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ORIGINAL RESEARCH ARTICLE

Use of the electronic tongue as a tool for the characterization of *Melipona scutellaris* Latreille honey

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This study aimed to characterize honey of *Melipona scutellaris* regarding its physicochemical parameters using the electronic tongue (e-tongue) technique combined with the multivariate statistical analysis for honey differentiation. Physicochemical parameters were evaluated following official methods of chemical analyses. A potentiometric electronic tongue with 16 cross-sensitivity sensors was used for the analysis. The Principal Component Analysis and the Cluster Analysis distinguished two groups for the sample set in the evaluation of physicochemical parameters, similar to results found using the electronic tongue. We verified a correlation greater than 0.70 between the profile of potentiometric signals and values of pH, ashes, electrical conductivity, HMF, diastase activity, reducing sugars, and apparent sucrose. The combined use of the electronic tongue with the statistical analysis showed the similarity between samples through the formation of two groups of the sample set. The electronic tongue may be used as a complement to traditional techniques of analyses to determine honey physicochemical parameters, constituting a promising tool in association with the multivariate statistical analysis.

Keywords: cluster analysis; stingless bees; e-tongue; sensors; potentiometric signal; PCA

Introduction

Researchers from different countries have studied methods for determining physicochemical parameters and metals in honey (Aghamirlou et al., 2015; Ahmida et al., 2017; Biluca et al., 2016; Frausto-Reyes et al., 2017; Khan et al., 2016; Manzanares et al., 2017; Nascimento et al., 2015a; Silici et al., 2016). However, these studies encompass honey of *Apis mellifera* Linnaeus, 1758 (Hymenoptera, Apidae, Apini). Stingless bees (Meliponini), especially from the genus *Melipona*, are also good honey producers, with different physicochemical and sensorial characteristics. Some of the main challenges in stingless bees breeding lie in understanding the legislation that regulates stingless bees breeding and determining the market quality of its products (honey, pollen, and geopropolis) (Carvalho et al., 2013).

Honey produced by *Melipona scutellaris* Latreille, 1811, presents sensorial and physicochemical characteristics different from the honey of *A. mellifera*, which is more popular and more consumed. Honey of *M. scutellaris* (stingless bee) has higher water content, higher acidity, and is less viscous than the honey of *A. mellifera*. *M. scutellaris* honey has lower sugar content, and the flora visited and enzymes of bees influence its aroma and flavor (Almeida-Muradian et al., 2013; Biluca et al., 2016; Carvalho et al., 2013; Nascimento et al., 2018; Tsutsumi & Oishi, 2010).

Honey of stingless bees is characterized in some regions in Brazil; however, these studies use different bees species and different numbers of samples from diverse floral origins, which limit commercialization of this honey, requiring studies for standardization, regulation, and knowledge of Meliponini honey composition (Biluca et al., 2016; Jaffé et al., 2015; Sousa et al., 2016). Given the great species diversity of this group of bees in Brazil (Jaffé et al., 2015; Michener, 2013), characterization of their products to determine quality standards is of utmost importance. In this sense, the electronic tongue (e-tongue) analysis is an important tool to generate information for the standardization of these products. The e-tongue comprises an analytical instrument to evaluate liquids made by a system of chemical multisensors (arrays of nonspecific chemical sensors) low selective, with high stability and cross-sensitivity to different species in solution; nevertheless, it is still underused for the analysis of bee products (Vlasov & Legin, 1998; Vlasov et al., 2005).

The e-tongue was designed especially for flavor analyses; however, it has applications in several industries, such as the pharmaceutical, food, and beverage (Latha & Lakshmi, 2012). This e-tongue analysis provides a

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Table 1. Conditions of analyses of ICP OES for metal quantification in *Melipona scutellaris* honey.

Parameters – ICP OES		Conditions of analysis
Potency RF		1150 W
Nebulization flow		0.70 L/min
Gas flow auxiliary		0.50 L/min
Internal Standard		Yttrium (Y)
Integration time and reading		15 s
Purity of Gas (Argon)		99.999%
Metal	Wavelength (nm)	Limit of detection (LD) (mg/kg)
Cd – cadmium	226.5 axial	0.005
Cr – chromium	267.7 radial	0.002
Cu – copper	324.7 radial	0.005
Ni – nickel	231.6 axial	0.002
Pb – lead	220.3 axial	0.010
Zn – zinc	213.8 axial	0.002

general signal profile representative of the sample analyzed, allowing to correlate with qualitative and quantitative information obtained from traditional analyses, such as the physicochemical analyses (Dias et al., 2015; Ha et al., 2015; Juan-Borrás et al., 2017; Veloso et al., 2018). In addition, the simplified procedure of sample preparation is one of the main advantages of this analytical technique, as it does not require the use of chemical products (Major et al., 2011).

The e-tongue uses a range of sensors that respond to sensory parameters on salty, sour, sweet, and bitter tastes. Interpretation of complex data set generated by sensors of this analytical instrument is performed by the multivariate statistical analyses, such as the Principal Component Analysis (PCA), the Cluster Analysis, the discriminant function analysis, the non-linear regression, among others (Baldwin et al., 2011; Kalit et al., 2014). Thus, this study assessed honey of *M. scutellaris* in terms of physicochemical parameters using the e-tongue analytical technique combined with the multivariate statistical analyses to evaluate the possibility of differentiation or association of honeys by the technique used.

Materials and methods

Sampling

Samples were collected at a meliponary in the municipality of Lauro de Freitas, metropolitan region of Salvador, Bahia, Brazil (S′12°50′38.1″; W′038°21′12.1″, altitude 59 m). Nine honey samples of *M. scutellaris* Latreille, 1811 (Hymenoptera, Apidae, Meliponini) were collected, each composed of approximately 250 g. The sampling was carried out from August/2014 to July/2015.

Sampling occurred in 12 months, and in May (autumn), June, and July (winter) there was no honey production in the colonies of *M. scutellaris* in the study site for the collection of honey needed for the analyses in these months, due to the lower food availability in the field as well as, possible intrinsic bioecological aspects of this stingless bee species.

Physicochemical analyses and determination of metals concentration

Initially, the Lugol reaction adulteration test was performed according to the methodology of Instituto Adolfo Lutz (IAL) (2008). We used a solution of each sample composed of 10 g of honey and 20 mL of distilled water. The sample was homogenized on IKA C MAG-H57 shaker plate with a magnetic bar inserted into the sample solution. After homogenization, we added 0.5 mL of Lugol solution and checked for a change in the solution color. This reaction indicates the presence of dextrin and starch and is considered positive when the final color is violet or blue.

We used the official analysis method of the Association of Official Analytical Chemists (AOAC) (1990), International Honey Commission (IHC) (Bogdanov, 2009), and Codex Alimentarius Commission (CAC) (2001) to evaluate the physicochemical parameters. The color of honeys was determined using a spectrophotometer (WPA Lightwave II model) operating at an absorbance range of 560 nm, in a quartz cell, used as blank glycerin. The value found (absorbance, nm) was used for color classification by the Pfund scale (mm) (Marchini et al., 2004; Vidal & Fragosi, 1984).

Additionally, we analyzed the parameters of moisture (AOAC, 1990 method number 969.38), free acidity, and pH (AOAC, 1990 method number 962.19). We also checked the diastase activity (AOAC, 2005 method 920.180, with modifications in the reading time of samples in a spectrophotometer, performed at intervals of 1 h), hydroxymethylfurfural (AOAC, 1990 method number 980.23), reducing sugars, and apparent sucrose (CAC, 2001), with adaptation from the methods described by Lane and Eynon (1934) and Marchini et al. (2004). For conductivity and ashes, a conductivity meter (Tecnal, R-Tec-04P-MP) according to the Harmonized Methods of the European Honey Commission and the ashes content (%) was calculated using the equation, total ash (%) $=$ 0.083*EC-0.092. All physicochemical parameters were analyzed in triplicate.

Cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), nickel (Ni), and zinc (Zn) were selected for this study. The samples were prepared following the acid digestion method (nitro-perchloric) proposed by Malavolta et al. (1989) and Krug (2008). We used 2 g mass of each honey sample. All glassware used was placed in 10% HNO_3 for 24 h for decontamination. We used a standard solution (blank solution) containing only acids, which were also submitted to the same procedures for honey samples digestion.

For the analyses, we used reagents of certified analytical grade. We used nitric acid 65% (E. Merck, Darmstadt, Germany) and concentrated perchloric acid from Sigma Chemicals Co. (St Louis, MO, EUA) for acid digestion of the samples. For dilution, we used ultrapure water (Milli-Q Millipore 18.2 MΩ.cm). Standard solutions of metals used for calibration were produced by diluting 1000 mg/L solution of each Sigma metal Chemicals Co. (St Louis, MO, USA). Metals concentration in the samples was determined using the Inductively Coupled Plasma Optical Emission Spectrometry technique (ICP OES). The ICP (Spectrometer) Thermo Scientific iCAP 6000 Series, Model 6300 Duo, was used to determination of metals. The conditions of the analyses of ICP OES are presented in Table 1.

E-tongue analysis

A potentiometric e-tongue with a sensor array comprising 16 cross-sensitivity indicator electrodes and a double junction Ag/AgCl reference electrode was used for the honey analyses. The indicator electrodes were built with lipidic polymeric membranes, which were prepared using combinations of four different lipid additives (octadecylamine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid; 3%) with each of the four different plasticizers (2-nitrophenyl-octylether, tris(2-ethylhexyl)phosphate, bis(1-butylpentyl) adipate, dibutyl sebacate; 65%) and high molecular weight polyvinyl chloride (PVC; 32%). All reagents were from Fluka (minimum purity $\geq$ 97%). The sensor array used in this study follows the schematic presented in the work of Dias et al. (2015). The multi-sensor system was connected to a data logger (Campbell Scientific CR1000) to obtain the potentiometric signal of each sensor through a computer using the software BenchLink Data Logger. Honey solutions composed of 2 g of honey diluted in 50 mL of distilled water were prepared for e-tongue analysis. These solutions were analyzed in sequence and in triplicate, under stirring (IKA C MAG-H57 stirrer). The analysis time of each sample was 4 min for potentiometric signal stabilization, being the final potential values recorded for each e-tongue analysis. As a final analytical result, the mean of the three signal profiles obtained for each sample was calculated. In this analytical method, a data matrix constituted by 9 honey samples and 16 potentiometric signals was obtained.

Statistical analysis

The experiment was conducted in a completely randomized design. The analyses for each parameter were performed in triplicate. The descriptive statistical analysis was performed by calculating the mean and standard deviation. The Pearson correlation analysis was performed to verify the linear dependence between the pairs of variables. Based on the physicochemical parameters and analytical results of the electronic tongue, two techniques of multivariate, the unsupervised statistical analyses were used: the Principal Component Analysis (PCA) and Cluster Analysis (CA). Both PCA and the CA can be used together to check the grouping of separated or overlapping clusters.

The PCA, based on the Pearson correlation matrix (auto-scaled), was used to verify the variability of honey samples. The correlation matrix was also used for the diagnosis of multicollinearity between the parameters. The Principal Components (PC) was calculated by linear combinations of the original variables with eigenvectors. The first Principal Component (PC1) explained the highest percentage of the total variance, the second Principal Component (PC2) explained the second highest percentage and so on until all variance was explained. In a dataset with p variables, the random vector $\boldsymbol{x}' = [x_1,\ x_2,\ \ldots, x_p]$ has a correlation matrix R with eigenvalue-eigenvector $\lambda_1 \geq \lambda_2 \geq \ \ldots \geq \ \lambda_p \geq 0$ (Johnson & Wichern, 2007). The ith Principal Component is given by linear combinations:

$$PC_1 = \mathbf{e}_1^t\boldsymbol{x} = e_{11}x_1 + e_{12}x_2 + \ldots + e_{1p}x_P$$

$$PC_2 = \mathbf{e}_2^t\boldsymbol{x} = e_{21}x_1 + e_{22}x_2 + \ldots + e_{2p}x_P$$

$$\vdots$$

$$PC_i = \mathbf{e}_i^t\boldsymbol{x} = e_{i1}x_1 + e_{i2}x + \ldots + e_{ip}x_P$$

Where: e_{ip} is the p^{th} eigenvector and x_P is the p^{th} value of the original variable. The choice of the Principal Components that explain most data variation was determined with the scree plot that shows the relative variance proportion of all components in decreasing order.

The hierarchical Cluster Analysis method of Ward, based on the dissimilarity matrix generated by Euclidean distance among the samples, was used as a formation criterion of groups in the dendrogram (Johnson & Wichern, 2007). The cophenetic correlation coefficient (CCC) proposed by Sokal and Rohlf (1962) was used to evaluate the cluster consistency. All statistical analyses were performed using packages of open source of the statistical program R version 3.4.4 (R Core Team, 2018), at 5% significance level.

Table 2. Descriptive analysis of the physicochemical parameters and concentration of metals determined in honey samples of *Melipona scutellaris*.

Sample/Month of collection	Color (mm)*	Moisture (%)	pH	Acidity (meq/kg)	EC (μS/cm)	Ashes (%)	HMF (mg/kg)	Diastase (Göthe)	RS (%)	Suc (%)
1- Aug 2014	LA	27.43	3.86	56.00	337.70	0.22	33.83	0.11	61.27	1.58
2- Sep 2014	LA	29.17	3.71	88.33	241.53	0.05	1.80	0.04	68.44	2.05
3- Oct 2014	LA	29.50	3.29	47.00	318.23	0.13	1.25	0.06	70.25	2.75
4- Nov 2014	ELA	28.17	3.73	69.00	226.67	0.14	9.53	0.07	71.66	1.64
5- Dec 2014	LA	30.50	4.06	65.00	306.90	0.15	9.63	0.08	73.90	0.86
6- Jan 2015	LA	29.13	4.10	12.00	615.37	0.30	23.60	0.11	76.97	4.33
7- Feb 2015	LA	28.37	4.73	22.33	537.63	0.24	27.69	0.11	80.08	2.06
8- Mar 2015	ELA	29.80	4.28	59.00	623.90	0.47	20.06	0.15	88.52	0.17
9- Apr 2015	LA	30.43	4.17	37.33	610.43	0.43	39.87	0.12	56.31	0.97
Mean	–	29.17	3.99	50.67	424.26	0.24	18.58	0.10	71.93	1.82
±SD	–	1.03	0.41	23.84	169.13	0.14	13.88	0.03	9.64	1.21
Min	–	27.43	3.29	12.00	226.67	0.05	1.25	0.04	56.31	0.17
Max	–	30.50	4.73	88.33	623.90	0.47	39.87	0.15	88.52	4.33
Brasil (2000)[a]	ww – DA	Max. 20	–	Max. 50	–	Max. 0.6	Max. 60	Min. 8.0	Min. 65	Max. 6.0
ADAB (2014)[a]	ww – DA	20–35	–	Max. 50	–	Max. 0.6	Max. 10	Max. 3.0	Min. 60	Max. 6.0

Sample/Month of collection	Metal (mg/kg)						
	Cd	Cu	Cr	Ni	Pb	Zn	–
1- Aug 2014	<LD	0.55	0.29	0.08	<LD	0.68	–
2- Sep 2014	<LD	0.24	0.40	0.02	<LD	0.83	–
3- Oct 2014	<LD	0.53	0.56	0.02	<LD	1.93	–
4- Nov 2014	<LD	0.38	0.40	0.03	<LD	1.04	–
5- Dec 2014	<LD	0.22	0.17	0.03	<LD	2.01	–
6- Jan 2015	<LD	0.49	0.55	0.02	<LD	0.48	–
7- Feb 2015	<LD	0.40	1.83	0.01	<LD	0.50	–
8- Mar 2015	<LD	0.31	0.90	0.00	<LD	0.96	–
9- Apr 2015	<LD	0.47	0.22	0.02	<LD	0.29	–
Mean	–	0.40	0.59	0.03	–	0.97	–
±SD	–	0.12	0.51	0.02	–	0.62	–
Min	–	0.22	0.17	0.00	–	0.29	–
Max	–	0.55	1.83	0.08	–	2.01	–
Brazilian legislation[b]	0.50	10.00	0.10	5.00	0.50	50.00	–
WHO[c] (mg/kg/dia)	0.007	0.05–0.50	0.00003–0.00013	0.50	0.05	0.30–1.00	–

Color = Pfund scale (mm), DA = Dark amber, EC = Electrical conductivity, ELA = Extra light amber, HMF = Hydroxymethylfurfural, LA = Light amber, Max. = maximum, Min. = minimum, RS = Reducing sugars, SD = Standard deviation of the mean, Suc = Apparent sucrose, ww = White water, and LD = Detection limit, Cd (LD <0.005 mg/kg) and Pb (LD <0.010 mg/kg),

[a]Brazilian legislation;

[b]Brasil (1965, 1998, 2009) (mg/kg),

[c](Recommended Ingestion – WHO = World Health Organization (mg/kg/dia), 1982, 1991, 1993, 1996).

Results

Physicochemical analyses and determination metals concentration

The honeys analyzed by the Lugol reaction showed a negative result for the presence of dextrin and starch. The samples presented color, moisture, ashes content, diastase activity (DA), and apparent sucrose in accordance with the Brazilian legislation specific for *Melipona* honey (Agência Estadual de Defesa Agropecuária da Bahia [ADAB], 2014). For hydroxymethylfurfural (HMF), five samples presented values according to the threshold established by Brazilian legislation (ADAB, 2014) and one sample did not meet the requirements established for reducing sugars by Brasil (2000) and ADAB (2014) (Table 2).

Metals Cu, Cr, Ni, and Zn were detected in the samples, and only Cr showed an average concentration above the limit (0.10 mg/kg) established by Brazilian legislation (Brasil, 1965). Cadmium (LD <0.005 mg/kg) and lead (LD <0.010 mg/kg) showed concentrations below the detection limit (Table 2).

The Principal Component Analysis (PCA) in Figure 1 shows that two groups were formed (group I – samples 2 to 5 and group II – samples 6 to 9) for the average values for the physicochemical parameters, and only sample 1 did not clearly integrate the groups formed. The first two PC explained 58.98% of the total variability of the data analyzed. Only group I had a contribution of five physicochemical parameters (acidity, color, apparent sucrose, zinc, and nickel metals), while group II was influenced by nine parameters (reducing sugars, apparent sucrose, electrical conductivity (EC), ashes, pH, DA, HMF, Cu, and Cr) (Figure 1). Group II presented higher levels for pH, EC, ashes, DA, and HMF than group I.

Sample 7 showed the greater influence of ashes content, diastase activity (DA), electrical conductivity (EC), pH and Cr concentration. Sample 8 showed greater contribution of the content of reducing sugars. Samples 9 and 6 showed greater similarity, possibly influenced by HMF values, and similarity between these samples was confirmed by the correlation coefficient of the Euclidean distance matrix ($d = 4.299$) (Table 3). The other samples were influenced by different physicochemical parameters.

E-tongue analysis

The potentiometric signals of the electronic tongue varied in amplitude from 2.50 to 5.31 V. The profiles obtained were similar showing that sensors responded similarly to all samples (Table 4), as sensor 1 presented a variation from 69.55 to 74.75 V with an average of 71.19 V for the nine samples evaluated. Outliers presence was not detected (Figure 2). The results of each sensor through the correlation matrix showed that the correlation coefficient varied between $r = 0.33$ and $r = 0.99$, and 94.54% of the correlations between the sensors presented $r > 0.75$, as expected because they are cross-sensitivity sensors.

The PCA for potentiometric signals of the electronic tongue revealed the formation of two groups for the sample set (Figure 3). The first two Principal Components explained 98.87% of the total variability of the analyzed data, while the others (7 Principal Components) contributed to less than 1.00% for the final model. This behavior of the samples was also observed in the Cluster Analysis by the Ward method (Figure 4). The Euclidean distance matrix also revealed the similarity between the samples (Table 3).

Discussion

Physicochemical analyses and determination of metals concentration

The parameters evaluated in the samples reflect the quality as to purity (ashes), deterioration (DA and HMF), and maturity (moisture, reducing sugars, and apparent sucrose) of honey (Table 2). Thus, honey of *M. scutellaris* produced in the metropolitan region of Salvador, Bahia (urban environment), complies with quality requirements for the physicochemical parameters.

Moisture average in the samples meets the requirements of the Brazilian legislation (ADAB, 2014), specific for the honey of stingless bees of the genus *Melipona*, with values ranging from 20 to 35% for honeys kept under refrigeration (6 to 10 °C). The honeys evaluated in this study were kept under refrigeration (6 to 8 °C) until analyses. However, moisture in all samples was higher than the limit established for honey by Brasil (2000) (Table 2).

The high moisture content (average = 29.17%) among the samples shows a characteristic factor of honey of stingless bees (Meliponini), a bottleneck of meliponiculture (Camargo et al., 2017; Venturieri et al., 2012; Villas-Bôas, 2018), because honeys with moisture higher than 20% are more susceptible to deterioration due to the fermentation process (Almeida-Muradian et al., 2013; Pita-Calvo & Vázquez, 2017). Almeida-Muradian et al. (2013) and Biluca et al. (2016) also observed high water content in honey from stingless bees. The European Union establishes a limit of 20% of the water content in honey (EU Directive 110/2001). The water content is of great relevance to ensure honey shelf life because adequate water content enables honey to remain stable and resist deterioration due to the fermentation process caused by yeast (Pita-Calvo & Vázquez, 2017). Due to the high water content, honey from stingless bees is usually stored under refrigeration soon after harvest to avoid deterioration (Camargo et al., 2017).

Free acidity presented average values above the threshold 50.00 meq/kg established by Brazilian

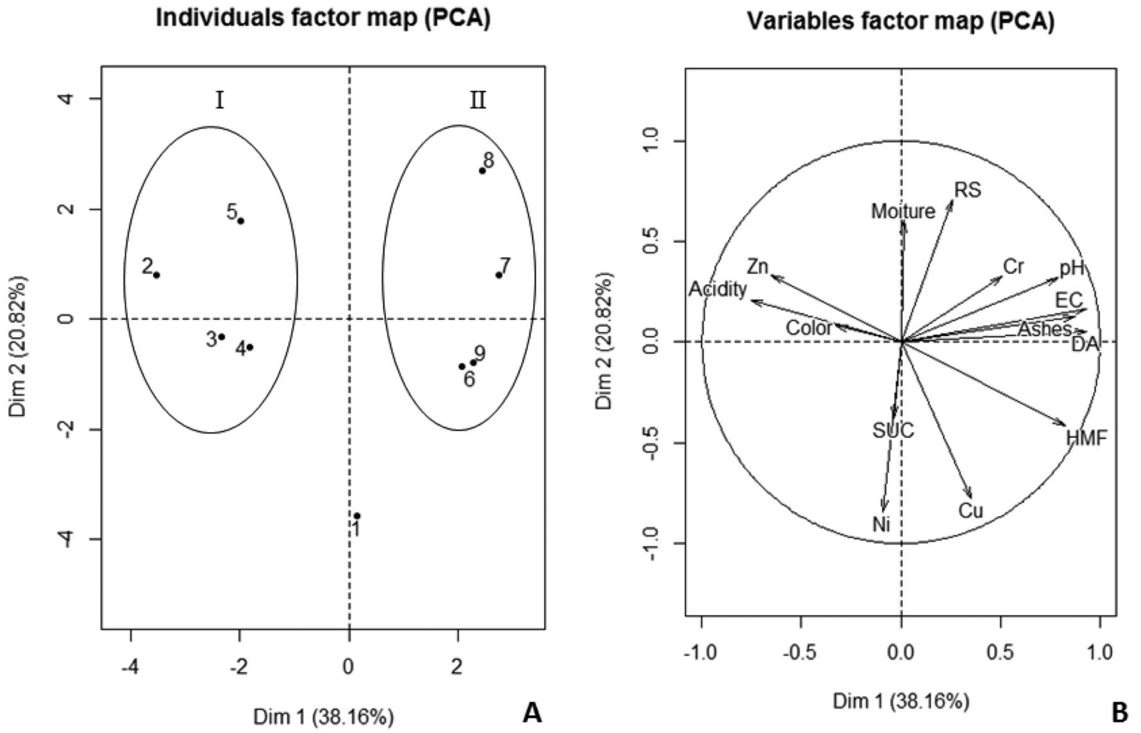

Figure 1. Principal Components Analysis (PCA) of physicochemical parameters and concentration of metals for *Melipona scutellaris* honey samples (A and B); Groups I and II. DA (Diastase Activity), EC (Electrical Conductivity), HMF (Hydroxymethylfurfural), RS (Reducing Sugars), Suc (Apparent Sucrose).

Table 3. Euclidean distance matrix between honey samples of *Melipona scutellaris*.

Euclidean distance matrix: Physicochemical								
Sample	1	2	3	4	5	6	7	8
2	6.305							
3	5.358	4.430						
4	3.934	4.160	3.299					
5	5.800	4.295	3.953	3.374				
6	5.031	6.467	4.836	4.948	5.771			
7	5.769	6.631	6.063	5.321	5.979	3.771		
8	6.440	6.870	6.235	5.366	4.875	4.976	4.241	
9	4.884	6.523	5.999	5.666	5.597	4.299	5.160	4.756
Euclidean distance matrix: e-tongue								
Sample	1	2	3	4	5	6	7	8
2	7.694							
3	5.885	4.474						
4	7.144	2.545	2.457					
5	6.155	2.815	2.178	1.052				
6	1.723	6.336	5.273	6.162	5.211			
7	1.607	8.217	6.988	8.068	7.108	1.941		
8	1.688	8.709	7.319	8.475	7.502	2.412	0.583	
9	1.692	8.978	7.445	8.677	7.701	2.693	0.947	0.454

legislation in five samples (Table 2). This indicates the possible occurrence of a fermentative process in samples considering that food acidity is related to the presence of organic acids. High food acidity can indicate the beginning of the fermentation process by yeasts that deteriorate organic acids (Hassan et al., 2015; Hazan et al., 2004). Our results for free acidity differ from values recorded by other authors, such as Biluca et al. (2016) and Nascimento et al. (2015b), who reported lower average values. Our results are also different from those found by Sousa et al. (2013), who reported an average free acidity of 86.20 meq/kg, in the honey of *M. scutellaris*.

In samples 2 to 5, HMF values comply with the threshold allowed by the legislation for *Melipona* honey (ADAB, 2014). HMF is used as indicator of honey freshness. High HMF levels in honey indicate possible excessive heating during processing or storage conditions. In

Table 4. Mean profile of potentiometric signals (V) of the electronic tongue for *Melipona scutellaris* honey samples.

Sensors (S)	Mean	±SD	Median	Min	Max	Range
S1	71.19	1.75	70.59	69.55	74.75	5.20
S2	69.33	1.84	68.66	67.34	71.90	4.56
S3	45.75	1.04	45.50	44.65	47.15	2.50
S4	66.04	1.07	65.68	64.87	67.50	2.63
S5	14.43	1.19	14.02	13.14	15.96	2.82
S6	27.41	1.74	26.73	25.49	29.63	4.14
S7	48.00	1.80	47.20	46.23	51.06	4.83
S8	63.85	1.73	63.23	61.97	65.94	3.97
S9	−34.88	1.83	−35.36	−36.63	−31.32	5.31
S10	−43.36	1.81	−43.86	−45.19	−40.03	5.16
S11	5.08	1.77	4.39	3.16	7.23	4.07
S12	−10.75	1.49	−11.33	−12.35	−9.10	3.25
S13	80.10	1.77	79.49	78.21	82.72	4.51
S14	79.89	2.00	78.98	77.71	82.17	4.46
S15	53.95	1.70	53.14	52.16	55.96	3.80
S16	63.87	1.77	63.02	62.01	65.99	3.98

SD = Standard deviation of the mean, Min = minimum, Max = maximum, S1–S4 = electrodes with 4 additives (fixed sequential order of octadecylamine, oleyl alcohol, methyltrioctylammonium chloride and oleic acid) and plasticizer 2-nitrophenyl-octylether, S5–S8 = electrodes with 4 additives and plasticizer tris(2-ethylhexyl)phosphate, S9–S12 = electrodes with 4 additives and plasticizer bis(1-butylpentyl), S13–S16 = electrodes with 4 additives and plasticizer adipate, dibutyl sebacate.

addition, freshly harvested honey has lower HMF content (Biluca et al., 2016; Pita-Calvo & Vázquez, 2017). The Codex Alimentarius and the European Union establish a threshold of HMF in the honey of 40.00 mg/kg, and for honeys from tropical countries, the threshold is 80.00 mg/kg. The honey of *M. scutellaris* evaluated in our study presents quality for this physicochemical parameter, indicating the need to evaluate the threshold established by ADAB (2014) for this important parameter for honey quality.

The metals Cd and Pb had concentrations below the limit of detection (LD $<$0.005 mg/kg) in the honeys evaluated. These results are relevant, as Cd and Pb are not required for the metabolism of humans and animals (Dhahir & Hemed, 2015; Nazari, 2011). The metals Cd and Pb are of great concern for environmental pollution and their contamination usually has an anthropogenic source. Additionally, these metals are listed as potentially toxic and their presence in food is prohibited in some countries (Akbari et al., 2012).

The PCA for the physicochemical variables and metals concentration revealed the formation of two groups for the sample set (Figure 1). The Euclidean distance matrix also revealed the similarity between the samples (Table 3). The samples were collected in different months throughout the sampling period, and despite belonging to the same bee species (*M. scutellaris*), the floral origin of samples was possibly diversified. According to Pita-Calvo and Vázquez (2017), nectar composition influences honey physicochemical characteristics. Sample 1 did not clearly integrate into the groups formed due to its botanical origin and climatic conditions of the honey production period, which may have conferred different characteristics to other samples. This honey was collected in August 2014 and this month represents a transition period between the rainy season and dry season in northeastern Brazil. In the PCA, sample 1 showed greater influence of physicochemical parameters of apparent sucrose, Cu, and Ni.

E-tongue analysis

The PCA revealed that the e-tongue formed two sample groups, indicating distribution in two-dimensional spaces in the pair of the PC used (Figure 3), with similar results found for physicochemical parameters (Figure 1).

Sample 1 in the PCA for the analysis by e-tongue presented an integral part of samples of group II (Figure 3). The PCA for the analysis of physicochemical parameters and metals, however, showed no clear integration of any of the groups (Figure 1). The other samples presented similar behavior in the PCA for the analysis by e-tongue and physicochemical parameters. Possibly, the e-tongue sensors were able to associate similar characteristics of the samples, thus, the complex data set generated by the sensors allowed more evident grouping of samples that formed group II.

The Euclidean distance matrix for the samples analyzed by the e-tongue technique showed that sample 1 was more similar to samples 6 ($d = 1.72$), 7 ($d = 1.60$), 8 ($d = 1.67$) and 9 ($d = 1.69$), as indicated in the PCA (Figure 3). When comparing the samples belonging to the sample set formed by groups I and II, we observed a low similarity between these groups with linear coefficient $d \leq 5.21$ (Table 3).

The fact that the samples were collected in different months, possibly with different botanical origin, could explain the low similarity in physicochemical parameters in some samples analyzed, confirmed by the e-tongue analysis (Table 3). The distinct profile of each sample may be related to the contribution to nectar volume of each plant species for honey composition, besides climatic conditions that influence honey characteristics (Pita-Calvo & Vázquez, 2017).

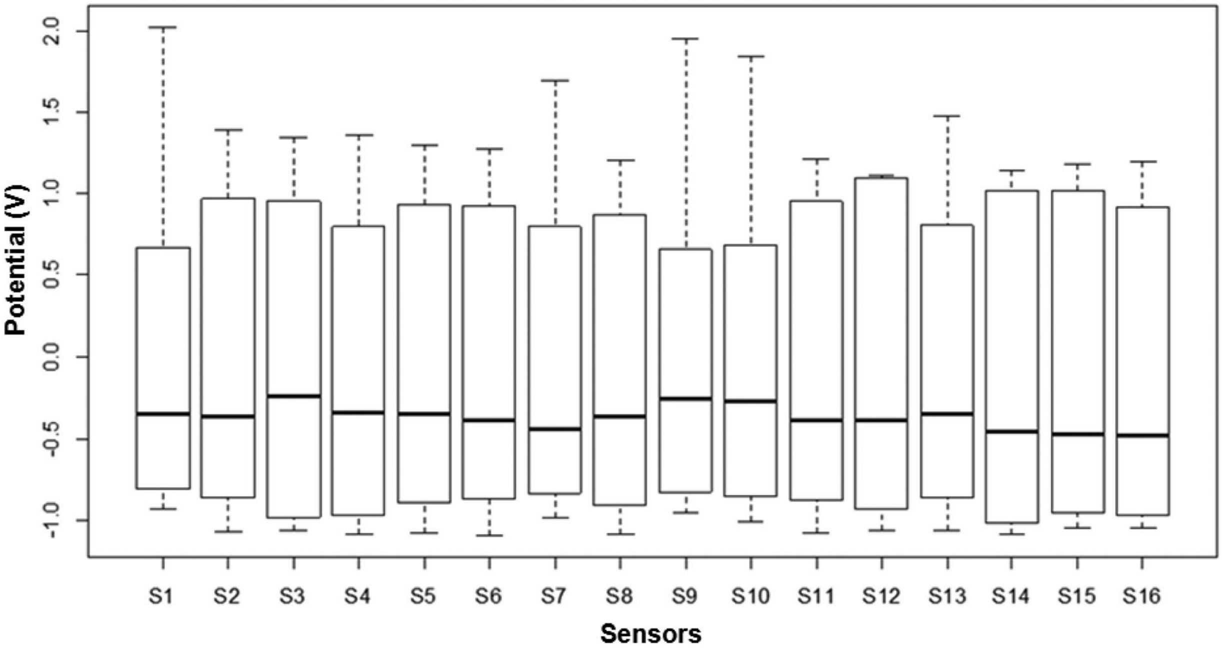

Figure 2. Boxplot of the potentiometric signals (V) of the electronic tongue for honey samples of *Melipona scutellaris*.

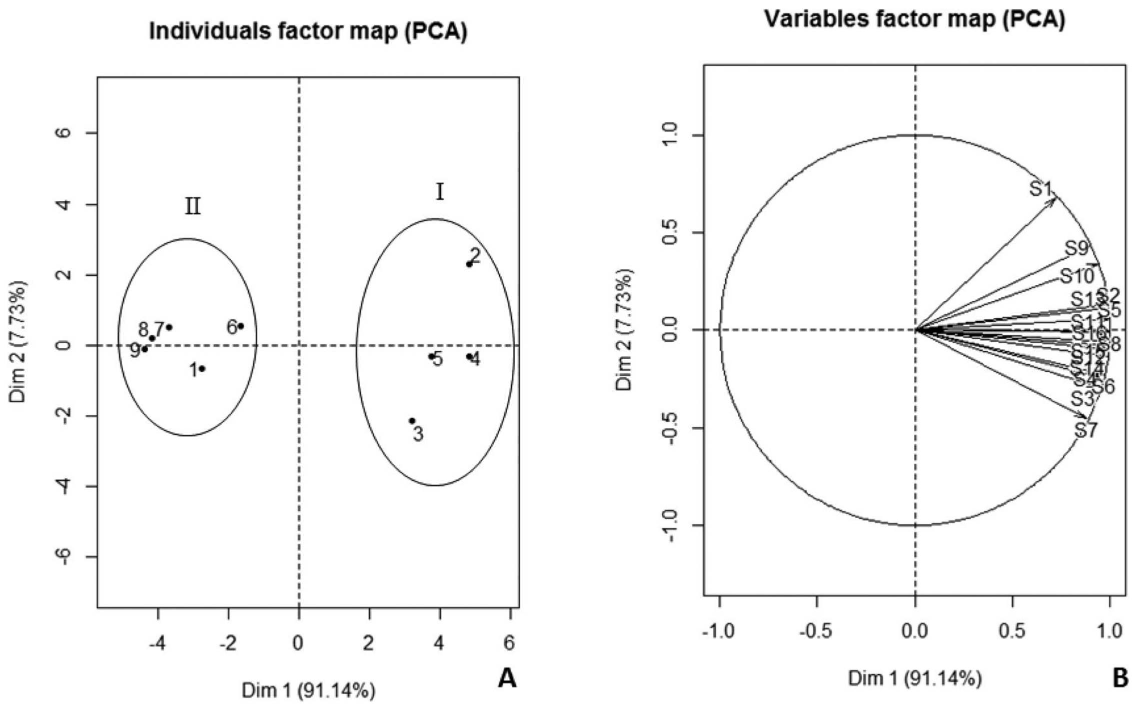

Figure 3. Principal Components Analysis (PCA) of potentiometric signals of the electronic tongue for samples of *Melipona scutellaris* honey, A = samples; B = e-tongue sensors and S = sensors.

To verify this behavior, the formation of two groups was related to physicochemical parameters and concentration of metals in samples and we searched for correlations between each PC of the potentiometric signals of the e-tongue and 17 variables analyzed (physicochemical and metals). In this study, high correlations ($p < 0.05$), $r > 0.70$ occurred between pH values in PC1 ($r = 0.79$); color PC4 ($r = 0.81$); ashes PC1 ($r = 0.87$); electrical conductivity PC1 ($r = 0.93$); HMF PC1 ($r = 0.82$); diastase activity PC1 ($r = 0.93$); reducing sugars PC2 ($r = 0.71$) and apparent sucrose PC3 ($r = 0.78$). In the other linear relations, values of correlation coefficient were lower than $r = 0.70$.

In addition, we performed the Cluster Analysis by the Ward method for honey samples of *M. scutellaris* in relation to the variables evaluated (physicochemical, metals, and e-tongue analysis). The sample set presented again the formation of two groups (Figure 4). For the analysis of physicochemical parameters using the e-tongue technique, samples 2, 3, 4, and 5 form

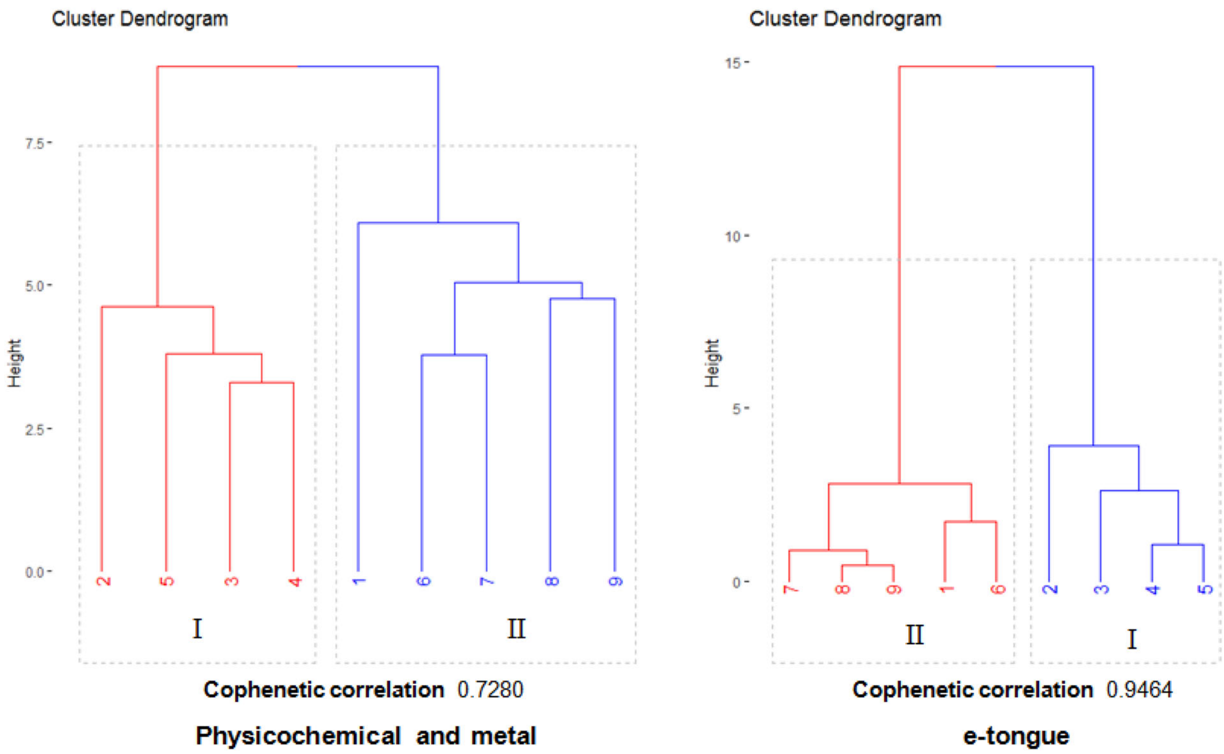

Figure 4. Cluster Analysis by the Ward method for *Melipona scutellaris* honey samples.

group I, and the others constitute group II, confirming that the samples share similar physicochemical characteristics, although they were collected in different sampling months (Table 2).

The results show that the potentiometric signals from the analysis of honey samples are related to their chemical content in reducing sugars, ashes, diastase activity, moisture, and HMF, since they presented high correlations ($r > 0.70$). Possibly other compounds may have influenced the signal profile of the e-tongue analysis, thus, complementary chemical analyses, such as the determination of phenolic compounds, aided the discussion of the results. However, the results indicate the possibility of differentiating honey samples according to their physicochemical characterization and metals using the e-tongue analysis.

The physicochemical parameters of acidity, electrical conductivity, and pH stand out in the analysis of authenticity and adulteration of honey, along with the analysis with electronic tongue, considered a fast, easy and accurate method for detecting honey adulteration that could be used *in situ* by beekeepers (Oroian et al., 2018; Oroian & Ropciuc, 2019). In our study, the pH, electrical conductivity, and diastase activity showed high correlations with the potentiometric signals of e-tongue, indicating the relevance of this analytical instrument for honey characterization.

The PCA and the Cluster Analysis by the Ward method showed that the methodology (e-tongue combined with the statistical analysis) differentiated the honeys evaluated (Figures 3 and 4). Dias et al. (2015) also observed that most honeys evaluated in their study could be accurately classified in terms of botanical origin as monofloral or polifloral honey with the use of the e-tongue allied to the statistical analysis methods of multiple linear regression. The authors report results that indicate the possible use of this analytical technique as a complement to the pollen analysis traditionally used.

According to Juan-Borrás et al. (2017), the use of e-tongue to differentiate honeys of botanical origin (orange blossom, rosemary, thyme, sunflower, winter savory, and honeydew honey) could be a quick, easy, and less costly option for the honey-packaging sector. It is an assessment to confirm honey authenticity regarding its botanical origin, which should be correctly mentioned in the product label.

This analytical instrument (e-tongue) allows verifying the authenticity of floral honey combined with the physicochemical analysis, constituting a complementary and rapid mechanism of honey evaluation (Oroian & Ropciuc, 2019). In addition, e-tongue may be useful for honey differentiation in sensory evaluations to infer preferences of consumers by certain honey types. According to Wang and Liu (2019), e-tongue could mimic the sensory perception of human taste and compensate for the weaknesses of panels in sensory evaluation. E-tongue could also be used in assessments of food safety to determine physicochemical parameters and metals.

We observed the possible use of e-tongue to differentiate *M. scutellaris* honey through the determination of physicochemical parameters and metals (Table 3 and

Figures 3 and 4). Di Rosa et al. (2018) found a similar result for *A. mellifera* honey. However, these authors used this analytical equipment along with the melissopalynological analysis, and were able to discriminate the different honey types by the pollen profile of each sample.

As honey prices depend on the characteristics of color and floral origin, and monofloral honey is more valued than polifloral honey, Sousa et al. (2014) constructed an e-tongue for floral classification of honey and found that the use of e-tongue combined with the Linear Discriminant Analysis (LDA) allowed 100% of accurate classifications of monofloral honey. The results of our study, as well as those performed by other authors (Di Rosa et al., 2018; Oroian et al., 2018; Oroian & Ropciuc, 2019; Sousa et al., 2014; Wang & Liu, 2019), demonstrate the versatility of e-tongue for characterization and determination of quality standards of beehive products, along with analyses to determine physicochemical, metal, palynological, or sensory parameters.

Dias et al. (2008) studied the development of alternative techniques for honey classification using the e-tongue analysis and found that this analysis allows differentiating honey samples, not perfectly, however, in accordance with the honey botanical origin. According to the authors, e-tongue is promising as a complementary analysis for honey quality control. In this study, we also observed the possibility of differentiating honey samples in terms of physicochemical parameters (Figures 1, 2, and 4 and Table 3). Thus, the e-tongue analysis, a simple method based on metallic potentiometric electrodes, may be useful as a complementary tool to the traditional methods used for honey analyses. In addition, the PCA and the Cluster Analysis are important tools to recognize different patterns between honeys (Escriche et al., 2012; Ulloa et al., 2013; Veloso et al., 2018; Wei et al., 2009).

Conclusions

The e-tongue analysis could be used as complementary to the traditional analytical techniques to determine honey physicochemical parameters. In association with multivariate statistical analyses, e-tongue constitutes a promising tool for determining quality standards of stingless bees honey.

In our study, the combined use of e-tongue with the statistical analysis evidenced the similarity between the samples with the formation of two groups of the sample set. The results showed a greater correlation between the profile of potentiometric signals and the values of pH, ashes, electrical conductivity, HMF, diastase activity, reducing sugars, and apparent sucrose, indicating that these physicochemical parameters are relevant for differentiating honey samples using the e-tongue analysis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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