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Artigo

Physicochemical Characterization and Determination of Metals in *Apis mellifera* L. Honey Produced in a Region Contaminated By Lead

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Caracterização Físico-Química e Determinação de Metais no Mel de *Apis mellifera* L. Produzido em uma Região Contaminada por Chumbo

Abstract: Neste estudo avaliou-se a qualidade do mel de *Apis mellifera* L. produzido em uma região com histórico de contaminação por chumbo há mais de 30 anos. Os parâmetros físico-químicos foram avaliados seguindo os métodos oficiais de análises químicas. As concentrações de metais nas amostras foram determinadas pela técnica de Espectrometria de Emissão Óptica com Plasma Acoplado Indutivamente. As amostras apresentaram diferença estatisticamente significativa. Em relação aos parâmetros físico-químicos, os níveis de metais em todas as amostras atenderam os padrões estabelecidos pela legislação vigente no Brasil. Os metais Cd, Cr, Cu, Fe, Pb e Zn foram detectados nas amostras. Somente alumínio apresentou concentrações abaixo do limite de detecção em todas as amostras. Entre os metais avaliados, apenas o cromo apresentou concentrações em todas as amostras acima do limite estabelecido pela legislação. O mel avaliado atendeu aos requisitos de qualidade quanto aos parâmetros físico-químicos. Os metais determinados e quantificados apresentaram concentrações de acordo com os limites estabelecidos pelas legislações brasileira e internacional, exceto o cromo.

Palavras-chave: Apicultura; controle de qualidade; ICP OES; contaminação ambiental.

Abstract

This study evaluated the quality of *Apis mellifera* L. honey produced in a region with lead contamination for more than 30 years. Physicochemical parameters were evaluated following official methods of chemical analyses. Metal concentrations in samples were determined using the technique Inductively Coupled Plasma/Optical Emission Spectrometry. Samples showed significant differences. Regarding the physicochemical parameters, metal levels in all samples complied with standards established by current legislation in Brazil. The metals Cd, Cr, Cu, Fe, Pb and Zn were detected in samples. Only aluminum showed concentrations below the detection limit in all samples. Among the metals evaluated, only chromium showed concentrations in all samples above the threshold established by the legislation. The honey evaluated complied with quality requirements for physicochemical parameters. Metals determined and quantified presented concentrations in compliance with limits established by Brazilian and international legislations, except for chromium.

Keywords: Beekeeping; quality control; ICP OES; environmental contamination.

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Physicochemical Characterization and Determination of Metals in *Apis mellifera* L. Honey Produced in a Region Contaminated by Lead

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1. Introduction

Honeybees and honey products are known worldwide with a broad spectrum of consumers and beekeeping is an important means for subsistence of small farmers. The best-known and most used bee product is honey[1] and its quality depends on its chemical properties, which are related to the floral origin.[2-3] Thus, physicochemical characterization and determination of metals in honey play an important role in honey quality. Determination of honey physicochemical parameters is used to compare results obtained with standards established by international and national institutions, aiming to ensure honey quality both for domestic consumption and exports.[3,4-7]

Requirements of honey quality refer to sensorial characteristics (color, flavor, aroma and consistency), physicochemical characteristics of maturity (reducing sugars, apparent sucrose, moisture), purity (ash, insoluble solids in water, pollen), and deterioration (acidity, diastase activity, hydroxymethylfurfural).[8] Analyses of honey quality are essential, as honey adulteration is not only illegal, but also a practice that can cause problems of public health. Adulterated honey may contain ingredients prohibited for human consumption, due to their toxic or allergenic potential.[6,9] In addition, honey adulteration compromises reliability of the product domestically and internationally.[10-11]

In their foraging activity, bees collect nectar, honeydew, pollen, plant exudates, and have contact with plants, water, air and soil within their radius of action. If these trophic resources are contaminated, metals can be carried to hive products. The presence of metals (environmental pollutants) at high levels in beehive products compromise product integrity, posing a contamination risk to consumers.[12-15]

Determining metal concentrations is of utmost relevance to evaluate honey quality. The honey evaluated in our study comes from the municipality of Santo Amaro, Bahia State, Brazil. The site has a history of environmental contamination by lead and has been studied for approximately 30 years, since evidence of lead presence was found in the Subaé River and in blood samples from workers of Companhia Brasileira de Chumbo (Brazilian Lead Company).[16] Thus, determining metal concentrations in honey samples from this site can provide information on quality and safety of this product for consumption. Researchers from several countries have evaluated metal concentrations in beehive products. Studies have investigated the level of environmental pollution in areas where bees are reared.[12-15,17] The site where the beekeeping activity is carried out is a very important factor to determine the quality of its products in terms of metal concentrations caused by anthropogenic activities. The presence of metals in honey and pollen, products derived from beekeeping, can threaten human health.[1,18]

Beekeeping is predominant in the region of Santo Amaro, Bahia, and it has been professionalized with the support of the technical assistance of the National Service of Rural Learning, which helped to double honey production in 2017.[19,20] However, honey quality produced in the region is not monitored, especially regarding metal levels. In this context, we evaluated the quality requirements and determined metal concentrations in *Apis mellifera* L. honey produced an area contaminated by lead.

2. Experimental

2.1. Sampling

Samples (n= 11) of approximately 250 g of honey were collected from apiaries (n= 8) in the municipality of Santo Amaro, Bahia State, Brazil (12°32’48”S; 38°42’43”W, altitude 42m). Each sample was collected from a different beehive during the month of February in 2016.

2.2. Physicochemical analyses

Official methods of analyses of Association of Official Analytical Chemists (AOAC),[21] International Honey Commission (IHC)[22] and Codex Alimentarius Commission (CAC)[23] were used to determine the physicochemical parameters. The analyses were performed in triplicate and the following parameters were evaluated:

Moisture: recorded with a portable digital refractometer Atago PAL-22S, expressed in percentage (%).[21]

Color: honey color was classified using a WPA Lightwave II model spectrophotometer at 560 nm to read the samples. Pure glycerin as white was used. The value found (absorbance, nm) was used for color classification by the Pfund scale.[24]

pH: determined with a solution containing 10 g of honey dissolved in 75 mL of distilled water, homogenized and subjected to reading in a Limatec pH meter (model mPA-210).[21]

Acidity: determined by neutralizing the honey solution (10 g of honey dissolved in 75 mL of distilled water) using a solution of sodium hydroxide (NaOH) 0.1 M.[21] for titration, and results expressed in mEq/kg.

Hydroxymethylfurfural: determined by readings at different absorbance scales (wavelengths 284 and 336 nm) in a spectrophotometer of a honey solution, as described by the Association of Official Analytical Chemists,[21] and results expressed in mg/kg.

Diastase activity: determined by spectrometry according to the methodology of Codex Alimentarius Commission (CAC) and Official Methods of Analysis.[21,25] A buffered starch-honey solution was prepared and kept in water bath at 40 °C until absorbance lower than 0.235 nm with a spectrophotometer operating at wavelength

660 nm. The follow-up readings were performed every 5 min. The diastase activity corresponds to the Göthe scale number by dividing 300 by the time spent in minutes to obtain an absorbance lower than 0.235 nm.

Reducing sugars and apparent sucrose: determined according to the method described in CAC, Copersucar and in Nascimento *et al.*,[7,22,26] and results expressed as percentage (%).

Ashes: determined using a conductivity meter (Tecnal, R-Tec-04P-MP) according to manufacturer's recommendations. A solution with 14 g of honey diluted in 50 mL of distilled water was prepared, the reading was performed, and results expressed as percentage (%).

Electrical conductivity: obtained from a solution containing 10 g of honey dissolved in 50 mL of distilled water for each sample. The reading was performed in a model conductivity meter Tecnal (R-Tec-04P-MP), results expressed in µS/cm.

2.3. Phenolic compounds

Phenols and total flavonoids were quantified by spectrometry, respectively, using the methods of Folin-Ciocalteau and aluminum chloride according to Feás *et al.*[27] and the methodologies described by Singleton *et al.*[28] and Woisky and Salatino.[29]

Total phenols: a standard solution of Gallic acid was used for the calibration curve ($y= 0.0099x + 0.0119$; $R^2 = 0.9976$). For the analysis, we used a 0.5 mL aliquot of the honey solution and added 2.5 mL of the Folin-Ciocalteau reagent (10 %) and 2 mL of sodium carbonate (4 %) (v/v). Subsequently, the samples were kept in the dark for 2 h at room temperature (25 °C). Afterward, we performed the absorbance reading at 760 nm in WPA Lightwave II spectrophotometer. We used deionized water as white. The results were expressed in mg equivalent of Gallic acid per kg of honey (mg EAG/Kg).

Total Flavonoids: a standard solution of quercetin was used for the calibration curve ($y= 0.0326x - 0.0244$; $R^2= 0.9983$). For the analysis, we added 3.0 mL of aluminum chloride (10 %) to a 2.0 mL aliquot of honey solution, which was kept at rest for 40 min. The absorbance readings were performed at 420 nm in a WPA Lightwave II spectrophotometer. The control sample was submitted to the same procedure, except for the addition of aluminum chloride. The results were expressed in mg equivalent of quercetin per kg of honey (mg EQ/Kg).

Lugol reaction: performed according to methodology of the Adolfo Lutz Institute.[30] We used a solution of each sample containing 10 g of honey and 20 mL of distilled water. After homogenization, we added 0.5 mL of the Lugol solution and we observed 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.

2.4. Determining metal concentrations

Aluminum (Al), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), iron (Fe) and zinc (Zn) were selected for this study due to their relevance for environmental contamination, mainly Pb, due to contamination caused by a mining company that used to operate in the site. The samples were prepared following the nitro-perchloric digestion method.[31-32] We used 2 g of each honey sample and the analysis was performed in triplicate. We used a standard solution (white solution) containing only acids, submitted to the same digestion procedures of honey samples.

For the analysis, reagents with an analytical grade certificate were used. The acidic digestion of the samples was performed with 65 % of nitric acid and concentrated perchloric acid. For dilution, we used ultra-pure water (18.2 MΩ.cm). Standard solutions with metals used for calibration were produced by diluting a stock solution of 1000 mg/L of each metal. Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) was used to determine metal concentrations in the samples. The ICP (Spectrometer Thermo Scientific iCAP 6000 Series, model 6300 Duo) was used to identify the metals. Conditions of analyses of ICP OES are presented in Table 1. The accuracy of the analytical method was evaluated by repeatability of the experimental results of real samples (in triplicate) and expressed as mean and standard deviation. The accuracy was verified by the calibration curve (using standard solutions of each metal studied).

2.5. Statistical analysis

For the variables obtained, we performed the descriptive statistical analysis with calculation of the minimum and maximum values, mean and standard deviation. The data were submitted to analysis of variance (ANOVA) and the means were compared by the Scott-Knott clustering test at 5 %

Table 1. Conditions of analyses of ICP OES for metal quantification in *Apis mellifera* L. honey (Hymenoptera: Apidae)

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)		100.00 %
Metal	**Wavelength (nm)**	**Limit of detection (LD) (mg/kg)**
Al (aluminum)	308.2 axial	0.005
Cd (cadmium)	226.5 axial	0.005
Cr (chromium)	267.7 radial	0.002
Cu (copper)	324.7 radial	0.005
Fe (iron)	259.9 radial	0.010
Pb (lead)	220.3 axial	0.010
Zn (zinc)	213.8 axial	0.002

probability. We performed the normality test of Shapiro-Wilks, in addition to the Pearson linear coefficients. The analyses were performed by statistical software R (R Core Team).[33]

3. Results and Discussion

3.1. Physicochemical analysis

The samples evaluated by the Lugol reaction showed a negative result for dextrin and starch. The ANOVA showed a significant difference at 1 % probability of the honey samples analyzed (Table 2). Table 3 shows the descriptive analysis and the comparison of means of the physicochemical parameters evaluated in the honey samples (n=11). The statistical difference between the samples for the variables evaluated is possibly attributed to their botanical origin, as each sample was collected from a different hive. Although the samples came from the same site, the bees may have explored different floral resources, conferring different physicochemical and sensorial characteristics to honey.

Moisture

We observed that 100 % of the honey samples complied with the threshold for moisture established by the current legislation in Brazil[8] – 20 % maximum for the moisture content (Table 3). Moisture levels in the samples ranged from 18.27 to 20.40 %. The moisture content is critical for honey quality, as it refers to honey maturity and shelf life. However, honeys with a moisture content higher than 20 % are prone to fermentation, because the osmotic pressure of sugar is not sufficient to prevent proliferation of osmophilic yeast (sugar tolerant). Commonly, moisture in honey is adequate when honeycombs in the hive are closed[7, 34-36]. The influence of water content on rheological properties in honey is also important, such as viscosity, an important parameter for the sensory quality of honey. Viscosity affects a number of technological operations, namely mixing, filtering, hydraulic transport and product packaging[37]. Thus, honey harvest must be properly carried out to avoid collecting honey from honeycombs that are not closed yet. The moisture content in honey is also related to environmental and geographic conditions and handling procedures at the apiary and at honey storage, therefore, the results for moisture suggest that beekeepers are using adequate handling and storage conditions of honey.[38]

Color

Honey color ranged from light to dark amber, where 54.54 % of the samples were amber, 36.36 % light amber and 9.10 % dark amber, according to the Pfund classification scale (Table 3). Color is an attractive physical property quickly observed by the consumer. Honey color is related to its botanical origin, besides phenolic compounds and pollen grains. Commonly, dark honey has more minerals than light honey does.[37,39] Studies indicate a positive relationship between color and minerals that compose honey.[40-43] The correlation between color and the ash content was positive; however, the correlation was higher between color and phenolic compounds between the physicochemical parameters for total flavonoids r=0.93 and total phenols r=0.42 (Table 4).

pH

In the honey samples evaluated, the pH varied from 3.30 to 4.41. The Brazilian legislation[8] for honey does not establish a threshold for this parameter. However, honey pH is commonly evaluated in physicochemical studies,[7,35,44] because the pH also characterizes the acidic nature of honey, helping understand the results of acidity determination. The acidic nature of honey is attributed to the presence of organic acids, and acidity variation between different honey types can result from variation of organic acids.[37] We observed a negative correlation between the pH and acidity in the samples evaluated (r=-0.03) and a positive correlation of pH with ash (r=0.86), with Cu (r=0.80) and with electrical conductivity (r=0.50) (Table 4). Oroian *et al.*[11] reported a negative correlation between the pH and acidity (r=-0.42) and a positive correlation with the ash content and electrical conductivity.

Acidity

Acidity values in the samples ranged from 30.33 to 43.67 mEq/kg. The threshold established by the Brazilian legislation[8] for acidity is 50 mEq/kg. For all samples evaluated, acidity value was lower than the threshold, complying with quality requirements. Acidity is an important parameter related to honey deterioration, indicating the beginning of the fermentation process, which is associated to many factors, namely floral sources, mineral content, harvest time, and the amount of gluconic acid resulting from glucose enzymolysis.[11,23,45]

Hydroxymethylfurfural

In this study, the hydroxymethylfurfural (HMF) content, widely recognized as an indicator of honey freshness, ranged from 18.86 to 47.90 mg/kg (Table 3). This result was expected, since

Table 2. Analysis of variance (ANOVA) of physicochemical parameters and metals evaluated in honey samples of *Apis mellifera* L. from a region contaminated by lead, Bahia State, Brazil

VF	DF	MS [a]								
		Moisture (%)	Color (nm)	pH	Acidity (mEq/kg)	HMF (mg/kg)	DA (Göthe)	RS (%)	Suc (%)	Ashes (%)
Sample	10	1.440**	0.149**	0.398**	51.158**	240.066**	17.803**	36.849**	4.519**	0.039**
Error	22	0.040	0.000	0.003	1.758	6.720	0.000	0.070	0.149	0.000
Total	32									
CV (%)		0.57	1.46	1.53	3.54	6.69	0.08	0.34	13.68	2.43
Mean		19.45	0.52	3.80	37.48	38.74	18.32	77.12	2.82	0.29
VF	DF	MS								
		EC (µS/cm)	Phe (mgEAG/kg)	Flav (mgEQ/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Sample		36.396*	28539.525**	1237.133**	0.000**	0.180**	0.172**	54.642**	0.034**	2.145**
Error	10	16.259	746.355	9.434	0.000	0.000	0.000	0.002	0.000	0.002
Total	22									
CV (%)	32	1.31	5.88	3.84	11.57	0.82	1.06	0.31	3.47	1.89
Mean		417.82	464.29	79.89	0.03	0.52	0.64	13.59	0.17	2.26

[a] ** Highly significant at 1 % significance by the F test; Mean square (MS); Coefficient of Variation (CV); DA = diastase activity, RS = reducing sugars, EC = electrical conductivity, Phe = total phenols, Flav = total flavonoids, HMF = hydroxymethylfurfural, and Suc = apparent sucrose. Metal: Cd= cadmium, Cr= chromium, Cu= copper, Pb= lead, Fe= iron and Zn= zinc

Table 3. Comparison of means of physicochemical parameters evaluated in honey samples of *Apis mellifera* L. from a region contaminated by lead, Bahia State, Brazil

Sample	Physicochemical parameters[a]						
	Moisture (%)	Color(Λ) (nm)	Color-classification	pH	Acidity (meq/kg)	HMF (mg/kg)	DA (Göthe)
A001	18.27d	0.342h	light amber	4.01b	41.67a	43.51a	20.75c
A002	19.03c	0.351h	light amber	4.00b	30.33d	18.86d	18.68e
A003	19.80b	0.856b	amber	4.10b	40.67a	45.36a	16.13i
A004	20.00a	0.293i	light amber	3.32f	37.33b	47.90a	19.89d
A005	18.47d	0.451f	amber	3.30f	34.67c	38.52b	18.33f
A006	19.10c	0.570c	amber	3.42e	41.67a	34.03c	17.49g
A007	19.53b	0.526d	amber	3.51e	33.33c	43.81a	16.17h
A008	19.27c	0.984a	dark amber	3.81d	43.67a	47.21a	14.80l
A009	20.13a	0.375g	light amber	4.41a	34.33c	43.96a	15.95j
A010	20.00a	0.474e	amber	3.92c	38.00b	30.54c	21.52b
A011	20.40a	0.555c	amber	4.03b	36.67b	32.39c	21.86a
Mean	19.45	0.525	-	3.80	37.48	38.74	18.32
Min.	18.27	0.293	-	3.30	30.33	18.86	14.80
Max.	20.40	0.984	-	4.41	43.67	47.90	21.86
SD	0.69	0.217	-	0.36	4.13	8.95	2.44
Legislation[b]	Max. 20.00	<0.030 to >945	Water white to Dark amber	**	Max. 50.00	Max. 60.00	Min. 8.00

Sample	Physicochemical parameters[a]					
	RS (%)	Suc (%)	Ashes (%)	EC (µS/cm)	Phe (mgEAG/kg)	Flav (mgEQ/kg)
A001	78.69c	2.53c	0.37c	611.97a	453.13c	70.67e
A002	74.71g	3.93a	0.41b	608.20b	359.70d	65.00f
A003	79.83b	1.45d	0.45a	605.33b	436.46c	108.87b
A004	76.49f	4.78a	0.12j	308.03b	340.51d	55.80g
A005	79.50b	3.26b	0.14i	311.37a	420.30c	79.42d
A006	84.40a	4.27a	0.21g	312.47a	390.51d	65.77f
A007	74.68g	1.18d	0.19h	306.40b	452.12c	75.74d
A008	77.07e	1.26d	0.30f	315.23a	567.27b	124.36a
A009	73.61h	3.29b	0.32e	306.60b	450.10c	71.44e
A010	77.65d	2.90b	0.34d	305.80b	586.46b	72.82e
A011	71.68i	2.21c	0.40b	604.63b	650.61a	88.93c
Mean	77.12	2.82	0.29	417.82	464.29	79.89
Min.	71.68	1.18	0.12	305.80	340.51	55.80
Max.	84.40	4.78	0.45	611.97	650.61	124.36
SD	3.50	1.23	0.11	150.45	97.54	20.31
Legislation[b]	Min. 65.00	Max. 6.00	Max. 0.60	**	**	**

Means followed by the same lowercase letters in the column belong to the same group by the Scott Knott test at 5 % probability.

[a] DA= diastase activity, RS= reducing sugars, EC= electrical conductivity, Color(Λ)= absorbance, SD= standard deviation, Phe= total phenols, Flav= total flavonoids, HMF= hydroxymethylfurfural, Max.= maximum, Min.= minimum and Sac= apparent sucrose.

[b] Brazilian legislation;8 ** Not established by Legislation

Table 4. Correlation matrix (Pearson) of the physicochemical parameters and metals determined in honey samples of *Apis mellifera* L. from a region contaminated by lead, Bahia State, Brazil

	Moi	Color	pH	Acidity	HMF	DA	RS	Suc	Ashes	EC	Phe	Flav	Cd	Cr	Cu	Fe	Pb	Zn
Moi	1.00																	
Color	0.10	1.00																
pH	0.32	0.09	1.00															
Acidity	-0.13	0.58	-0.03	1.00														
HMF	0.06	0.30	-0.13	0.48	1.00													
DA	0.08	-0.56	-0.02	-0.10	-0.43	1.00												
RS	-0.52	0.22	-0.44	0.57	0.10	-0.16	1.00											
Suc	-0.06	-0.69	-0.30	-0.25	-0.34	0.35	0.22	1.00										
Ashes	0.17	0.26	0.86	0.09	-0.34	0.12	-0.25	-0.38	1.00									
EC	-0.11	0.01	0.50	-0.02	-0.33	0.33	-0.19	-0.19	0.78	1.00								
Phe	0.39	0.42	0.37	0.27	-0.04	0.26	-0.37	-0.58	0.40	0.08	1.00							
Flav	0.07	0.93	0.25	0.49	0.33	-0.48	-0.01	-0.78	0.37	0.14	0.53	1.00						
Cd	-0.62	-0.40	0.05	0.01	-0.22	0.45	0.24	0.31	0.20	0.47	-0.31	-0.24	1.00					
Cr	-0.43	-0.21	-0.42	-0.15	0.16	-0.26	0.39	0.34	-0.63	-0.55	-0.38	-0.21	0.13	1.00				
Cu	0.43	0.04	0.80	0.03	-0.21	0.46	-0.50	-0.27	0.78	0.54	0.68	0.25	0.16	-0.47	1.00			
Fe	-0.50	0.14	-0.29	0.52	0.49	-0.36	0.79	-0.01	-0.27	-0.13	-0.47	-0.02	0.10	0.35	-0.53	1.00		
Pb	0.37	-0.01	0.11	-0.14	-0.34	0.57	-0.45	-0.08	0.25	0.37	0.61	0.11	-0.10	-0.24	0.57	-0.58	1.00	
Zn	0.03	-0.11	-0.61	-0.01	-0.32	0.44	0.25	0.37	-0.49	-0.44	0.20	-0.20	-0.04	0.35	-0.15	-0.21	0.39	1.00

Moi= moisture, DA= diastase activity, RS= reducing sugars, EC= electrical conductivity, Phe= total phenols, Flav= total flavonoids, HMF= hydroxymethylfurfural and Suc= apparent sucrose. Metal: Cd= cadmium, Cr= chromium, Cu= copper, Pb= lead, Fe= iron and Zn= zinc.

the samples evaluated did not undergo thermal processing and were stored at room temperature (≈25 °C) and analyzed one month after collection. In honey, HMF is related to its quality and extension of thermal processing, especially when crystallized, as well as storage conditions that preserve its sensory characteristics.[37] The maximum HMF allowed for honey by the Brazilian legislation[8] is 60 mg/kg and HMF is also associated to honey deterioration.

Diastase activity

The diastase activity, indicator of exposure to high temperatures, for the samples analyzed ranged from 14.80 to 21.86 Göthe, complying with the threshold established by the Brazilian legislation,[8] which indicates a minimum of 8 Göthe. The satisfactory diastase activity rates of this enzyme in honey could be related to the tropical climate of Santo Amaro region, Bahia State, with an average annual temperature of 24.7 °C. The diastase activity is a quality factor to determine honey freshness and is influenced by botanical origin, regional climate, product storage, and heating.[10] The studies conducted by Oroian,[37] Nayik and Nanda[41] and Al-Farsi *et al*.[10] reported values for the diastase activity of *A. mellifera* honeys similar to those observed in our study; however, in different climatic conditions. In addition, a positive correlation was observed between the diastase activity and the ash content (Table 4). According to Oroian[37], these two parameters could be used to classify honey of different floral origins, as observed in our study.

Reducing sugars

Values of reducing sugars in the samples ranged from 71.68 to 84.40 %, in compliance with the standards proposed by the Brazilian legislation.[8] Reducing sugars (glucose and fructose) are a requirement for honey maturity and the main carbohydrates in honey composition, responsible for its quality and properties, such as antibacterial activity, granulation, hygroscopicity, energetic value, and viscosity.[46] The composition of sugars in honey is related to the floral sources used by the bees, as well as the climatic.[7,47] The determination of this physicochemical parameter is fundamental, since it helps evaluate honey maturity, which influences honey shelf life. In addition, we observed a positive correlation (r=0.79) between reducing sugars and iron (Fe). Charley *et al*.[48] and Christides and Sharp[49] report that simple sugars, such as glucose and fructose, affect Fe bioavailability because these sugars can chelate inorganic iron.

Apparent sucrose

The sucrose content in the samples analyzed ranged from 1.18 to 4.78 %, in compliance with the threshold proposed by the Brazilian legislation,[8] which establishes a maximum of 6.00 % (Table 3). A high sucrose content may indicate inadequate procedures, such as premature honey harvest, that is, extracting honey from the hive with sucrose that has not yet been totally transformed into glucose and fructose by the enzyme invertase.[7,50] Determining the sucrose content is important to assess honey quality, as it allows identifying honey adulteration by addition of sucrose or supply of commercial sugar to the colony in the event of food shortage.[51]

Ashes

The ash content is an indicator of mineral content in the honey sample. In the samples evaluated, the ash percentage ranged from 0.12 to 0.45 %, and 0.6 % is the threshold allowed by the Brazilian legislation.[8] Almeida-Muradian *et al*.[35] and El Sohaimy *et al*.[40] obtained results for the ash content similar to our results. The ash content is a quality criterion for honey and is related to its botanical and geographical origin. The ash content is also related to honey color, because the darker the honey, the higher its ash content. Floral honeys have a lower mineral content (0.1 and 0.3 %) when compared to honeydew (up to 1.0 %).[3,52] The correlation between the ash content and color was positive in this study (Table 4).

Electrical conductivity

Electrical conductivity values for all samples complied with the threshold (800 µS/cm) established by the international legislation for floral honeys.[53] The Brazilian legislation[8] does not establish a threshold for this physicochemical parameter. Among the samples evaluated, electrical conductivity values ranged from 305.80 to 611.97 µS/cm (Table 3). Electrical conductivity in honey is related to ash content and honey acidity, reflecting the presence of ions, organic acids, and proteins. This parameter is frequently used to classify floral honeys and honeydew. For the European legislation,[53] floral honey must have values equal to or lower than 800µS/cm.[3,54] Orioan[37] evaluated honeydews and reported higher electrical conductivity values due to the high mineral content (ash content, %). Likewise, electrical conductivity values presented a high positive correlation between mineral content and honey ash content (r=0.76). This was also verified for samples in our study, where a positive correlation (r=0.78) was recorded between these physicochemical parameters (Table 4).

3.2. Phenolic compounds

Total phenols

Values of total phenols in the samples ranged between 340.51 and 650.61 mg EAG/kg. The total phenol content is influenced by the botanical origin of honey and is one of the main factors responsible for the biological activity of honey.[37,55] Oliveira *et al*.[56] reported that darker honeys, with higher mineral content, presented higher levels of total polyphenols. The correlation of total phenols was positive in relation to the diastase activity, ashes, and electrical conductivity, with low values (r<0.50) (Table 4). Orioan[37] reported that the content of phenols showed a high positive correlation with the diastase activity (r=0.84), ashes (r=0.91), and electrical conductivity (r=0.78). The same author stated that the diastase activity, ash, and electrical conductivity are widely known as parameters to evaluate quality of honeys and classify them as floral or honeydew. The content of phenols is a good indicator for honey classification.

Total Flavonoids

The content of flavonoids in the honey samples ranged from 55.80 to 124.36 mg QE/kg (Table 3). Flavonoids are phenolic compounds with low molecular weight that affect aroma and antioxidant properties of honey. The content of total flavonoids varies for different honeys, because it is related to the geographic origin of different floral sources used by the bees to collect pollen and nectar.[57] We found values for total flavonoids in honey similar to other studies[58-59] and mean values higher than those reported by Ahmed *et al*.[57] and Hussein *et al*.[60]

3.3. Determination of metal concentrations

The ANOVA showed a significant difference at 1 % probability level between the honey samples analyzed for Cd, Cr, Cu, Fe, Pb and Zn (Table 2). All metals evaluated were detected in the samples and the mean concentrations are presented in Table 5. Among the metals evaluated, only Cr showed concentrations above the threshold established by the Brazilian legislation[61-62] in all samples (A001-A011) and Pb in one sample (A011) (Table 5). Fe and Zn showed the highest concentrations in the samples.

Aluminum (Al) had concentrations below the limit of detection (LD<0.005 mg/kg) in all samples. Considering that Al is not required by the human body, it is relevant that Al concentration in the samples from Santo Amaro, Bahia, was below the threshold established by National Council for the Environment (CONAMA),[63] which determines a maximum of 0.10 mg/L for fresh water. No specific limits are established for honey. In the last decades, Al has been appointed as a major environment contaminant, a concern for the public health due to neurotoxic action, linked, for example, to the Alzheimer's disease. Human activities, such as the burning of fossil fuels that result in acid rain, intensive agriculture that produces soils with sulfate acid, as well as mining and production of Al salts, increase the availability of Al to the environment.[64-65]

The mean cadmium (Cd) concentration in the samples ranged from 0.0110 to 0.0610 mg/kg (Table 5). These values comply with the requirements in the Brazilian legislation.[61] Cadmium is considered potentially toxic and is not required in any quantity for human metabolism. Studies show that metals in honey have higher Cd concentrations when compared to the values obtained in our study.[14,66-67] The variation in metals concentrations in samples from different sites is influenced by the botanical origin and climatic conditions.[17]

Cadmium plays an important role in environmental protection due to its cumulative toxicity. Its natural occurrence in the environment comes from volcanic emissions. The main cause of Cd contamination is the anthropogenic activity, such as industrial production, wastewater, mining, waste incineration and combustion of coal and oils. In addition, phosphate fertilizers are important Cd sources in agricultural soils. If ingested at high concentrations through contaminated food, Cd may accumulate in the kidney, where it impairs blood filtering to eliminate harmful substances in the human body.[68-69]

Lead (Pb), along with Al and Cd, is a potentially toxic metal. In the samples evaluated, Pb concentration ranged from 0.1123 to 0.5038 mg/kg and only sample A011 had concentration with a value above the threshold established for honey by the Brazilian legislation.[61] Considering the history of Pb contamination in the region of Santo Amaro, Bahia, this result was not expected. Studies on Pb contamination in Santo Amaro, Bahia, have been carried out for approximately four decades and Pb concentrations have been identified in environmental compartments and samples of biological material of miners that worked for the mining company, which operated in the municipality for 30 years.[16] Thus, it was expected that Pb concentration in the samples presented values higher than the threshold established by the Brazilian legislation.[61]

Nevertheless, the lower values of Pb concentration may be related to the physiology of plants used by the bees as source material for collecting nectar. Pb is a heavy metal and accumulates in plant roots due to the higher affinity of Pb for the negative charges, resulting from the dissociation of the carboxylic galacturonic groups and glucuronic acids from the cell wall into cells of root tissues. This mechanism restricts Pb access to the xylem and, consequently, reduces its translocation to the aerial parts of the plant.[70-71] Lead is present in most environments and is recognized by the great risk to the health of humans and animals. Lead can be absorbed by air, water and/or contaminated food. Lead intoxication can cause anemia, headache, muscle aches and irritations. Chronic exposure causes nephritis and shrinkage of renal tissues. Industrial activity is a source of Pb contamination to the environment.[69,72-73]

A study carried out by Naccari *et al*.[67] to monitor environmental pollution showed that Pb concentration in eucalyptus honey was 0.1500 mg/kg similar to the values recorded in our study. A higher Pb concentration (1.6278 mg/kg) was found in honeys evaluated by Aghamirlou *et al*.[17] Czipa *et al*.[76] found a lower concentration (0.0748 mg/kg) than that found in our study (Table 5). These results indicate that the variation in Pb concentration in honey is influenced by degree of environmental pollution in the place where the bees are managed.

Chromium (Cr) presented concentration in samples ranging from 0.2418 to 1.1243 mg/kg (Table 5), above the threshold stablished by the Brazilian.[62] This reflects the anthropic degree of the origin site where the samples were collected. Cr is a natural element in the earth crust and is released into the environment from natural and anthropogenic sources. The industrial activity is the main form of Cr release, especially in the production process of chrome iron.[74] Cr is an essential mineral for the human body, as it actively participates in carbohydrates metabolism, mainly boosting insulin production, improving glucose tolerance. Thus, Cr concentrations observed in the samples (Table 5) need to be carefully evaluated, because when ingested at extremely high dosages, Cr may become toxic. However, there are two forms of Cr: Cr^{+3} (co-acting with insulin) and Cr^{+6} (toxic to living things). Cr negative effects are related to Cr^{+6} intoxication, which is usually inhaled in industrial settings and may cause nasal septum ulceration, inflammation of nasal mucosa, chronic bronchitis, and emphysema.[75] The analysis performed in our study did not allow to discriminate the form of Cr occurrence (Cr^{+3} or Cr^{+6}) in the samples.

Iron (Fe) had the highest concentrations in the samples (Table 5) with values ranging from 6.8780 to 20.4655 mg/kg. Fe also had higher values among the metals evaluated in the studies conducted by Batista *et al.*,[66] Czipa *et al*.[76] and Altun *et al*.[77] Fe is an essential metal for living things. In the human body, Fe plays an important role in metabolism, such as protein synthesis and oxidative metabolism. The mean daily Fe intake for men is estimated at 17.00 mg and for women, at 9-12.00 mg.[78-79]

Copper (Cu) concentrations ranged from 0.3073 to 1.0560 mg/kg thus complying with the threshold established by Ordinance No. 685/98.[80] Cu is an essential mineral for living things; however, at high concentrations, Cu may cause adverse effects. Therefore, it is necessary to consider the

Table 5. Comparison of means of metal concentrations in honey samples of *Apis mellifera* L. from a region contaminated by lead, Bahia State, Brazil

Sample[a]	Metal						
	Al	Cd	Cr	Cu	Fe	Pb	Zn
	(mg/kg)						
A001	<LD	0.0610a	0.4380f	0.7623d	18.7280b	0.1248e	1.2895h
A002	<LD	0.0480b	0.3605h	0.6260e	8.3043j	0.1573d	1.7932g
A003	<LD	0.0360c	0.3668h	0.7598d	17.5280c	0.1336e	1.2832h
A004	<LD	0.0360c	0.4968d	0.4173h	13.1405f	0.1373e	2.5932e
A005	<LD	0.0480b	1.1243a	0.4510g	14.4530e	0.2123b	3.5182a
A006	<LD	0.0230d	0.6855c	0.3073j	20.4655a	0.1323e	3.0232c
A007	<LD	0.0110e	0.4543e	0.3448i	15.0155d	0.1123f	1.8095g
A008	<LD	0.0230d	0.4368f	0.5798f	12.5155h	0.1261e	2.0507f
A009	<LD	0.0230d	0.7418b	0.8023c	12.7530g	0.1173f	1.2870h
A010	<LD	0.0360c	0.4030g	0.8823b	9.7005i	0.1761c	3.3870b
A011	<LD	0.0230d	0.2418i	1.0560a	6.8780l	0.5038a	2.7920d
Mean	-	0.0330	0.5227	0.6353	135.893	0.1748	22.570
Min.	-	0.0110	0.2418	0.3073	68.780	0.1123	12.832
Max.	-	0.0610	11.243	10.560	204.655	0.5038	35.182
SD	-	0.0150	0.2450	0.2396	42.677	0.1097	0.8448
Legislation[b]	-	0.5000	0.1000	10.000	-	0.5000	50.000

[a] Means followed by the same lowercase letters in the column belong to the same group by the Scott Knott test at 5 % probability; LD= limit of detection; Min.= Minimum and Max.= Maximum; SD= standard deviation; Al= aluminum, Cd= cadmium, Cr= chromium, Cu= copper, Pb= lead, Fe= iron and Zn= zinc.

[b] value maximum permitted.[61-62,80]

daily Cu intake from different food sources.[81] The Cu detected in the samples possibly comes from anthropogenic and industrial activities and brake pads of motor vehicles are considered significant Cu sources [82], one of the main sources of Cu pollution.[83]

Zinc (Zn) concentration in the samples analyzed ranged from 1.2832 to 3.5182 mg/kg (Table 5). The maximum permissible concentration for Zn established by the Brazilian legislation is 50.00 mg/kg.[73] Zn is an essential mineral for metabolism in the human body. The World Health Organization establishes 0.30 to 1.00 mg/kg as a tolerable limit for daily intake.[80] Studies on metals in honey presented values similar to those recorded in our research.[14,17]

4. Conclusion

The physicochemical parameters in the honey samples evaluated complied with the quality standards required. The metals determined and quantified presented concentrations comply with the thresholds established by Brazilian and international legislations, except for chromium. The results indicate quality requirements for honey from Santo Amaro, State of Bahia, which can help to add commercial value to honey.

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References

[1] Tsutsumi, L. H.; Oishi, D. E.; In: *Specialty Crops for Pacific Island Agroforestry*, Elevitch, C. R. ed.. Permanent Agriculture Resources: Holualoa, 2010. [Link]

[2] Corvucci, F.; Nobili, L.; Melucci, D.; Grillenzoni, F. V. The discrimination of honey origin using melissopalynology and Raman spectroscopy techniques coupled with multivariate analysis. *Food Chemistry*, **2015**, *169*, 297. [CrossRef] [PubMed]

[3] Pita-Calvo, C.; Vázquez, M. (2017). Differences between honeydew and blossom honeys: A review. *Trends in Food Science & Technology*, **2017**, *59*, 79. [CrossRef]

[04] Aljuhaimi, F.; Ozcan, M. M.; Ghafoor, K.; Babiker, E. E. Determination of physicochemical properties of multifloral honeys stored in different containers. *Journal of Food Processing and Preservation,* **2018**, *42*, 1. [CrossRef]

[05] Naila, A.; Flint, S. H.; Sulaiman, A. Z.; Ajit, A.; Weeds, Z. Classical and novel approaches to the analysis of honey and detection of adulterants. *Food Control*, **2018**, *90*, 152. [CrossRef]

[06] Oroian, M.; Ropciuc, S.; Paduret, S.; Todosi, E. Rheological analysis of honeydew honey adulterated with glucose, fructose, inverted sugar, hydrolysed inulin syrup and malt wort. *LWT - Food Science and Technology*, **2018**, *95*, 1. [CrossRef]

[07] Nascimento, A. S.; Marchini, L. C.; Carvalho, C. A. L.; Araújo, D. F. D.; Silveira, T. A.; Olinda, R. A. Physical chemical parameters of honey of stingless bee (Hymenoptera: Apidae). *American Chemical Science Journal*, **2015**, *7*, 139. [CrossRef]

[08] Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa 11, 2000. [Link]

[09] Sobrino-Gregorio, L.; Vargas, M.; Chiralt, A.; Escriche, I. Thermal properties of honey as affected by the addition of sugar syrup. *Journal of Food Engineering*, **2017**, *213*, 69. [CrossRef]

[10] Al-Farsi, M.; Al-Belushi, S.; Al-Amriahlam, A.; Al-Hadhrami, A.; Al-Rusheidi, M.; Al-Alawi, A. Quality evaluation of Omani honey. *Food Chemistry*, **2018**, *262*, 162. [CrossRef]

[11] Oroian, M.; Ropciuc, S. Honey authentication based on physicochemical parameters and phenolic compounds. *Computers and Electronics in Agriculture*, **2017**, *138*, 148. [CrossRef]

[12] Bonsucesso, J. S.; Gloaguen, T. V.; Nascimento, A. S.; Carvalho, C. A. L.; Dias, F. S. Metals in geopropolis from beehive of *Melipona scutellaris* in urban environments. *Science of the Total Environment*, ***2018***, *634*, 687. [CrossRef] [PubMed]

[13] Matin, G.; Kargar, N.; Buyukisik, H. B. Bio-monitoring of cadmium, lead, arsenic and mercury in industrial districts of Izmir, Turkey by using honey bees, propolis and pine tree leaves. *Ecological Engineering*, **2016**, *90*, 331. [CrossRef]

[14] Nascimento, A. S.; Marchini, L. C.; Carvalho, C. A. L.; Araújo, D. F. D.; Silveira, T. A.; Olinda, R. A. Determining the levels of trace elements Cd, Cu, Pb and Zn in honey of stingless bee (Hymenoptera: Apidae) using voltammetry. *Food and Nutrition Sciences*, **2015**, *6*, 591. [CrossRef]

[15] Porrini, C.; Sabatini, A. G.; Girotti, S.; Ghini, S.; Medrzycki, P.; Grillenzoni, F.; Bortolotti, L.; Gattavecchia, E.; Celli, G. Honey bees and bee products as monitors of the environmental contamination. *Apiacta*, **2003**, *38*, 63. [CrossRef]

[16] Andrade, M. F.; Moraes, L. R. S. Contaminação por chumbo em santo amaro desafia décadas de pesquisas e a morosidade do poder público. *Ambiente & Sociedade*, **2013**, *16*, 63. [CrossRef]

[17] Aghamirlou, H. M.; Khadem, M.; Rahmani, A.; Sadeghian, M.; Mahvi, A.H.; Akbarzadeh, A.; Nazmara, S. Heavy metals determination in honey samples using inductively coupledplasma-optical emission spectrometry. *Journal of Environmental Health Science & Engineering*, **2015**, *13*, 2. [CrossRef]

[18] Ru, Q. M.; Feng, Q.; He, J. Z. Risk assessment of heavy metals in honey consumed in Zhejiang province, southeastern China. *Food and Chemical Toxicology*, **2013**, *53*, 256. [CrossRef]

[19] Loureiro, S.; Spínola, A. G.; Carvalho, F. M.; Barreto, M. L. Lead poisoning and hookworm infection as multiple factors in anaemia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **1983**, *77*, 321. [CrossRef] [PubMed]

[20] Senar - Serviço Nacional de Aprendizagem Rural. Apicultores da Bahia dobram a produção depois da chegada da ATeG do SENAR, 2017. [Link]

[21] AOAC - Association of Official Analytical Chemists. Official methods of Analysis. 15 th. ed. Supl 2., Official Methods of Analysis, method number 969.38, 1090.

[22] Bogdanov, S. Harmonised methods of the International Honey Commission, 2009. [Link]

[23] CAC - Codex Alimentarius Comission. Revised codex standard for honey, 24th session of the Codex Alimentarius, 2001. [Link]

[24] Vidal, R.; Fragosi, E. V. *Mel:* características, análises físico-químicas, adulteração e transformação. Barretos, SP: Instituto Tecnológico Científico "Roberto Rios". 1984.

[25] AOAC - Association of Official Analytical Chemists. 16th ed, Official methods of Analysis. 2005.
[26] Copersucar - Cooperativa dos Produtores de Cana, Açúcar e Álcool do Estado de São Paulo; Em: *Copersucar. Manual de controle químico de produção de açúcar*, Copersucar: Piracicaba, 1987.
[27] Feás, X.; Vázquez-Tato, M. P.; Estevinho, L.; Seijas, J. A.; Iglesias, A. Organic bee pollen: botanical origin, nutritional value, bioactive compunds, antioxidante activity and microbiological quality. *Molecules*, **2012**, *17*, 8359. [CrossRef] [PubMed]
[28] Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, **1999**, *299*, 152. [CrossRef]
[29] Woisky, R. G.; Salatino, A. Analysis os propolis: some parameters ond prodecore for chemical fuality control. *Journal Apicultural Research*, **1998**, *37*, 99. [CrossRef]
[30] IAL - Instituto Adolfo Lutz.; *Métodos físico-químicos para análise de alimentos*, Instituto Adolfo Lutz: São Paulo, 2008.
[31] Krug, F. J.; *Métodos de preparo de amostras*: Fundamentos sobre métodos de preparo de amostras orgânicas e inorgânicas para análise elementar,.Calq: Piracicaba, 2008.
[32] Malavolta, B.; Vitti, G. C.; Oliveira, S. A.; *Avaliação do estado nutricional das plantas*: princípios e aplicações, Potafos: Piracicaba, 1989.
[33] R Core Team, A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, 2018. [Link]
[34] De-Melo, A. A. M.; Almeida-Muradian, L. B.; Sancho, M. T.; Pascual-Maté, A. Composition and properties of *Apis mellifera* honey: A review. *Journal of Apicultural Research*, **2017**, *1*, 1. [CrossRef]
[35] Almeida-Muradian, L. B.; Stramm, K. M.; Horita, A.; Barth, O. M.; Freitas, A. S.; Estevinho, L. M. Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera*. *International Journal of Food Science and Technology*, **2013**, *48*, 1698. [CrossRef]
[36] Bogdanov, S.; Martin, P. Honey authenticity: A review. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*, **2002**, *93*, 232. [CrossRef]
[37] Oroian, M. Physicochemical and Rheological Properties of Romanian Honeys. *Food Biophysics*, **2012**, *7*, 296. [CrossRef]
[38] García-Tenesaca, M.; Navarrete, E. S.; Iturralde, G. A.; Villacrés Granda, I. M.; Tejera, E.; Beltrán-Ayala, P.; giampieri, F.; Battino, M.; Alvarez-Suarez, J. M. Influence of botanical origin and chemical composition on the protective effect against oxidative damage and the capacity to reduce in vitro bacterial biofilms of monofloral honeys from the Andean Region of Ecuador. *International Journal of Molecular Sciences*, **2018**, *19*, 1. [CrossRef] [PubMed]
[39] Silva, T. M. S.; Santos, F. P.; Evangelista-Rodrigues, A.; Silva, E. M. S.; Silva, G. S.; Novais, J. S.; Santos, F. A. R.; Camara, C. A. Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. *Journal of Food Composition and Analysis*, **2013**, *29*, 10. [CrossRef]
[40] El Sohaimy, S. A.; Masry, S. H. D.; Shehata, M. G. Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, **2015**, *60*, 279. [CrossRef]
[41] Nayik, G. A.; Nanda H. V. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from kashmir valley of india with a multivariate approach. *Polish Journal of Food and Nutrition Sciences*, **2015**, *65*, 101. [CrossRef]
[42] Liberato, M. T. C.; Morais S. M.; Magalhães, C. E. C.; Magalhães, I. L.; Cavalcanti, D. B.; Silva, M. M. O. Physico-chemical properties, mineral, and protein content of honey samples from Ceara state, Northeastern Brazil. *Food Science and Technology*, **2013**, *33*, 38. [CrossRef]
[43] Couto, R. H. N.; Couto, L. A.; *Apicultura: manejo e produtos*. Jaboticabal, Funep: São Paulo. 2002.
[44] Biluca, F. C.; Braghini, F.; Gonzaga, L. V.; Costa, A. C. O.; Fett, R. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey (Meliponinae). *Journal of Food Composition and Analysis*, **2016**, *50*, 61. [CrossRef]
[45] Karabagias, I. K.; Vavoura, V. M.; Nikolaou, C.; Badeka, A. V.; Kontakos, S.; Kontominas, M. G. Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International*, **2014**, *62*, 753. [CrossRef]
[46] CRANE, E.; *Honey: a comprehensive survey.* Heinemann: London, 1975. [CrossRef]
[47] Mateo, R.; Bosch-Reig, F. Classification of Spanish unifl oral honeys by discriminant analysis of electrical conductivity, color, water content, sugars and pH. *Journal of Agricultural and Food Chemistry*, **1998**, *46*, 393. [CrossRef] [PubMed]

[48] Charley, P. J.; Sarkar, B.; Stitt, C. F.; Saltman, P. Chelation of Iron by Sugars. *Biochim Biophys Acta*, **1963**, *69*, 313. [CrossRef]
[49] Christides, T.; Sharp, P. Sugars increase non-heme iron bioavailability in human epithelial intestinal and liver cells. *PLoS One*, **2013**, *8*, 1. [CrossRef]
[50] Azeredo, M. A. A.; Azeredo, L. C.; Damasceno, J. G. Physicochemical characteristics of the honeys from São Fidélis county-RJ. *Ciência e Tecnologia de Alimentos*, **1999**, *19*, 3. [CrossRef]
[51] Mendes, E.; Proença, E. B.; Ferreira, I. M. P. L. V.; Ferreira M. A. Quality evaluation of Portuguese honey. *Carbohydrate Polymers*, **1998**, *37*, 219. [CrossRef]
[52] Buba, F.; Gidado, A.; Shugaba, A. Analysis of Biochemical Composition of Honey Samples from North-East Nigeria. *Biochemistry & Analytical Biochemistry*, **2013**, *2*, 1. [CrossRef]
[53] EU - Commission Regulation. Regulation nº 2015/1005 of 25 June 2015 Amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs. OJ EU L161, 9-13, 2015. [Link]
[54] Fechner, D. C.; Moresi A. L.; Díaz, J. D. R.; Pellerano, R. G.; Vazquez, F. A. Multivariate classification of honeys from Corrientes (Argentina) according to geographical origin based on physicochemical properties. *Food Bioscience*, **2016**, *15*, 49. [CrossRef]
[55] Chakir, A.; Romane, A.; Marcazzan, G. L.; Ferrazzi, P. Physicochemical properties of some honeys produced from different plants in Morocco. *Arabian Journal of Chemistry*, **2016**, *9*, 946. [CrossRef]
[56] Oliveira, P. S.; Müller, R. C. S.; Dantas, K. G. F.; Alves, C. N.; Vasconcelos, M. A. M.; Venturieri, G. C. Ácidos fenólicos, flavonoides e atividade antioxidante em méis de *Melipona fasciculata*, *M. flavolineata* (Apidae, Meliponini) e *Apis mellifera* (Apidae, Apini) da Amazônia. *Química Nova*, **2012**, *35*, 1728. [CrossRef]
[57] Ahmed, M.; Khiati, B.; Meslem, A.; Aissat, S.; Djebli N. Evaluation of Physicochemical and antioxidant properties of raw honey from Algeria. Journal of Microbial & Biochemical Technology, **2014**, *S4*, 2. [CrossRef]
[58] Cabrera, M.; Perez, M.; Gallez, L.; Andrada, A.; Balbarrey, G. Colour, antioxidant capacity, phenolic and flavonoid content of honey from the Humid Chaco Region, Argentina. *International Journal of Experimental Botany*, **2017**, *86*, 124. [CrossRef]
[59] Moniruzzaman, M.; An, C. Y.; Rao, P. V.; Hawlader, M. N. I.; Azlan, S. A. B. M.; Sulaiman, S. A.; Gan, S. H. Identification of Phenolic Acids and Flavonoids in Monofloral Honey from Bangladesh by High Performance Liquid Chromatography: Determination of Antioxidant Capacity. *BioMed Research International*, **2014**, *1*, 1. [CrossRef]
[60] Hussein, S. Z.; Yusoff, K. M.; Makpol, S.; Yusof, Y. A. Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules*, **2011**, *16*, 6378. [CrossRef] [PubMed]
[61] Ministério da Agricultura, Pecuária e Abastecimento, Instrução Normativa n° 14, de 25 de maio de 2009. Programas de Controle de Resíduos e Contaminantes em Carnes, Leite, Mel, Ovos e Pescado, 2009. [Link]
[62] Brasil. Ministério da Saúde. Decreto nº55.871, de 26 de março de 1965. Modifica o Decreto nº50.040, de 24 de janeiro de 1961, referente as normas reguladoras do emprego de aditivos para alimentos, alterado pelo Decreto nº691, de 13 de março de 1962. Diário Oficial da União*,* Brasília. 1965.
[63] Conama - Conselho Nacional do Meio Ambiente. Resolução CONAMA n° 357, Classificação das águas, de 17 de março de 2005, Diário Oficial da União: Brasília, 2005.
[64] Exley, C.; Rotheray, E.; Goulson, D. Bumblebee pupae contain high levels of aluminium. *PLoS One*, **2015**, *10*, 1. [CrossRef]
[65] Exley, C. Human exposure to aluminium. *Environmental Science: Processes & Impacts*, **2013**, *15*, 1807. [CrossRef] [PubMed]
[66] Batista, B. L.; Silva, L. R. S.; Rocha, B. A.; Rodrigues, J. L.; Berretta-Silva, A. A.; Bonates, T. O.; Gomes, V. S. D.; Barbosa, R. M.; Barbosa, F. Multi-element determination in Brazilian honey samples by inductively coupled plasma mass spectrometry and estimation of geographic origin with data mining techniques. *Food Research International*, **2012**, *49*, 209. [CrossRef]
[67] Naccari, C.; Macaluso, A.; Giangrosso, G.; Naccari, F.; Ferrantell, V. Risk assessment of heavy metals and pesticides in honey from Sicily (Italy). *Journal of Food Research*, **2014**, *3*, 107. [CrossRef]
[68] Akinola, M. O.; Njoku, K. L.; Ekeifo, B. E. Determination of lead, cadmium and chromium in the tissue of an economically important plant grown around a textile industry at Ibeshe, Ikorodu area of Lagos State, Nigeria. *Advances in Environmental Biology*, **2008**, *2*, 25. [CrossRef]

[69] Dhahir, S. A.; Hemed, A. H. Determination of heavy metals and trace element levels in honey samples from different regions of Iraq and compared with other kind. *American Journal of Applied Chemistry*, **2015**, *3*, 83. [CrossRef]
[70] Soares, C. R. F. S.; Accioly, A. M. A.; Marques, T. C. L. S. M.; Siqueira, J. O.; Moreira, F. M. S. Content and distribution of heavy metals in roots, stems and leaves of tree seedlings in soil contaminated by zinc industry wastes. *Revista Brasileira de Fisiologia Vegetal*, **2001**, *13*, 302. [CrossRef]
[71] Alves, J. C.; Souza, A. P.; Pôrto, M. L.; Arruda, J. A.; Tompson Júnior, U. A.; Silva, G. B.; Araújo, R. C.; Santos, D. Absorption and distribution of lead in vetiver, mimosa and mesquite plants. *Revista Brasileira de Ciência do Solo*, **2008**, *32*, 1329. [CrossRef]
[72] Nazari, S. Determination of trace amounts of lead by modified graphite furnace atomic absorption spectrometry after liquid phase microextraction with pyrimidine-2-thiol. *American Journal of Analytical Chemistry*, **2011**, *2*, 757. [CrossRef]
[73] Formicki, G.; Gren, A.; Stawaez, R.; Zysk, B.; Gal, A. Metal content in honey, propolis, wax, and bee pollen and implications for metal pollution monitoring. *Polish Journal of Environmental Studies*, **2013**, *22*, 99. [CrossRef]
[74] ATSDR - Agency for Toxic Substances and Disease Registry; *Toxicological profile for Manganese*, Department of Health and Human Services: Atlanta, 2012.
[75] Gomes, M. R.; Rogero, M. M.; Tirapegui, J. Considerations about chromium, insulin and physical exercise. *Revista Brasileira de Medicina do Esporte*, **2005**, *11*, 262. [CrossRef]
[76] Czipa, N.; andrási, D.; Kovács, B. Determination of essential and toxic elements in Hungarian honeys. *Food Chemistry*, **2015**, *175*, 536. [CrossRef]
[77] Altun, S. K.; Dinç, H.; Paksoy, N.; Temamoğullar, F. K.; Savrunlu, M. Analyses of mineral content and heavy metal of honey samples from south and east region of Turkey by using ICP-MS. *International Journal of Analytical Chemistry*, **2017**, *1*, 1. [CrossRef]
[78] Hofvander, Y. Hematological investigations in Ethiopia with special reference to a high iron intake. *Acta Medica Scandinavica*, **1968**, *494*, 1. [PubMed]
[79] WHO - World Health Organization. Toxicological evaluation of certain food additives and food contaminants. FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, 18. Geneva: World Health Organization, 1983.
[80] Ministério da Saúde, Secretaria de Vigilância Sanitária. Portaria nº685, de 27 de agosto de 1998. Aprova o Regulamento Técnico: "Princípios Gerais para o Estabelecimento de Níveis Máximos de Contaminantes Químicos em Alimentos" e seu anexo: "Limites máximos de tolerâncias para contaminantes inorgânicos". Área de atuação Mercosul. Diário Oficial da União: Brasília, 1998.
[81] WHO - World Health Organization. Toxicological evaluation of certain food additives. FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series, 17. Geneva: World Health Organization, 1982.
[82] Zarić, N. M.; Ilijević, K.; Stanisavljević, L.; Gržetić, I. Metal concentrations around thermal power plants, rural and urban areas using honeybees (*Apis mellifera* L.) as bioindicators. *International Journal of Environmental Science and Technology*, *2016*, *13*, 413. [CrossRef]
[83] Silva, L. T.; Pinho, J. L.; Nurusman, H. Traffic air pollution monitoring based on an air-water pollutants deposition device. *International Journal of Environmental Science and Technology*, **2014**, *11*, 2307. [CrossRef]