



RESEARCH ARTICLE - BEES

Physico-Chemical Properties of Brazilian Eastern Amazon Honey Assessed by Multivariate Principal Components Analysis

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Abstract

Honey from the *Apis mellifera* bee (*Hymenoptera*, *Apidae*) is one of the most consumed beehive products in the world, mainly due to its nutritional and therapeutic value. In poorer regions, its commercialization can be an essential source of income for families and communities. However, the quality of honey can change both during harvesting and processing, as well as by climatic conditions and the type of vegetation. Therefore, implementing good production practices, with quality control in collection and processing, can add value to this product, promoting the development of the region in which it is produced. Thus, the objectives of this work were to characterize the honey of Africanized honeybees produced in the Eastern Amazon region in the cities of Nova Olinda do Maranhão (NO), Santa Luzia do Paruá (SL) and verify its correlation with a commercial honey sample (CH) – from São Luiz-MA. The physicochemical properties (color, moisture, solids insoluble in water, minerals, and ash, hydroxymethylfurfural, acidity, and diastatic activity) were analyzed according to the methods of CODEX Alimentarius and the Association of Official Analytical Chemists, and these data were evaluated by the Principal Component Analysis (PCA). The results showed that commercial honey presented higher levels of insoluble solids, HMF, minerals, and ash than samples from NO and SL. Variables that differentiated NO and SL honey were HMF, moisture, and diastase index. Although the samples have different characteristics, the data comply with Brazilian legislation, which can strengthen the development of apiculture in the region.

Introduction

Honey is a natural bee product and is appreciated for its sweet taste and nutritional and therapeutic value (Schramm et al., 2003). It is made from floral nectar, plant secretions, and insect excretions that bees collect and begins the process of dehydration

and transformation by the action of specific enzymes. It is processed and matured in honeycombs (Crane, 1983), where it becomes a complex nutritional sweetener composed mainly of carbohydrates (60–85%) and water (12–23%), in addition to minerals, vitamins, amino acids, proteins, phenolic compounds, and flavonoids (de Almeida-Muradian et al., 2013).



The composition and characteristics of honey change according to its origin, as the nectar produced by plants varies with climatic and environmental factors (Abu-Jdayil et al., 2002; Anthony & Balasuriya, 2016). Due to its territorial extension, Brazil has great potential for apiculture, as the climate and diversity of vegetation favor the production of honey all year with different compositions (Costa et al., 2018).

The major honey producers are *Apis mellifera* (Hymenoptera, Apidae) bees. Today 80% of the 200 thousand tons currently produced annually in Brazil are exported to the United States and Europe (Paula et al., 2017). The characteristics of their product are determined mainly by chemical, physical, microbiological, and sensory characteristics (da Costa Leite et al., 2000). Differences in flora type, climatic conditions, and geographical location influence honey's physicochemical properties. In addition, due to its commercial value, honey is often adulterated, so methods are required to ensure product quality. (Braga et al., 2020; Soares et al., 2017). For quality control and commercialization of *A. mellifera* honey, the parameters of physical-chemical analysis determined by Brazilian legislation involve maturity (reducing sugars, moisture, apparent sucrose), purity (water-insoluble solids, minerals or ash, pollen) and deterioration (free acidity, diastase activity, and hydroxymethylfurfural) (BRASIL, 2000).

The Eastern Amazon region was chosen for the development of the study, specifically, the municipalities of Nova Olinda do Maranhão, and Santa Luzia do Paruá, as they are located in the main region where the association of local beekeepers operates.

This region has predominantly humid tropical forest vegetation (Alvares et al., 2013). It has a well-defined climate with a rainy season from January to July and a dry season between August and December. Such characteristics make apiculture activities more favorable.

Taking into account these factors, the region of Nova Olinda do Maranhão and Santa Luzia do Paruá presents factors that favor honey production, such as i) diversified flora that provides a peculiar flavor to honey (Marques et al., 2011) ii) a privileged geographic position which facilitates the flow of the product for commercialization (Bentabol Manzaneres et al., 2011); iii) excellent resistance to colony diseases due to Africanized bees; iv) honey free from pesticide contamination due to being distant from arable areas (Haddad et al., 2015).

Government incentives and partnerships with other organizations and foundations have also favored honey production in this region, bringing social inclusion, environmental preservation, and income generation to municipalities. However, even with all the beneficial characteristics of the region, toxins, pollutants, and contaminants introduced during processing can alter honey benefits due to the environment and/or agricultural and apicultural practices (Kujawski & Namieśnik, 2008).

Thus, this work aimed to profile the honey of local Africanized bees to initiate a regional characterization according to its physicochemical characteristics. We also used Principal Component Analysis to identify which physicochemical properties differed from commercial honey samples. These data can help producers improve the quality of their products and, consequently, increase their consumption in the domestic and foreign markets, aiding the development of apiculture in the region.

Material and Methods

Sample Collecting and Storage

Honey samples produced by Africanized bees of the species *A. mellifera* were collected in apiaries in the Eastern Amazon (Figure 1) between 08/2011 and 09/2012. The study region is composed of 10 apiaries located in Nova Olinda do Maranhão (02° 50' 50" S, 45° 41' 41" W) and 12 apiaries located in Santa Luzia do Paruá (02° 30' 02" S; 45° 46' 30" W), which are cities that stand out in the production of honey in the northwest of Maranhão State. The apiaries comprise small informal producers with a cooperative where the entire honey extraction process is carried out.

The region's average temperatures during the collections varied between 26 and 27 °C. Of the total of 22 apiaries, honey was sampled in 11 (5 in Santa Luzia do Paruá and 6 in Nova Olinda do Maranhão); the samples were taken in the same apiaries in both years. In each apiary, three 50 mL amber bottles of honey were collected, wrapped in aluminum foil, and kept at room temperature until arrival at the laboratory, where the samples were subsequently analyzed. The three bottles from each apiary were analyzed in a pool of samples.

The commercial multifloral honey (CH) samples were acquired from a São Luiz-MA producer in September 2012. The producer provided a total of 9 samples, where three pools were made for the physical-chemical analysis, making up three groups with similar floral characteristics according to the information provided by the supplier. Commercial honey was kept in its original packaging and kept on ice. The analyzes of both the collections from the apiary and the commercial samples were performed as soon as they arrived at the laboratory; that is, they practically did not remain stored.

Physical and chemical parameters

Physical and chemical parameters of the honey samples were analyzed in triplicate, right after collection, according to a method recommended by the Association of Official Analytical Chemists (Horwitz, 2010).

Color

The color was measured using a spectrophotometer (BioSpectro, Curitiba, Paraná, Brazil) at 560 nm. Pure glycerin (PA) was used as a blank, and the results were converted using the scale of Pfund as a reference (Fell, 1978).

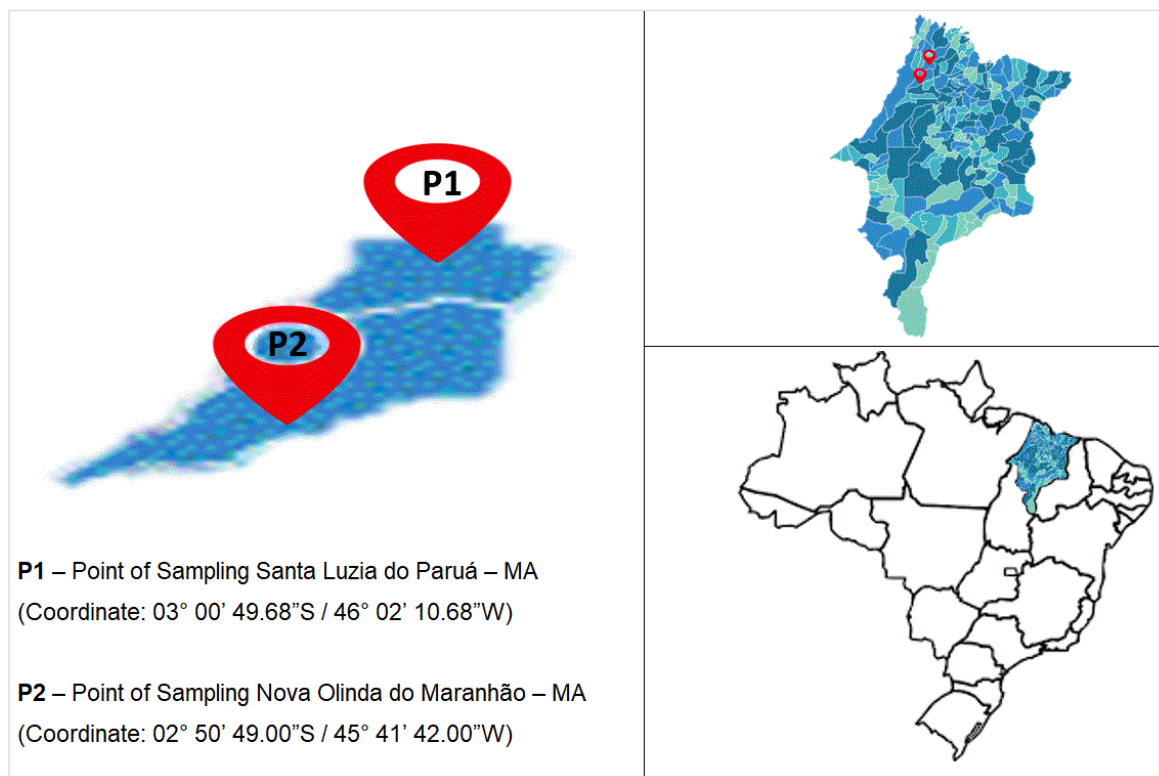


Fig 1. (A) City (P1) Santa Luzia do Paruá-MA and (P2) Nova Olinda-MA where the honeys studied in this work were sampled; **(B)** Map of Brazil showing the location of the State of Maranhão and the map of Maranhão showing the location of cities P1 and P2 in its territory.

Moisture

Moisture was determined using the refractive method of Chataway (1932). Samples of refractive indexes were measured using a refractometer (Atago, Ribeirão Preto, São Paulo, Brazil). The refractometric data were converted to moisture content (%) using the Chataway table. Considering that the honey temperature was 25 °C, 0.00023 was added to the refractive index result for each degree above 20 °C before converting by the mentioned table.

Water-insoluble solids content

According to Codex Alimentarius, the content of water-insoluble solids was determined by the gravimetric method, where the honey was diluted with distilled water at 80 °C and filtered in porous crucibles. The weight of the residue remaining from the wash is determined, and the proportion of the constituent is calculated from the initial weight of the sample, according to the Codex Alimentarius (1989 – item 7.4) (BRAZIL, 2000).

Mineral Analysis

Five grams of honey were weighed in a melting pot (pre-treated at 600 °C for 30 min) which was heated to start carbonization on the hot plate (Quimis, Diadema, São Paulo, Brazil). Afterward, the samples were transferred to muffle at 600 °C and kept for five hours. Finally, the melting pots were cooled and weighed. Results were expressed as a percentage of minerals and ashes (Bogdanov et al., 2002).

Hydroxymethylfurfural (HMF)

HMF determination was carried out by taking 5 g of each sample mixed with 25 mL of water in a 50 mL flask. After adding 0.5 mL of Carrez 1 solution (potassium ferricyanide) and 0.5 mL of Carrez 2 solution (zinc acetate), the flask was completed with water. Then, the mixture was filtered on Qualitative Whatman (GE) Grade 1 Filter Paper. (Merck Darmstadt, German), and discard the first 10 mL. Five milliliters of filtered samples were pipetted into tubes. 5 ml of water was added in one tube and 5 ml of sodium bisulfite in another tube (blank). Samples' absorbance was measured at 284 and 336 nm (Bogdanov et al., 2002).

$$HMF \left(\frac{mg}{Kg} \text{ honey} \right) = \frac{(A_{284} - A_{336}) \cdot (149,7) \cdot (5) \cdot (D)}{M (g \text{ of sample})}$$

A₂₈₄ and A₃₃₆ - Absorbances; D - dilution samples; M - samples mass

Equation 1: Formula to calculate HMF values in samples

Acidity

Acidity was determined by weighing 10 g of honey sample, mixing it with 75 mL of decarbonated water, and adding phenolphthalein. This solution was titrated using 0.05 mol/L sodium hydroxide until pH 8.5. To obtain lactic acid, 10 mL of 0.05 mol/L sodium hydroxide was added to the honey sample and titrated with 0.05 mol/L hydrochloric acid until pH 8.9. Free acidity, lactic acidity, and total acidity were calculated according to AOAC (Horwitz, 2010).

Diastase Activity

Diastase activity was determined by mixing 10 g of honey, 5 mL of 1.5 mol/L sodium acetate, 3 mL of 0.5 mol/L sodium chloride, and organic-free water. This mix was divided into two tubes containing 10 mL. 5 mL of 2% starch solution was added. Both tubes were heated at 40 °C for 15 min. The solutions were mixed, 1 mL aliquots were taken every 5 min, and the absorbance was measured on a spectrophotometer (BioSpectro, Curitiba, Paraná, Brazil). Thus, analytical curves relating to absorbance and time were built (Bogdanov et al., 2002).

Statistical analysis

SIMCA-P software (Umetrics) was applied for statistical data analysis employing the multivariate Principal Component Analysis (PCA) method. This method describes data variability and discriminates between groups according

to the physical and chemical analyses performed. It is mainly used to compress data due to a correlation between several measured variables.

SIMCA analyses were performed using raw data without any mathematical transformation and UV scaling. The variables used were color, moisture, HMF, total acidity, diastase activity, insoluble solids, minerals, and ashes. As color is a qualitative parameter, it was coded to be used in the PCA matrix. The same numbers were considered for a PCA to recognize this parameter as a variable for equal colors.

Results and Discussion

From all the measured parameters, moisture was the most similar among all the samples, ranging from 16.2 to 21%. The commercial sample had 19.5% moisture. In all other parameters, the commercial honey presented values much greater or smaller than the fresh samples collected in NO and SL (Table 1).

Table 1. Physical and chemical properties of honey samples from Santa Luzia do Paruá (SL), and Nova Olinda do Maranhão (NO) – Collect (A – collected in 2012) and (B – collected in 2013), compared to commercial honey from São Luís, capital of Maranhão CH – Commercial honey (collected in 2012).

Samples	Color	Moisture (%)	HMF (mg kg ⁻¹)	Total Acidity (meq kg ⁻¹)	Diastase Index	Insolubles Solids (%)	Minerals and Ashes (%)
SL1A	Extra white	17.2	3.2	50.2	21.4	0.013	0.024
SL2A	White	17.4	3.7	53.4	23.9	0.015	0.061
SL3A	Extra Light Amber	21.0	3.9	47.8	14.3	0.076	0.014
SL4A	White	16.2	3.4	47.6	7.5	0.032	0.037
SL5A	Extra White	17.4	3.3	47.9	18.1	0.034	0.027
SL1B	Light Amber	18.0	4.3	51.8	22.2	0.046	0.070
SL2B	Light Amber	19.0	2.5	64.6	23.2	0.060	0.090
SL3B	Light Amber	16.6	2.0	46.1	16.1	0.026	0.080
SL4B	Extra Light Amber	17.6	2.0	52.4	22.5	0.004	0.100
SL5B	Extra Light Amber	18.0	1.4	44.9	11.4	0.006	0.006
NO1A	White	18.0	2.8	52.2	26.7	0.002	0.150
NO2A	White	17.4	3.3	49.4	25.0	0.036	0.061
NO3A	Extra White	17.7	3.4	49.8	14.4	0.008	0.010
NO4A	Extra Light Amber	21.0	3.4	59.8	19.5	0.029	0.166
NO5A	Extra Light Amber	16.4	6.1	52.4	16.9	0.008	0.034
NO6A	White	17.2	3.8	50.8	28.0	0.010	0.084
NO1B	Light Amber	20.8	3.9	58.9	59.2	0.040	0.045
NO2B	Light Amber	18.7	4.5	63.0	44.8	0.005	0.064
NO3B	Light Amber	20.2	6.2	64.4	58.1	0.010	0.087
NO4B	Extra Light Amber	20.8	3.8	51.7	46.9	0.005	0.045
NO5B	Extra Light Amber	20.6	4.1	50.5	58.7	0.097	0.042
NO6B	Light Amber	21.0	5.0	53.9	30.2	0.052	0.068
CH1	Light Amber	19.5	16.2	37.7	13.7	0.350	0.290
CH2	Light Amber	19.6	16.1	37.7	13.8	0.350	0.260
CH3	Light Amber	19.4	16.2	37.8	14.0	0.820	0.200

PC1 x PC2 explained 85% of the total data variance and allowed the characterization of the trends among the samples. PCA's main discrimination, promoted by component 1 of the PCA (65.7% of the total variability), is between commercial honey and all honey collected in NO and SL apiaries (Fig 2). The score plot (Fig 3) indicates that this discrimination occurs mainly due to the variables Insoluble Solids, HMF, and Minerals and Ashes.

In the commercial sample, the HMF content is about four or five times higher than the average found in honey harvested in Nova Olinda and Santa Luzia, possibly due to storage time, although it is within the parameters established internationally. HMF is a six-carbon heterocyclic organic compound containing aldehyde and alcohol functional groups formed during the dehydration of hexoses through the Maillard reaction. It is a parameter used to evaluate the freshness and quality of honey (Crane, 1983).

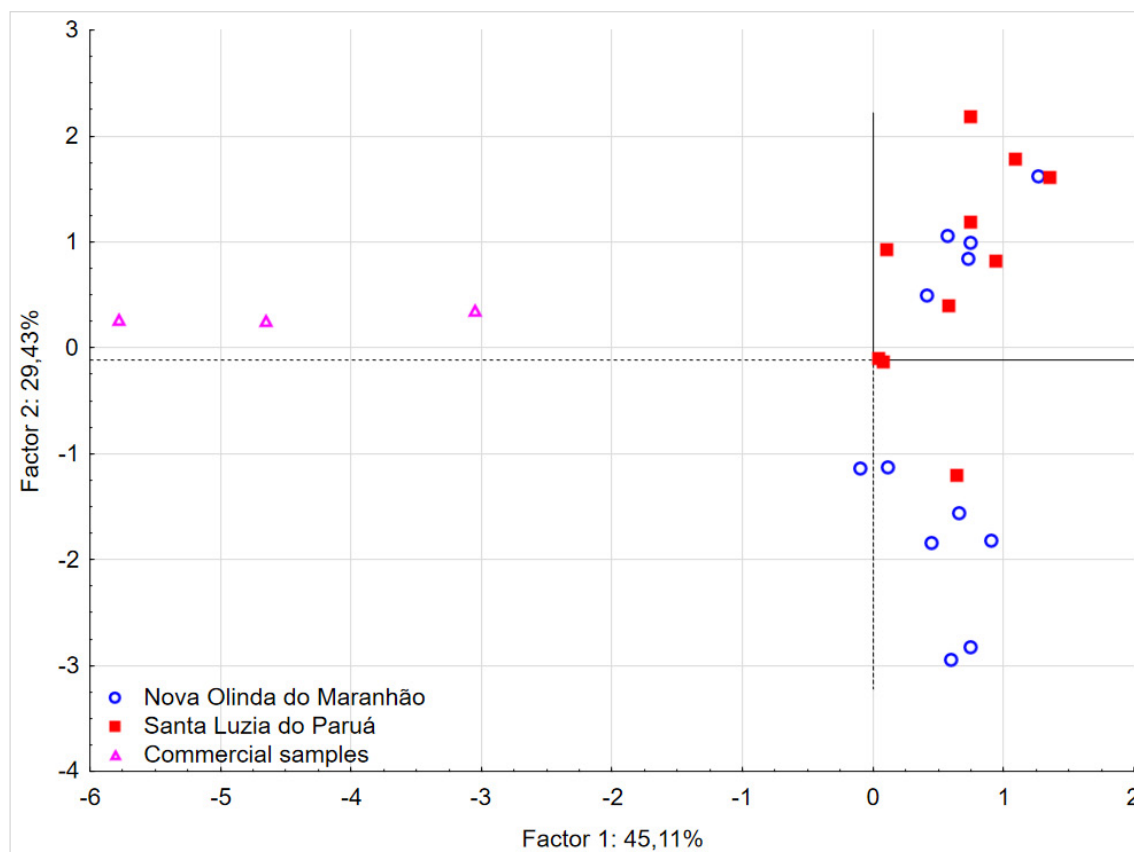


Fig 2. Scatterplot of the Principal Components Analysis of honey chemical composition from different origins.

In general, HMF values in freshly harvested honey are usually in the 1 mg/kg low (Pacias et al., 2018), and higher values indicate significant changes resulting from prolonged storage at high temperatures and/or overheating (Khan et al., 2015; Pacias et al., 2018). It may also indicate adulteration by sugar addition, such as corn syrup (Amiry et al., 2017). In our data, the highest HMF concentration (6.2 mg/kg from sample NO3B) is directly related to the highest acidity and ash values; 64.4 meq/kg and 0.087%, respectively.

Although the HMF values differ, they are within those established as acceptable by Brazilian legislation, which is 60 mg/kg (Mel, 2000). The HMF in commercial honey was 16.2 mg/kg, a value below that stipulated by Brazilian legislation; This parameter shows an adequate indication of good quality of commercial honey in the city of São Luiz-MA, as the temperature to which the honey is submitted during the storage period until consumption can change the HMF values.

The mineral content was another important discriminant parameter. In NO and SL, honey ranges from 0.006 to 0.100%, while commercial ones presented a value of 0.29%, about 3 to 48 times higher than the freshly collected samples. According to Crane (Crane, 1983), the mineral content in honey is generally low, ranging between 0.02 and 0.3% in blossom types of honey. Bee species, climate, soil, and botanical origin can influence the mineral content in different kinds of honey. Mineral contents were also related to the color of the honey, a variable that also contributed to the discrimination of commercial honey, although on a smaller scale. Lighter colors are related to low mineral content, and dark colors are related to high mineral content, as suggested in the Pfund scale (Fell, 1978).

The ash content of these samples (NO and SL) ranged from 0.006 to 0.100%, indicating low mineral concentration and light color in these samples (Table 1). From the commercial point of view, this is a desirable feature since Brazilian

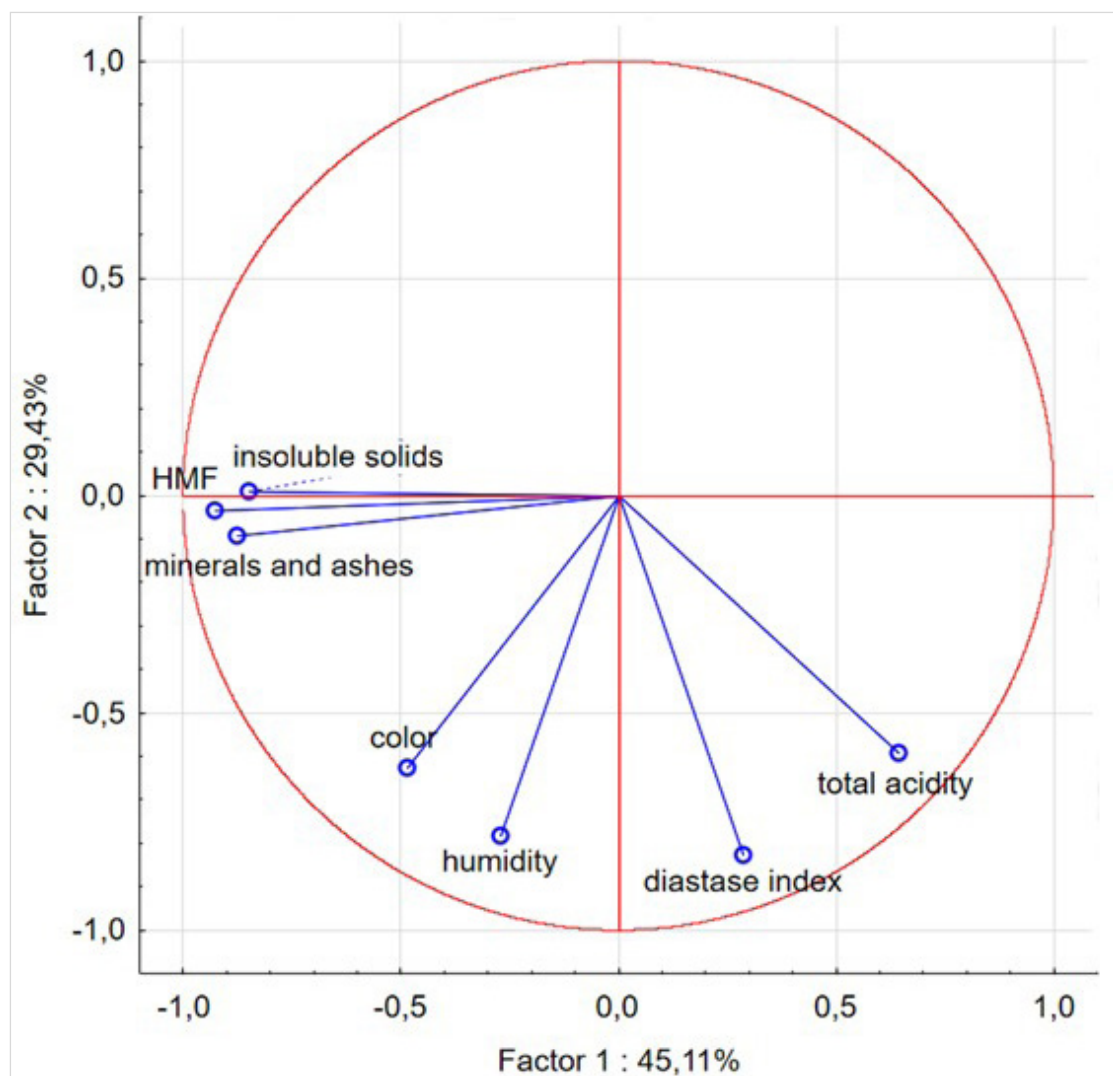


Fig 3. Score plot grouping samples according to their physical and chemical properties. $r = 0.975$ and $Q = 0.811$.

consumers prefer lighter colors of honey (Delmoro et al., 2010). Besides mineral content, color is also related to floral origin, climatic conditions during the nectar flow, and hive temperature. Furthermore, storage time, light, heat, and possible enzyme reactions can affect honey coloring (Baltrušaitytė et al., 2007; Terrab et al., 2003).

The content of water-insoluble solids found in fresh samples was less than 0.1%, and in commercial honey was 0.35% (Table 1). This concentration indicates an absence of contaminations from honey harvesting and processing. Water-insoluble solids are particles larger than 15.40 μm and not soluble in water at 80 $^{\circ}\text{C}$ (de Almeida-Muradian et al., 2013). Thus, water-insoluble solids content indicated that the studied honey had acceptable quality of hygienic-sanitary conditions to which honey was submitted during harvesting and processing.

Honey moisture influences viscosity, maturity, crystallization, flavor, conservation, and, consequently, the commercial value of the final product (Kadri et al., 2017). High moisture content facilitates the growth of microorganisms and the transformations that occur in food. Under high humidity

conditions, honey can ferment by osmophilic yeast action. Of the 23 samples, 16 of them presented values below 20%, 4 had values slightly above 20%, and 3 had a value of 21% of humidity. These values between 20 and 21% may have occurred due to the samples being fresh; they may be indicative of honey from an early harvest, which was in the process of finalizing dehumidification, some producers leave the honey to be dehumidified, which would not make this honey with a 21% unfit for consumption and would not be an indication of maturity or deterioration.

On the other hand, the main variables that contributed to the grouping of Santa Luzia and Nova Olinda honey compared to commercial honey were the total acidity and diastase activity (Figure 3). Honey acidity may occur due to factors such as *i*) variation of organic acids from different nectar sources, *ii*) glucose-oxidase enzyme action that originates gluconic acid, *iii*) bacteria action during honey maturation; *iv*) minerals present in honey; *v*) presence of organic acids in equilibrium with their corresponding lactones or internal esters and some inorganic ions such as phosphate (Finola et al., 2007; Crane, 1975).

Among the acids present in honey, gluconic acid is the most representative, as it is synthesized from the glucose-oxidase enzyme present in bees (Bucekova et al., 2018), and this enzyme remains active during storage and even after honey processing (Apriceno et al., 2018). Furthermore, the acidity of honey can be affected by the presence of internal esters and some inorganic ions (Finola et al., 2007). Organic acids contribute to the characteristic flavor of honey and stability to microbial deterioration (da Silva et al., 2016).

Bee species also influence honey acidity. The acidity in the honey of *A. mellifera* from the Tocantins, also in the North region, ranged from 35.0 to 59 mEq/kg (Abadio Finco et al., 2010). In the region of Goiás, in the Brazilian Midwest, acidity analyses ranged from 19.9 to 78.1 mEq/kg (Ananias, et al., 2013). *Melipona beecheii* from Mexico produces honey with an acidity of around 41 mEq/kg, while *Tetragona melanoleuca* from Thailand produces honey with higher acidity, around 590 mEq/kg (de Sousa et al., 2016; Nordin et al., 2018; Campos & Hincapié, 2023).

The value of diastase activity in commercial honey was lower than that observed for freshly harvested honey (Table 1). Diastase is an enzyme found in the hypopharyngeal glands of bees and pollen grains to a lesser extent. It can digest starch, in addition to being highly sensitive to heat. It can be used as an indicator of product conservation degree (Bucekova et al., 2018; Öner et al., 2018). Low values of this enzyme reflect procedures or adulterations that honey may have undergone during its processing, such as the use of temperatures above 60 °C, inverted sugar addition, or inadequate storage conditions (above six months and high temperatures).

Diastase activity is reduced by partial or total denaturation of amylases as a temperature effect (Ajilouni & Sujirapinyokul, 2010). According to the Brazilian law, permitted diastase activity is at least eight on the Göthe scale. However, honey with low enzyme content must have at least three Göthe, and the HMF concentration should not exceed 15 mg/Kg (Mel, 2000). HMF contents between 40 and 80 mg/kg are accepted for honey from countries or regions with tropical temperatures.

This standard ensures that honey has not been heated during processing (Amiry et al., 2017; Machado De-Melo et al., 2018). One sample from Santa Luzia showed less than eight diastases activities, but its HMF was 3.4 mg/kg. Thus, all samples met the standards defined for these parameters. However, the diastase activity of some samples from Nova Olinda is superior to that of the other samples, which justifies the separation in an opposite quadrant (Figure 2).

In this work, among the physicochemical parameters analyzed, few samples were inadequate to the Brazilian and international honey quality standards; this fact allows greater commercial use of this product in the eastern Amazon, as the product has been marketed since 2004, and 80% of the 200 tons currently produced annually are exported to the United States and Europe (Paula et al., 2017).

This is an important characteristic since this is one of the poorest regions in Brazil, and this source of income is a fine addition to the annual budget of the families. In addition, PCA proved to be an efficient data analysis tool when applied to the physicochemical parameters proposed in this work and allowed the differentiation of commercial samples collected in the city of São Luiz-MA, from samples from Nova Olinda do Maranhão and Santa Luzia do Pará. It can also be useful to compare honey from different origins and to have different patterns according to the origin, geographical region, and storage conditions.

The next steps involve sampling honey from different floral sources and more regions to verify the possibility of creating patterns for honey classification based on these parameters. These results are also important for strengthening local productivity related to apiculture and meliponiculture since this region, known as the Eastern Amazon, was recently included in the “Honey Route” of the Amazon and had the potential to become one of the Brazilian regions with the greatest honey production.

Authors' Contributions

TMAO: Conceptualization, Writing Original Draft, Writing-Review & Editing, Data Curation, Methodology, Visualization, Investigation, Formal analysis.

VJCM: Writing Original Draft, Methodology, Investigation.

LTDC: Writing-Review & Editing, Methodology, Investigation.

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OPAJ: Writing Original Draft, Writing-Review & Editing, Methodology.

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