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Article

# Classification of Honeys from Pará State (Amazon Region, Brazil) Produced by Three Different Species of Bees using Chemometric Methods

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Vinte e sete amostras de mel, produzidas em dez cidades do Estado do Pará (Região Amazônica, norte do Brasil) por três espécies diferentes de abelhas (*Apis mellifera*, *Melipona fasciculata* e *Melipona flavoneata*), foram analisadas em seus teores de elementos minerais (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sr e Zn) e alguns parâmetros fisicoquímicos (cor, umidade, densidade, pH, sólidos insolúveis e solúveis totais, cinzas, condutividade elétrica, índice de formol, acidez livre, hidroximetilfurfural, açúcares redutores e totais e sacarose). Os teores minerais foram determinados via espectrometria de emissão atômica por plasma acoplado indutivamente (ICP OES) e as análises dos parâmetros físico-químicos seguiram metodologias oficiais. Os resultados das análises físico-químicas apresentaram-se de acordo com a legislação nacional e internacional, bem como com outros trabalhos similares ao redor do mundo. A análise estatística multivariada (análise por agrupamento hierárquico (HCA) e por componentes principais (PCA)) foi aplicada aos resultados dos teores metálicos e aos parâmetros físico-químicos, sendo possível a separação das amostras de mel conforme a espécie produtora.

Twenty seven samples of honey produced in ten cities of Pará State (Amazon region, North of Brazil) by three different species of bee (*Apis mellifera*, *Melipona fasciculata* and *Melipona flavoneata*) were characterized based on mineral composition (Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Sr and Zn), and some physicochemical parameters, namely color, moisture, density, pH, total soluble solids, insoluble solids, ash, electrical conductivity, formol index, free acidity, hydroxymethylfurfural, reducing sugars, total sugars and sucrose. The mineral content was determined using inductively coupled plasma-optical emission spectrometry (ICP OES), and the physicochemical parameters were determined according to officially approved methods. The results of the physicochemical analysis were in agreement with national and international regulations, and with the results of similar studies from around the world. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied in the study of the results of the mineral contents and physicochemical parameters, and it was possible to distinguish the honey samples produced by each of the three different species of bee.

**Keywords**: honey, Amazon region, physicochemical parameters, mineral content, HCA, PCA

## Introduction

Honey is a natural product produced by bees from nectar collected from nectaries and stored as food in the hive. It is defined as a pure product that does not include any other substances, such as added water or sweeteners, and is a definition that has been widely accepted in the food standards regulations of most nations, including Brazil.[1-3]

Honey has a limited availability as a commodity and is relatively costly, encouraging the practice of its adulteration. This process is usually performed with the addition of other carbohydrates, particularly commercially available sugars (disaccharides), commercial glucose solution or sucrose syrup and inverted sucrose solution. The most widely used form of adulteration is the addition of sugar cane juice, with the mixture then being heated to thicken it. The appearance of the mixture is improved by adding iodine (for color) and chemical additives (for viscosity).[4]

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The physicochemical properties of honey have been increasingly studied across the world in recent years because these parameters are important in the certification process that determines the quality and origins (geographical, floral or entomological) of the honey.[4] Globally, there are more studies of the geographical and floral origins of honey than there are about its entomological origins.[5]

According to Pohl,[6] the assessing of the metal content in honey is a widely used method for determining the floral and geographical origins of honey, but also for investigating honey quality and as an indicator of environmental pollution.

Fernández-Torres *et al.*[7] noted that different procedures for determining the mineral content of honey have been proposed in earlier works, some for classification purposes, but always according to geographical origin. These authors studied the mineral concentrations in Spanish honey with the objective of establishing their botanical origin, and concluded that this was a factor on which zinc, manganese, magnesium and sodium concentrations were strongly dependent.

Santos *et al.*,[8] working with samples of honey produced in Bahia State (Northeastern Brazil), concluded that the mineral content values obtained from honey of different geographical and botanical origins are strongly dependent on the origin of the honey, and that sodium, potassium, copper and calcium were the most sensitive to these origins.

Multivariate methods of analysis, such as hierarchical cluster analysis (HCA) and principal component analysis (PCA), have always been used in the statistical treatment of the data in order to build models, enabling honey samples to be classified according to the physicochemical and metal concentrations found in different groups of geographical or botanical origins.[5-11]

Pará State is located in the North of Brazil (Amazon region) and is the second largest state in the country. It is one of the most promising regions in Brazil for the production of distinct types of honey, including the honey of the native stingless bees. These native bees are strongly adapted to this region because of the different soils, geographical location, good climatic conditions and the large diversity of plants and, in particular, flowering plants.

There are more than three hundred of species of native bees in the Amazon region.[5] However, in the Northeast of Pará State, where the samples for this study were collected, only three bee species (*Apis mellifera*, *Melipona flavoneata* and *Melipona fasciculate*) are used for the purpose of marketing their honey. These are the honeys that are the most favored by regional consumers.

Despite the large diversity of bee species in the studied region, there is still very little research into the quality of honey they produce, or into discriminating their botanical and geographical origins, especially with regard to the species used for commercial honey production.

The aim of this research was to investigate the physicochemical parameters, using official analytical methods of analysis, and mineral contents obtained using inductively coupled plasma-optical emission spectrometry (ICP OES), that might enable the differentiation, by applying HCA and PCA, of honeys produced by three different species of bees (*A. mellifera*, *M. fasciculata* and *M. flavoneata*) in Pará State.

## Experimental

### Honey samples

Twenty seven honey samples, representing honey from three different species of bees (*A. mellifera*, *M. fasciculata* and *M. flavoneata*), were used in this work. Fifteen honeys were produced by *A. mellifera* and six from each of the two other bee species. The number of honey samples obtained from *A. mellifera* was so large because this species is established in all cities where the samples were collected, while only a few cities hosted populations of the other two bee species studied.

The samples came from ten cities in the Northwest of Pará State. They were collected directly from beekeepers (*A. mellifera*) or directly from hives (other honeys) using a 10 mL syringe, previously cleaned and sterilized, in the period from September 2008 to January 2009. Honey samples were stored in glass or plastic bottles and stored at 4 ºC until analysis.

### Reagents and solutions

All reagents were of analytical grade. Nitric acid was of analytical grade (Merck). Laboratory glassware was kept overnight in 10% nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in a dust-free environment. All metal solutions were prepared by diluting 1000 μg $mL^{-1}$ standard solutions (Merck) with 1% hydrochloric acid solution.

### Chemical analysis

Fourteen physicochemical parameters were studied in this work and determined according to AOAC methods.[1-3,12]

Color was determined using a spectrophotometer (Cecil Instruments, model CECIL 1010) and cuvettes with optical glass, a volume of 3.5 mL and a 1 cm optical path, using pure glycerin as a reference, and recording the absorbance

value of the samples for a wavelength of 560 nm. The Pfund scale was then used to classify the honey and its color.

Ash content was measured by calcination, overnight at 550 ºC in a furnace, until constant mass was achieved. Free acidity (FA) was determined by the titrimetric method, i.e., by adding 0.05 mol $L^{-1}$ NaOH and stopping at pH 8.5.

Density, moisture and total soluble solids (TSS) were measured with an Abbe refractometer specifically for honey (Instrutherm Instruments, São Paulo, Brazil). The sample compartment and window of the refractometer were first cleaned with acetone. The measurements were taken at room temperature (30 ºC).

The pH was measured using a pH meter (Quimis-SC09, Belém-Brazil), for a 10% (m/v) solution of honey prepared in distilled water.

The electrical conductivity (EC) measurement was done at 30 °C using conductivity meter Model CD-880 (Instrutherm Instrument) placed in a solution of 20 g honey in 100 mL distilled water.

Hydroxymethylfurfural (HMF) was determined after clarifying samples with Carrez reagents (I and II) and the addition of sodium bisulfite. Five g of honey were dissolved in 25 mL of water, transferred quantitatively into a 50 mL volumetric flask; 0.5 mL of Carrez I solution and 0.5 mL of solution II were then added and the solution was made up to 50 mL with the addition of water. The solution was filtered through paper, discarding the first 10 mL of the filtrate. Aliquots of 5 mL were placed in two test tubes; 5 mL of distilled water (sample solution) were added to one tube, and 5 mL of 0.2% sodium bisulfite solution (reference solution) to the second one. The absorbance was determined at 284 and 336 nm in a spectrometer (Genesys 10uv, Belém-Brazil).

In order to determine the insoluble solids (IS) in the honey samples, 20 g of honey were weighed and dissolved with 10 mL of water at 80 ºC, filtered through quantitative filter paper and then washed with distilled water until no sugars were left. The filter paper was dried in an oven at 105 ºC for 1 h or until constant weight was achieved.

A 20% (m/v) honey solution was prepared for analyzing the reducing sugar for each sample; it was withdrawn at a rate of 5.0 mL and transferred to a 100.0 mL volumetric flask. This solution was titrated with another solution containing 5.0 mL of Fehling solution A and 5.0 mL of Fehling solution B, with 20.0 mL water and one drop of a 1% solution of methylene blue as indicator.

Total sugar was determined using a 20% (m/v) honey solution. An aliquot of 5.0 mL was then pipetted into a 125.0 mL Erlenmeyer flask, to which 40.0 mL of distilled water and 1.0 mL of concentrated HCl were added. This solution was placed in a water bath at 45 ºC for 25 min, and then cooled. The solution was neutralized with 30% (m/v) sodium carbonate solution. The final volume was completed to 100 mL with distilled water in a volumetric flask. This solution was titrated with a solution containing 5.0 mL of Fehling solution A, plus 5 mL of Fehling solution B, 20 mL of water and one drop of a 1% solution of methylene blue as indicator.

The concentration of sucrose (%) in the honey samples was determined by calculating the stoichiometric difference between total sugars (TS) and reducing sugars (RS), using the equation:

$$\text{Sucrose (\%)} = 0.95\ (TS - RS) \quad (1)$$

In order to determine the formol index, 10 g of honey were weighed in a 250 mL Erlenmeyer flask, and 75 mL of deionized water were added. The solution obtained was homogenized and the pH was controlled with a pH electrode. Afterwards, a solution of 0.01 mol $L^{-1}$ NaOH was added slowly until a pH of 8.0 was reached, and 10 mL of 35% formol (previously neutralized to pH 8.0) were also added. The solution was stirred, and was cleaned and titrated with 0.1 mol $L^{-1}$ NaOH solution until the pH returned to 8.0.

### Instrumentation

Nineteen mineral elements were investigated with ICP OES equipment (Varian Vista Pro, axial view). The spectrometer was operated in the transient signal acquisition mode. A cyclonic spray chamber and concentric nebulizer were used. The metal determinations were carried out using the manufacturer recommended conditions for power (1.2 kW), plasma gas flow (15 L $min^{-1}$), auxiliary gas flow (1.5 L $min^{-1}$), nebulizer gas flow (0.7 L $min^{-1}$) and also nebulizer pressure (200 kPa). The emission intensity scan duration was 60 s. The following analytical wavelengths (nm) were selected: Al (308.215), As (188.980), Ba 455.403), Be (313.042), Bi (223.061), Ca (317.933), Cd (228.802), Co (238.892), Cr (276.653), Cu (327.395), Fe (238.204), K (766.471), Li (670.783), Mg (279.553), Mn (257.610), Na (588.995), Ni (221.648), Sr (407.771), Zn (213.857).

One g of honey was weighed in a digestion tube and 2.0 mL of concentrated $HNO_3$ were added; this mixture was put in a block digester overnight for approximately 12 h. Then, 1.0 mL of 30% (v/v) $H_2O_2$ was added, and the mixture was heated at 150 ºC for 2 h, and at 200 ºC for 30 min. The resulting solution was then diluted with deionized water to 20.0 mL in a volumetric flask before being analyzed. Blank solutions were prepared under identical conditions and the average signal was subtracted from the analytical signals of the honey samples.

Limits of detection (defined as (blank + 3 SD), where SD is the standard deviation of the blank determination) were determined for all the mineral elements analyzed: Al and Na (0.047 mg $kg^{-1}$), As, Bi and Ni (0.013 mg $kg^{-1}$), Ba (0.041 mg $kg^{-1}$), Be (0.011 mg $kg^{-1}$), Ca (0.120 mg $kg^{-1}$), Cd (0.060 mg $kg^{-1}$), Co and Zn (0.030 mg $kg^{-1}$), Cr (0.019 mg $kg^{-1}$), Cu (0.018 mg $kg^{-1}$), Fe and K (0.090 mg $kg^{-1}$), Li (0.568 mg $kg^{-1}$), Mg (0.292 mg $kg^{-1}$), Mn (0.014 mg $kg^{-1}$) and Sr (0.015 mg $kg^{-1}$).

The method of analyte addition and recovery was applied using standard solutions previously prepared for each of the studied elements, and the obtained recovery values ranged between 84.16% (Zn) and 108.83% (Bi).

### Statistical analysis

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were used for the data analyses, using the software Meet Minitab Release 14 for Windows.

## Results and Discussion

### Physicochemical parameters

The results of the physicochemical analysis are given in Table 1, showing the average and the standard deviation for all the physicochemical parameters studied. Table 2 presents the results for the physicochemical parameter analysis for each kind of honey studied.

The predominant color of the honey samples was light amber (64%), and the second most predominant color was amber (21%). A total of 6% of the samples was dark amber and a further 6% were extra light amber, with only 3% being classified as white. The *A. mellifera* honeys analyzed tended to be darker than the *Melipona* honeys, especially honeys derived from *M. fasciculata*. A similar result was found by Alves *et al.*[13] This parameter (color) is used by consumers in selecting honey, with lighter-colored honeys being preferred.[5] Honey color is determined by its botanical source and by the local climate and soil conditions. Storage, light, heat and potential enzymatic reactions may also affect color.[11,14]

Seventeen samples were above the legal limit for moisture content in honey.[2,15,16-17] However, legislation from around the world has been based only on the *A. mellifera* honey, with no consideration given to honey derived from other bee species. Five *A. mellifera* honey samples were above 20% moisture content, with other research[14,18-21] yielding similar results. The results for *M. flavoneata* and *M. fasciculata* agreed with other studies.[5,13,22-24] This moisture variation could be explained by the composition and floral origin of the honey samples. The hyperosmotic nature of honey prevents the growth of bacteria and yeasts by drawing water out of the organism, killing them by dessication.[24] Considering only this parameter, the honeys of native bees are more subject to degradation via microorganism than the *A. mellifera* honeys, and also show lower quality.

There are no density limits in either Brazilian or international legislation, and there are few works in the literature that have studied this parameter. The *A. mellifera* honey densities agreed with other studies.[4,25] Densities of honeys from *M. fasciculata* and *M. flavoneata* were somewhat lower than those for *A. mellifera* honey, which is probably connected with the higher water content for the *M. fasciculata* and *M. flavoneata* honeys.

There are no limits for TSS in Brazilian or international legislation,[1-3] and other authors have found similar results.[25-28]

The average value found for IS was above the maximum limit established by national and international law,[1-3] but these laws are based only on the *A. mellifera* honeys. Considering fifteen *A. mellifera* honey samples separately, only four samples were above the legal limit, and the average value conformed to the regulations. Some other works have obtained results that are lower than the results determined in the present study,[18,26] but Ordóñez *et al.*,[29] working with *A. mellifera* in Yucatan, México, obtained averages of 0.16, 0.32 and 0.21% for three different kinds of honey, being higher than the results of the present study. Considering IS it was found that the mean values for honeys produced by the two native bees studied were above legal limits,[2] but these legal limits were established on the basis only of the *A. mellifera* honey.

Only one sample studied did not conform to the legislative requirements pertaining to the ash content of honey, but this was not a sample of the *A. mellifera* honey, on which national and international laws for honey are based. Many researches in Brazil and other countries have focused on this parameter and obtained values similar to the present results.[5,8,14,18,23,25-27,30-36] According to Vit *et al.*,[5] ash represents a direct measure of the inorganic residues left after honey carbonization, and this variability in the ash content can be explained by the floral origin of the honey.

The mean determined for electrical conductivity (EC) was below the maximum limit established in international law,[3] but for many samples (41.18%), it was below the minimum limit set by international legislation. Brazilian laws have established no limits for EC in honey. All results of EC from the present study agreed with values presented in the literature.[10,33-37] Electrical conductivity depends on the mineral content of the honey. As the differences in EC

**Table 1.** Physicochemical parameters of honey samples analyzed[a]

| Sample | Species | Color | Moi. / % | Density / (g mL$^{-1}$) | TSS / % | IS / % | Ash / % | EC / (mS cm$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 1 | *M. fasciculata* (1) | amber light | 25.00 ± 0.00 | 1.368 ± 0.000 | 73.00 ± 0.00 | 0.02 ± 0.00 | 0.39 ± 0.08 | 0.17 ± 0.00 |
| 2 | *M. fasciculata* (1) | amber light | 24.00 ± 0.00 | 1.374 ± 0.000 | 74.00 ± 0.00 | 0.16 ± 0.02 | 0.08 ± 0.00 | 0.27 ± 0.05 |
| 3 | *M. fasciculata* (1) | amber extra light | 24.17 ± 0.29 | 1.374 ± 0.000 | 74.00 ± 0.00 | 0.07 ± 0.01 | 0.28 ± 0.09 | 0.19 ± 0.01 |
| 4 | *M. fasciculata* (1) | white | 25.00 ± 0.00 | 1.368 ± 0.000 | 73.00 ± 0.00 | 0.15 ± 0.01 | 0.11 ± 0.01 | 0.30 ± 0.01 |
| 5 | *M. flavoneata* (2) | amber | 26.00 ± 0.00 | 1.362 ± 0.000 | 72.00 ± 0.00 | 0.40 ± 0.13 | 0.20 ± 0.03 | 0.19 ± 0.01 |
| 6 | *A. mellifera* (3) | amber | 19.50 ± 0.29 | 1.406 ± 0.004 | 79.00 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.01 | 0.21 ± 0.00 |
| 7 | *M. fasciculata* (1) | amber light | 23.23 ± 0.25 | 1.383 ± 0.004 | 76.00 ± 0.00 | 0.31 ± 0.02 | 0.34 ± 0.01 | 0.18 ± 0.01 |
| 8 | *M. flavoneata* (2) | amber light | 26.17 ± 0.29 | 1.362 ± 0.000 | 72.00 ± 0.00 | 0.17 ± 0.05 | 0.34 ± 0.01 | 0.21 ± 0.01 |
| 9 | *A. mellifera* (3) | amber | 20.33 ± 0.29 | 1.401 ± 0.000 | 78.33 ± 0.58 | 0.15 ± 0.00 | 0.16 ± 0.06 | 0.17 ± 0.00 |
| 10 | *A. mellifera* (3) | amber light | 21.00 ± 0.00 | 1.394 ± 0.000 | 77.17 ± 0.29 | 0.05 ± 0.00 | 0.18 ± 0.02 | 0.20 ± 0.00 |
| 11 | *A. mellifera* (3) | amber light | 21.50 ± 0.50 | 1.394 ± 0.000 | 77.00 ± 0.00 | 0.20 ± 0.02 | 0.03 ± 0.00 | 0.15 ± 0.01 |
| 12 | *A. mellifera* (3) | amber light | 19.80 ± 0.93 | 1.406 ± 0.004 | 79.17 ± 0.29 | 0.22 ± 0.01 | 0.57 ± 0.09 | 0.18 ± 0.00 |
| 13 | *A. mellifera* (3) | amber light | 19.60 ± 0.17 | 1.404 ± 0.000 | 79.00 ± 0.00 | 0.05 ± 0.01 | 0.50 ± 0.01 | 0.20 ± 0.00 |
| 14 | *A. mellifera* (3) | amber light | 19.00 ± 0.00 | 1.408 ± 0.000 | 79.00 ± 0.00 | 0.04 ± 0.00 | 0.28 ± 0.08 | 0.15 ± 0.01 |
| 15 | *A. mellifera* (3) | amber | 19.00 ± 0.00 | 1.410 ± 0.004 | 79.17 ± 0.29 | 0.09 ± 0.01 | 0.20 ± 0.03 | 0.18 ± 0.01 |
| 16 | *A. mellifera* (3) | amber light | 19.13 ± 0.11 | 1.408 ± 0.000 | 79.00 ± 0.00 | 0.16 ± 0.03 | 0.02 ± 0.00 | 0.16 ± 0.01 |
| 17 | *A. mellifera* (3) | amber | 19.00 ± 0.00 | 1.408 ± 0.000 | 79.00 ± 0.00 | 0.03 ± 0.00 | 0.05 ± 0.01 | 0.22 ± 0.00 |
| 18 | *M. flavoneata* (2) | amber light | 25.00 ± 0.00 | 1.368 ± 0.000 | 73.00 ± 0.00 | 0.08 ± 0.01 | 0.26 ± 0.03 | 0.24 ± 0.00 |
| 19 | *M. flavoneata* (2) | amber extra light | 19. 00 ± 0.00 | 1.408 ± 0.000 | 79.50 ± 0.00 | 0.45 ± 0.04 | 0.14 ± 0.03 | 0.22 ± 0.00 |
| 20 | *A. mellifera* (3) | amber light | 19.00 ± 0.00 | 1.412 ± 0.004 | 79.50 ± 0.00 | 0.08 ± 0.01 | 0.17 ± 0.03 | 0.14 ± 0.01 |
| 21 | *M. flavoneata* (2) | amber | 26.00 ± 0.00 | 1.362 ± 0.000 | 72.00 ± 0.00 | 0.06 ± 0.00 | 0.09 ± 0.00 | 0.30 ± 0.02 |
| 22 | *A. mellifera* (3) | amber light | 20.67 ± 0.29 | 1.401 ± 0.007 | 79.17 ± 0.29 | 0.23 ± 0.01 | 0.11 ± 0.01 | 0.20 ± 0.01 |
| 23 | *M. fasciculata* (1) | amber light | 25.33 ± 0.29 | 1.362 ± 0.000 | 72.67 ± 0.29 | 0.22 ± 0.01 | 0.38 ± 0.09 | 0.22 ± 0.01 |
| 24 | *M. flavoneata* (2) | amber light | 28,00 ± 0.00 | 1.349 ± 0.000 | 70.50 ± 0.00 | 0.03 ± 0.00 | 0.98 ± 0.18 | 0.33 ± 0.04 |
| 25 | *A. mellifera* (3) | dark amber | 18.50 ± 0.00 | 1.415 ± 0.000 | 80.00 ± 0.00 | 0.02 ± 0.00 | 0.31 ± 0.06 | 0.25 ± 0.01 |
| 26 | *A. mellifera* (3) | amber | 20.00 ± 0.00 | 1.401 ± 0.000 | 78.00 ± 0.00 | 0.05 ± 0.01 | 0.06 ± 0.01 | 0.16 ± 0.01 |
| 27 | *A. mellifera* (3) | amber light | 20.33 ± 0.29 | 1.401 ± 0.000 | 78.00 ± 0.00 | 0.04 ± 0.01 | 0.29 ± 0.06 | 0.18 ± 0.02 |

| Sample | Species | pH | FI / (mL kg$^{-1}$) | FA / (meq kg$^{-1}$) | HMF / (mg kg$^{-1}$)[b] | RS / % | TS / % | Suc. / % |
|---|---|---|---|---|---|---|---|---|
| 1 | *M. fasciculata* (1) | 3.39 ± 0.05 | 5.33 ± 0.58 | 15.25 ± 3.87 | 6.10 ± 2.34 | 61.01 ± 0.01 | 63.70 ± 0.00 | 2.55 ± 0.01 |
| 2 | *M. fasciculata* (1) | 3.17 ± 0.05 | 4.33 ± 0.58 | 21.45 ± 1.52 | 1.84 ± 0.70 | 65.24 ± 0.01 | 65.83 ± 0.03 | 2.24 ± 0.07 |
| 3 | *M. fasciculata* (1) | 3.12 ± 0.03 | 5.00 ± 1.00 | 15.18 ± 0.94 | 9.21 ± 3.82 | 67.05 ± 0.05 | 70.52 ± 0.01 | 4.51 ± 0.02 |
| 4 | *M. fasciculata* (1) | 4.06 ± 0.04 | 7.00 ± 1.00 | 12.37 ± 1.25 | 2.75 ± 0.52 | 65.34 ± 0.00 | 67.52 ± 0.02 | 2.08 ± 0.02 |
| 5 | *M. flavoneata* (2) | 3.99 ± 0.02 | 5.67 ± 0.58 | 16.85 ± 1.35 | 0.27 ± 0.01 | 60.51 ± 0.00 | 67.40 ± 0.01 | 5.91 ± 0.01 |
| 6 | *A. mellifera* (3) | 3.20 ± 0.05 | 10.00 ± 1.00 | 39.18 ± 1.12 | 95.66 ± 2.96 | 70.37 ± 0.01 | 75.47 ± 0.01 | 4.84 ± 0.02 |
| 7 | *M. fasciculata* (1) | 4.95 ± 0.08 | 3.67 ± 1.15 | 11.69 ± 0.70 | 0.86 ± 0.08 | 61.72 ± 0.06 | 63.60 ± 0.00 | 3.30 ± 0.04 |
| 8 | *M. flavoneata* (2) | 3.79 ± 0.01 | 4.00 ± 1.00 | 33.26 ± 1.11 | 4.93 ± 0.59 | 56.14 ± 0.02 | 65.10 ± 0.02 | 8.51 ± 0.01 |
| 9 | *A. mellifera* (3) | 3.11 ± 0.01 | 7.67 ± 2.08 | 27.95 ± 0.50 | 31.17 ± 0.86 | 68.59 ± 0.01 | 76.57 ± 0.01 | 7.58 ± 0.01 |
| 10 | *A. mellifera* (3) | 3.18 ± 0.02 | 9.33 ± 0.58 | 43.44 ± 2.77 | 40.71 ± 2.61 | 71.62 ± 0.01 | 76.87 ± 0.02 | 4.99 ± 0.01 |
| 11 | *A. mellifera* (3) | 3.18 ± 0.01 | 6.33 ± 0.58 | 27.30 ± 4.74 | 6.02 ± 1.19 | 64.49 ± 0.00 | 72.70 ± 0.00 | 7.80 ± 0.03 |
| 12 | *A. mellifera* (3) | 3.37 ± 0.01 | 6.67 ± 1.15 | 21.72 ± 0.75 | 87.64 ± 7.69 | 68.60 ± 0.02 | 73.32 ± 0.01 | 4.48 ± 0.01 |
| 13 | *A. mellifera* (3) | 3.34 ± 0.04 | 7.67 ± 0.58 | 39.36 ± 1.78 | 104.79 ± 7.42 | 71.31 ± 0.02 | 76.03 ± 0.02 | 4.49 ± 0.03 |
| 14 | *A. mellifera* (3) | 3.46 ± 0.12 | 15.33 ± 1.53 | 31.46 ± 1.22 | 3.76 ± 0.07 | 70.23 ± 0.01 | 70.75 ± 0.01 | 0.49 ± 0.05 |
| 15 | *A. mellifera* (3) | 3.14 ± 0.04 | 9.33 ± 0.58 | 35.45 ± 0.50 | 88.34 ± 9.86 | 68.17 ± 0.02 | 70.49 ± 0.03 | 2.21 ± 0.02 |
| 16 | *A. mellifera* (3) | 3.72 ± 0.02 | 5.33 ± 0.58 | 46.02 ± 2.24 | 32.29 ± 3.25 | 71.11 ± 0.01 | 71.27 ± 0.01 | 0.29 ± 0.03 |
| 17 | *A. mellifera* (3) | 3.26 ± 0.03 | 7.67 ± 0.58 | 47.74 ± 3.21 | 87.89 ± 1.12 | 66.50 ± 0.01 | 68.08 ± 0.00 | 1.50 ± 0.01 |
| 18 | *M. flavoneata* (2) | 3.77 ± 0.02 | 5.00 ± 1.00 | 46.61 ± 0.75 | 2.18 ± 0.28 | 61.50 ± 0.00 | 61.98 ± 0.00 | 0.44 ± 0.01 |
| 19 | *M. flavoneata* (2) | 3.31 ± 0.01 | 4.00 ± 1.00 | 33.19 ± 0.25 | 0.82 ± 0.82 | 71.36 ± 0.01 | 72.27 ± 0.01 | 0.87 ± 0.01 |
| 20 | *A. mellifera* (3) | 2.96 ± 0.01 | 9.67 ± 0.58 | 30.92 ± 2.08 | 6.11 ± 1.54 | 72.84 ± 0.00 | 73.58 ± 0.01 | 0.71 ± 0.01 |
| 21 | *M. flavoneata* (2) | 4.29 ± 0.00 | 4.00 ± 1.00 | 25.99 ± 0.90 | 7.10 ± 2.22 | 63.74 ± 0.01 | 66.51 ± 0.00 | 2.63 ± 0.01 |
| 22 | *A. mellifera* (3) | 3.04 ± 0.02 | 17.33 ± 2.52 | 46.51 ± 3.05 | 1.99 ± 0.96 | 76.16 ± 0.01 | 76.82 ± 0.02 | 1.01 ± 0.04 |
| 23 | *M. fasciculata* (1) | 4.44 ± 0.01 | 5.33 ± 0.58 | 8.24 ± 0.27 | 0.92 ± 1.09 | 69.64 ± 0.01 | 71.90 ± 0.06 | 3.46 ± 0.04 |
| 24 | *M. flavoneata* (2) | 3.41 ±0.01 | 5.67 ± 1.15 | 76.72 ± 1.16 | 29.33 ± 3.66 | 53.27 ± 0.00 | 58.39 ± 0.02 | 4.86 ± 0.02 |
| 25 | *A. mellifera* (3) | 2.96 ± 0.02 | 3.67 ± 0.58 | 68.99 ± 1.73 | 2.84 ± 1.143 | 62.65 ± 0.00 | 66.94 ± 0.01 | 4.07 ± 0.01 |
| 26 | *A. mellifera* (3) | 3.01 ± 0.01 | 5.67 ± 0.58 | 40.87 ± 3.84 | 174.40 ± 28.77 | 62.05 ± 0.02 | 65.03 ± 0.01 | 2.83 ± 0.01 |
| 27 | *A. mellifera* (3) | 3.15 ± 0.01 | 11.00 ± 2.65 | 32.37 ± 2.31 | 26.93 ± 0.80 | 69.30 ± 0.02 | 73.13 ± 0.01 | 3.64 ± 0.01 |

[a]Average of three determinations ± standard deviation (n = 3). [b]Average of two determinations ± standard deviation (n = 2). Moi.: moisture, TSS: total soluble solids; IS: insoluble solids; EC: electrical conductivity; FI: formol index; FA: free acidity; HMF: hydroxymethylfurfural; RS: reducing sugar; TS: total sugar; Suc.: sucrose contents.

**Table 2.** Results of physiochemical parameters according to the species of honeybees studied

| Bee species | Parameter | Moi. / % | Density / (g $mL^{-1}$) | TSS / (degree Brix) | IS / % | Ash / % | EC / (mS $cm^{-1}$) | pH | FI / (mL $kg^{-1}$) | FA / (meq $kg^{-1}$) | HMF / (mg $kg^{-1}$) | RS / % | TS / % | Suc. / % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. mellifera* | Mean | 19.80 | 1.405 | 78.70 | 0.10 | 0.19 | 0.18 | 3.21 | 8.63 | 38.62 | 46.53 | 68.91 | 72.47 | 3.40 |
| | SD | 0.88 | 0.006 | 0.83 | 0.09 | 0.17 | 0.03 | 0.20 | 3.71 | 11.38 | 48.99 | 3.91 | 3.82 | 2.53 |
| | CV | 4.44 | 0.43 | 1.03 | 90.00 | 89.47 | 16.67 | 6.23 | 42.99 | 29.47 | 105.29 | 5.67 | 5.27 | 74.41 |
| | Min. | 18,50 | 1.394 | 77.00 | 0.02 | 0.02 | 0.18 | 2.94 | 3.00 | 20.98 | 1.32 | 60.48 | 63.93 | 0.00 |
| | Max. | 22.00 | 1.415 | 80.00 | 0.25 | 0.63 | 0.25 | 3.74 | 20.00 | 70.33 | 194.75 | 77.42 | 78.73 | 8.09 |
| | Limit | 20.00[b] | NL | NL | 0.10[b] | 0.60[b] | 0.80[a] | 3.3-4.6 | NL | 40.00[b] | 60[b]/80[a] | 80.00[+] | NL | 6.00[+] |
| *M. fasciculata* | Mean | 24.46 | 1.372 | 73.78 | 0.16 | 0.26 | 0.22 | 3.86 | 5.11 | 13.50 | 3.81 | 65.17 | 66.60 | 3.98 |
| | SD | 0.76 | 0.017 | 1.15 | 0.13 | 0.14 | 0.05 | 0.70 | 1.28 | 3.98 | 3.49 | 4.45 | 2.55 | 2.89 |
| | CV | 3.11 | 1.24 | 1.56 | 81,25 | 53.85 | 22.73 | 18.13 | 25.05 | 29.48 | 91.60 | 6.83 | 3.83 | 72.61 |
| | Min. | 19.00 | 1.362 | 72.50 | 0.01 | 0.08 | 0.17 | 3.10 | 3.00 | 7.98 | 0.15 | 53.34 | 62.45 | 0.31 |
| | Max. | 25.50 | 1.388 | 76.00 | 0.49 | 0.44 | 0.32 | 5.04 | 8.00 | 21.32 | 11.91 | 75.79 | 72.37 | 9.64 |
| *M. flavoneata* | Mean | 23.78 | 1.369 | 73.17 | 0.18 | 0.33 | 0.25 | 3.76 | 4.67 | 38.77 | 7.44 | 61.09 | 65.27 | 3.87 |
| | SD | 2.99 | 0.019 | 3.01 | 0.16 | 0.32 | 0.05 | 0.34 | 1.14 | 15.76 | 10.60 | 6.00 | 4.62 | 3.09 |
| | CV | 12.53 | 1.39 | 4.11 | 88.89 | 96.97 | 20.00 | 9.04 | 24.41 | 40.65 | 142.47 | 9.82 | 7.08 | 79.84 |
| | Min. | 19.00 | 1.349 | 70.50 | 0.01 | 0.08 | 0.18 | 3.30 | 3.00 | 15.63 | 0.24 | 53.20 | 56.66 | 0.05 |
| | Max. | 28.00 | 1.408 | 79.50 | 0.49 | 1.11 | 0.36 | 4.29 | 7.00 | 78.04 | 31.92 | 72.34 | 73.02 | 9.59 |
| All species | Mean | 21.56 | 1.390 | 76.74 | 0.11 | 0.24 | 0.21 | 3.51 | 7.10 | 34.95 | 30.07 | 66.37 | 69.65 | 3.37 |
| | SD | 2.77 | 0.019 | 2.95 | 0.09 | 0.03 | 0.05 | 0.51 | 3.48 | 17.37 | 43.08 | 5.30 | 5.05 | 2.59 |
| | CV | 12.85 | 1.37 | 3.84 | 81.82 | 12.50 | 23.81 | 14.53 | 49.01 | 49.69 | 143.26 | 7.98 | 7.25 | 76.85 |
| | Min. | 18.50 | 1.349 | 72.50 | 0.02 | 0.01 | 0.14 | 2.94 | 3.00 | 7.98 | 0.15 | 53.20 | 56.66 | 0.00 |
| | Max. | 28.00 | 1.415 | 80.00 | 0,49 | 1.11 | 0.36 | 5.49 | 20.00 | 78.04 | 194.75 | 77.42 | 79.19 | 9.64 |

[a]International legislation; [b]national legislation. SD: standard deviation; CV: coefficient of variation; Min.: minimum value; Max.: maximum value; NL: there is not a limit in national and international legislation; Moi.: moisture; TSS: total soluble solids; IS: insoluble solids; EC: electrical conductivity; FI: formol index; FA: free acidity; HMF: hydroxymethylfurfural; RS: reducing sugar; TS: total sugar; Suc.: sucrose contents.

of the various honeys are attributable to their differing geographical and botanical origins, this can serve to characterize different varieties of honey.[34]

Brazilian legislation[2] suggests a suitable range of pH for honey between 3.3 and 4.6. Fourteen of the samples analyzed (51.85%) were below this range, and only one sample (3.70%) was above it. The pH of the *M. fasciculata* and *M. flavoneata* honeys was higher than that of *A. mellifera*, but these results conform to the pH obtained in other studies.[22,37,38] Honey has a low enough pH to slow down or prevent the growth of many species of bacteria, but this acidity may be neutralized in the body by buffering liquid fluids.[34]

No limit exists for formol index in Brazilian and international law,[1-3] and the results of this parameter determined in the present study agree with the values in the literature for the three species studied.[13,14,20,23,36,39]

Values of FA found in eight *A. mellifera* honeys were above the legal limit, but much of the honey research in Brazil has obtained ranges that do not agree with Brazilian legislation, in agreement with the results found in the present work for the *A. mellifera* honeys.[11,18,20,23,26,32,35,38] FA for the *M. fasciculata* and *M. flavoneata* honeys found in this work agree with some of the results found in the literature.[5,13,22,36,38] All the results were above the maximum limit established by Brazilian and international law.[2-3,13]

The HMF results determined for some of the *A. mellifera* honey samples were not in accordance with either national law[2] or international laws.[3,13] However, many works have yielded similar results.[10,14,18,25,27,33,35] The HMF results for the *M. fasciculata* and *M. flavoneata* honey samples were in agreement with Brazilian and international laws,[2-3,13] and conform to the results of other studies in Brazil and in other countries.[5,13,36,39] According to White Jr.,[40] the honey produced in subtropical regions can contain higher HMF concentrations without either overheating or adulteration because of the typically high local temperatures.

The results for RS relating to three *A. mellifera* honeys were below the minimum limit established by Brazilian and international legislation.[2,3,13] These results were lower than results established by other studies.[14,19,26,31,35,38] The RS results for the *M. fasciculata* and *M. flavoneata* honeys were in agreement with works conducted in Brazil and other countries.[5,13,22,23]

All the results determined for TS agreed with those of other studies.[10,14,19,22,23,25]

Four samples had somewhat higher values than the maximum limit established in Brazilian and international law for the sucrose content in honey. However, these results were similar to those obtained by other researchers.[35,38] All results

for the *M. flavoneata* and *M. fasciculata* honeys conformed to the findings of studies in Brazil and other countries.[5,14,39]

### Mineral contents

Eight of the elements studied (As, Ba, Be, Bi, Cd, Co, Cr and Li) were not detected in any samples, and the results obtained for the other mineral elements studied are shown in Table 3, which gives the average and SD for all mineral elements studied. Table 4 presents the results obtained for mineral content for every kind of honey studied.

K was the most abundant element in the honeys studied, in agreement with other works.[4,7,14,33,35] It comprised 80.53% of the total mineral contents in the *A. mellifera* honey, 48.76% in the *M. fasciculata* honey and 86.41% in the *M. flavoneata* honey.

Na was the second most abundant element found in the honeys studied; in the *A. mellifera* honeys, this metal comprised 8.46% of the total mineral content, in agreement with other studies.[4,7,33,35] Na comprised 34.68 and 4.49% of the total mineral content in the *M. fasciculata* and *M. flavoneata* honeys, respectively.

Ca was the third most abundant element, comprising 7.84, 12.71 and 6.75% of the total mineral content in the *A. mellifera*, *M. fasciculata* and *M. flavoneata* honeys, respectively; similar results have been found across the world.[4,7,14,33,35]

The results found in this work for the Al, Mg, Mn and Sr contents of the *A. mellifera* honeys were in agreement with other studies.[4,7,14,33,35]

The Fe content determined in this study for the *A. mellifera* honeys corroborates other studies.[4,14,33,35] According to the standard values determined by the Codex Alimentarius Commission,[3] the maximum Fe values that must be found in sweet nutrients, such as sugar and honey, is reported as 15 mg $g^{-1}$; all Fe values obtained in this study did not exceed this limit.

All samples had a Cu level below the maximum limit established by Brazilian law,[2] similar results have been found in many other studies.[4,7,14,33,35,40]

The maximum limit for Zn and Ni in Brazilian law[2] is 5.00 mg $kg^{-1}$, all values obtained were below this limit.

Cr was detected only in three samples. In Brazilian law,[2] the maximum limit for Cr is 0.10 mg $kg^{-1}$, and one sample yielded a value above this limit.

### Multivariate analysis

From a total of fourteen physicochemical parameters, only eleven mineral contents were used in the discrimination between honey samples of the three different bee species because As, Ba, Bi, Be, Cd, Co, Cu and Li were not detected in any of the samples analyzed.

The twenty seven samples were divided into three groups: group 1, representing the *M. fasciculata* honeys, group 2, formed by the samples of the *M. flavoneata* honey, and group 3, representing *A. mellifera* honey.

After autoscaling, the more relevant physicochemical parameters and metal content descriptors were evaluated. For this, a correlation matrix between the calculated variables and the variance, and the weighted Fisher's exact test were used. This procedure determines the relative importance of each variable, and a separation of the three groups according to honeybee species was obtained for only four metal contents (Ca, Mg, Na and Sr) and only three physicochemical parameters (moisture, total soluble solids and free acidity).

HCA was applied to the autoscaled data, Euclidean distance with the complete linkage method was used to calculate the sample similarities, and a hierarchical agglomerative procedure was employed to establish cluster. The obtained results are shown as a dendrogram in Figure 1: in this graphic, vertical lines represent honey samples and horizontal lines represent similarities between samples in terms of the Euclidian distances that originate from the cluster analysis between samples and a group of samples, and between groups of samples.

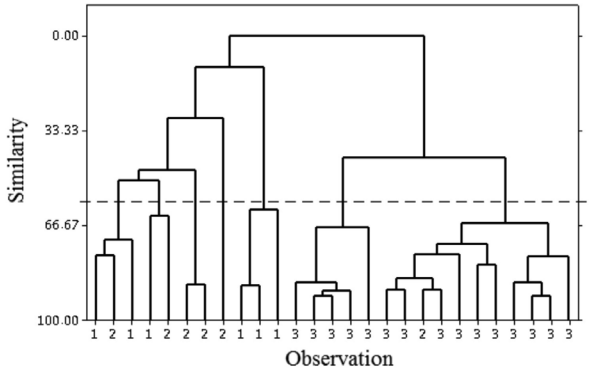


**Figure 1.** Dendrogram of cluster analysis of physicochemical parameters and mineral contents.

Seven clusters were found at a similarity level of 60%. From the left, the first cluster is composed of three samples that are samples of *M. fasciculata* (two samples) and only one sample of the *M. flavoneata* honey; the second cluster is composed of only two samples (one sample of the *M. flavoneata* honey and one of the *M. flavoneata* honey); the third cluster has only two *M. flavoneata* samples; the fourth cluster is composed of only one *M. flavoneata* honey sample; the fifth cluster is composed of three samples of the *M. fasciculata* honey; the sixth cluster is formed only of samples of the *A. mellifera* honey (five samples); and the

**Table 3.** Honey sample mineral contents[a]

| Sample | Species | Al / (mg kg$^{-1}$) | Ca / (mg kg$^{-1}$) | Cu / (mg kg$^{-1}$) | Fe / (mg kg$^{-1}$) | K / (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | *M. fasciculata* (1) | 5.743 ± 0.048 | 51.457 ± 0.226 | 0.107 ± 0.035 | 4.177 ± 0.455 | 127.860 ± 7.114 |
| 2 | *M. fasciculata* (1) | < LOD | 15.150 ± 0.654 | < LOD | 0.439 ± 0.033 | 122.736 ± 14.352 |
| 3 | *M. fasciculata* (1) | < LOD | 12.331 ± 0.186 | < LOD | 0.422 ± 0.038 | 67.429 ± 1.457 |
| 4 | *M. fasciculata* (1) | < LOD | 20.385 ± 0.654 | < LOD | 2.278 ± 0.125 | 131.979 ± 6.198 |
| 5 | *M. flavoneata* (2) | < LOD | 37.902 ± 3.753 | 0.358 ± 0.010 | 0.720 ± 0.071 | 580.074 ± 0.992 |
| 6 | *A. mellifera* (3) | < LOD | 10.280 ± 0.394 | < LOD | 0.100 ± 0.014 | 415.588 ± 51.465 |
| 7 | *M. fasciculata* (1) | < LOD | 92.503 ± 5.249 | 0.257 ± 0.058 | 0.387 ± 0.022 | 883.260 ± 64.353 |
| 8 | *M. flavoneata* (2) | < LOD | 50.474 ± 6.233 | 0.181 ± 0.041 | 0.341 ± 0.023 | 706.419 ± 2.152 |
| 9 | *A. mellifera* (3) | 0.771 ± 0.021 | 43.491 ± 0.389 | 0.302 ± 0.007 | 0.664 ± 0.002 | 490.113 ± 40.034 |
| 10 | *A. mellifera* (3) | 5.809 ± 0.072 | 38.486 ± 0.601 | 0.360 ± 0.069 | 1.905 ± 0.051 | 462.850 ± 17.523 |
| 11 | *A. mellifera* (3) | < LOD | 49.717 ± 1.828 | 0.461 ± 0.056 | 0.472 ± 0.016 | 477.749 ± 1.981 |
| 12 | *A. mellifera* (3) | 3.428 ± 0.054 | 50.716 ± 1.458 | 0.457 ± 0.006 | 2.006 ± 0.045 | 628.017 ± 6.452 |
| 13 | *A. mellifera* (3) | 4.201 ± 0.053 | 41.287 ± 0.478 | 0.317 ± 0.035 | 0.676 ± 0.070 | 459.533 ± 40.144 |
| 14 | *A. mellifera* (3) | 1.025 ± 0.005 | 40.571 ± 4.169 | 0.314 ± 0.022 | 1.585 ± 0.098 | 336.702 ± 7.179 |
| 15 | *A. mellifera* (3) | < LOD | 33.844 ± 0.458 | 0.292 ± 0.001 | 0.570 ± 0.043 | 345.487 ± 2.281 |
| 16 | *A. mellifera* (3) | < LOD | 24.178 ± 2.867 | 0.219 ± 0.009 | 0.558 ± 0.016 | 245.618 ± 4.955 |
| 17 | *A. mellifera* (3) | < LOD | 20.068 ± 0.226 | 0.294 ± 0.019 | 0.794 ± 0.024 | 613.587 ± 3.171 |
| 18 | *M. flavoneata* (2) | 0.558 ± 0.002 | 76.301 ± 3.850 | 0.252 ± 0.023 | 0.392 ± 0.012 | 845.514 ± 29.851 |
| 19 | *M. flavoneata* (2) | 0.885 ± 0.002 | 29.799 ± 0.102 | 0.313 ± 0.059 | 0.739 ± 0.017 | 647.910 ±55.017 |
| 20 | *A. mellifera* (3) | < LOD | 29.471 ± 0.151 | 0.333 ± 0.023 | 0.916 ± 0.010 | 292.663 ± 7.412 |
| 21 | *M.* flavoneata (2) | < LOD | 40.741 ± 0.097 | 0.213 ± 0.016 | 0.202 ± 0.021 | 596.571 ± 2.909 |
| 22 | *A. mellifera* (3) | 0.331 ± 0.009 | 18.436 ± 0.295 | 0.129 ± 0.018 | 0.825 ± 0.006 | 383.412 ± 21.128 |
| 23 | *M. fasciculata* (1) | 0.288 ± 0.001 | 50.495 ± 1.322 | 0.261 ± 0.039 | 0.393 ± 0.019 | 494.104 ± 1.144 |
| 24 | *M. flavoneata* (2) | 2.296 ± 0.023 | 93.364 ± 2.114 | 0.321± 0.048 | 1.799 ± 0.038 | 907.829 ± 23.740 |
| 25 | *A. mellifera* (3) | 1.578 ± 0.010 | 22.969 ± 0.252 | < LOD | 1.092 ± 0.025 | 42.834 ± 0.511 |
| 26 | *A. mellifera* (3) | < LOD | 22.702 ± 0.664 | 0.299 ± 0.087 | 0.758 ± 0.008 | 266.884 ± 15.630 |
| 27 | *A. mellifera* (3) | 1.965 ± 0.017 | 32.706 ± 1.032 | 0.288 ± 0.056 | 1.448 ± 0.001 | 344.618 ± 5.949 |

| Sample | Species | Mg / (mg kg$^{-1}$) | Mn / (mg kg$^{-1}$) | Na / (mg kg$^{-1}$) | Ni / (mg kg$^{-1}$) | Sr / (mg kg$^{-1}$) | Zn / (mg kg$^{-1}$) | Total |
|---|---|---|---|---|---|---|---|---|
| 1 | *M. fasciculata* (1) | 13.343 ± 0.382 | 0.271 ± 0.047 | 94.583 ± 6.741 | < LOD | 0.142 ± 0.007 | < LOD | 297.683 |
| 2 | *M. fasciculata* (1) | 6.566 ± 0.450 | 0.118 ± 0.023 | 103.907 ± 2.143 | < LOD | 0.098 ± 0.001 | < LOD | 249.014 |
| 3 | *M. fasciculata* (1) | 5.724 ± 0.179 | 0.015 ± 0.001 | 149.110 ± 1.325 | 0.045 ± 0.008 | 0.093 ± 0.007 | < LOD | 235.228 |
| 4 | *M. fasciculata* (1) | 10.859 ± 0.539 | 0.073 ± 0.008 | 256.693 ± 10.299 | < LOD | 0.178 ± 0.005 | < LOD | 422.445 |
| 5 | *M. flavoneata* (2) | 25.636 ± 0.375 | 0.527 ± 0.050 | 50.533 ± 3.867 | < LOD | 0.206 ± 0.006 | < LOD | 695.956 |
| 6 | *A. mellifera* (3) | 6.503 ± 0.870 | 0.698 ± 0.084 | < LOD | < LOD | < LOD | 0.199 ± 0.009 | 433.368 |
| 7 | *M. fasciculata* (1) | 9.078 ± 0.555 | 0.366 ± 0.039 | 24.886 ± 0.162 | < LOD | 0.245 ± 0.015 | < LOD | 1,010.982 |
| 8 | *M. flavoneata* (2) | 8.034 ± 0.270 | 0.497 ± 0.021 | 17.335 ± 0.213 | < LOD | 0.049 ± 0.002 | < LOD | 783.330 |
| 9 | *A. mellifera* (3) | 7.727 ± 0.081 | 1.600 ± 0.067 | 23.038 ± 0.159 | < LOD | 0.038 ± 0.002 | < LOD | 567.744 |
| 10 | *A. mellifera* (3) | 9.812 ± 0.075 | 1.086 ± 0.019 | 21.779 ± 1.827 | 0.167 ± 0.012 | 0.082 ± 0.005 | < LOD | 542.336 |
| 11 | *A. mellifera* (3) | 13.843 ±0.071 | 2.185 ± 0.038 | 5.582 ± 0.026 | < LOD | 0.048 ± 0.003 | 0.913 ± 0.013 | 550.970 |
| 12 | *A. mellifera* (3) | 12.742 ± 0.291 | 1.390 ± 0.017 | 29.569 ± 0.053 | < LOD | 0.080 ± 0.004 | < LOD | 728.405 |
| 13 | *A. mellifera* (3) | 11.695 ± 0.794 | 2.116 ± 0.204 | 9.964 ± 1.300 | < LOD | 0.054 ± 0.002 | < LOD | 529.843 |
| 14 | *A. mellifera* (3) | 7.280 ± 0.036 | 2.513 ± 0.162 | 23.282 ± 0.397 | 0.076 ± 0.009 | 0.052 ± 0.011 | < LOD | 413.400 |
| 15 | *A. mellifera* (3) | 8.692 ± 0.947 | 2.795 ± 0.059 | 4.270 ± 0.039 | < LOD | 0.045 ± 0.009 | < LOD | 395.995 |
| 16 | *A. mellifera* (3) | 8.236 ± 0.530 | 0.734 ± 0.010 | 7.706 ± 0.142 | 0.094 ± 0.017 | 0.056 ± 0.009 | < LOD | 287.399 |
| 17 | *A. mellifera* (3) | 8.944 ± 0.380 | 1.165 ± 0.046 | 2.572 ± 0.175 | < LOD | 0.035 ± 0.006 | 0.134 ± 0.034 | 647.593 |
| 18 | *M. flavoneata* (2) | 9.936 ± 0.462 | 0.157 ± 0.005 | 24.355 ± 1.037 | < LOD | 0.288 ± 0.005 | < LOD | 957.753 |
| 19 | *M. flavoneata* (2) | 7.281 ± 0.650 | 1.274 ± 0.028 | 17.610 ± 0.202 | < LOD | 0.062 ± 0.012 | < LOD | 705.873 |
| 20 | *A. mellifera* (3) | 6.920 ± 0.976 | 1.850 ± 0.245 | 3.705 ± 0.264 | < LOD | 0.044 ± 0.007 | < LOD | 335.902 |
| 21 | *M. flavoneata* (2) | 8.377 ± 0.072 | 0.262 ± 0.025 | 4.644 ± 0.674 | < LOD | 0.097 ± 0.005 | < LOD | 651,107 |
| 22 | *A. mellifera* (3) | 6.707 ± 0.341 | 2.182 ± 0.142 | 10.902 ± 0.678 | < LOD | 0.029 ± 0.002 | < LOD | 422.953 |
| 23 | *M. fasciculata* (1) | 17.966 ± 0.474 | 0.218 ± 0.084 | 163.331 ± 13.197 | 0.052 ± 0.010 | 0.269 ± 0.005 | < LOD | 727.421 |
| 24 | M. flavoneata (2) | 31.399 ± 0.238 | 0.824 ± 0.019 | 91.749 ± 3.338 | < LOD | 0.331 ± 0.005 | 0.498 ± 0.049 | 1,130.432 |
| 25 | *A. mellifera* (3) | 5.855 ± 0.698 | 0.052 ± 0.002 | 20.849 ± 0.331 | < LOD | 0.055 ± 0.007 | < LOD | 95.284 |
| 26 | *A. mellifera* (3) | 7.353 ± 0.371 | 1.127 ± 0.069 | 19.704 ± 0.956 | < LOD | 0.040 ± 0.001 | < LOD | 308.867 |
| 27 | *A. mellifera* (3) | 7.559 ± 0.061 | 1.935 ± 0.001 | 23.707 ± 0.372 | < LOD | 0.066 ± 0.003 | < LOD | 414.342 |

[a]Average of two determinations ± standard deviation. LOD: limit of detection.

**Table 4**. Results of mineral contents according to the species of honeybees studied

| Bee specie | Parameter | K / (mg kg$^{-1}$) | Na / (mg kg$^{-1}$) | Ca / (mg kg$^{-1}$) | Mg / (mg kg$^{-1}$) | Mn / (mg kg$^{-1}$) | Fe / (mg kg$^{-1}$) | Al / (mg kg$^{-1}$) | Cu / (mg kg$^{-1}$) | Sr / (mg kg$^{-1}$) | Cr / (mg kg$^{-1}$) | Ni / (mg kg$^{-1}$) | Zn / (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. mellifera* | Mean | 387.058 | 13.109 | 32.059 | 8.666 | 1.559 | 0.960 | 1.273 | 0.278 | 0.048 | 0.050 | 0.045 | 0.784 |
| | SD | 146.889 | 10.085 | 19.790 | 2.484 | 0.780 | 0.607 | 2.461 | 0.125 | 0.024 | 0.017 | 0.057 | 1.023 |
| | CV | 37.95 | 76.93 | 61.73 | 28.67 | 50.03 | 63.23 | 193.23 | 44.96 | 50.00 | 34.00 | 126.67 | 130.48 |
| | Min. | 39.220 | < LOD | 7.490 | 5.360 | 0.040 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| | Max. | 636.010 | 30.140 | 96.218 | 13.890 | 2.840 | 2.320 | 10.929 | 0.501 | 0.128 | 0.121 | 0.176 | 5.188 |
| | Total / % | 80.53 | 8.46 | 7.84 | 2.24 | 0.31 | 0.37 | 0.33 | < 0.10 | 0.10 | < 0.10 | < 0.10 | 0.10 |
| | Limit | – | – | – | – | – | 15.000 | – | 10.000 | – | 0.100 | 5.000 | 5.000 |
| *M. fasciculata* | Mean | 304.560 | 132.411 | 40.393 | 10.591 | 0.177 | 1.351 | 1.005 | 0.228 | 0.171 | – | – | 0.080 |
| | SD | 310.329 | 76.244 | 30.521 | 4.735 | 0.129 | 2.076 | 2.647 | 0.288 | 0.073 | – | – | 0.180 |
| | CV | 101.89 | 57.58 | 75.53 | 44.70 | 72.88 | 153.66 | 263.38 | 126.31 | 42.69 | – | – | 225.00 |
| | Min. | 66.342 | 20.533 | 12.200 | 5.600 | 0.011 | 0.149 | < LOD | < LOD | 0.088 | < LOD | < LOD | < LOD |
| | Max. | 928.760 | 265.900 | 96.860 | 21.320 | 0.393 | 7.401 | 9.137 | 0.861 | 0.315 | < LOD | < LOD | 0.530 |
| | Total / % | 48.76 | 34.68 | 12.71 | 2.55 | 0.03 | 0.25 | 0.30 | < 0.10 | < 0.10 | < 0.10 | – | < 0.10 |
| *M. flavoneata* | Mean | 714.048 | 34.370 | 50.087 | 15.110 | 0.590 | 0.700 | 0.843 | 0.273 | 0.182 | – | – | – |
| | SD | 145.495 | 30.941 | 23.411 | 10.191 | 0.399 | 0.617 | 1.357 | 0.071 | 0.121 | – | – | – |
| | CV | 20.37 | 90.02 | 46.74 | 67.44 | 67.63 | 88.14 | 160.97 | 26.00 | 66.48 | – | – | – |
| | Min. | 554.209 | 3.460 | 25.480 | 6.120 | 0.154 | 0.059 | < LOD | 0.151 | 0.036 | < LOD | < LOD | < LOD |
| | Max. | 924.620 | 94.110 | 94.860 | 31.570 | 1.427 | 2.071 | 3.923 | 0.365 | 0.335 | < LOD | < LOD | < LOD |
| | Total / % | 86.41 | 4.49 | 6.75 | 1.90 | 0.14 | 0.14 | 0.13 | < 0.10 | < 0.10 | < 0.10 | – | – |
| All species | Mean | 454.169 | 50.398 | 39.673 | 11.238 | 1.069 | 1.023 | 1.390 | 0.260 | 0.108 | 0.050 | 0.071 | 0.744 |
| | SD | 240.429 | 65.689 | 21.889 | 7.078 | 1.585 | 0.803 | 1.576 | 0.134 | 0.085 | 0.017 | 0.038 | 0.693 |
| | CV | 52.94 | 130.34 | 55.17 | 62.98 | 148.26 | 78.49 | 113.38 | 51.54 | 78.70 | 34.00 | 53.52 | 93.14 |
| | Min. | 42.829 | < LOD | 10.280 | 2.012 | 0.002 | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD | < LOD |
| | Max. | 907.829 | 256.936 | 298.235 | 33.251 | 8.831 | 4.177 | 5.809 | 0.861 | 0.331 | 0.121 | 0.167 | 3.050 |
| | Total / % | 78.39 | 10.85 | 7.64 | 2.16 | 0.22 | 0.26 | 0.38 | < 0.10 | < 0.10 | < 0.10 | < 0.10 | 0.10 |

SD: standard deviation; CV: coefficient of variation; Min.: minimum value; Max.: maximum value.

seventh cluster is the largest, composed of ten *A. mellifera* honey samples and one *M. flavoneata* honey sample.

PCA was performed on the autoscaled and standardized data. From the loadings of the original variables in the first two principal component considered, it was established that principal component one (PC1) represents 56.00% of the total variance, and is given by the expression

PC1 = −0.479 (Moi.) + 0.168 (FA) + 0.477 (TSS) − 0.279 (Ca) + 0.358 (Mn) – 0.341 (Na) – 0.438 (Sr);

while principal component two (PC2) explains up to 20.00% of the total variance and is given by the expression

PC2 = −0.048 (Moi.) – 0.520 (FA) + 0.014 (TSS) − 0.619 (Ca) – 0.218 (Mn) + 0.468 (Na) – 0.279 (Sr).

The first two principal components account for 76.00% of the total variance and were considered to be sufficient for the data.

Figure 2 shows that the Na and Ca contents and free acidity are the most important variables explaining the separation between the honey samples in PC2, and those values of moisture and total soluble solids are the most important variables explaining the separation between honey samples in PC1.

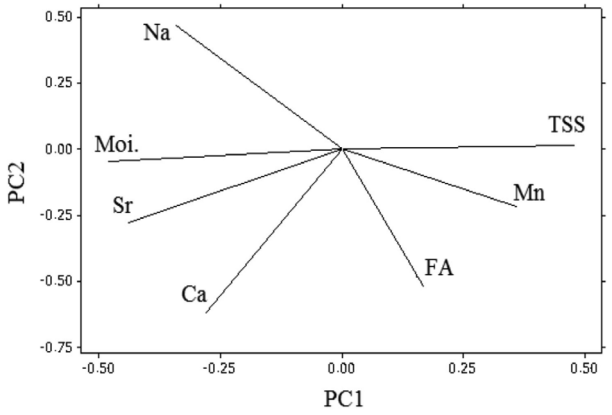


**Figure 2.** Graphic of the loading for principal component.

High Na values resulted in positive PC2 scores with a smaller negative score contribution from moisture values and a smaller positive contribution from total soluble solids.

On examining the score plot of the objects in the space defined by the two principal components (Figure 3), it was found the three honey groups could be separated according to different entomological origin. A very compact group composed of all the *A. mellifera* honey samples can be observed on the right-hand side of the biplot. The *M. fasciculata* honeys form a dispersive group on the top left hand side of the biplot. The *M. flavoneata* honey samples appear at the bottom left hand side as a very dispersive group. Despite the *M. flavoneata* and *M. fasciculata* samples appearing very close together in the biplot, they can be considered to form two different groups. Just one sample of *M. flavoneata* and one sample of the *M. fasciculata* honey were dispersed within the other groups. A total of 25 samples (92.6% of samples) was therefore correctly grouped, with only two samples not being properly separated.

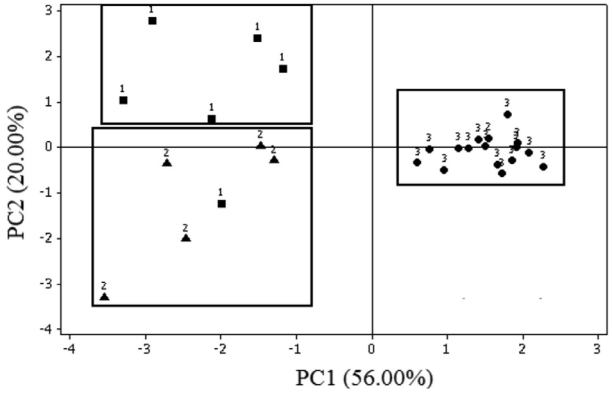


**Figure 3.** Principal component score plot of the separation of honey samples by entomological origin. 1: *Melípona fasciculate*, 2: *Melípona flavoneata* and 3: *Apis mellifera*.

Based on the classification obtained using HCA and PCA, it is possible establish that free acidity, total soluble solids, moisture, and Na, Sr, Mn and Ca contents are responsible for the separation between the honeys produced by the three different species studied.

## Conclusion

All the fourteen physicochemical parameters studied in the present work all presented values were in agreement with national and international laws for the *A. mellifera* honey or, in which the values were outside the limits established by Brazilian and internationals law for honey, they were nonetheless in conformity with the results of other similar works in Brazil and from around the world.

Honeys produced by native species exhibit some physicochemical parameters (moisture, density, ash, total soluble solids) with values that are distinct from those produced by the species *A. mellifera*. This suggests that there should be specific legislation for these honeys.

The most abundant mineral element determined in all the samples studied was potassium, followed by sodium, calcium and magnesium, in agreement with results from the literature. Conversely, arsenic, beryllium, barium, bismuth, cadmium, cobalt and lithium were not detected in any of the samples analyzed.

The sodium content found in the *M. flavoneata* honeys was higher than for the other kinds of honey considered in the present study.

HCA and PCA methods, using only three physicochemical parameters (free acidity, soluble solids and moisture) and only four mineral contents (Na, Ca, Mn and Sr), showed that honey samples from three different species of bees could be aligned into three groups. However, one sample of *M. flavoneata* was placed in the *A. mellifera* group, and one sample of *M. fasciculata* was placed in the *M. flavoneata* group.

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