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Microbiological quality of honey market in North-Centre of Rio de Janeiro

Qualidade microbiológica do mel comercializado na região Centro-Norte Fluminense

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RESUMO

O presente trabalho teve como objetivo realizar o diagnóstico dos principais agentes microbiológicos associados ao perfil higienicossanitário de méis de *Apis mellifera* comercializados em diferentes municípios do Centro-Norte do Estado do Rio de Janeiro, Brasil. As análises microbiológicas realizadas em 25 amostras de méis foram: contagem de coliformes totais e termotolerantes, fungos filamentosos e leveduras, *Staphylococcus* spp., *Clostridium* spp. e *Bacillus* spp. e detecção de *Salmonella* spp.. Os resultados obtidos nas análises microbiológicas detectaram a presença mais significativa por parte dos fungos filamentosos e leveduras, das bactérias mesófilas, *Bacillus cereus* e *Staphylococcus* spp.. Apesar da maioria das amostras de méis apresentarem isolamentos positivos para marcadores microbiológicos, os resultados das contagens estavam abaixo dos limites máximos estabelecidos pelas legislações nacionais. Do total avaliado, apenas três (12%, 3/25) amostras de méis apresentaram padrões de contagem acima do limite preconizado para fungos filamentosos, leveduras e bactérias mesófilas, refletindo um padrão higienicossanitário insatisfatório para consumo. A detecção destes marcadores microbiológicos em amostras de méis comercializadas na região Centro-Norte do Rio de Janeiro aponta para a necessidade do fortalecimento de políticas públicas de apoio a apicultura e produção de alimentos seguros.

Palavras-chave: Alimento seguro; apicultura; microbiota bacteriana; segurança alimentar.

ABSTRACT

The present work aimed to diagnose honey microbiological profile of samples obtained in the Center-North region of Rio de Janeiro, Brazil. A total of 25 honey samples were analyzed for: counts of total and thermotolerant coliforms, filamentous fungi and yeasts, *Staphylococcus spp*., *Clostridium spp*. and *Bacillus spp*. and detection of *Salmonella spp*. The results of microbiological analyzes detected the most significant presence of filamentous fungi and yeasts, mesophilic bacteria, *Bacillus* cereus and *Staphylococcus* spp. Although the majority of honey samples presented positive isolations for microbiological markers, the counting results were in accordance with the limits established by national legislation. Only three (12%, 3/25) honey samples presented counts above the recommended limit for filamentous fungi, yeasts and mesophilic bacteria, reflecting an unsatisfactory hygienic-sanitary pattern for consumption. The detection of these microbiological markers in honey samples sold in the Center-North region of Rio de Janeiro points to the strengthening of public policies to support beekeeping and food safety.

KEYWORDS: food safety; beekeeping; bacterial microbiota; food security.

INTRODUCTION

In Brazil, beekeeping stands out as a viable employment and income option, being one of the most significant sustainable economic growth activities. It can be practiced in virtually all regions of the country, thanks to Brazil's diverse flora, vast territory, and varied climate, allowing for year-round honey production (AGUIAR et al. 2023, POSTELARO et al. 2021). According to recent data from the Brazilian Institute of Geography and Statistics (IBGE 2022), Brazil's honey production has been steadily rising, reaching an estimated 15,5 tons annually. In other words, there was an increase in the sales of this product, leading to a price surge, which contributed to a 26.2% rise in production value, with the North American market being the main destination for Brazilian honey (CNA 2020). Beekeeping thus generates significant interest across various sectors of society, as it is a low-maintenance venture with minimal startup costs, while also contributing to species conservation (LOURENÇO & CABRAL 2016). It's one of the few agricultural activities that meets all three pillars of sustainability: economic, social, and environmental. It generates income for farmers, employs family labor, and preserves and enriches native flora and fauna (DE OLIVEIRA SILVA et al. 2023).

According to Brazil's Ministry of Agriculture, Livestock, and Supply (MAPA) Normative Instruction No. 11 of 2000, honey is defined as a food product made by honey bees from flower nectar or plant secretions, or from insect excretions derived from plant sap. Bees collect, transform, and combine these substances with their own specific secretions, then store and allow them to mature in the honeycomb (BRASIL 2000).

Honey is a concentrated solution of various sugars, primarily glucose and fructose, which are monosaccharides, as defined by the Codex Standard for Honey (CODEX 2001). It also contains a complex blend of other carbohydrates, proteins, enzymes, amino acids, organic acids, minerals, aromatic compounds, pigments, and pollen grains, and may include beeswax from the extraction process. In addition to other substances, such as sucrose, maltose, melezitose and other oligosaccharides, including dextrins and small concentrations of yeasts, fungi, and algae.

Although honey is considered a nutritious food, it may contain chemical or microbial contaminants that could pose health risks to consumers(BANDINI & SPISSO 2017). The physicochemical and microbiological quality of honey is influenced by various factors, including geographical and climatic conditions, the plants foraged by bees, the bee subspecies, the colony's physiological state, and the hygienic processing and storage conditions (GREGÓRIO et al. 2021, PRADO et al. 2023). Honey must not contain added sugars or other substances that alter its original composition, and any contaminants present must not exceed the limits set by Mercosur Technical Regulations (MERCOSUR GMC88/89 2000).

Honey's microorganisms can be categorized into two types: those naturally present and those introduced through contamination. The key microorganisms of concern are mainly yeasts, molds, and spore-forming bacteria (SILVA et al. 2008). These are crucial for public health, as honey is often given to children and is considered one of the main risk factors for infant botulism (ABREU et al. 2023).

Brazil has specific honey regulations that set quality control standards for the product, including required analyses and testing methods(BRASIL 2000). However, current Brazilian honey regulations lack specific microbiological standards. The only reference values were set by 2001 ANVISA's Resolution RDC 12, which was replaced by RDC 331 and Normative Instruction 60 in 23 December 2019. These new regulations only specify maximum limits for mold, yeast, and coliform counts at 35°C and 45°C in honey produced by *Apis mellifera* bees.

Food safety is an increasingly important public health concern, and governments worldwide have stepped up their efforts to improve it (WTO 2022). Regarding this scenario, this scientific article aimed to investigate the hygienic-sanitary profile of A. *mellifera* honey samples sold in the municipalities of Rio das Ostras, Macaé, Casimiro de Abreu, Búzios, and Nova Friburgo, the types of packaging, the presence of nutritional labeling, and certification by the Sanitary Inspection Service, in order to generate data that support research and development of beekeeping in the interior of the State of Rio de Janeiro.

MATERIAL AND METHODS

This study evaluated a total of 25 *Apis mellifera* honey samples collected between October 2021 and June 2022. Samples were collected in various-sized containers, ranging from 250 mL flasks to 1-liter glass bottles, from diverse retail locations across several municipalities in Rio de Janeiro State. These included farmers' markets, street vendors, bakeries, local markets, and family-run producers. Rio das Ostras, Macaé, Casimiro de Abreu, Búzios, and Nova Friburgo. During sample collection, packaging type, sealing, nutritional labeling, and health inspection certification were observed (Table 1). The samples were sent to the Food Microbiology Laboratory at the Ajuda Campus, UFRJ-Macaé Multidisciplinary Center, for microbiological

testing.

The honey samples were handled in a biosafety cabinet, with the surface of the honey packaging disinfected using cotton soaked in 70% alcohol and opened aseptically. Next, 25 g of honey sample was weighed and transferred to an Erlenmeyer flask containing 225 mL of 0.1% peptone water. This mixture was then homogenized for three minutes. From this dilution (1:10), the suspensions were plated onto media specific for the targeted microorganisms. Microbiological analyses were conducted using methods recommended by the American Public Health Association (APHA 2001) and the International Organization for Standardization (ISO 6579, 2002). The microbiological analyses included tests for counting viable aerobic and facultative heterotrophic mesophilic bacteria, molds and yeasts, total and thermotolerant coliforms, *Clostridium* spp., *Bacillus* spp., coagulase-positive *Staphylococcus*, and detection of *Salmonella* spp.

For the standard count of viable aerobic and facultative heterotrophic mesophilic bacteria, serially diluted samples were plated using the Pour Plate technique on Standard Plate Count Agar (PCA/OXOID®). The plates were incubated at 36 ± 1°C for 48 hours (ISO 4833, 2003). Results were reported as Colony Forming Units (CFU) per gram (g) of sample, in accordance with APHA (2001) guidelines. Similarly, for fungal and yeast enumeration, serial decimal dilutions of honey samples were plated using the Spread Plate technique on 2% potato dextrose agar acidified to pH 3.5 (PDA). The plates were incubated at 25 ± 1 °C for 5-7 days. Results were reported as CFU per mL (ISO 21527-1, 2008).

Samples of honey in serial decimal dilutions were plated using the Spread Plate technique on Baird Parker Agar and Mannitol Salt Agar with 7.5% NaCl (OXOID®) to count and identify *Staphylococcus* spp. Gram staining, coagulase test, and other phenotypic tests were performed to characterize the genus and species (ISO 6888-1, 2003).

For the presumptive test, total coliform count was performed using Lauryl Sulfate Tryptose Broth (LST/OXOID®), with three aliquots of three sample dilutions incubated at 35°C for 24 to 48 hours. Presumptive positive dilutions, indicated by color change and gas production, underwent confirmatory testing for total coliforms using tubes with 10 mL of 2% Brilliant Green Bile Lactose Broth (BGBLB/OXOID®), incubated at 35°C for 24/48 hours. Simultaneously, for thermotolerant coliforms, test tubes containing 10 mL of EC Broth (OXOID®) were incubated in a water bath at 44.50 ± 0.2 °C for 24/48 hours (BRASIL 2019, KONEMAM et al. 2008).

To detect *Salmonella* spp., samples were homogenized in 0.1% alkaline peptone water (APW) and incubated for 16-20 hours at 36 ± 1 °C. Then, 1 mL and 0.1 mL aliquots were transferred to Selenite Cystine, Rappaport Vassiliadis, and Sodium Tetrathionate broths (OXOID®). After incubation for 24-30 hours at 41 ± 0.5°C in a water bath, selective media isolation was performed using Hektoen agar, XLD, and SS (OXOID®). The plates were incubated for 18-24 hours at 36 ± 1°C to observe typical *Salmonella* spp. characteristics. (ISO 6579, 2002).

Bacillus cereus spores were heat-activated, then 0.1 mL aliquots of diluted honey samples were plated in duplicate on selective *Bacillus cereus* medium (MYP/OXOID®) and incubated at 30°C for 24-48 hours for enumeration and identification. Typical colonies with precipitation zones indicating lecithinase production were transferred to slanted Nutrient Agar (OXOID®) tubes and incubated at 30°C for 24 hours, then subjected to identification tests (APHA 2001, KONEMAN et al. 2001, RHODEHAMEL and HARMON 2020).

Sulfite-reducing *Clostridium* spp. were pre-enriched in trypticase-peptone-glucose-yeast extract broth (TPGY/OXOID®) for enumeration. 2mL aliquots of each honey sample were inoculated in duplicate into 15 mL of each enrichment broth. The tubes were promptly placed in a hot water bath (90°C) for 15 minutes, then cooled in an ice bath. Serial decimal dilutions were plated using the Pour Plate technique on Sulfite Polymyxin-Sulfadiazine Agar (SPS/OXOID®) supplemented with 5% egg yolk emulsion to obtain isolated colonies (MONETTO et al. 1999) Incubated in an anaerobic jar at 35°C for 48 hours. Typical colonies (curved or flat, smooth or rough, with precipitation zone) were re-isolated in duplicate on SPS Agar and subjected to biochemical characterization (RALL et al. 2003, SOLOMON & LILLY 2001, KÜPLÜLÜ et al. 2006).

Statistical data were analyzed using Excel® software, with results presented as percentages and averages.

RESULTS AND DISCUSSION

During the collection of 25 *Apis mellifera* honey samples, deficiencies were noted regarding the lack of nutritional labeling on the final product, as well as issues with packaging type and sealing (Table 1). A majority of samples (60% or 15 out of 25) were stored improperly, using inadequate or reused containers with poor sealing, some even sealed with corks. Glass containers are preferable to plastic ones, as plastic containers often have poorly sealing lids. This can lead to moisture absorption from the environment, creating conditions for microbial growth and product fermentation (EMBRAPA 2008).

Table 1. Gathered honey samples from 25 distinct sites across Rio de Janeiro's North-Central area.

Sample no.	Municipalities	Collection point	Labeling/ S.I.M/S.I.F.	Type of packaging and sealing
1	Rio das Ostras	Apiary	Present/*	Plastic packaging with proper sealing
2	Rio das Ostras	Apiary	Present/*	Plastic packaging with proper sealing
3	Rio das Ostras	Apiary	Absent	Reusable glass container with stopper
4	Rio das Ostras	Apiary	Present/*	Plastic packaging with proper sealing
5	Rio das Ostras	Apiary	Absent	Reusable glass container with stopper
6	Rio das Ostras	Apiary	Absent	Inadequately sealed plastic packaging
7	Macaé	Road	Present/*	Glass packaging with proper sealing
8	Macaé	Road	Present/*	Plastic packaging with proper sealing
9	Macaé	Fair	Absent	Inadequately sealed plastic packaging
10	Nova Friburgo	Trade	Present/*	Glass packaging with proper sealing
11	Búzios	Apiary	Absent	Reusable glass container with stopper
12	Macaé	Fair	Absent	Reusable glass container with stopper
13	Macaé	Fair	Absent	Inadequately sealed plastic packaging
14	Macaé	Fair	Absent	Improperly sealed glass packaging
15	Casimiro Abreu	Fair	Absent	Glass packaging with proper sealing
16	Macaé	Road	Absent	Improperly sealed glass packaging
17	Macaé	Trade	Present/*	Plastic packaging with proper sealing
18	Rio Bonito	Road	Present/S.I.F.	Plastic packaging with proper sealing
19	Macaé	Apiary	Present/*	Glass packaging with proper sealing
20	Macaé	Apiary	Present/*	Glass packaging with proper sealing
21	Macaé	Apiary	Present/*	Glass packaging with proper sealing
22	Macaé	Apiary	Present/*	Glass packaging with proper sealing
23	Macaé	Apiary	Present/*	Glass packaging with proper sealing
24	Macaé	Apiary	Present/*	Glass packaging with proper sealing
25	Macaé	Apiary	Present/*	Glass packaging with proper sealing

*Incomplete product labels, missing federal, state or municipal inspection quality seals.

Of all the labeled honey packages identified (60%, 15/25), only one sample displayed a label with the Federal Inspection Service (SIF) quality seal. The lack of certification found on most packaging highlights the need to strengthen health inspection services for local family beekeepers to ensure improved hygiene and sanitary standards throughout the entire production chain.

All 25 honey samples tested negative for *Salmonella* spp. and thermotolerant coliforms in the microbiological quality analysis (Table 2). Mesophilic bacteria, yeasts, filamentous fungi, *Staphylococcus* spp., *Bacillus* spp., and *Clostridium* spp. were successfully isolated, with counts below the recommended limit of 25 CFU/g. In three (8%, 3/25) honey samples from Macaé and Búzios, the counts exceeded the recommended limit for molds, yeasts, and mesophilic bacteria, indicating unsatisfactory hygiene standards for consumption.

Table 2. Microbiological analyses of 25 honey samples collected from various municipalities in the North-Central region of Rio de Janeiro.

Sample no.	Municipalities	CM	BAC	CLO	COL	SAL	CFL	STA
1	R. Ostras*	0,1 x10^1	0,1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	0,1x10^1
2	R. Ostras*	0,1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	0,1x10^1
3	R. Ostras*	0,4x10^1	0,1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	0,1x10^1
4	R. Ostras*	0,3x10^1	0,1x10^1	< 1x10^1	< 1x10^1	Absence	0,7x10^1	< 1x10^1
5	R. Ostras*	0,2x10^1	0,1x10^1	< 1x10^1	< 1x10^1	Absence	1,4x10^1	0,1x10^1
6	R. Ostras*	0,1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	1,5x10^1	< 1x10^1
7	Macaé	< 1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,6x10^1	< 1x10^1
8	Macaé	0,2x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,2x10^1	< 1x10^1
9	Macaé	1,3 x10^2	0,9x10^1	0,5x10^1	< 1x10^1	Absence	0,1x10^1	0,9x10^1
10	N. Friburgo *	0,1x10^1	0,1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	< 1x10^1
11	Búzios	0,6x10^1	0,6 x10^1	< 1x10^1	< 1x10^1	Absence	7,2x10^2	0,2x10^1
12	Macaé	0,1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,5x10^1	< 1x10^1
13	Macaé	0,2x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,6x10^1	< 1x10^1
14	Macaé	0,3x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	7,8x10^4	0,2x10^1
15	C. Abreu*	< 1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,8x10^1	< 1x10^1
16	Macaé	0,2x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	< 1x10^1
17	Macaé	1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	< 1x10^1
18	Rio Bonito	0,1x10^1	0,3x10^1	< 1x10^1	< 1x10^1	Absence	0,2x10^1	< 1x10^1
19	Macaé	0,1x10^1	0,2x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	< 1x10^1
20	Macaé	0,8x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	0,6x10^1	< 1x10^1
21	Macaé	< 1x10^1	< 1x10^1	< 1x10^1	< 1x10^1	Absence	< 1x10^1	0,1x10^1
22	Macaé	0,2x10^1	0,1x10^1	< 1x10^1	0,2x10^1	Absence	< 1x10^1	< 1x10^1
23	Macaé	0,2x10^1	< 1x10^1	< 1x10^1	0,1x10^2	Absence	< 1x10^1	< 1x10^1
24	Macaé	0,1x10^1	< 1x10^1	< 1x10^1	0,1x10^2	Absence	< 1x10^1	0.1x10 ^{1}
25	Macaé	0,2x10^1	< 1x10^1	< 1x10^1	0,1x10^1	Absence	< 1x10^1	< 1x10^1

Note:*R. Ostras: Rio das Ostras; N. Friburgo: Nova Friburgo; C. Abreu: Casimiro de Abreu; CM: standard count of viable aerobic and facultative heterotrophic mesophilic bacteria (UFC/g); BAC: *Bacillus* spp. count; CLO: *Clostridium* spp. sulfite-reducing count (UFC/g); CFL: filamentous fungi and yeast count (UFC/g); COL: total coliform count by the Most Probable Number (MPN) method; SAL: *Salmonella* spp. detection; STA: *Staphylococcus* spp. count. (UFC/g).

Compared to Table 1 data, these samples also showed inadequate packaging with poor sealing, missing labels, and no health inspection certification (Table 1) The improper packaging of the evaluated honey samples may have led to quality losses after processing, allowing moisture absorption from the environment and creating conditions conducive to microbial growth.

Positive results were found in 84% (21/25) of samples for the standard count of viable strict and facultative aerobic heterotrophic mesophilic bacteria, with values ranging from 0.1 to 0.8 x 10 CFU/g. Only 4% (1/25) of samples exceeded the recommended limit of 25 UFC/g, with a count of 1.3 x 10^2 CFU/g. In the study conducted by SOUZA et al. (2020) A study analyzing 36 A. *mellifera* honey samples from the Brazilian Amazon region found that 19.4% (7/36) of the samples had unsatisfactory counts of mesophilic bacteria.

According to ICMS (2002), the presence of mesophilic bacteria in food is widely used as a key microbiological indicator of quality, reflecting the effectiveness of cleaning and disinfection procedures, as well as the control of time and temperature during processing, transportation, and storage. This importance is justified by the fact that most foodborne pathogenic bacteria belong to this group. This microbial indicator is also linked to deteriorative changes and reduced shelf life (LIRA et al. 2001, SILVA 2002).

Of the 25 honey samples analyzed for mold and yeast counts, 8% (2/25) exceeded 10^2 UFC/mL, surpassing the limit set by the Ministry of Agriculture, Livestock and Supply's Ordinance No. 367 of September 4, 1997 (BRAZIL 1997), which establishes a maximum limit of 1.0 x 10^2 CFU/g. This ordinance was superseded by Normative Instruction No. 11 of October 20, 2000, which includes the Technical Regulation on Honey Identity and Quality as an attachment. However, this document does not provide microbiological standards for honey.

The presence of filamentous fungi in the final product may be due to their ability to tolerate high sugar concentrations, acidity, and the antimicrobial properties of honey. The most commonly found filamentous fungi in honey are from the genera *Penicillium*, *Mucor*, and *Aspergillus*, which can produce toxic metabolites.

Yeasts can thrive in acidic environments and aren't hindered by sucrose. Certain factors like high

humidity, moderate temperatures, granulation, and elevated yeast counts promote honey fermentation (PEREIRA 2008). The counts for these microorganisms in this study were lower than those reported in previous research. Sodré et.al (2007) detected a count of 1.7 x10^4 and SCHLABITZ et al. (2010) who reported a value of 2.7 x 10^2 UFC/g when assessing the microbiological quality of A. *mellifera* honey. NERIS et. al (2013) detected mold and yeast presence with results exceeding 7.38 x 10^2 (UFC/g) in honey sold in the state of Maranhão.

For the coliform count analysis of the 25 samples tested using the Most Probable Number (MPN) method, results showed counts below <3.0 MPN/g for both total coliforms and thermotolerant coliforms, meeting the standards set by MAPA's Normative Instruction No. 11 of October 20, 2000, which requires absence (<3.0 MPN/g) of total coliforms. Simultaneously, a coliform count was conducted using MacConkey agar plates incubated at 36^0C for 18-24 hours. The plate counting technique yielded more sensitive results, detecting 8% (2/25) positive samples at 50 UFC/g. Similar findings were reported by PIRES et al. (2015), which examined the microbiological quality of A. *mellifera* honey produced in Piauí.

No coliform bacteria were found in any of the honey samples tested. Souza et al. (2012) when assessing the microbiological characteristics of 21 honey samples from Northeast Bahia, values < 3.0 MPN/g were found. Silva (2016), examining honey samples from Roraima, found no coliforms present in any of the analyzed samples. Wanderley et al. (2015) microbiologically characterized A. *mellifera* honey samples produced in the Sousa-PB region, and no coliform group microorganisms were detected in any of them.

Coliform microorganisms can indicate the microbiological quality of products regarding shelf life or safety. The presence of this microbiota in honey may result from improper handling during harvesting and packaging, unsuitable temperature conditions during production or storage, or the use of non-sterilized containers (LIMA 2012).

In the standardized analyses for *Salmonella* spp. detection conducted on all 25 samples, the result was absence in 25 g, complying with Brazilian legislation (BRASIL 2000). The findings of this study align with other published research, confirming the absence of *Salmonella* spp. in honey samples and compliance with legal microbiological standards.

Research has shown honey's antibacterial properties against various bacteria, including *Salmonella* species. Pimentel et al. 2013, Nishio et. al 2016). The antibacterial properties of honey, such as its acidic pH, low water activity, low protein content, and high sugar concentration, can inhibit or stop bacterial growth, leading to an extended shelf life of the product (EUROPEAN COMMISSION 2002, MONTE et al.). 2013).

Bacillus spp. count revealed that 40% (10/25) of the samples tested positive in selective culture medium, with counts below 10^1 UFC/g. Biochemical analysis of the positive samples identified the following species: *Bacillus cereus, B. thuringiensis, B. mycoides and B. megaterium.*

In a study conducted in Argentina by IURLINA et. al. (2006), it was detected 38.6% of 70 analyzed honey samples contained *Bacillus* bacteria, with 23% specifically contaminated by *Bacillus cereus*. LÓPEZ & ALIPPI (2010) found B*. cereus* contamination in 27% of Argentine honey samples. MARTINS et. al. (2003), in a study of 80 honey samples found only six samples with B*. cereus* counts exceeding 10^3 spores per gram.

In the analyses for detection and enumeration of sulfite-reducing *Clostridium* spp., typical growth was observed on selective culture media in 4% of the samples (1/25). Biochemical analyses revealed that the typical sample of sulfite-reducing *Clostridium* spp. corresponded to the species *Clostridium perfringens.* Multiple studies report low prevalence of *Clostridium* spp. in honey samples, aligning with this study's findings of its absence in most samples.

RALL et al. (2003) detected *Clostridium botulinum* in 3% of 100 samples analyzed in São Paulo State. SCHOCKEN-ITURRINO et al. (1999) in a study of 80 Brazilian honey samples revealed that six (7.5%) were contaminated with *C. botulinum.* In international studies, such as those by KÜPLÜLÜ et. al (2006) study of 88 Turkish samples, 6 (6.8%) were found to contain *C. botulinum* spores. In France, DELMAS et al. (1994) isolated *C. botulinum* in 6.7% of the analyzed samples.

NEVAS et al. (2005) examined 529 honey samples from Nordic countries, detecting *C. botulinum* in 83 (15.69%) of them. Due to contamination and adulteration risks, which pose a threat to public health, honey is not recommended for children under two years old, as their protective gut bacteria and antibodies are not yet fully developed (CERESER et al. 2008).

A total of 36% (9/25) of the honey samples showed a count lower than 10^1 UFC/g of typical *Staphylococcus* colonies. Subsequently, the isolates underwent biochemical characterization, with negative results for the coagulase test and biochemical detection of *Staphylococcus* aureus species. The honey's typically low pH may have inhibited these microorganisms, as *Staphylococcus* spp. thrive best in pH ranges

between 4.2 and 9.3 (SILVA et al. 2010). *Staphylococcus aureus* poses a food safety risk when counts exceed 10^6 CFU/g, often causing food poisoning (SALOTTI et al. 2006).

The presence of *S. aureus* in honey may be linked to the harvesting process, with equipment and honey handling techniques considered the main sources of contamination by this microorganism (DÜMEN et al. 2013, PUCCIARELLI et al. 2014). *S. aureus* contamination in honey primarily stems from secondary sources during post-processing, mainly due to human contact with processed honey or exposure to improperly sanitized surfaces (PINHEIRO et al. 2018).

Ensuring microbiological quality in honey production through good beekeeping practices, from hive to consumer, is crucial for safe, hygienic output and preventing *Staphylococcus* contamination.

Key hygiene control measures include proper handling practices, equipment sanitation, and packaging cleanliness as the main critical control points for preventing contamination by this bacterial species (OLIVEIRA et al. 2017). This species colonizes both the skin and mucous membranes of humans and animals, allowing honey contamination through direct or indirect contact from secretions, aerosols, droplets, infected wounds, and poor hand hygien (KADARIYA et al. 2014).

Therefore, proper hygiene practices including regular handwashing, sanitizing contact surfaces, wearing face masks, and regular medical check-ups for beekeepers are essential rules to ensure safe honey production. Equipment must be sanitized daily before use, with disinfection following the cleaning process to reduce microbial load.

Cleaning products should be neutral and unscented, and the water used in sanitization processes must be potable and of excellent quality (GARCIA 2003). Glass or plastic packaging may be used, but must be food-grade and comply with the labeling regulations for packaged animal products as specified in MAPA's Normative Instruction No. 22, dated November 24, 2005. Labeling should occur at processing facilities, and storage must be in contamination-free areas (BRAZIL 2005).

Macaé, Rio das Ostras, and Casimiro de Abreu have a history of supporting family farming initiatives, including beekeeping best practices. Notable programs include Rio Rural and the work of EMATER-RJ, which offers farmers access to training courses and project funding to promote good production practices. However, the economic downturn in the oil industry led to a decrease in royalty revenues for these municipalities, limiting available resources, including those that funded programs supporting and developing family farming.

CONCLUSION

The microbiological markers found in most of the analyzed honey samples were filamentous fungi, yeasts, mesophilic bacteria, *Bacillus cereus*, and *Staphylococcus spp*., with count results conforming to the standards set by national regulations. Only in three samples from Macaé and Búzios were filamentous fungi, yeast, and mesophilic bacteria counts detected above the maximum acceptable hygienic-sanitary standard for consumption.

The detection of microbial contaminants in honey samples sold in the North-Central region of Rio de Janeiro highlights the need to strengthen food safety oversight and implement public policies supporting local beekeeping to improve hygienic and sanitary quality throughout the production chain.

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REFERENCES

ABREU SM et al. 2023. Rotulagem de mel: Uma análise qualitativa quanto ao cumprimento da legislação em embalagens comercializadas em diferentes cidades do RJ e RS. Research, Society and Development 12: 1-12.

AGUIAR A et al. 2023. A produção de mel apícola: importância socioeconômica e aspectos da cadeia produtiva. Facit Business and Technology Journal 41: 229-245.

APHA. 2001. AMERICAN PUBLIC HEALTH ASSOCIATION. Compendium of Methods for the Microbiological Examination of Foods. Washington: APHA. 4.ed.

BANDINI TB & SPISSO BF. 2017. Risco sanitário do mel no Brasil em relação a novas ameaças: resíduos e contaminantes químicos emergentes. Vigilância Sanitária em Debate: Sociedade, Ciência & Tecnologia 5: 116-126.

BRASIL. 2019. Agência Nacional de Vigilância Sanitária- ANVISA. Instrução Normativa nº 60, de 23 de dezembro de 2019. Estabelece as listas de padrões microbiológicos para alimentos.

BRASIL. 2005. Ministério da Agricultura, Pecuária e do Abastecimento (MAPA), Regulamento técnico para rotulagem de produto de origem animal embalado, Instrução Normativa nº 22, de 24 de novembro de 2005.

BRASIL. 2000. Ministério da Agricultura, Pecuária e do Abastecimento (MAPA), Qualidade dos Produtos - Regulamento técnico de identidade e qualidade do mel, Instrução Normativa nº11, de 20 de outubro de 2000.

BRASIL. 1997. Ministério da Agricultura, Pecuária e do Abastecimento (MAPA), Qualidade dos Produtos - Regulamento técnico de identidade e qualidade do mel, Portaria nº 367, de 4 de setembro de 1997.

CERESER ND et al. 2008. Botulismo de origem alimentar. Ciência Rural 38: 280-287.

CODEX ALIMENTARIUS COMMISSION. 2001. Revised codex standard for honey. CXS 12-1981. Rev.2.

COMISSÃO EUROPEIA. 2002. Opinion of the scientific committee on veterinary measures relating to public health on honey and microbiological hazards. Health & Consumer Protection Directorate-General.

DELMAS C et al. 1994. Survey of Honey for *Clostridium botulinum* Spores in Eastern France. Food Microbiology 11: 515-518.

DE OLIVEIRA SILVA EL et al. 2023. O potencial do mercado internacional de mel a partir da legislação e normas para exportação. Revista de Gestão e Secretariado (Management and Administrative Professional Review)14: 9395-9419.

DÜMEN E et al. 2013. Microbiological and parasitological quality of honey produced in Ístanbul. Turkish Journal of Veterinary and Animal Sciences 37: 602-607.

EMBRAPA MEIO NORTE. 2002. Apicultura: Sistema de Produção 3: 138.

EMBRAPA. 2008. Extração e Processamento do Mel 2:60.

GARCIA W. 2003. Guia de Buenas Prácticas de Apícolas y de Manufactura. Buenos Aires: Secretaría de Agricultura, Ganadería, Pesca y Alimentos. 35 p.

GOLYNSKY A. et al. 2004. Apicultura como alternativa econômica para os pequenos produtores rurais da região norte do Rio Grande do Sul. In: CONGRESSO DA SOBER, Anais eletrônicos. Cuiabá: Sober. p.42.

GREGÓRIO A et al. 2021. Antimicrobial activity, physical-chemical and activity antioxidant of honey samples of *Apis mellifera* from different regions of Paraná, Southern Brazil. Food Science and Technology 41: 583-590.

IBGE. 2022. INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. Pesquisa pecuária municipal. Brasília: IBGE.

ICMSF. 2002. INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS. Microorganisms in foods 7: Microbiological testing in food safety management. New York: Kluwer Academic/Plenum Publishers.

ISO 6579. 2002. INTERNATIONAL ORGANIZATION FOR STANDARDIZATION. Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. Geneva Amd 1:2007, annex D.

ISO 21527-1. 2008. INTERNATIONAL STANDARD ORGANIZATION. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeast and moulds Part 2: Colony count technique in products with water activity less than or equal to 0,95.

ISO 6888-1. 1999. INTERNATIONAL STANDARD ORGANIZATION. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species).

IURLINA MO et al. 2006. Prevalence of *Bacillus* spp. in different food products collected in Argentina. Food Science and Technology 39: 105-110.

KADARIYA J et al. 2014. *Staphylococcus aureus* and Staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Research International 2014: 1-9.

KONEMAN EW et al. 2008. Diagnóstico Microbiológico: Texto e atlas colorido. 6.ed. Rio de Janeiro: Guanabara Koogan p.1565.

KÜPLÜLÜ Ö et al. 2006. Inidence of *Clostridium botulinum* spores in honey in Turkey. Food Control 17: 222-224.

LIMA JBA. 2012. Condições Higiênico-Sanitárias Do Mel Produzido Por Apis Melífera no Estado do Maranhão. Laboratório de Microbiologia de Alimentos e Água. São Luís: UEMA.

LIRA GM et al. 2001. Avaliação da qualidade de peixes comercializados na cidade de Maceió - Al. Revista Higiene Alimentar 15: 67-74.

LÓPEZ AC & ALIPPI AM. 2010. Enterotoxigenic gene profiles of Bacillus cereus and *Bacillus megaterium* isolates recovered from honey. Revista Argentina Microbiología 42: 216-225.

LOURENÇO MSM & CABRAL JEO. 2016. Apicultura e Sustentabilidade: Visão dos Apicultores de Sobral (Ce). Revista em Agronegócio e Meio Ambiente 9: 93-115.

MARTINS HM et al. 2003. Bacillaceae spores, fungi and aflatoxins determination in honey. Revista Portuguesa de Ciências Veterinárias 98: 85-88.

MERCOSUL GMC88/89. 2000. Regulamento Técnico de Identidade e Qualidade do Mel.

MONTE AM et al. 2013. Quality of honey from stingless bees native of Piaui, Brazil. Revista Brasileira de Medicina Veterinária 35: 48-54.

NERIS MS et al. 2013. Ocorrência de bolores e leveduras em méis comercializados informalmente no Estado do Maranhão. Nutrire 38: 439-439.

NEVAS M et al. 2005. Prevalence and diversity of *Clostridium botulinum* types A, B, E and F in honey produced in the Nordic countries. International Journal of Food Microbiology 105:145– 151.

NISHIO EK et al. 2016. Antibacterial synergic effect of honey from two stingless bees: *Scaptotrigona bipunctata* Lepeletier, 1836, and *S. postica* Latreille, 1807. Scientific Reports 6: 21641.

OLIVEIRA FDC et al. 2017. Análise de mel de abelha coletado em comércio informal na cidade de Teresina, PI. Higiene alimentar 3: 268-269.

PEREIRA APR 2008. Caracterização de mel com vista a produção de hidromel. Dissertação (Mestrado em Qualidade e

Segurança Alimentar). Bragança: Instituto Politécnico de Bragança, Escola Superior Agrária de Bragança. 66p.
PIMENTEL RBQ et al. 2013. Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona* compressipes manaosensis and commercial honey. BMC Complementary and Alternative Medicine 13:151.
PINHEIRO CGME et al. 2018. Qualidade microbiológica do mel da abelha sem ferrão jandaíra (*Melipona subnitida*) da região semi-árida do Brasil. Ciência Rural 48: 9.
PIRES RMC et al. 2015. Evaluation of hygienic-sanitary quality of honey from Apis mellifera L. obtained in semi-arid region of Piauí, Brazil. African Journal of Microbiology Research 9: 1806-1813.
POSTELARO ER et al. 2021. Apicultura Familiar: sua importância no cenário econômico, social e ecológico. Revista Interface Tecnológica 18: 298-307.
PRADO A et al. 2023. Honey bees change the microbiota of pollen. Botanical Sciences 101: 127-133.
PUCCIARELLI AB et al. 2014. Microbiological and physicochemical analysis of yateí (*Tetragonisca angustula*) honey for assessing quality standards and commercialization. Revista Argentina de Microbiología 46: 325-332.
RALL VLM et al. 2003. Incidência de esporos de *Clostridium botulinum* e análise da qualidade microbiológica do mel no Estado de São Paulo. In: CONGRESSO BRASILEIRO DE MICROBIOLOGIA. Anais Foz do Iguaçu: Sociedade Brasileira de Microbiologia. p.403.
RHODEHAMEL EJ & HARMON SM. 2020. *Bacillus cereus*. In: Bacteriological Analytical Manual. 8.ed. Cap.14.
SALOTTI BM et al. 2006. Qualidade microbiológica do queijo minas frescal comercializado no município de Jaboticabal, SP, Brasil. Arquivos do Instituto de Biologia 73: 171-175.
SCHLABITZ C et al. 2010. Avaliação de parâmetros físico-químicos e microbiológicos em mel. Revista brasileira de tecnologia agroindustrial 4: 80-90.
SCHOCKEN-ITURRINO RP et al. 1999. Study of presence of the *Clostridium botulinum* in honey in Brazil. FEMS Imunology and Medical Microbiolgy 24: 379-382.
SILVA MBL et al. 2008. Qualidade microbiológica de méis produzidos por pequenos apicultores e de méis de entrepostos registrados no serviço de inspeção federal no Estado de Minas Gerais. Alimentos e Nutrição 19: 417-420.
SILVA N et al. 2010. Manual de métodos de análise microbiológica de alimentos e água. 4.ed. São Paulo: Livraria Varela.
SILVA BPPC 2016. Caracterização Físico-química de mel produzido em Santo Antônio do Tauá In: 14º Encontro de profissionais da química do Amazonas. Belém: UFRA. p. 39-43.
SODRÉ GS et al. 2007. Caracterização Físico-química de Amostras de Méis de *Apis mellifera* L. (Hymenoptera: Apidae) do Estado do Ceará. Revista Ciência Rural 37: 1139-1144.
SOLOMON HM & LILLY T. 2001. *Clostridum botulinum*. In: Bacteriological Analytical Manual. 8.ed. Cap. 17.
SOUZA FG et al. 2012. Análise do mel de pequenos produtores do vale do Médio Araguaia-Tocantins. Enciclopédia Biosfera. Centro Científico Conhecer 8: 1001.
SOUZA BFS et al. 2020. Qualidade sanitária do mel de *Apis mellifera* em relação a forma de coleta. Brazilian Journal of Development 6: 79959-79972.
WANDERLEY ROS et al. 2015. Avaliação dos parâmetros de qualidade e estabilidade térmica de méis produzidos na região de Sousa-PB. ACTA Apicola Brasilica 3: 10-16.
WTO. 2022. WORLD TRADE ORGANIZATION. Ministerial decision on world food programme food purchases exemption from export prohibitions or restrictions. Geneve 1:1.