


Physicochemical, bioactive properties and antioxidant of *Apis mellifera* L. honey from western Paraná, Southern Brazil

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Abstract

The Southern region of Brazil is known for its floral diversity, presenting great potential for bee products such as honey, pollen, propolis and royal jelly. In this study, 67 honey samples provided by beekeepers from 14 municipalities of western Paraná were evaluated. Physicochemical parameters, bioactive compounds and antioxidant activity were analyzed. The physicochemical parameters of the samples were in compliance with the specifications by national and international standards, presenting average values of 3.26, 34.54 meq.kg⁻¹, 18.75%, 10.79 mg.kg⁻¹, 0.14% and 340.10 μS.cm⁻¹, for pH, acidity, moisture, hydroxymethylfurfural, ash content and electrical conductivity, respectively. The nutritive values of honey, on average, achieved 0.28% of protein from 69.09% of total sugars, 64.57% of reducing sugars and 4.28% of sucrose. Bioactive compounds showed average values of 34.83 mg GAE/100 g⁻¹ of total phenols and 16.26 mg EQ/100 g⁻¹ of flavonoids, enabling antioxidant activity of 2.68 μmol FE (II)/g⁻¹ of FRAP, 1.01 μmol ET/g⁻¹ from ABTS and 0.12 μmol ET/g⁻¹ of DPPH. This first study evaluated the parameters through cluster analysis and observed nine groups formed, as well as the characteristics between the samples with similar reaction. We concluded that the honey samples have high similarity and confirmed the quality of the honey produced.

Keywords: Africanized honeybee; denomination of origin; honeybee; NMDS; multivariate analysis.

Practical Application: This study presents data on honey with geographical identification and in the process of Denomination of Origin. It expresses the results on the physicochemical and bioactive characteristics of the honey produced in the region in question. Through the application of NMDS, it was possible to identify homogeneity of the samples among the different groups.

1 Introduction

Honey cannot be used as a nutritionally complete option of food for humans, but it has a good potential to serve as food supplement. It has been used in medicine against various diseases since ancient times in Egypt, Greece and Rome, and also in western, eastern, Chinese and Ayurveda medicines (Kuropatnicki et al., 2018). In traditional medicine, it has effects on the treatment of burns, diarrhea and ulcers (Ahmed et al., 2018). In addition, it boosts the immune system by measuring the effects of anti-inflammatory, antibacterial, antifungal and antiviral activities. Also, its positive effects against breast, colorectal, renal, prostate, cervical and oral cancer have already been proven (Ahmed et al., 2018). The medicinal effects of honey are due to its antioxidant action as it has intrinsic compounds, such as glucose oxidase enzymes, catalase and peroxidase. Other compounds are carotenoids, organic acids, ascorbic acid, amino acids and proteins. (Machado De-Melo et al., 2018). Phenolic acids and flavonoids are listed as the main reason why honey has an antioxidant capacity (Baglio, 2018), due to their mechanism of action and reactions based on the transference of hydrogen atoms or simple electrons (Eteraf-

Oskouei & Najafi, 2013). Honey antioxidant capacity depends on its botanical origin, environmental conditions and seasonal changes (Machado De-Melo et al., 2018).

Brazilian honey has been recognized for its quality that also depends on the aforementioned factors and the region where it is produced (Braga et al., 2019). This quality presents variations in quantity and type of phenolic compounds, as well as in flavor and aroma. The country has a vast geographical area, with great diversity of honey bee species for apiculture products, which reflects on the wide variety of honey (Moraes et al., 2019; Sekine et al., 2019; Nascimento et al., 2018). Furthermore, its quality is also attributed to the resistance of Africanized bees to diseases and pests, so there is no need to use chemicals that contaminate honey to maintain hives.

Moreover, the increase in studies on the quality of honey has been showing the incidence of trace elements (Altunatmaz et al., 2018). These trace elements and pesticides had been monitoring, to seek an alternative and methods to improve the quality of

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the honey produced (Yaquib et al., 2020). Demonstrating, even more, the importance of the georeferencing of production (Camargo et al., 2014) and denomination of origin of honey.

Honey from the Western region of Paraná has been granted the “Indication of Origin” seal (Instituto Nacional da Propriedade Industrial, 2017), which gives it higher added value. For that reason, producers follow international quality standards. Being awarded the seal requires information on the relationship between honey characteristics and edaphoclimatic factors in the region, which is the object of this study, since the honey produced in western Paraná has not been a topic of research regarding physicochemical characteristics, phenolic compounds or antioxidant activity. Thus, the aim of this study was to characterize the honey produced by beekeepers from the aforementioned region, with regard to physicochemical parameters, phenolic compounds and antioxidant activity, as well as the degree of similarity between the samples from different municipalities.

2 Materials and methods

2.1 Geographical origin of the honey samples

A total of 67 honey samples of *A. mellifera* L. were collected from October 2016 to March 2017 and, then, analyzed. They were

provided by beekeepers from 14 municipalities of Western Paraná: Santa Helena (n = 27), Missal (n = 7), Terra Roxa (n = 7), Marechal Cândido Rondon (n = 7), Diamante do Oeste (n = 4), Entre Rios do Oeste (n = 3), Corbélia (n = 2), Matelândia (n = 2), Toledo (n = 2), Ramilândia (n = 2), Francisco Alves (n = 1), Itaipulândia (n = 1), Palotina (n = 1), and one from São José das Palmeiras (Figure 1).

2.2 Physicochemical analysis

Physicochemical analyses were performed according to Marchini et al. (2004), in triplicate, which allows higher reliability to the measured data. Moisture was determined following the Chataway refractometric method, which uses the refractive index measurement of the sample to be converted to moisture content by using an Abbe refractometer (Atago, Abbe refractometer, Tokyo, Japan). The pH was performed by diluting 10 g of honey in 75 mL of distilled water, determining the concentration of hydrogen ions contained in the honey solution used. Total acidity was determined based on neutralization of the honey acid solution, titrated with 0.05 N sodium hydroxide to the equivalence point. The ash content was determined by the gravimetric method after incineration in muffle furnace at 550°C for 5 hours. For honey color determination, honey samples' absorbance was read with a spectrophotometer at 560 nm

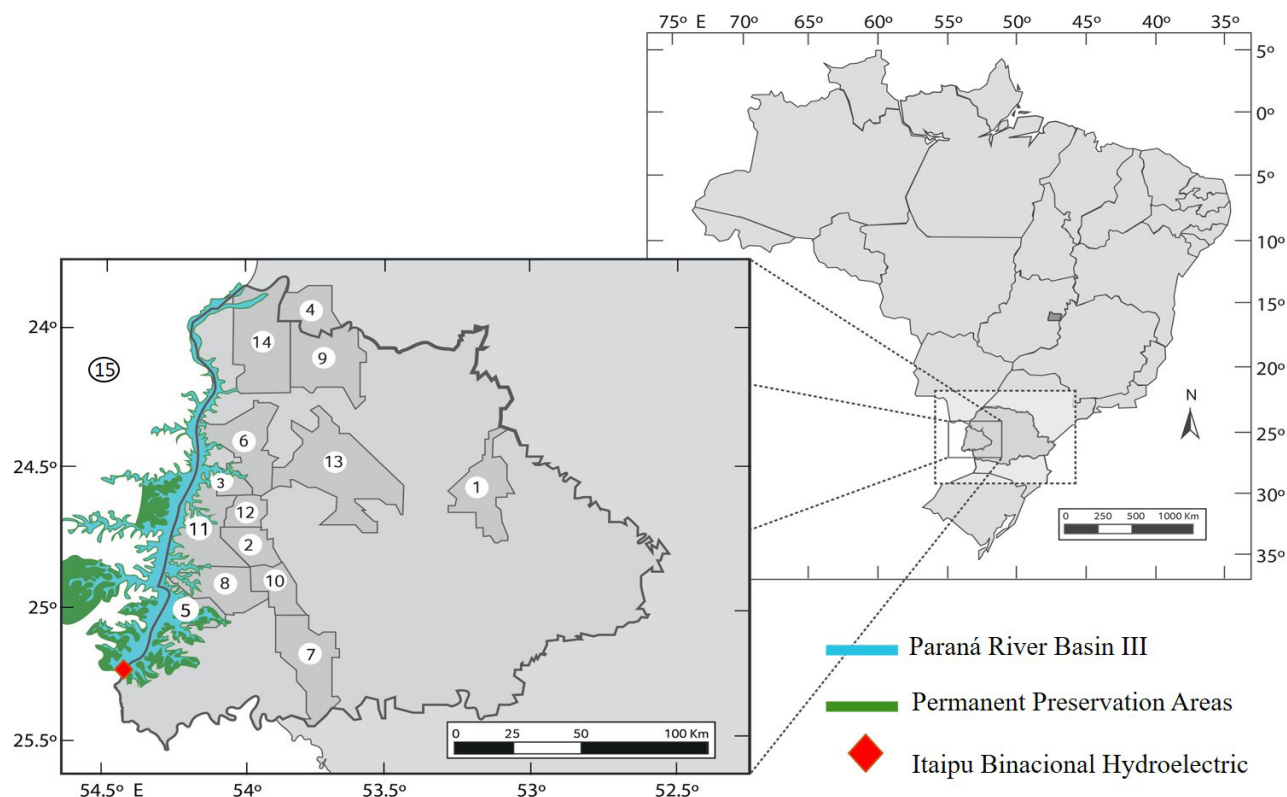


Figure 1. Location map of the study area: map showing the position of the State of Paraná in Brazil (right); map showing the position of the Western region of Paraná (left), with emphasis on sampling municipalities. 1 - Corbélia, 2 - Diamante do Oeste, 3 - Entre Rios do Oeste, 4 - Francisco Alves, 5 - Itaipulândia, 6 - Marechal Cândido Rondon, 7 - Matelândia, 8 - Missal, 9 - Palotina, 10 - Ramilândia, 11 - Santa Helena, 12 - São José das Palmeiras, 13 - Toledo, 14 - Terra Roxa, as well as Paraná River Basin III, which borders Paraguay (15).

(UV-1800, Shimadzu, Columbia, USA). The reading was transformed into color by using the Pfund scale. Electrical conductivity was measured in a solution with 20% honey dry matter, diluted in distilled water and quantified by using a conductivity meter (Tec-4MP, Tecnal, Piracicaba, Brazil). The total nitrogen content was determined by the Micro-Kjeldahl method using factor 6.25 to convert nitrogen into proteins. Determination of HMF content was based on White's reaction by spectrophotometry with readings at 284 nm and 336 nm. Determination of total sugars (%), reducing sugars (%) and apparent sucrose (%) was based on the procedure described by Sereia et al. (2017). The method was based on the ability of sugars, such as glucose and fructose, to reduce the copper found in the cuproalcaline (Fehling liquor) solution, changing from Cu^{2+} to Cu^+ (reduction of cupric ions in cuprous), and the sugars were oxidized into organic acids.

2.3 Phenolics and flavonoids

Total phenolic content (TPC) and Total flavonoid content (TFC)

Honey extract was obtained by diluting the honey in methanol (1:11, w / v) and stirring in Vortex for one minute. That was followed by an ultrasonic bath for 15 minutes. Then the extract was filtered through a qualitative paper filter for retention of larger particles. The extract remained refrigerated until the analyses were performed in triplicate. The total phenol content was determined according to Meda et al. (2005). Gallic acid ($0.05 - 0.08 \text{ mg mL}^{-1}$) was used as standard for constructing the calibration curve ($R^2: 0.9999$), expressed as mg GAE 100 g^{-1} of honey. Total flavonoid content was determined according to the method described by Meda et al. (2005), with some modifications. Quercetin (0.01 to 0.07 mg mL^{-1}) was used as standard for constructing the calibration curve ($R^2: 0.9972$), expressed as mg EQ 100 g^{-1} of honey.

FRAP, DPPH and ABTS antioxidant activities

The ferric reducing antioxidant potential (FRAP) was determined according to Benzie & Strain (1996), where the ability of honey extracts to reduce TPTZ reagent (2,4,6-tripyridyl-s-triazine) was tested. Results were obtained by adjusting the ferrous sulfate calibration curve ($250-2000 \mu\text{M}$; $R^2: 0.9988$). Results of FRAP antioxidant activity were expressed as μmol ferrous sulfate equivalent per gram of honey ($\mu\text{mol FeSO}_4/\text{g}^{-1}$ honey).

We determined the sequestering activity of the DPPH oxidizing radical from honey extracts (2,2-diphenyl-1-picrylhydrazyl), equivalent to the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), according to the parameters of Beretta et al. (2005), with modifications. Results were expressed as μmol trolox equivalent (ET) per gram of honey, according to the adjusted equation of the standard curve based on the synthetic antioxidant Trolox (20 to $140 \mu\text{mol}$ Trolox; $R^2: 0.9977$).

ABTS (2,2-azinobis (3-ethylbenzotiazolina-6-sulfonic acid) antioxidant activity was performed according to Re et al. (1999), with modifications. Results were expressed as μmol trolox equivalent (TE) per gram of honey, according to the adjusted

equation of the standard curve based on the synthetic antioxidant Trolox. (0.100 to $0.150 \mu\text{mol mL}^{-1}$; $R^2: 0.9953$).

2.4 Statistical analysis

Low and high values, medians, mean and quartiles were calculated for the physicochemical parameters, bioactive compounds and antioxidant activity. The data of the variables were analyzed by the multivariate factor analysis. Non-Metric Multidimensional Scaling (NMDS) was used to test the parameter differences between the different honey samples. Later, the Euclidean distance was used with the normalized data, using the "metaMDS" command to generate random and interactive processes, aiming to find the best possible solution. The measured NMDS adjustment value was assessed by the stress value. To estimate the adjustment between the dissimilarity matrix and the generated dendrogram, we calculated the cophenetic correlation coefficient (Estevinho et al., 2016). All statistical analyzes were performed using the R statistical software, version 3.0.2.

3 Results and discussion

3.1 Physicochemical parameters

The results of the physicochemical evaluations of the samples are presented in Table 1. The values found were compared with Brazilian (Brasil, 2000) and international (Food and Agriculture Organization, 2001) standards. The honey samples evaluated were characterized as acidic, because of the average pH value (3.26), which was lower than the minimum standard pH (3.30). In addition, the mean total acidity of the samples was $34.54 \text{ meq kg}^{-1}$, with only three samples with values above 60 meq kg^{-1} , which is the limit established by legislation (Brasil, 2000; Food and Agriculture Organization, 2001). Values above this may indicate fermentation of the product. The pH and free acidity of honey are also important in determining taste and stability against microbial development.

The moisture content of the honey samples analyzed presented an average of 18.75% (Table 1), but 7% of them presented moisture content above the allowed limit (20%) (Brasil, 2000). Several factors may influence moisture content, such as the degree of maturity, environmental conditions during the harvest, processing techniques and conditions of storage (Silva et al., 2016; Guo et al., 2019).

As for color, the honey analyzed presented an average value of $0.26 \pm 0.11 \text{ inc}$ (Table 1) and, according to a classification using the Pfund scale, 67.16% of the samples presented light amber color; 1.49%, white; 20.9% extra light amber; and 10.45%, amber. This information corroborates studies on honey samples from previous harvests in this region, which presented colors ranging from white to dark amber, with prevalence of lighter colors (Camargo et al., 2014). That is the reason why the honey in question has advantages, since light colors have higher acceptability by consumers (Silva et al., 2016; Kortensniemi et al., 2018). For this reason, warehouses from other regions of Paraná and Brazil purchase honey from this region to mix it with darker types, favoring commercialization. The low HMF values indicate the good quality of the honey produced, since all samples presented

values below 13.67 mg kg⁻¹ (Table 1). According to Machado De-Melo et al. (2018), the honey evaluated is considered fresh because the lower the HMF value, the higher the indicative of honey that was stored for a short time or did not undergo heating and fermentation process.

The ash contents of the samples presented an average value of 0.14% (Table 1). Variations of this parameter may be due to floral and geographical origin (Nascimento et al., 2018), honey management techniques applied and / or the type of extraction used (Nascimento et al., 2018; Baglio, 2018). All samples comply with current regulations (Brasil, 2000), which consider 0.6% of ash content for floral honey, whereas the limit tolerated for melate or melate honey and its mixtures with honey is 1.2%.

Silva et al. (2016) state that electrical conductivity in honey is directly related to ash content. The authors reported that this parameter was incorporated by Codex Alimentarius Standards, replacing the parameter of ash content. The legislation (Brasil, 2000) does not present low and high values for electrical conductivity, but Codex (Food and Agriculture Organization, 2001) considers the maximum value of 800.00 $\mu\text{S cm}^{-1}$. Thus, the values obtained, which ranged from 181.20 to 565.8 $\mu\text{S cm}^{-1}$ (Table 1), were well below the limit. Electrical conductivity of honey is a parameter that, together with evaluations of insoluble solids and minerals, is used to determine honey purity (Silva et al., 2016).

Protein percentage of honey samples presented an average value of 0.28% (Table 1). Honey protein levels vary due to different factors, such as bee species and nectar botanical origin (Alvarez-Suarez et al., 2018), as well as the proteins from secretions of the mandibular and hypopharyngeal glands that

are incorporated by bees in the process of transformation and dehydration of nectar into honey (Baglio, 2018).

The total sugar content is not standardized and, in this study, it presented an average value of 69.09% (Table 1). As for reducing sugars, there was a variation between 56.4 to 72.78% (Table 1), which is within the limits (Brasil, 2000; Food and Agriculture Organization, 2001), as well as the sucrose content that presented mean values of 4.28% (Table 1).

3.2 Phenolic compounds

The total phenolic compound content of the honey samples in this study ranged from 11.39 to 61.27 mg GAE 100 g⁻¹ (Table 2). These values were similar to honey samples from Portugal (Ferreira et al., 2009) in a way that an increase in the total phenol content of darker samples was detected: the white, amber and dark amber samples presented increasing values of 13.2, 16.8 and 20.4 mg GAE/100 g⁻¹, respectively. It may be the explanation for the amplitude of the low and high values observed in this study.

Flavonoid contents of the honey samples analyzed (Table 2) ranged from 7.97 to 44.99 mg EQ/100 g⁻¹. We also observed that all honey samples had higher total phenol content than flavonoids. This is due to the fact that flavonoids represented more than 50% of total phenols in plants and are transferred from plants to honey by bees (Silva et al., 2016). Flavonoid levels are related to the variation in honey color, as there is a significant increase of 12.6, 34.2 and 58.7 mg EQ/100 g⁻¹ honey, for white, amber and dark amber (Ferreira et al., 2009). The values show that the honey analyzed has bioactive compounds and can be

Table 1. Physicochemical parameters of honey from Western Paraná, South of Brazil.

Parameters	Standards ^b	Low	High	Median	Q1	Q3	Mean (SD) ^c
pH	3.30 to 4.60	2.65	4.44	3.10	2.92	3.48	3.26 ± 0.48
Acidity (meq kg ⁻¹)	60	6.00	83.50	34.00	23.25	41.50	34.54 ± 13.07
Moisture (%)	< 20	15.40	21.55	18.70	18.23	19.30	18.75 ± 1.02
Color (inc ^a)	-----	0.10	0.60	0.22	0.19	0.26	0.26 ± 0.11
HMF (mg kg ⁻¹)	< 60	10.12	13.67	10.65	10.48	10.91	10.79 ± 0.57
Ask (%)	< 0.60	0.03	0.45	0.14	0.11	0.16	0.14 ± 0.06
Conductivity ($\mu\text{S cm}^{-1}$)	-----	181.20	565.80	349.10	286.80	385.60	340.10 ± 70.94
Protein (%)	-----	0.13	0.60	0.26	0.22	0.31	0.28 ± 0.09
Total sugar (%)	-----	63.56	76.96	68.97	66.74	70.92	69.09 ± 3.06
Reducing sugar (%)	Low 65	56.40	72.78	64.39	62.76	66.67	64.57 ± 3.16
Sucrose (%)	High 6	0.42	13.07	3.92	2.19	5.42	4.28 ± 2.60

pH = Hydrogen potential; HMF = 5-hydroxymethylfurfural. ^aIncidence—absorbance at 560 nm; ^bBrasil (2000). Q1: 25% of the samples are below the median. Q3: 75% of the samples are above the median; ^cMean ± standard deviation.

Table 2. The bioactive compounds and antioxidant activity of honey samples. Western of Paraná, South of Brazil.

Parameters	Low	High	Median	Q1	Q3	Mean (SD) ^a
Total phenolic (mg GAE/100 g ⁻¹)	11.39	61.27	34.20	24.37	45.73	34.83 ± 13.12
Total flavonoid (mg EQ/100 g ⁻¹)	7.97	44.99	16.04	13.12	18.15	16.26 ± 5.15
FRAP ($\mu\text{mol FeSO}_4/\text{g}^{-1}$)	0.03	11.12	2.32	1.59	3.34	2.68 ± 1.82
ABTS ($\mu\text{mol ET}/\text{g}^{-1}$)	0.43	1.54	1.05	0.80	1.21	1.01 ± 0.27
DPPH ($\mu\text{mol ET}/\text{g}^{-1}$)	0.04	0.16	0.12	0.11	0.13	0.12 ± 0.02

Q1: 25% of the samples are below the median. Q3: 75% of the samples are above the ^astandard deviation. ABTS (2,2-azinobis (3-etilbenzotiazolina-6-sulfonic acid); DPPH (2,2-diphenyl-1-picryl-hydrazyl); (FRAP) ferric reducing antioxidant power (2,4,6-tripirydyl-s-triazine).

used as functional food or as a source of food antioxidants with nutraceutical properties (Ahmed et al., 2018).

3.3 Antioxidant activity

The antioxidant activity FRAP presented a variation from 0.03 to 11.12 $\mu\text{mol FeSO}_4/\text{g}^{-1}$ (Table 2). It showed higher values than ABTS methods (0.43 to 1.54 $\mu\text{mol ET}/\text{g}^{-1}$) and DPPH (0.04 to 0.16 $\mu\text{mol ET}/\text{g}^{-1}$), which may be due to the fact that FRAP is expressed as iron sulfate, while ABTS and DPPH are expressed as Trolox equivalent. FRAP antioxidant activity is in accordance with what was observed in studies with honey samples from Minas Gerais, Rio de Janeiro and Rio Grande do Sul, in Brazil (Sant'Ana et al., 2012; Nascimento et al., 2018). The observed gap is compatible with the FRAP, ABTS and the DPPH values of honey samples from countries such as Algeria (Khalil et al., 2012) and Cuba (Alvarez-Suarez et al., 2018). The use of different equivalents as ferric (II) and quercetin for FRAP makes it difficult to compare the values of work with data from other studies (Kuś et al., 2014; Duarte et al., 2012). The same is observed with ABTS and DPPH, whose results are expressed as percent inhibition of DPPH free radical (Sant'Ana et al., 2012; Nascimento et al., 2018) or in equivalent quercetin and gallic acid (Duarte et al., 2012).

Studies show that samples from the same region have different values, showing variations due to botanical, geographical origin and seasonality (Sant'Ana et al., 2012; Salgueiro et al., 2014). Honey antioxidant activity may increase with industrial honey processing (Machado De-Melo et al., 2018). Another factor that influences the antioxidant activity is storage time, because honey degradation processes occur by enzymatic or Maillard reaction, which releases intermediate chemical groups with reducing power.

During the collection period there were changes in the color intensity of honey, and the samples collected in October and November were characterized by lighter shades. During this period, there are flowering grapes from Japan (*Hovenia dulcis*), abundant species in the study area, mainly in the lakeside forests of Itaipu bi-national hydroelectric (Camargo et al., 2014). Moraes et al. (2019) verified that in honey samples from the lake area, there is a predominance of pollen type *H. dulcis* in most of the honey sampled, characterized as monofloral of light shades. Nascimento et al. (2018) found out that monofloral honey of this species has light shades, as well as a total phenol content of 30.5 mg GAE/100 g⁻¹ and 0.2 mg EQ/100 g⁻¹ of flavonoids. Between December and March, the honey got darker, which may be linked to the Eucalyptus flowering, quite prevalent in the region (Sekine et al., 2019; Camargo et al., 2014) and significantly present in the honey samples of the region (Sekine et al., 2019; Moraes et al., 2019). It causes an increase in the bioactive compound contents and antioxidant activity. (Nascimento et al., 2018).

The similarity relationship between the evaluated parameters and honey samples from the 14 municipalities was assessed by the Non-Metric Multidimensional Scaling (NMDS) (Figure 2A). The ordering produced a two-dimensional solution with a final stress value of 0.033. According to Estevinho et al. (2016), the ordering is representative because there was a low stress value.

The nine groups were formed by using the criterion of the mean silhouette widths, from the result of the cluster analysis of the honey samples (Figure 3). The correlation coefficient (CCC) was 0.73, a value considered reasonable as a factor of representativeness (Estevinho et al., 2016). NMDS showed that

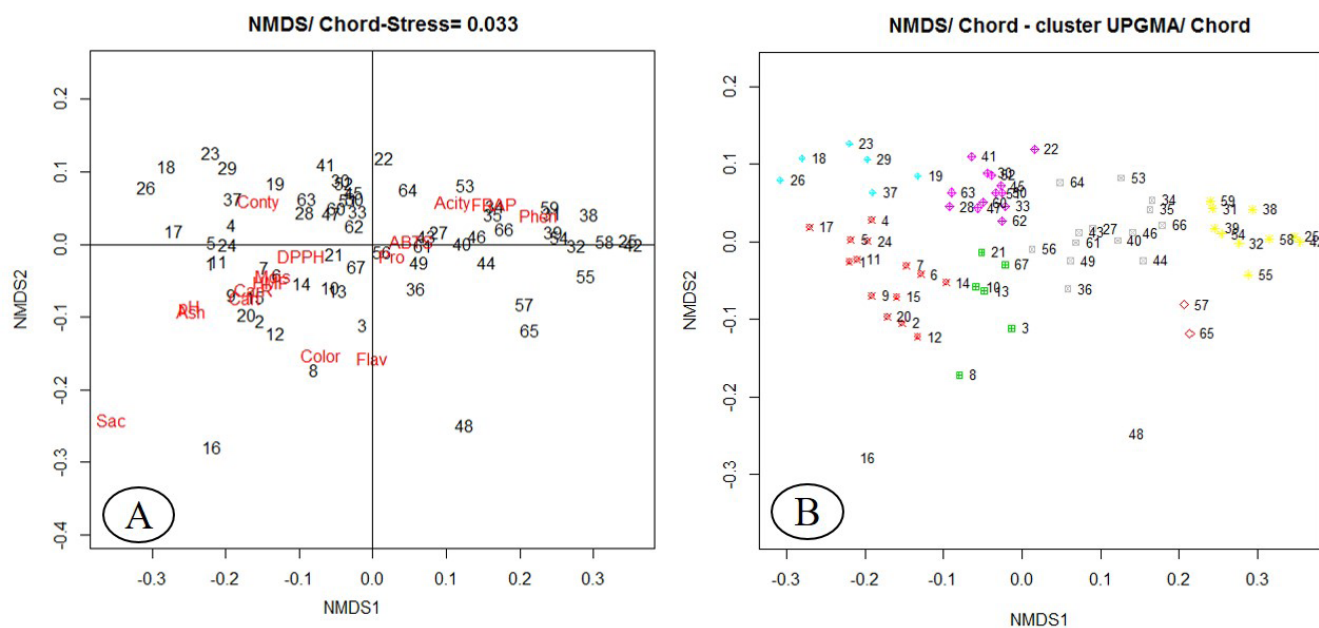


Figure 2. (A) Non-Metric Multidimensional Scaling (NMDS) for similarity of physicochemical data, bioactive compounds and antioxidant activity of 67 honey samples from Western Paraná. A chord distance from the data matrix was generated with a stress value of 0.033. Each number refers to a sample and the acronyms are the different parameters evaluated: Phen: Total phenols; Flav: Flavonoids; Sac: sucrose; Conty: conductivity; ASH: ashes; pH: pH; color: color; Acity: free acidity; Pro: protein; AR: reducing sugars; TA: total sugars; HMF: Hydroxymethylfurfural and Mo: Moisture; (B) UPGMA grouping of a chord distance matrix between groups in NMDS ordering for physicochemical data, bioactive compounds and antioxidant activity (CCC = 0.73).

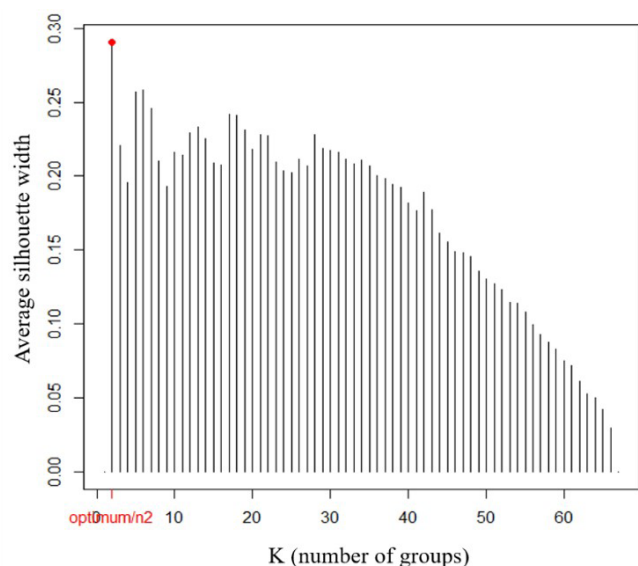


Figure 3. Bar portions showing the average width of the silhouette to $k = 9-67$ groups.

the studied samples were grouped according to their similarities in relation to the evaluated parameters (Figure 2B). The blue and purple groups have higher values for electrical conductivity. On the other hand, the samples of the yellow group presented the highest values for total phenols. The group in green presented the highest values for color and flavonoids, parameters that have a correlation of 0.96% according to Khalil et al. (2012).

The best partition by this criterion is the one with the highest average silhouette width. The group in red presented the highest values for pH, HMF, humidity, ash content, total sugars and reducing sugars. The gray group presented the highest values for FRAP, ABTS, acidity and protein. Khalil et al. (2012) reported that the antioxidant activity FRAP and Proline showed a high correlation (0.98%). The group with two red samples showed similarity for the vast majority of compounds and may indicate a relationship of bioactive compounds despite their different geographical origin. Finally, the two groups formed by only one sample each did not show similarity relationship with other samples. These results are important because they indicate that, within the nine groups, there was a homogeneous distribution of samples from different locations, showing that, in general, the honey samples evaluated present great similarity. As there is a differentiation of the samples according to their botanical origin, it would be necessary to analyze these honey samples coming from different municipalities to observe if they were grouped according to their botanical origins (Marchini et al., 2005), or if some pollen types interfere with the parameters evaluated (Estevinho et al., 2016).

4 Conclusion

The honey samples evaluated complied with national and international normative instructions. In addition, this first work quantifying bioactive compounds and antioxidant activity in honey samples from Western Paraná found relevant concentrations of phenolic acids and flavonoids, as well as antioxidant activity, which means that the product has nutraceutical properties.

The analyses show similarity between the honey samples of the region in relation to the parameters that were evaluated. This study demonstrates that the types of honey analyzed have quality, commercial maturity, and bioactive properties that may be related to the phytogeographic origin of the product.

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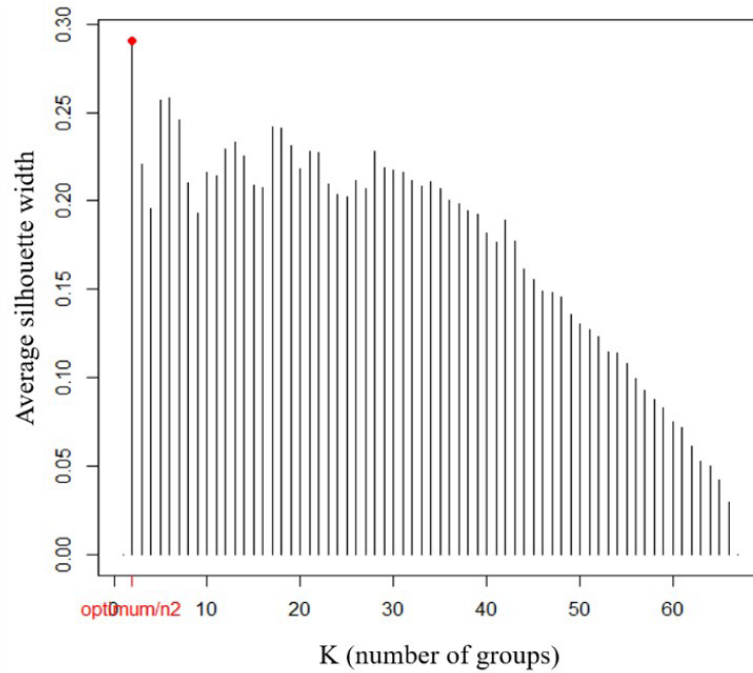
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Erratum

In the article “Physicochemical, bioactive properties and antioxidant of *Apis mellifera* L. honey from western Paraná, Southern Brazil”, DOI <https://doi.org/10.1590/fst.11720>, published in Food Science and Technology, ISSN Print version ISSN 0101-2061 On-line version ISSN 1678-457X, 2021, vol.41, suppl.1, pp.247-253. Epub Sep 28, 2020.

Where it reads: Figure 3. Change



It Should be read:

