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CHEMICAL SCIENCES

Multi-element composition, physicochemical and pollen attributes of honeys from the Paraguaçu River (Bahia, Brazil) by inductively coupled plasma-optical emission spectrometry (ICP OES)

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Abstract: Honey is a food of nutritional, medicinal and commercial importance. The physicochemical characteristics, pollen spectrum and mineral composition of eighteen honey samples obtained from regions (Cachoeira, Coqueiros, Maragojipe and Santiago do Iguape) near the Paraguaçu River, Bahia, Brazil were evaluated. Botanical families Asteraceae, Leguminosae, Malvaceae, Myrtaceae and Palmae were most frequently found. Five samples had water contents above the maximum limit established by the Brazilian legislation (> 20%). The mineral composition was determined by ICP OES, after microwave digestion. Ca, K, Mg and Na were measured (mg Kg^{-1}) in the range from: 18.85 to 79.61; 366.74 to 1214.98; 12.46 to 44.59 and 11.56 to 85.39, respectively. Cu, Fe, Mn and Zn had variable concentration ranges, between 0.05 and 6.13 mg Kg^{-1}. Al, Ba, Cd, Co, Cr, Ni, Pb, Se and V showed values below the LOD. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) demonstrated that there are no similarities of mineral composition among honey samples.

Keywords: Honey, physicochemical analysis, pollen spectrum, minerals, multivariate analysis.

INTRODUCTION

Honey is a food of nutritional, medicinal and commercial importance, produced by honeybees and other social insects from the nectar of flowers, secretions of living parts of plants or excretions of sucking insects of plants by water evaporation and the addition of enzymes (Samarghandian et al. 2017, Miguel et al. 2017). It consists of water and sugar (99%) and other substances, in small quantities, but important for its characterization - enzymes, organic acids, minerals, amino acids and pollen grains (Ajibola et al. 2012). The contents of its main constituents may vary according to plant species, environmental conditions (climate, soil, humidity, wind, etc.) and other factors related to beekeeping techniques (Bogdanov et al. 2008).

The pollen analysis of honey samples is based on the frequency of classes of pollen grains (Barth 2004). The pollen spectrum is also important to evaluate the quality of honeys, which may be mono or polyfloral; the latter is more commonly used due to cost, organoleptic properties and pharmacological activities (Martins et al. 2011). In the literature, some studies discuss pollen spectrum of bee species (Jaafar et al. 2017, Ouchemoukh et al. 2007).

Honey is a concentrated sugar solution with a predominance of glucose and fructose. The addition of sugars or other substances that alter its original composition is not allowed. The main requirements for the marketing of honey are: color, taste, aroma, self-consistency, presence of pollen grains; as well as physicochemical properties: reducing sugars, humidity, apparent sucrose, insoluble solids in water, minerals, acidity, diastase activity and hydroxymethylfurfural content (Wei et al. 2012, Azeredo et al. 2003). Several honeys, commercially distributed, have undergone some degree of physical processing such as filtration, centrifugation and decanting, in order to remove parts of insects, pollen grains and wax particles. Several studies have been developed in Brazil and in the world to characterize the honey produced in different regions, indicating various physical and chemical parameters (Oliveira et al. 2017, Batista et al. 2012, Viuda-Martos et al. 2010, Silva et al. 2009).

Some minerals are present in honey at low concentrations (Cu, Fe, Mn, Zn, etc) and others, such as Ca, K, Na and Mg, can have their values increased depending on geographical and environmental differences (BRASIL 2000). The determination of the elemental composition of foods rich in sugar has been an analytical challenge, since its high content may cause interference in analytical techniques. Therefore, pretreatment procedures with partial or total destruction of the sample are necessary. Analytical methods using atomic spectrometry are available for determining the elemental composition of honey samples (Altundag et al. 2016, Aghamirlou et al. 2015, Vanhanen et al. 2011, Madejczyk & Baralkiewicz 2008, Tuzen et al. 2007, Hernández et al. 2005). Inductively coupled plasma optical emission spectrometry (ICP OES) is an analytical technique widely used for the determination of trace metals (concentration of <1 mg L^{-1}), due to the high sensitivity, versatility, multi-element analysis, ruggedness and speed of analysis (Korn et al. 2008, Ioannidou et al. 2005, Fernandez-Torres et al. 2005).

In general, a great amount of chemical results are generated from the multi-element analysis of food samples. In order to facilitate the interpretation of these results, multivariate statistical techniques have been used successfully in recent years. The multivariate data analysis allows for a simple and rapid reduction in the multidimensionality of the data set, verifying the similarities among samples, tendencies to form clusters, classification, among others. Among these multivariate techniques, principal component analysis (PCA) and hierarchical cluster analysis (HCA) have been used successfully to evaluate and characterize several food matrices, such as honey (Callao & Ruisánchez 2018, Martins et al. 2008).

The aims of this study were to evaluate the botanical origin of honeys from municipalities in the micro-region of the Paraguaçu River/Bahia/Brazil, their physicochemical properties and to determine macro- and micro-nutrients by ICP OES. PCA and HCA were also applied to evaluate the mineral composition data obtained from the analysis of the honey samples. In this region, there is the vegetation of Caatinga, typical of semi-arid regions, characterized by great diversity of plant species, and beekeeping has become an economic activity alternative for farmers and ranchers (Carvalho & Marchini 1999).

MATERIALS AND METHODS

Materials (Chemicals/reagents and samples)

Eighteen honey samples produced in areas of the municipalities of the micro-region (Cachoeira, Coqueiros, Maragojipe and Santiago do Iguape) of the Paraguaçu River, in Bahia,

Brazil were analyzed. The samples were collected from honeys sold by local people in stalls and supermarkets, in the summer, with a hot and humid climate. The samples were numbered by Roman numerals and kept in their original containers (glass bottles, capped with cork stoppers) in a dark place at room temperature (±20 °C). The processing of pollen and physicochemical analysis occurred immediately after each sample collection period. No sample showed signs of fermentation or deterioration (up to one month of storage).

All the chemical reagents used were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade. The water was purified using the Milli-Q purification system from Millipore (Bedford, MA, USA).

Palynological analysis

Pollen analysis was carried out using the methods established by the International Commission of Bee Botany (Iwama & Melhem 1979). The determination of the botanical origin of honeys was based on the frequency of classes of pollen types. About 10 g of honey sample was dissolved in 20 mL of warm water. The mixture was centrifuged at 2.500 rpm for 15 minutes (Fanem 206BL - São Paulo, Brazil) and the supernatant was discarded. The pollen pellet was dried in glacial acetic acid, subsequently subjected to the process for acetolysis for best microscopic observation of the pollen grains. Then, using the resulting pellet, slides were mounted performing up counting a minimum of 1500 pollen grains per sample for the determination of the percentage of each species, using a light microscope (Zeiss Axioskop 2 - Bloomfield, Connecticut, USA).

Physicochemical analysis

Physicochemical analysis consisted in analyzing the moisture content, reducing sugars, presence of starch, free acidity and presence of albumin and proteins. Physicochemical parameters were analyzed using The Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) and The Harmonised Methods of the European Honey Commission (Cunnif 1998, IHC 2009). All experiments were performed in triplicate.

- Starch, dextrins and/or commercial sugars (Lugol Reaction): about 10 mL of honey were dissolved in 10 mL of distilled water, shaking it vigorously for 15 minutes. Subsequently, 5 mL of 0.1% Lugol solution (w v^{-1}). In case of pure honey, the final color is mantained nevertheless, in the presence of starch or dextrin, it is black.

- Albumin and proteins in suspension (Lund reaction): About 2 g of honey sample was dissolved in 10 mL of distilled water, followed by stirring for 15 minutes. 5 mL of 0.5% (w v^{-1}) tannic solution were added to this mixture. Water was added to reach a total volume of 40 mL. The mixture was homogenized and kept at rest for 24 hours. In the case of pure honey, it forms a flocculated precipitate or deposit on the bottom of the container.

- Moisture content was determined by the refractive index of honey at 20 °C, using a refractometer, Quimis Q 107 A1 (Diadema, São Paulo, Brazil), in which it was converted to the moisture content using a reference table which gives the concentration-refractive index.

- Free acidity was determined by neutralization volumetry, titrating the honey sample with 0.05 mol L^{-1} sodium hydroxide and 1% phenolphthalein alcohol solution (w v^{-1}) as an indicator. pH was measured using a pH meter, Cientec - CT pH 2 (Piracicaba, São Paulo, Brazil), which should not exceed 8.5.

- The method used for quantification of reducing sugars (mainly fructose and glucose) and apparent sucrose in honey was by reducing

Soxhlet modification of Fehling method (Areda 2015).

Preparation and multi-element analysis

The acid digestion of honey samples was performed using a commercial high-pressure laboratory microwave oven (Milestone Ethos 1600 Microwave Labstation, Sorisole, Italy) operating at a frequency of 2450 Hz, with an energy output of 900 W. This microwave digestion system was equipped with ten 100-mL tetrafluoromethoxy vessels and a ceramic vessel jacket. The maximum operating temperature and pressure were 180 °C, 1000 W and 35 bar, respectively, in 30 minutes. The method used to determine the mineral composition of the honey samples was quick and simple, according to the following procedure: approximately 0.5g of each honey sample was inserted directly into a microwave-closed vessel. Seven mL of 65% (m m^{-1}) HNO_3 and 1 milliliter of 30% (m m^{-1}) H_2O_2 were added to each vessel. After cooling, the samples were then diluted to 10 mL with distilled water plus 1 mL of 0.01 (v v^{-1}) Triton X-100. Metal contents of the final solution were determined by ICP OES.

In multi-element analysis, the samples I, II, III, VI, VIII, XV and XVIII were not analyzed due to the small quantities of samples. An inductively coupled plasma optical emission spectrometer (ICP OES, VISTA PRO, Varian - Mulgrave, Australia) with axial viewing and a solid state detector was used for the simultaneous determination of the analytes of interest (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, V and Zn). The optical system was calibrated using a multi-element stock solution for ICP OES, whereas a 5.0 mg L^{-1} Mn standard solution was used for optical alignment. The spectral lines were selected according to the absence of spectral interference and appropriate sensitivity for determining elements at high and low concentrations, by studying the emission lines of the elements to be investigated. The lines that exhibited low interference, high analytical signal and background ratios were selected. The optimized parameters of ICP OES were: radio frequency power (RF) generator power (1300 W); Plasma gas rate (mL min^{-1}); Auxiliary gas rate (1.5 mL min^{-1}); Nebulizer gas rate (0.7 mL min^{-1}); Sample uptake rate (0.8 mL min^{-1}); Injector tube diameter (2.4 mm) and Signal integration time (1s). The analytical wavelengths (nm) used for multi-elemental analysis were: Al (396.152); Ba (455.403); Ca (396.847); Cd (228.802); Co (238.892); Cr (267.716); Cu (324.754); Fe (238.204); K (769.897); Mg (279.553); Mn (257.610); Na (589.592); Ni (227.021); Pb (217.000); Se (196.026); V (309.310); Zn (213.857).

Validation studies

The method was validated by performance parameters: stability, linearity, precision, accuracy, limit of detection, limit of quantification and matrix effect (ICH 2007).

Honeys and multi-element solution stability were evaluated for 24 hours at room temperature and kept at 37 °C for 2 hours in 0.01 and 0.1 mol L^{-1} HCl by checking changes in the analytical signal after analyzed by ICP OES. Linearity was evaluated by linear regression of analytical curves (1.0 to 100.0 µg mL^{-1} of macro- and microelements). Precision was evaluated through relative standard deviation (RSD) from the obtained data by analyzing solutions of macro- and microelements with a working concentration of 5 and 10 µg mL^{-1}, in six replicates.

The accuracy of the measurements was assessed using spinach leaves Certified Reference Material 1570a (CRM 1570a) from the National Institute of Standards and Technology (Gaithersburg, MD, USA). In addition, a recovery study was conducted by adding known amounts (5 and 10 µg) of each mineral in honeys samples, for evaluation of accuracy. Limits of detection

(LOD) and quantification (LOQ) were calculated using the concentration equivalent to three times the standard deviation (3σ) of the signal (n = 10) of the analytical blank solution, and the LOQ was calculated using 10σ criterion (n = 10), respectively.

The matrix effect was evaluated by comparing the slopes of curves obtained by external calibration with standard solutions in acid medium (2 mol L^{-1} HNO_3), from solutions of the digested samples. After the measurements of emission intensities by ICP OES were performed, the slope coefficients were obtained from analytical curves.

Multivariate analysis

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to evaluate the mineral composition data obtained from the analysis of the honey samples by ICP OES. For this statistical evaluation, the samples were taken as objects and arranged in rows, whereas the analytes were taken as variables and arranged in columns. A data matrix of 11 x 8 was generated. The data were auto-scaled and processed by using the Statistica 8.0 software. For HCA, the Ward's method and Euclidean distance were used to establish groups and generate the dendrogram.

RESULTS AND DISCUSSION

Analytical performance

Honeys and multi-element solutions were stable within 24 hours. There were no changes in the analytical signal of the ICP OES. Linearity was evaluated and a good linearity was obtained for all observed lines, with determination coefficients (R^2) in the range from 0.9996 to 0.9999. Precision was assessed and the RSD values were lower than 10%, indicating the good precision of this method. The results showed good agreement with the reference values of the CRM sample (Table I). The obtained recovery values, in the range of 90 to 110%, showed good accuracy of the method. In a recovery study, acceptable recoveries (> 95%) were obtained for the studied elements. The LOD and LOQ obtained for Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, V and Zn determined by ICP OES are shown in Table II. The validation parameters were comparable with Fernandez-Torres et al. (2005), who analyzed metals in honey by ICP OES.

In an evaluation of matrix effect, the slopes of the calibration curves for each element do not show significant variations, at 95% confidence level. Therefore, that matrix effect is not significant for the measures in ICP OES under the selected operating conditions. The

Table I. Analysis of spinach leaves (Certified Reference Material - CRM 1570a) by ICP OES after microwave radiation procedures (μg g^{-1} or %, mean ± standard deviation, n = 3, 95% confidence level).

Element	Certified value (μg g^{-1})	Determined (μg g^{-1})	Recovery (%)
Cu	12.2 ± 0.60	12.3 ± 0.05	101
Mn	75.9 ± 1.90	75.6 ± 0.80	100
Zn	82.00 ± 3.00	83.7 ± 0.15	102
Element	**Certified value (%)**	**Determined (%)**	**Recovery (%)**
Ca	1.527 ± 0.041	1.472 ± 0.103	96
K	2.903 ± 0.052	2.603 ± 0.087	90
Na	1.818 ± 0.043	1.992 ± 0.244	110

Table II. LOD and LOQ, in μg g^{-1}, for macro- and microelements determined by ICP OES after the microwave radiation procedure.

ELEMENTS	LOD	LOQ
Al	0.004	0.011
Ba	0.009	0.024
Ca	3.096	10.77
Cd	0.008	0.027
Co	0.022	0.108
Cr	0.005	0.009
Cu	0.003	0.005
Fe	0.002	0.006
K	0.441	1.877
Mg	0.165	0.489
Mn	0.044	0.127
Na	1.983	5.971
Ni	0.002	0.005
Pb	0.003	0.006
Se	0.038	0.123
V	0.001	0.004
Zn	0.002	0.003

slopes of the analytical curves (Table III) for each element were obtained using different media: M1 (2 mol L^{-1} HNO_3) and M2 (honeys after microwave radiation procedures).

Palynological and physicochemical analysis

Despite the large number of plant species (especially native plants from savanna vegetation), it was possible to indicate the wild condition of the majority of samples, as a source of nectar. Most pollen grains were identified to the genus level, being originated mainly from plants of the following families: Asteraceae, Leguminosae, Malvaceae, Myrtaceae and Palmae.

The main pollen types are shown in Table IV, as well as the physicochemical parameters investigated for the 18 honey samples analyzed. The results were evaluated by central trend analysis (average), variability (standard deviation) and statistically analyzed, adopting the 95% confidence level.

Currently, Brazil has an important participation in the world Beekeeping scenario due, among other factors, to the diversity of its natural and wild blossoms originated from the native flora. The palynological analysis of Brazilian honeys began in the 1960s, especially in the Southeast. In the Brazilian Northeast, there are still few studies involving the question (Alves et al. 2011, Freitas 1994). Knowledge about the sources of floral resources exploited by bees is crucial to design strategies for the preservation and multiplication of honey potential of plants, aiding to establish a sustainable beekeeping. Therefore, pollen analysis of honey samples is intended to achieve a botanical and geographic

Table III. The slopes of the analytical curves for Al, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Se, V and Zn by ICP OES in M1 (2 mol L^{-1} HNO_3) and M2 (honeys after microwave radiation procedures).

Elements	M1	M2
Al	16210	15651
Ca	24156	22598
Cd	5247	5009
Co	6543	6109
Cr	3857	3114
Cu	1247	1194
Fe	13578	12129
K	20504	19316
Mg	22149	21352
Mn	59475	54178
Na	206086	197106
Ni	3985	3497
Pb	2476	2225
Se	6453	6002
V	4517	4128
Zn	28741	25124

characterization of this bee product. As noted, the most representative botanical families regarding the number of pollen types present in the samples were Asteraceae, Fabaceae, Malvaceae, Myrtaceae and Palmae, five families with high species richness in the semi-arid area, and also with great potential to obtain honeys (Santos et al. 2005). From the pollen analysis, it was possible to determine the botanical origin of honey, which is important for a better understanding of the physical and chemical analysis, which may vary according to the botanical origin of honeys (Bogdanov 2004).

Physical and chemical tests were conducted in order to assess the quality of honeys marketed by the population of the Paraguaçu-Ba River micro-region. The most common adulteration is made from honey bee itself, to which a sugar syrup made of water is added, increasing the volume of the final product. . No adulteration by commercial sugar or starch was found, since the reaction with Lugol was negative, in all samples.

Regarding the reaction of Lund, all samples showed precipitation of proteins and albumin, but the sample XV showed a precipitate volume (0.1 mL) below the minimum established by the law (0.6 mL). This indicates that honey may have been partially adulterated. The variation for the results is similar to that presented by honeys from other regions of the country (Campos et al. 2001).

The amount of water in honey is presented within a lower limit in which their constituents can be dissolved and an upper limit above which honey is subject to fermentation. The Brazilian legislation sets the maximum rate at 20% (BRASIL 2000). Whereas all honeys contain osmophilic yeast, increasing moisture favors their multiplication and, consequently, the fermentation process (Bogdanov et al. 1999). In Algeria, a study revealed water contents

Table IV. Palynological and pysicochemical analysis of honey samples marketed in municipalities in the region Paraguaçu River, Bahia.

Sample	Main Types pollen	Lugol reaction	Lund reaction (mL)	Moisture (%)	Reducing sugars (%)	Free acidity (meq Kg^{-1})
I	*Syagrus coronata* (PA) *Vernonia* sp. (PA)	Absent	1.2	<13.0	57.0	24.65
II	*Syagrus coronata* (PA) *Herissatia* sp. (PA)	Absent	1.5	13.0	39.4	43.95
III	*Mimosa scabrella* (PA) *Vernonia* sp. (PA)	Absent	1.8	17.6	58.8	42.10
IV	Type Asteraceae (PD) *Mimosa scabrella* (PA)	Absent	2.2	17.6	59.4	45.50
V	*Mimosa gemmulata* (PA) Type Unidentified (PA)	Absent	0.9	<13.0	66.7	40.25
VI	*Mimosa gemmulata* (PA)	Absent	1.1	>20.8	58.0	28.85
VII	*Piptadenia moniliformis* (PD)	Absent	1.8	13.0	47.0	32.55
VIII	*Mimosa arenosa* (PD)	Absent	1.6	>20.8	55.0	26.50
IX	*Syagrus coronata* (PD)	Absent	1.1	20.8	69.8	29.85
X	*Vernonia* sp.(PD)	Absent	2.2	20.6	48.0	34.00
XI	*Mimosa arenosa* (PA)	Absent	1.7	<13	46.5	25.05
XII	*Syagrus coronata* (PA) *Mimosa gemmulata* (PA)	Absent	1.0	>20.8	47.0	30.70
XIII	*Myrcia* sp. (PD)	Absent	1.1	17.8	58.0	25.60
XIV	*Syagrus coronata* (PD)	Absent	0.9	19.6	50.5	26.65
XV	*Syagrus coronata* (PA) *Eupatorium* sp. (PA)	Absent	0.1	16.6	61.8	53.80
XVI	*Mimosa arenosa* (PD) *Mimosa scabrella* (PA)	Absent	1.6	<13	55.0	27.42
XVII	*Syagrus coronata* (PD)	Absent	1.1	18.4	66.2	28.70
XVIII	*Mimosa arenosa* (PD)	Absent	1.8	18.0	50.5	30.00

Legend: PD - dominant pollen (above 45%); PA - accessory pollen (15-44%).

between 14.64% and 19.04% (Ouchemoukh et al. 2007). As well, in Portugal, the percent moisture in honeys ranged from 13.53 to 19.70% (Silva et al. 2009). The samples VI, VIII, IX, X and XII showed moisture contents above the maximum limit set by the Brazilian legislation. This can lead to fermentation, altering the quality of honeys for fresh consumption. These values can be

attributed to the conditions of the containers in which the collected honey samples were stored.

Samples V, IX, XV and XVII showed to be consistent with the values determined by the legislation (65%), for reducing sugars; it is not necessary to perform other more specific tests for adulteration check for added sugars (Campos et al. 2001).

Free acidity is a parameter frequently determined in honeys, as an indicator for the evaluation of fermentation process and transformation of alcohol into organic acid. The most typical acid found in honey is gluconic acid, produced by the action of the enzyme glucose oxidase on glucose. According to the legislation, the maximum acidity concentration allowed is 40 mEq kg^{-1} (BRASIL 2000). Samples II, III, IV, V, and XV showed acidity above the established limit values, which may be an indicative of fermentation processes. It is emphasized that these samples had moisture contents consistent with the expectations. Thus, if there is a fermentation process, this can be attributed to the possibility of contamination and not to the high water level.

Multi-element analysis

The multi-element determination in honey is made to ensure quality and authenticity as healthy food for consumption. In this sense, the concentrations of present metals act primarily as an environmental indicator and sources of dietary supplementation (Mendes et al. 2009). The multi-element composition of the samples is shown in Tables V and VI. High and varying concentrations of Ca, K and Mg in the samples were observed. Micronutrients such as Cu, Fe, Mn, Na and Zn were detected in the analyzed samples. Some elements such as Al, Ba, Cd, Co, Cr, Ni, Pb, Se and V showed values below the LOD.

The metal content (about 11 elements) was determined in Botanical Spanish honeys, using ICP OES with axial viewing (Fernandez-Torres et al. 2005). In the same year, traces of toxic metals were determined, in sugars and honey samples of Croatia and Greece, directly by the same analytical technique (Bilandžić et al. 2017, Ioannidou et al. 2005). The mineral content present in the honey samples analyzed in this study were consistent with the results obtained by the above authors. In Argentina, the quantified elements, in mg Kg^{-1}, in honeys were: Fe (1.13-10.32), Mn (0.07-0.68), Zn (0.14-3.87), Cu (0.05-0.68), Ca (18.60-136.14), Mg (6.01-46.57), Na (6.10-89.98) and K (90.92-1955.75), according to the authors (Cantarelli et al. 2008, Conti 2000). In this study, the concentrations of Ca, Cu, Mg, Na were concordant. The contents of Fe and Zn were lower, while the concentration of K was higher, in accordance with others authors who quantified potassium: > 75% of the total mineral (Silva et al. 2009, Fernandez-Torres et al. 2005). Ribeiro et al. (2015) investigated the seasonal variation of trace elements and microelements in samples of Brazilian honey. K, Ca, Fe, Mn, Zn, Cr, Ni, Cu, Se, Br, Sr and Ti were determined by total reflection X-ray fluorescence spectroscopy (TXRF). The authors reported that seasonal variations may have a significant influence on honey composition, mainly affecting K and Ca contents in the analyzed samples, with higher values in spring and summer.

In this study, the concentration ranges of Ca, K, Mg, Fe and Zn in samples are large and intercalate. Samples IV, V, IX and XII showed the highest levels of Ca, K, Mg and Na, which makes these samples as additional sources of these macronutrients. These results may be useful for obtaining further information on national honeys for the formation of a mineral composition database of this food.

Table V. Composition of macronutrients (Ca, K, Mg and Na) in honey samples marketed in municipalities of Paraguaçu River/Bahia region (mg Kg^{-1}).

Sample	Main Types pollen	Ca 396.847 nm	K 766.461 nm	Mg 279.553 nm	Na 589.592 nm
I	*Syagrus coronata* (PA) *Vernonia* sp. (PA)	na	na	na	na
II	*Syagrus coronata* (PA) *Herissatia* sp. (PA)	na	na	na	na
III	*Mimosa scabrella* (PA) *Vernonia* sp. (PA)	na	na	na	na
IV	Type Asteraceae (PD) *Mimosa scabrella* (PA)	54.96 ± 1.18	723.23 ± 10.86	26.91 ± 0.27	16.95 ± 0.11
V	*Mimosa gemmulata* (PA) Type Unidentified (PA)	79.61 ± 1.03	683.87 ± 24.65	44.59 ± 2.43	85.39 ± 1.22
VI	*Mimosa gemmulata* (PA)	na	na	na	na
VII	*Piptadenia moniliformis* (PD)	32.77 ± 0.18	695.71 ± 3.99	12.46 ± 0.10	8.27 ± 0.19
VIII	*Mimosa arenosa* (PD)	na	na	na	na
IX	*Syagrus coronata* (PD)	62.95 ± 1.90	1214.98 ± 11.46	34.48 ± 0.45	73.22 ± 0.74
X	*Vernonia* sp.(PD)	39.83 ± 1.62	786.02 ± 33.50	17.29 ± 0.75	9.23 ± 0.67
XI	*Mimosa arenosa* (PA)	18.58 ± 6.18	366.74 ± 14.71	12.57 ± 4.45	9.94 ± 2.54
XII	*Syagrus coronata* (PA) *Mimosa gemmulata* (PA)	58.62 ± 1.35	694.33 ± 6.36	28.63 ± 0.16	16.38 ± 0.54
XIII	*Myrcia* sp. (PD)	45.34 ± 2.78	377.26 ± 24.11	18.42 ± 1.18	11.56 ± 1.25
XIV	*Syagrus coronata* (PD)	40.66 ± 2.15	768.39 ± 1.64	26.76 ± 0.07	26.84 ± 0.47
XV	*Syagrus coronata* (PA) *Eupatorium* sp. (PA)	na	na	na	na
XVI	*Mimosa arenosa* (PD) *Mimosa scabrella* (PA)	28.83 ± 2.21	385.67 ± 41.16	18.83 ± 1.58	15.35 ± 1.33
XVII	*Syagrus coronata* (PD)	42.22 ± 0.93	507.59 ± 2.16	21.57 ± 0.01	30.58 ± 0.81
XVIII	*Mimosa arenosa* (PD)	na	na	na	na

PD - dominant pollen (above 45%); PA - accessory pollen (15-44%) / na = not analyzed

Table VI. Composition of micronutrients (Cu, Fe, Mn, and Zn) in honey samples marketed in municipalities of Paraguaçu River/Bahia region (mg Kg^{-1}).

Sample	Main Pollen Types	Cu 327.395 nm	Fe 238.204 nm	Mn 257.610 nm	Zn 213.857 nm
I	*Syagrus coronata* (PA) *Vernonia* sp. (PA)	na	na	na	na
II	*Syagrus coronata* (PA) *Herissatia* sp. (PA)	na	na	na	na
III	*Mimosa scabrella* (PA) *Vernonia* sp. (PA)	na	na	na	na
IV	Type Asteraceae (PD) *Mimosa scabrella* (PA)	0.15 ± 0.05	4.22 ± 0.03	2.52 ± 0.04	0.14 ± 0.01
V	*Mimosa gemmulata* (PA) Type Unidentified (PA)	< 0.003	1.42 ± 0.02	1.48 ± 0.09	0.22 ± 0.03
VI	*Mimosa gemmulata* (PA)	na	na	na	na
VII	*Piptadenia moniliformis* (PD)	0.13 ± 0.04	1.38 ± 0.05	0.45 ± 0.01	0.09 ± 0.01
VIII	*Mimosa arenosa* (PD)	na	na	na	na
IX	*Syagrus coronata* (PD)	0.06 ± 0.01	1.28 ± 0.03	1.18 ± 0.02	0.19 ± 0.04
X	*Vernonia* sp.(PD)	0.08 ± 0.01	3.40 ± 0.14	0.79 ± 0.04	0.39 ± 0.03
XI	*Mimosa arenosa* (PA)	0.12 ± 0.05	0.22 ± 0.09	0.78 ± 0.31	0.08 ± 0.06
XII	*Syagrus coronata* (PA) *Mimosa gemmulata* (PA)	0.51 ± 0.06	5.74 ± 0.19	1.47 ± 0.01	0.19 ± 0.02
XIII	*Myrcia* sp. (PD)	0.29 ± 0.02	0.73 ± 0.06	0.53 ± 0.04	0.05 ± 0.02
XIV	*Syagrus coronata* (PD)	0.09 ± 0.03	2.31 ± 0.01	0.58 ± 0.01	0.30 ± 0.01
XV	*Syagrus coronata* (PA) *Eupatorium* sp. (PA)	na	na	na	na
XVI	*Mimosa arenosa* (PD) *Mimosa scabrella* (PA)	0.08 ± 0.01	1.27 ± 0.07	0.83 ± 0.08	0.30 ± 0.01
XVII	*Syagrus coronata* (PD)	0.06 ± 0.02	6.13 ± 0.09	1.57 ± 0.05	0.21 ± 0.06
XVIII	*Mimosa arenosa* (PD)	na	na	na	na

PD - dominant pollen (above 45%); PA - accessory pollen (15-44%) / na = not analyzed.

Multivariate analysis

From the evaluation of mineral composition data by PCA, score and loading plots were obtained, as shown in Figure 1. According to this analysis, the two first principal components (PC1 and PC2) account for 65.68% of total data variance. From the score plot (Figure 1a), it was not possible to observe clear trends of separation of the samples in groups, according to the mineral composition present in the honey samples. In relation to PC1, it was possible to verify that Mg is the variable with higher discrimination power of data, with the highest positive loading, followed by Ca, Na and K, as shown in the loading plot (Figure 1b). In relation to PC2, it was observed that Fe and Cu were the variables with the highest positive loadings. According to these results, samples V and IX presented the highest levels of Mg, Ca, Na and K, since they have the highest positive scores and are positively correlated with these elements, considering PC1. In a similar way, sample XII presented the highest levels of Fe and Cu, once it has the highest positive score and it is positively correlated with these elements, in relation to PC2.

The mineral composition data of the honey samples were also evaluated by hierarchical cluster analysis (HCA). Ward´s method and Euclidean distance were used to calculate similarities between samples. The data were auto-scaled prior to evaluation by HCA. The dendrogram obtained as the result of HCA demonstrated that there is not formation of clusters, considering the pollen types that comprise the honeys (Figure 2).

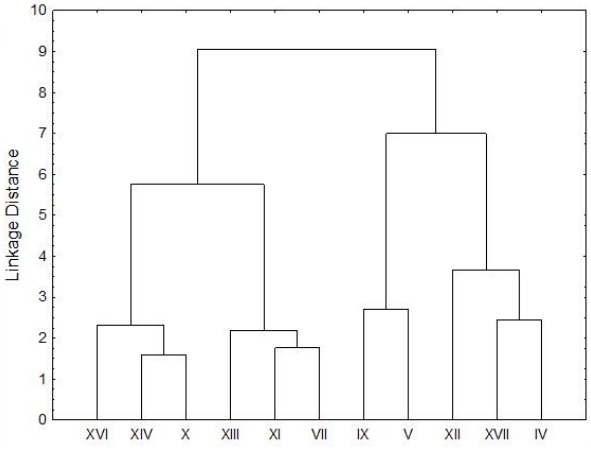

Figure 2. Dendrogram obtained from the evaluation of the mineral composition data by HCA.

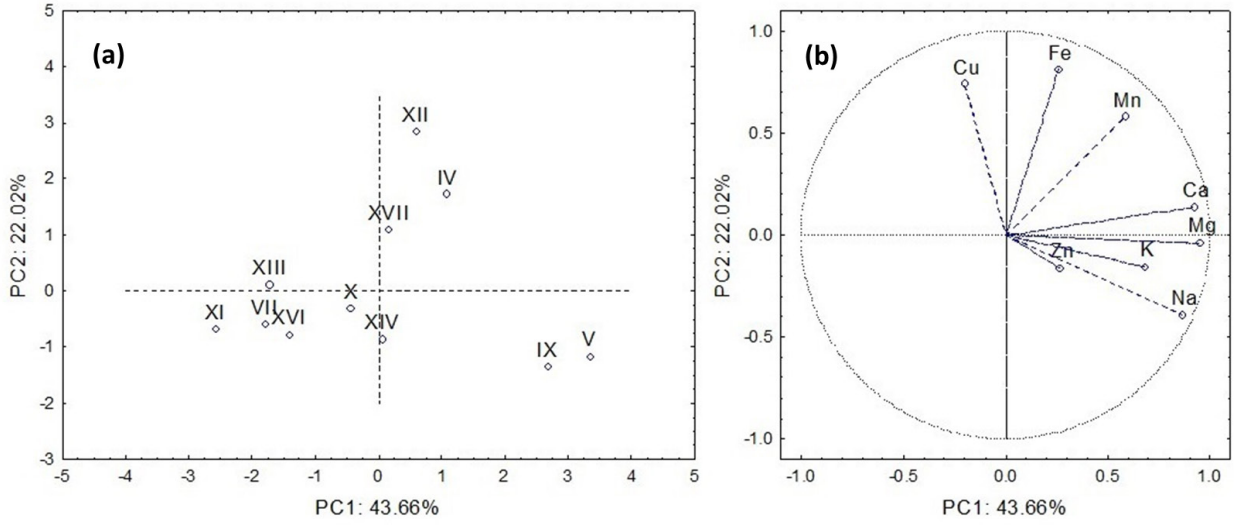

Figure 1. Score (a) and loading (b) plots of the two first principal components obtained from the evaluation of the mineral composition data by PCA.

CONCLUSIONS

From the data obtained, it was possible to trace the palynological profile of honey samples marketed in the micro-region of the Paraguaçu River, in the state of Bahia. This procedure is of relevant interest in identifying pollen and honey production in semi-arid regions. Regarding the physicochemical characterization, it was observed that some samples showed variability in the content of the parameters evaluated. Despite these differences, most honey fresh samples had to be suitable for human consumption.

The determination of the element composition of honey samples using ICP OES with axial viewing required a simple pretreatment procedure of the sample, featuring simple and reproducible method results. The multivariate statistical evaluation of these data by PCA and HCA revealed that there are not similarities among the samples, in relation to mineral composition of honeys. This article can contribute to the evaluation of honeys commercialized in Brazil and can be applied in the control of the physicochemical and toxicological quality of these foods.

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AFSJ, FARS and MGAK designed the study. MCSJ and FARS collected and processed the honey samples. AFSJ, GCB and MGAK carried out honey digests, analysis by ICP OES and multivariate analysis. AFSJ, GCB, MCSJ, HIFM, FARS and MGAK wrote the manuscript. All authors read and approved the manuscript.

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