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Analysis of the Physical and Chemical Properties of Honey Sold in the Free Fair of a Municipality Located in the Legal Amazon – Brazil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Honey is a food of natural origin produced by bees, which stands out as one of the most valued products. The practice of beekeeping and the increase in honey consumption by the population has been shown to be a promising and profitable activity for producers and the economy of Brazil. This research aimed to conduct analysis of the physicochemical properties of honey samples sold at the free fair of a city in the region of the Legal Amazon, located in the interior of Rondônia. Six samples from different sources were selected to evaluate their physical and chemical properties, including color, water content, hydroxymethylfurfural content (HMF), gray content, lund reaction and lugol reaction. The results were compared to the standards set by Normative Instruction No. 11 of the Ministry of Agriculture, Livestock and Supply (Map) of 2000, using the methodologies proposed by

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the Adolfo Lutz Institute. The results presented obtained variations between the samples, since the composition of honey differs according to the floral species and region in which it comes from, in addition to their storage and management conditions. It is observed that some of the honey samples, without proper inspection, marketed at free markets in Brazil, do not meet the standards required by Brazilian legislation for honey.

Keywords: Honey quality control; physicochemical properties of honey; adulteration.

1. INTRODUCTION

Bee honey is a food with high biological value, containing sugars, water, minerals and vitamins. It has typical sweet flavor, viscous texture and predominant aroma [1].

The United Nations (UN), instituted May 20 as World Bees Day, to portray the importance of pollination for sustainable development.

In Brazil, honey production between 2014 and 2019 reached a percentage of 19.61% (MAPA, 2022). Currently, there are five geographical indications (GI), registered relating bee products. These indications refer to products that have a strong bond with their home territory. But only two of these regions are recognized as an indication of origin and only one honey producer has record as a denomination of origin.

Regarding the export of honey, in 2020 the country exported 45.7 thousand tons of the product, earning a total of US \$ 98.560 million, and 72% of these exports to the United States (MAPA, 2022).

The federal legislation is based on changes and fraud in raw materials of origin to animal. Decree No. 10,468, of August 18, 2020, which provides for the regulation of the industrial and sanitary inspection of animal products, foods are considered fraudulent or counterfeit when they have substances that replace the originals or that there is an addition of another to hide defects or to increase product performance [2,3].

2. THEORETICAL FOUNDATION

Honey is considered a complete food as it has a rich variety of nutrients. It is produced by bees to start from the nectar collected from the flowers, the secretions of living parts of plants or the excretions of insects that suck as plants [4]. This food is rich in carbohydrates, vitamins, minerals and antioxidants, making it a great choice for those looking for a healthy lifestyle [5].

In addition, honey is also known for its antibacterial and anti-inflammatory properties, which makes it a popular option for the treatment of certain health conditions. In short, honey is a versatile and nutrient -rich food that offers a wide range of health benefits [6].

The physicochemical analysis of honey is necessary to ensure product quality and verify if it is in accordance with Brazilian law [7]. This verification is important to prevent fraud, overheating or poor storage, which can negatively affect honey quality and harm the consumer. Therefore, it is vital that all peers analyzed are in accordance with the legislation to ensure product safety and quality [8,9].

The quality assessment and properties of honey is performed by various techniques, including sensory, chemical, physicochemical analysis, species identification and pesticide analysis. These analyzes are important to ensure the quality, purity and safety of honey for consumers. Sensory analysis evaluates sensory aspects (odor, taste, color), chemistry measures specific components and physicochemical combines both to offer a comprehensive view of honey properties. The identification of species determines by which bee honey was produced and pesticide analysis verifies the presence of harmful waste [10], (Brazilian Society of Pharmacognosy [s.d.]).

Normative Instruction No. 11, established by the Ministry of Agriculture and Livestock, is the legal reference for assessing honey quality and its compliance with physicochemical and microbiological standards [11,12]. This standard establishes six parameters that are considered indicative of honey quality, they are: acidity, reducing sugars (glucose + fructose), gray, hydroxymethylfurfural (HMF), amylase index (distressed activity) and moisture. Sacarose is also mentioned as a parameter. These parameters are used to evaluate the purity and quality of honey, and ensure that the product is safe for human consumption [13].

The data presented by the Brazilian Institute of Geography and Statistics (IBGE) and the National Beekeeping Cooperative (CONAP) show that honey production in Brazil in 2015 was 37,815 tons, with revenues of R \$ 358.8 million. Of this total, 59% was exported, ie 22,205 tons. Rio Grande do Sul leads the production, with 9.2 thousand tons, followed by Paraná (8.4 thousand) and Piauí (6.9 thousand).

Already, honey production in the northern region, concentrated in the states of Rondônia, Amazonas, Roraima, Pará and Tocantins, is important for the economy of the Legal Amazon, because it is a source of income for the honey producers in the region, which often They have no other affordable sources of income. In addition, the marketing of honey in the Legal Amazon contributes to health, well-being and the environment [14].

The beekeeping chain is an initial low-cost activity with product diversification and income. However, the lack of qualified labor, lack of association and cooperative, weak logistics, lack of awareness, lack of native areas and organization, makes it difficult in the countryside. It is necessary to improve the safety of agrochemical application, to have support from the municipality in the donation of land to cooperative, partnerships with institutions for free practical courses to increase profitability and production [15]. Due to this scenario, it is essential to designate supervision to ensure the quality standard of the marketed honey, as there are challenges to be faced in production and marketing.

3. METHODOLOGY

3.1 Obtaining the Sample

The 6 honey samples were purchased at the farmer's free fair, located in a municipality of the Jamari Valley in the state of Rondônia.

The different samples of honey were collected taking into account their origin and quality pierced by traders in the region. They were then taken to the Faema University Center Laboratory-UNIFAEMA, to be packed in the refrigerator under constant temperature of (how many degrees) for their conservation during the processes of physicochemical analysis that occurred during the April periods (2022) to June (2022).

3.2 Analyzes Carried

The study was conducted at the Faema University Center Bromatology Laboratory - UNIFAEMA, where the following analyzes were performed: color determination, brix grade, refractive index (IR), humidity, pH, ash content, hydroxymethylfurfural content (HMF), lund reaction, lugol reaction. All tests were performed on triplicates, the samples were prepared according to the Food Analysis Standards of the Adolfo Lutz Institute [10].

3.3 Color

To determine honey color intensity, Bianchi's methodology [16] was used, which is based on the Pfund scale. The method consists of diluting 50% of the sample with distilled water and centrifugates at 3200 rpm/5min. Subsequently, absorbance should be measured at 635 nm using spectrophotometer. Lastly, the color intensity must be determined by the Pfund scale using the equation: $Pfund = -38.70 + 371.39 \times Abs$

3.4 Brix Degree, Refractive Index (RI) and Humidity

To determine the Brix degree, refractive index (IR) and humidity, the refractometric method of bhataway was applied, revised by Wedmore where it uses the sample refractive index measure to be converted into a moisture percentage [10].

3.5 pH

Following the Analytical Standards of the Adolfo Lutz Institute [10], pH measurement was performed by weighing 10 grams of the sample on a Gehaka AG: 200 model balance. The pH was determined by direct immersion of the electrode into the sample using a digital pH meter Qualxtron Qx 1500 model, previously calibrated with pH buffer solutions of 4 and 7.

3.6 Ash Content

The percentage of ashes was identified by the 4.8 method described in the Adolfo Lutz Institute Analytical Code (1985), which uses the burning of samples at 550°C to destroy organic matter without causing significant decomposition or loss of residual mineral components.

3.7 HMF Content

The Hydroxymethylfurfural (HMF) quantification test is used to determine the heating or changes from poor product storage. The HMF is the main secondary compound analyzed in relation to the quality of honeys. The HMF production speed is accelerated depending on the temperature and time exposed, direct exposure to light can lead to the HMF acceleration process [17].

The quantification of the hydroxymethylfurfural content (HMF) was performed through the quantitative method, in which the samples were taken for spectrophotometer tests and analyzed the transmittance and absorbance results in the spectrophotometric lengths at 320 nm and 372 nm.

3.8 Lund Reaction

Lund assessment is a way to measure the amount of protein present in honey. It provides important information about the floral origin of honey and the possibility of tampering. Morsairs with higher protein content are considered more nutritious. The test is based on the reaction of honey with tannic acid, resulting in the precipitation of albuminoid substances (proteins or their precursors) (IAL, 2005). The analysis is performed visually, by observing the precipitate formed in the beaker, which contains a sample of

honey and water. The ideal value for the amount of precipitate is between 0.6 and 3.0 ml.

3.9 Lugol Reaction

Lugol examination is used to detect tampering as the addition of commercial glucose or starch generates a reaction. The mixture of iodine and glucose potassium iodide produces a colorful solution, ranging from blue red to blue, with the intensity of color depending on the amount of dextrans in glucose [10].

3.10 Statistical Analysis

The data were processed in a simple way in the Excel software (Microsoft), using its standard tools for calculation and organization.

4. RESULTS AND DISCUSSION

The findings, which align with the characteristics that define honey, have been presented in the form of figures, graphs, and tables, as specified in Normative Instruction n. 11/00 of MAPA [4]. Fig. 1 expresses the percentage of color variations for all test samples. Through the results found (Fig. 1), it is observed that there was a predominance of the amber color with a relative percentage (40%) to the total of the samples present.

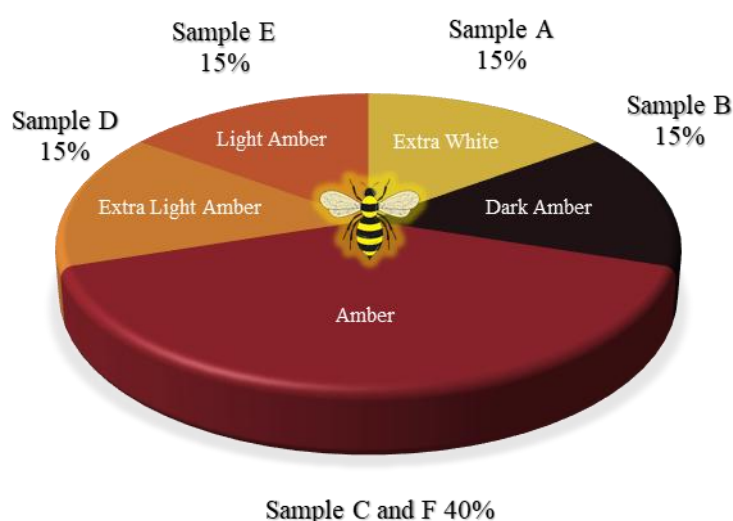


Fig. 1. COR determination

Source: Authors (2023).

Table 1. Determination of the Brix Degree, Refractive Index (RI) and Humidity

Parameters	Leg	Samples					
		A	B	C	D	E	F
°Brix (%)		82	81	95	79,20	80	76
IR		1,492	1,492	1,496	1,485	1,491	1,481
Humidity (%)	Max 20%	17,80	17,80	16,20	20,40	18,20	22,20

Subtitle: Leg=Legislation; Max=Maximum

Source: Authors (2023).

The color of honey is an important aspect for its characterization and market price definition. It varies according to the floristic nature of the origin of the bee, where the nectar is collected (Moreti, Augusta Carolina de Camargo Carmello et al. (2006). Also, the amino acid content is generally higher in dark honeys than light honeys. The mineral content of the soil, which is transmitted to the nectar of the plant, also influences the color of the honey. The variation in color can be affected by different factors, such as acidity, nitrogen content and fructose content [18]. According to Negueruela; Perez-Arquillue [19] the use of old honeycomb and heat treatment also darken the product.

The results shown in Table 1 indicate that honey samples (B, D and F) have a similar concentration of soluble sugars, approximately 80%, while another sample (C) has a significantly higher amount, around 95%. Also, Refractive Index values range between 1.4810 and 1.4961. The legislation establishes that the maximum humidity allowed is 20%. The samples (A, B, C and E) are below this limit, with 17.80%, 16.20% and 18.20% of humidity, respectively. However, samples (D and F) show moisture values above the limit, being 20.40% and 22.20%, respectively.

As noted in Table 1, the levels of dissolved sugars in the sample (C) could have been impacted by a variety of factors, including the bee species that produced the honey, the nectar sources, the weather conditions during the harvest, the extraction and storage, among others.

According to Pereira, Elka Odila Leita [20], honeys are composed of a significantly higher amount of sugars than water, with a ratio of 4.5:1. It is this high concentration of sugars that gives honey its high quality and enhances its energy value. However, the presence of excess dissolved sugars in honey can affect its quality and flavor, making it sweeter or more acidic, depending on the proportion of different types of

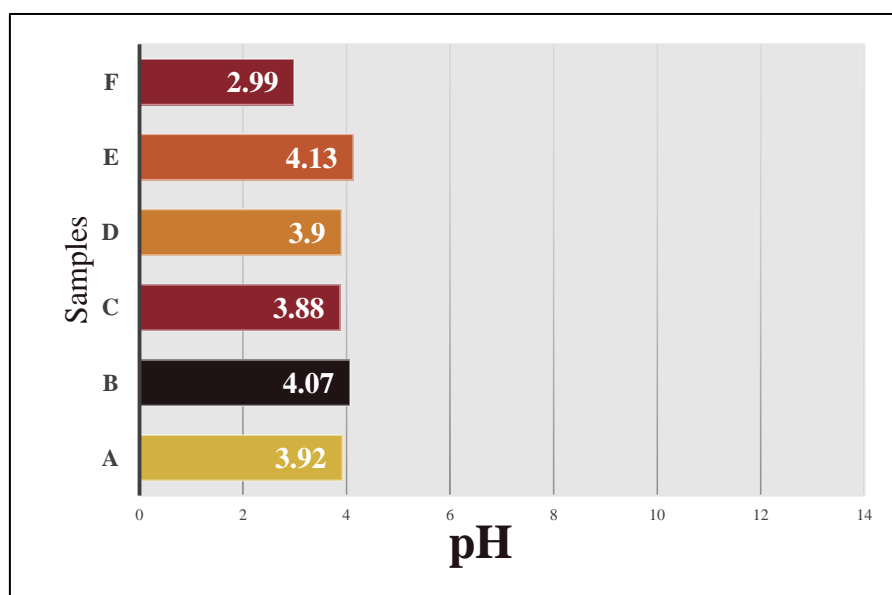
sugars present. Therefore, it is important to evaluate the amount of dissolved sugars in honey before commercialization.

A study carried out in a city in the Jamari valley compared two different samples and obtained an average of 80.05% of soluble solids in honey (Damasceno, 2012). Another study carried out by Amud et al. [21], observed mean values of 74.5% for true honeys and 77.38% for honeys with adulteration.

Current legislation does not include reference to the value of water-soluble solids (°Brix). However, its value is crucial to determine the moisture content, depending on the technique used. The analysis guide of Instituto Adolfo Lutz [10] presents a table where the value of soluble solids is directly linked to the value of moisture [22,23].

Góis [24] points out that water is a vital component in the composition of honey. Water content has a significant impact on honey characteristics, including flavor, maturity, specific weight, viscosity and preservation. According to Welke et al. [25] it is essential to know the moisture content of honey to ensure its quality and efficient marketing, as moisture is closely linked to quality and conservation. It is, therefore, crucial that the moisture content of the honeys is within the limits established by Brazilian legislation.

Two studies carried out in the Legal Amazon region found that most honey samples had adequate moisture levels, with average values of 17.52 and 18.75 (DAMASCENO, 2012); [21]. On the other hand, a study carried out in the state of Paraíba found that all honey samples analyzed had moisture levels above 20%, making them unsuitable for commercialization [26]. Moisture level can have a significant impact on the quality of the honey, hence its shelf life. Some scholars believe that honey with a moisture content above 21% can ferment in 3 or 4 months [27].



Graph 1. Determination of pH

Source: Authors (2023).

In the case of the values presented for the hydrogen ion potential (pH), all the samples (Graph 1) have a pH below 7, which indicates that they are all acidic. Sample (F) has the lowest pH (2.99), which means it is the most acidic of the samples.

The acidity of honey is determined by its botanical origin. In general, floral honeys have a pH below 4.0, while honeydew honeys have a pH above 4.5. In addition, the acidity of honey can also be influenced by the concentration of different acids, such as calcium, sodium, potassium, and other components present in the ashes [28].

According to Meireles and Cançado [22] the pH of honey bees indicates its conservation status. Honey is naturally acidic, with a pH of between 3.3 and 4.6 (on average).

Brazilian legislation does not specify specific pH levels, however, measuring this value is considered relevant due to its potential to indicate fraud or microbial contamination in food and beverage production [29]. In other words, the lack of specification of pH values in Brazilian legislation does not diminish the importance of this indicator to guarantee the safety and quality of food products.

The results presented (Table 2) indicate that the ash content in all samples is within the

acceptable range of 0.6%. Furthermore, all results have low variance, indicating that the analysis was performed accurately.

Some writers have found similar and intermediate results to those obtained in this study. According to Venturini, Sarcinelli and Silva [30], the ash content in honey is an indicator of the presence of minerals and can influence the color of the substance. Values that are too high or too low may indicate that the honey has been adulterated. In 2021, Souza et al. [31] analyzed 8 samples and found that 50% of them were within the established standards, ranging from 0.71% to 0.60%. Ludwig et al. [32] found that, when examining 47 samples, 100% were within legal limits, with a range of 0.03% to 0.52%.

When interpreting the results of hydroxymethylfurfural (HMF) content in honey, it is important to consider the reference value of 60 mg/kg. In Graph 2, the samples (A, E & F) present HMF contents below or close to the reference value: This may suggest that these honey samples have an acceptable quality and that there were no degradation processes during production or storage. Samples (B, C & D) have HMF levels above the reference value: This may indicate that these honey samples were exposed to conditions that caused an accelerated degradation process, which may have affected the quality of the product. It is significant to

consider the possible causes of this increase, such as the presence of contaminants, inadequate storage conditions or problems with the hive.

In countries with a tropical climate, levels of HMF (hydroxymethylfurfural) can be high in honey, even if it has not been altered or heated. This is due to the hot climate in the region [29,33].

In the study carried out by Sodr  [34], 58 honey samples were analyzed for HMF content. The amounts found ranged from 1.74 to 126.50 mg.kg-1 in the State of Cear , with an average of 31.45 mg.kg-1. In Piau , the variation ranged from 1.53 to 115 mg.kg-1, with an average of 13.78 mg.kg-1. Only 4 samples from Cear  and one from Piau  showed values above what is allowed by the current standard.

According to a study in Petrolina (Borges et al. 2017), samples of *Apis mellifera* honey showed average HMF levels between 13.98 and 50.92 mL.kg-1, within the limits established by national legislation. The samples from Salvador ranged from 37.21 to 64.60 mL.kg-1. However, three of the seven samples, SSA4, SSA5 and SSA7, showed values above the standards established

by law, with values of 60.78, 64.60 and 62.06 mL.kg-1, respectively.

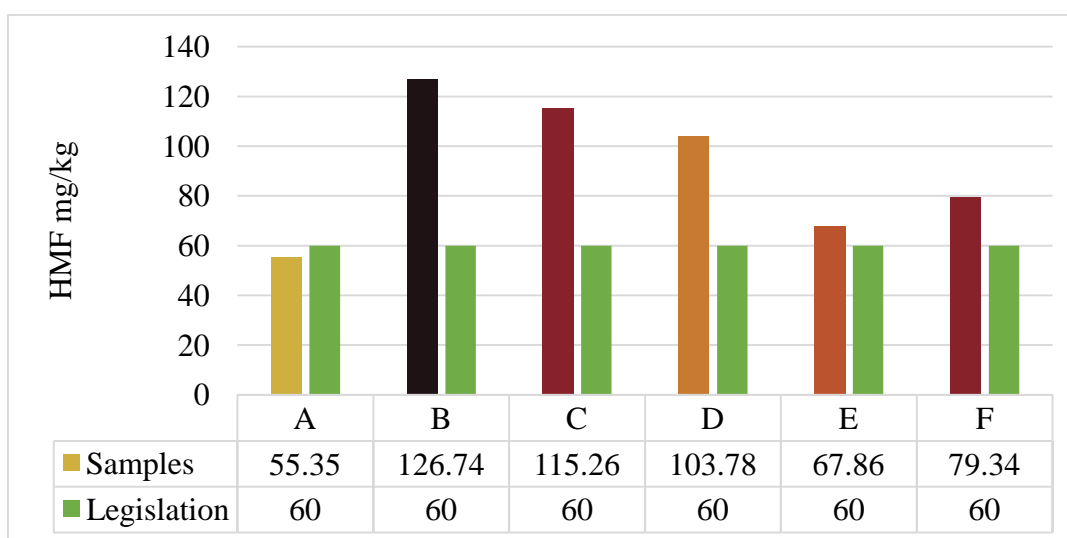
Table 3 shows Lund and Lugol's reaction for six samples (A, B, C, D, E, F) and compared with established legislation. In the Lund reaction, all samples showed a positive (+) result, indicating that they meet the legislation. In the Lugol reaction, all samples showed a negative result (-), which means that they also comply with the legislation. Finally, in the column for the sample (F), there is no result for the Lugol reaction, indicated as "n/a" due to the scarcity of the sample.

Bogo et al. [35], found similar results when analyzing seven honey samples for the presence of additives. Only one of the samples showed different results from the legislation in the Lund reaction, which suggests the presence of albuminoid substances, natural components of honey that are precipitated by the addition of tannic acid. Bera and Almeida-Muradian [36], also found in their samples values that varied between 0.5 and 2.0 mL, within the expected range for natural honey, indicating the absence of protein addition or loss during processing [37-40].

Table 2. Ash content

Parameters	Samples					
	A	B	C	D	E	F
% Ash	82	81	95	79,2	80	76

Source: Authors (2023).



Graph 2. HMF content

Source: Authors (2023)

Table 3. Lund and Lugol reaction

Parameters	Leg	Samples					
		A	B	C	D	E	F
Lund	+	+	+	+	+	+	-
Lugol	-	-	-	-	-	-	n/a

Subtitle: Leg=Legislation. Source: Authors (2023)

5. CONCLUSION

Honey samples, without some kind of inspection (municipal, state or federal) sold at the free markets of the cities of the state of Rondônia - Brazil, demonstrated low security and correspondence to the standards required by Brazilian law.

A fact like this does not negate the existence of high-quality honey sold in open-air markets. However, the lack of inspection and safety certification labels causes these honeys to have inferior physical-chemical properties.

With this, it is concluded that even the regularized samples may have some undue characteristic and this does not exempt the responsibility of the joint action of the public authorities, producers and the consumer market, to supervise the productivity of the commercialized honey, to avoid problems with the productive chain and, mainly, with adulterations, showing the need for greater quality control of certified honeys and inspection of non-certified products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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