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Physicochemical Analysis of Honey Produced and Sold in the Municipality of Senhor do Bonfim, Bahia

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ABSTRACT. The objective of this study was to analyze the physicochemical characteristics of *Apis mellifera* L. honey produced and sold in Senhor do Bonfim, Bahia, Brazil. A completely randomized design was adopted; it consisted of 6 treatments and 4 replicates, with the honey collection sites being considered as the treatments, namely: T1CMQ - Quicé Honey House, T2AMt - Maranata Apiary, T3FLv - Senhor do Bonfim Street Market, T4AMT - Monte Tabor Apiary, T5AJVC - Juvêncio Apiary and T6AS - Souza Apiary. The honeys in the different treatments analyzed showed similarity for moisture, total titratable acidity and Lund reaction ($p > 0.05$). The lowest ash and total soluble solids contents were obtained by the T3FLv honey samples ($p < 0.05$). The highest pH was obtained in the T1CMQ samples ($p < 0.05$). The honeys were classified as having a color between extra light amber and light amber. The Lugol test was negative. The results found show that the honey produced and sold in Senhor do Bonfim, BA, is of good quality and suitable for human consumption.

Keywords: Adulteration; food; beekeeping; bee product; quality.

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Introduction

A unique product with numerous therapeutic properties, honey is considered the purest food in nature, having a characteristic flavor and excellent nutritional value – hence its high economic value, which encourages adulteration (Fakhlaei et al., 2020). Several Brazilian researchers study honey as to its physicochemical, microbiological and sensory composition (Freitas et al., 2022; Mora Junior et al., 2021; Pereira et al., 2020), with a view to characterizing the product, based on the flowering periods, which vary according to the regions in which they occur.

The Brazilian Ministry of Agriculture, Livestock and Supply [Ministério da Agricultura, Pecuária e Abastecimento] (MAPA), through Normative Instruction No. 11, of October 20, 2000, sets forth the Technical Regulation for Honey Identity and Quality, which works to prevent the selling of adulterated honey and designates basic requirements for sensory characteristics (color, flavor, aroma and consistency), physicochemical characteristics (maturity, purity and deterioration) and packaging. Said Regulation also determines criteria aimed at hygiene practices for honey production, in addition to prohibiting additives and contaminants (BRASIL, 2000).

In this context, it becomes imperative to carry out physicochemical analyses on honey produced and sold in the most diverse Brazilian regions, since its nutritional characteristics can vary according to the environment in which it is produced, due to factors related to the environment, vegetation, climate, among others (Cucu et al., 2021; Freitas et al., 2022; Maicelo-Quintana et al., 2024).

As far as we know, beekeeping activity in the municipality of Senhor do Bonfim is still developing. The honey consumed is, for the most part, produced by beekeepers with less than 200 hives, and many of them are not regulated by a competent body, so they improvise places called “honey houses”, where they extract honey to sell without proper good beekeeping practices, which evidences the weaknesses of the municipality's beekeeping regarding honey management practices without quality assurance, generating uncertainty for the consumer (Macedo et al., 2002; Secretaria de Desenvolvimento Agropecuário, 2024).

In this sense, the objective of this study was to evaluate the physicochemical quality of honey produced and sold in the municipality of Senhor do Bonfim, Bahia.

Material and methods

Study site

The research was conducted in the geographic environment of the municipality of Senhor do Bonfim, BA, part of the Piemonte Norte do Itapicuru territory – TIPNI. The municipality of Senhor do Bonfim has a semi-arid climate, ranging from dry to sub-humid, with predominant Caatinga vegetation, an average temperature of around 27 ºC, and rainfall that does not exceed 800 mm/year (Reis & Souza, 2021).

Initial testing

The methodology consisted of three stages. In the first one, the research site was delimited. The area was chosen due to the practices of honey extraction and trading in the municipality of Senhor do Bonfim, Bahia, Brazil, as well as observations collected from 2014 to the present moment and the questions raised about the way honey is stored, the types of packaging used, the processing and the final quality of the honey produced and made available to the consumer market at the various commercial points in the municipality.

A semi-structured questionnaire (Figure 1) was then prepared to collect data and describe the treatments as to sample location and identification, apiaries, hive management, and honey processing and selling characterization. Subsequently, a Free and Informed Consent Form (FICF; BRASIL, 2012) was signed by the suppliers of the evaluated samples to guarantee the safety of the data obtained or recorded as images or photos and other elements that constitute the object of the research.

Research Title: Physicochemical Analysis of Honey Produced and Marketed in the Municipality of Senhor do Bonfim, Bahia		**Date:**	
Treatment		**Number of Samples:**	
Sample Identification/Supplier			
Collection Location:			
Latitude:	Longitude:		
Supplier Contact:			
Apiary/Location Characteristics			
Predominant flora:			
Are there exotic plants nearby? Yes ( ) No ( )	Which?		
Harvests number/year:	Honey produced/year (kg):		
Number of inhabited hives:	Number of abandoned hives:		
Hive Management Characteristics			
Is bee feeding carried out?	Yes ( )	No ( )	
Is honeycomb wax used?	Yes ( )	No ( )	
Is the honey super placed on the floor?	Yes ( )	No ( )	
Honey Processing Characteristics			
Extraction unit (family-owned, third-party, certified, or other)?			
Certified Yes ( ) No ( )	Centrifugation Yes ( ) No ( )		
Decantation Yes ( ) No ( )	Filtration Yes ( ) No ( )		
Commercialization Characteristics			
Formal Yes ( ) No ( )	Informal Yes ( ) No ( )		
Wholesale Yes ( ) No ( )	Retail Yes ( ) No ( )		
Cooperative ( ) Supermarket ( )	Own point of sale ( ) Online Commerce ( )		
Sample Characteristics			
Harvest date:	Processing date:		
Packaging date:	Sample coloration:		

Figure 1. Semi-structured questionnaire applied during sample collection.

Treatments and experimental design

The honeys analyzed in this study were named as T1CMQ (Quicé Honey House); T2AMt (Maranata Apiary); T3FLv (Senhor do Bonfim Street Market); T4AMT (Monte Tabor Apiary); T5AJVC (Juvêncio Apiary) and T6ASz (Souza Apiary) (Figure 2).

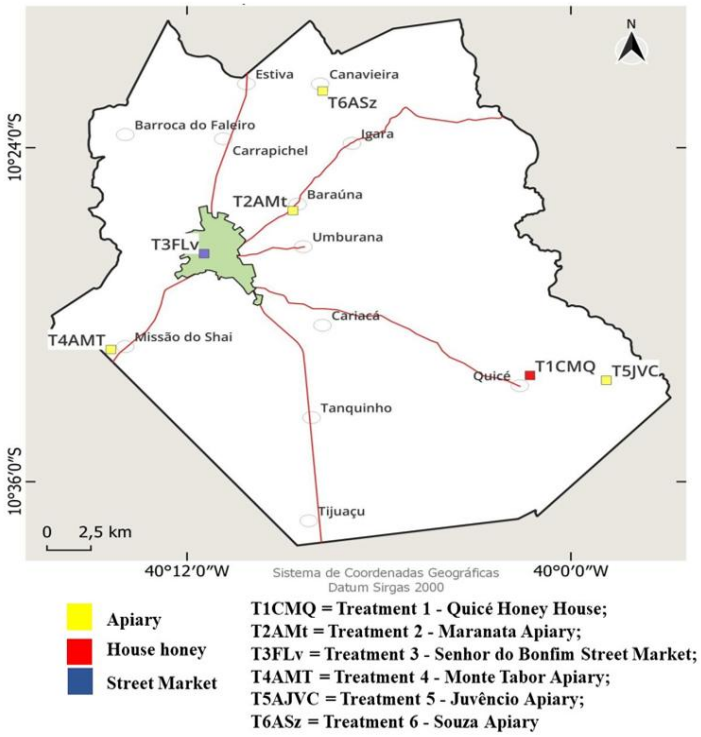

Figure 2. Map of the municipality with the honey harvest locations (Treatments).

To collect samples for the treatments, a honey packaging protocol was followed: 1st Step → choice of the container for the sample; 2nd Step → slow packaging of the honey to prevent air bubbles; 3rd Step → immediately closure of the container and repetition of the procedure for each honey sample. The collected samples were stored under natural conditions, at room temperature, in sterile, transparent plastic containers with a lid and a capacity of 500 g.

For each treatment, 4 samples were collected, making up a total of 24 experimental units. A completely randomized experimental design was adopted.

The T1CMQ samples came from a beekeeper who is a member of the Quicé Small Farmers Association [Associação dos Pequenos Agricultores de Quicé] (APAQ), which is legally responsible for maintaining the regulatory rigor of the health requirements of the Bahia state's regulatory body, as the HPU (Honey Processing Unit) is certified by it, so only the honey collected for this treatment is considered genuinely pure. The sample was collected directly from the decanter.

Unregistered honey samples are found at T2AMt. The apiary was located at the Caçuca Farm and its honey processing unit receives honey supers from several beekeepers in the municipality throughout the year, as it is located on BA-220 and is easily accessible. The processing unit is improvised and uncertified. The sample was collected directly from the decanter. For T3FLv, the samples were collected at the municipal street market, as this is considered the place that absorbs and sells a large part of the honey production in the municipality of Senhor do Bonfim, BA, and other family farming products. The honeys were sold in non-sterile plastic bottles.

At T4AMT, honey samples were obtained from a small family beekeeper who processes the product in an improvised place, but who seeks to comply with good processing practices. Regarding T5AJVC, the samples were obtained from a family apiary, whose beekeeper processes the honey in an improvised place, but uses the recommended equipment, made of stainless steel, seeking to comply with good manufacturing practices. At T6ASz, honey samples were collected from a small family apiary. The beekeeper processes and treats the honey, but the decanters are improvised.

Laboratory analysis

All laboratory analyses were performed in triplicate. Moisture was measured using a properly calibrated digital portable refractometer (AR200, Reichert, New York-USA). A drop of honey was inserted into the equipment, and the refractive index was subsequently read. The refraction value of the sample was converted into moisture percentage using the Chataway refractometric method (Instituto Adolfo Lutz [IAL], 2008).

The pH was measured with the aid of a digital benchtop pH meter (PHS-3, PHTEK) previously calibrated with pH 4 and 7 buffer solution. Ten grams of honey were diluted in 10 ml of distilled water. The solution remained under constant stirring until the particles were uniformly suspended, and, subsequently, the pH reading was performed (IAL, 2008).

To determine total titratable acidity (TTA), ten grams of the honey samples were diluted in 75 ml of distilled water, with NaOH solution (0.05M), until it reached pH 8.5. Next, for lactonic acidity, 10 ml of NaOH solution (0.05M) were pipetted, and, with an HCl solution (0.05M), a back titration was performed until pH 8.3 was obtained. The sum of free acidity and lactonic acidity results in TTA (IAL, 2008).

The total soluble solids (TSS) were determined with a previously calibrated digital portable refractometer (AR200, Reichert, New York-USA). Drops of honey were inserted into the refractometer receptacle operating in the Brix module (temperature compensated at 20 ºC). After a few moments, the TSS were read and the result was expressed in ºBrix (IAL, 2008).

For the Lund reaction, 2 g of honey were used – diluted in 20 ml of distilled water in a measuring cylinder with a lid. Then, 5 ml of a 5% (m/v) tannic acid solution was added and the cylinder was filled with distilled water to the 40 ml mark. The material was homogenized and left to rest for 24 hours. After this period, the samples were evaluated in relation to the volume of precipitate at the bottom of the cylinder (IAL, 2008).

For the Lugol reaction, the calorimetric method was carried out. Ten grams of honey were diluted in 20 ml of distilled water heated in a water bath (NI 1246, Belo Horizonte-MG, Brazil) for 1 hour. After cooling, 0.5 ml of 5% Lugol solution was added to the samples, and color change was checked. In the presence of commercial glucose, sugar syrups and starch, the solution has a hue ranging from reddish-brown to blue (IAL, 2008).

For ash analysis, 5g of honey were deposited in a porcelain capsule. The samples were heated in a water bath (NI 1246, Casalab, Belo Horizonte-MG, Brazil) for 1 hour and subsequently carbonized on a heating plate (MA 085, Marconi, Piracicaba-SP, Brazil) for 1 hour. After this period, the samples were incinerated in a muffle furnace (NI 1385, Casalab, Belo Horizonte-MG, Brazil), at 550°C, for 2 hours. After cooling, the samples were weighed and the ash content was calculated using this formula (Okaneku et al., 2020):

$$Ash = (W\ x\ 100)\ /\ W'$$

where: W = weight of white ash (W2 – W1); W1 = weight of empty crucible; W2 = weight after cooling; W' = weight of the sample.

The color of the honey samples was analyzed by the relative absorption of light, in a UV/Visible spectrophotometer (Cirrus 80, FEMTO, Brazil), with a wavelength of 560 nm of absorbance (Abs), taking as reference (white) the absorption of a pure glycerin sample. The value obtained in the readings was compared with the Pfund color scale (mm), classifying the honey color according to each of the color ranges: Water white (⩽0.030), Extra white (> 0.030 and ⩽0.060), White (> 0.060 and ⩽0.120), Extra light amber (> 0.120 and ⩽0.188), Light amber (> 0.188 and ⩽0.440), Amber (> 0.440 and ⩽0.945) and Dark amber (> 0.945) (Marsaro Júnior et al., 2022).

Statistical analysis

The data obtained was subjected to analysis of variance using the Sisvar software (version 5.6), with application of Tukey's test at a 5% probability level.

Results and discussion

Moisture contents were similar between treatments ($p > 0.05$), presenting a mean value of 17.2% (Table 1). All samples were in compliance with current Brazilian legislation (Brasil, 2000), which establishes a maximum value of 20% for honey from honey bees. In agreement, Seraglio et al. (2019) reinforce that values above 20% can be one of the factors that directly influence the quality of honey, its viscosity, specific weight, maturity, crystallization, flavor and conservation.

In view of this, it is possible to confirm that there is no evidence that the honey underwent inadequate practices, such as premature harvesting of honey supers with green honeycombs, that is, honeycombs with open alveoli, before its maturation point, which must have between 80 and 95% of its alveoli closed. And according to Guo et al. (2019), when most of the sucrose has been converted into glucose and fructose, the honey is mature. Similar results for moisture were found by Dantas et al. (2022) when analyzing honey from bees sold in the municipality of Frei Martinho, PB (17.0 to 17.2%).

Table 1. Physicochemical composition of honey collected in the municipality of Senhor do Bonfim-BA.

Variables	Treatments						SEM	Brazil (2000)*
	T1CMQ	T2AMt	T3FLv	T4AMT	T5AJVC	T6AS		
Moisture (%)	17.50	16.70	17.70	17.60	16.95	17.10	0.13	Max 20%
TA (mEq kg^{-1})	46.85	48.35	48.49	49.11	46.03	42.60	1.54	40 a 50 mEq kg^{-1}
Ash (%)	0.22^{a}	0.10^{c}	0.01^{c}	0.17^{ab}	0.11^{b}	0.09^{c}	0.01	0.04 a 0.6%
pH	3.7^{a}	3.3^{c}	3.2^{d}	3.5^{b}	3.3^{c}	3.3^{c}	0.03	3.3 a 4.6**
TSS (°Brix)	80.86^{ab}	81.17^{ab}	80.45^{b}	80.75^{ab}	81.66^{a}	81.42^{a}	0.20	-
Color (mm)	0.34^{a}	0.35^{a}	0.35^{a}	0.28^{ab}	0.40^{a}	0.17^{b}	0.03	Colorless to dark
Lund	0.29	0.29	0.30	0.29	0.31	0.29	0.01	3.0 a 0.6 mL**
Lugol	n	n	n	n	n	n	-	

T1CMQ: Quicé Honey House; T2AMt: Maranata Apiary; T3FLv: Senhor do Bonfim Street Market; T4AMT: Monte Tabor Apiary; T5AJVC: Juvêncio Apiary; T6ASz: Souza Apiary; TA: Total Titratable Acidity; TSS: Total Soluble Solids; n: Negative; SEM: Standard error of the mean; *: Normative Instruction No. 11/2000 (Brasil, 2000); **: SIPA Ordinance 006/85 (Brasil, 1985). Means followed by distinct letters differ statistically by Tukey's test at a 5% probability level.

Total titratable acidity also showed similarity between treatments, with mean values ranging from 42.60 to 49.11 mEq kg^{-1} of honey (Table 1), in accordance with current legislation (maximum 50 mEq kg^{-1}) (BRASIL, 2000). Acidity is an important component of honey that directly contributes to its stability and deterioration against the development of microorganisms, since the acidity of honey is caused by the variation of organic acids from the different nectar sources visited, by the action of the glucose-oxidase enzyme, which produces gluconic acid, by the action of bacteria during the maturation of honey and by the amount of minerals present in it, which directly influences its pH (Chou et al., 2020; Marsaro Júnior et al., 2022).

Ash content is a parameter that indicates the purity of honey. In this study, ash values between 0.01 and 0.22% were obtained, with a lower ash content (0.01%) for honey samples being found at street markets (T3FLv) (Table 1). The other evaluated samples presented an ash content within what is recommended in Brazilian legislation (0.04 to 0.6%; BRASIL 2000). According to Freitas et al. (2022), ash content in honey is small and varies according to the composition of the nectar of the plants that contribute to its formation. Furthermore, the percentage of ashes indicates the amount of minerals found in the honey, determining its color, as the lighter it is, the lower its ash amount (Krolow et al., 2020).

A pH analysis helps in evaluating the quality of honey, together with acidity analysis (Souza et al., 2021), as it influences its texture, stability and shelf life (Gois et al., 2015). Variation was found in the pH of the evaluated treatments, with mean values between 3.15 and 3.75, and with the lowest pH value being found in the T3FLv honey samples ($p < 0.05$; Table 1), which is below the limit recommended by the SIPA Ordinance 006/85 (BRASIL, 1985) – 3.3 to 4.6. According to Gois et al. (2015), pH values below the minimum one indicate that the honey possibly has a high acidity content.

At street markets, honey is commonly sold in reused plastic or glass packages. The containers are arranged on benches, at ambient temperature, and exposed to direct sunlight. Different suppliers meet the demands of market vendors, thus reducing certainty regarding the quality standards and uniformity of the product (Souza et al., 2021). Therefore, it is possible that these conditions may have influenced the pH value of honey from street markets (T3FLv) analyzed in this study, since pH values below those established in Brazilian legislation favor the growth of fungi and yeasts and reduce the shelf life of honey. Furthermore, pH values below the recommended level may indicate the occurrence of fermentation or adulteration of the product (Almeida et al., 2023). Thus, it is recommended that one always purchase bee products from reputable locations, whose products are certified and labeled, specifying what is contained in the food that will be consumed.

The concentration of Total Soluble Solids quantifies the sweetness of honey and is correlated with the amount of sugars contained in it (Mora Junior et al., 2021). The evaluated honeys presented a TSS content above 80%, with a higher TSS concentration in the T5AJVC and T6ASOs treatments ($p < 0.05$; Table 1). Similar results were obtained by Okaneku et al. (2020) when analyzing honey from Africanized bees (*Apis mellifera*). According to the authors, because honey consists mainly of sugars, high TSS values are considered normal.

The Technical Regulation for Honey Identity and Quality (Brasil, 2000) recommends that the color of honey should vary from almost colorless to dark brown. Thus, for this parameter, variation in the color of the honeys

studied was observed ($p < 0.05$; Table 1), with absorbance ranges between 0.17 and 0.40 nm, which classifies the honeys in extra light to light amber, according to the Pfund scale.

According to Vasconcelos et al. (2021), the clearest honeys are usually those with the highest market value, not because of the actual quality of the product, but rather because of the demand of consumers who prefer to purchase clear honey. Patruica et al. (2022) reinforce that honey color is influenced by factors such as the minerals present, flavonoid content, bee species and honey age; dark honey has a greater quantity of plant pigments, as well as greater quantities of minerals, being able to have four to six times more minerals than light honeys do (Olas, 2020).

The values found in this study for the Lund reaction stood between 0.29 and 0.31 ml, and it was found that no protein precipitate was formed in the analyzed honey samples. According to Erban et al. (2021) and Fakhlaei et al. (2020), there still is a scarcity of studies and little knowledge about the occurrence of protein in honey, since the quantification of this nutrient is used to detect adulteration of the commercial product. With proline being one of the most present amino acids in honey, representing around 50-85% of the total (Sun et al., 2017).

For the Lugol reaction, the results presented for the honeys were negative in all samples, indicating the absence of adulterating substances, and agreeing with the results found by Şen & Türkaslan (2021) when analyzing the physicochemical properties of multifloral honeys from different regions of Turkey, with a negative result for the Lugol reaction being obtained.

Although the samples comply with what is established in Brazilian legislation, it is necessary that Good Manufacturing Practices (GMP) measures be applied by honey extraction and processing units, in order to guarantee the sanitary quality and compliance of the product with current legislation, with a view to meeting the demands of the consumer market. It is also necessary that the entire honey production chain, from harvesting to delivery of the product for sale, be carried out with care and hygiene, using specific equipment and clothing, in addition to storage in a suitable place, so that the characteristics of honey are preserved and so that it becomes possible to control the risks of contamination, ensuring a safe product for consumption.

Therefore, educational measures and training must be carried out, seeking to raise awareness among beekeepers regarding the main hygiene measures of the facilities, equipment and utensils for the production, extraction and packaging of honey, and to raise awareness among those who handle it, showing their responsibility as to the safety and quality of honey. The adoption of techniques that strengthen beekeeping in Senhor do Bonfim, Bahia, are fundamental to strengthening beekeeping activity in the region.

Conclusion

The honeys evaluated in this study, produced and sold in the municipality of Senhor do Bonfim, Bahia, comply with Brazilian legislation on honey, being suitable for marketing and consumption.

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