicultural Research**, v. 57, n. 1, p. 38-74, 2018.
- RAMBO, J. M. C. *et al.* Analysis of lugol and labeling of *Apis mellifera* honey commercialized in Mato Grosso do Sul, Brazil. **Biosciences Journal**, v. 29, n. 2, 2023.
- RAWEH, H. S. A. *et al.* Physicochemical Composition of Local and Imported Honey Associated with Quality Standards. **Foods**, v. 12, n. 11, p. 2181, 2023.
- RAYPAH, M. E. *et al.* Identification of stingless bee honey adulteration using visible-near infrared spectroscopy combined with aquaphotomics. **Molecules**, v. 27, n. 7, p. 2324, 2022.
- RSTUDIO TEAM. **RStudio: Integrated Development for R**. Boston: RStudio, PBC, 2023.
- SANTOS, M. O. B.; RANGEL, V. P.; AZEREDO, D. P.; Adaptation of commercial restaurants to good practices. **Food Hygiene**, v. 24, n. 190/191, p. 44-49, 2010.
- SCHLABITZ, C.; SILVA, S. A. F.; SOUZA, C. F. V. Avaliação de parâmetros físico-químicos e microbiológicos em mel. **Revista brasileira de tecnologia agroindustrial**, v. 4, n. 1, 2010.
- SILVA, P. M. *et al.* Honey: Chemical composition, stability and authenticity. **Food Chemistry**, v. 196, p. 309-323, 2016.
- SILVA, F. R. **Avaliação da qualidade físico-química e microbiológica do mel de abelha (*Apis mellifera* Linnaeus, 1758) produzido em municípios do estado do Acre**. 2017. 58 f. Dissertação (Mestrado em Sanidade e Produção Animal Sustentável na Amazônia Ocidental) – Universidade Federal do Acre, Rio Branco, 2017.
- SNOWDON, J. A.; CLIVER, D. O. Microorganisms in honey. **International Journal Food Microbiology**, v. 31, n. 1-3, p. 1-26, 1996.
- SOUZA, A. C. **Panorama e perspectivas da cadeia produtiva do mel no Brasil**. 2018. 5 f Monografia (Graduação em Medicina Veterinária) – Universidade Federal de Uberlândia, Patos de Minas.
- TORRES, A. G.; BERTOLINI, D.; FRANCO, G. **Physicochemical methods for food analysis: Sugars and related products**. 4. ed. São Paulo: Instituto Adolfo Lutz, 2008. chap. VII. p. 271-301.
- VENTURINI, K. S.; SARCINELLI, M. F.; SILVA, L. C. 2007. **Characteristics of honey**. Available at: http://www.agais.com/telomc/b01107_caracteristicas_mel.pdf. Accessed on: 21 Apr. 2025.