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Floral Origin and Physical and Chemical Characteristics of Honey from Africanized Bees in Apiaries of Ubiratã and Nova Aurora, State of Paraná

ES Sekine[1], EH Takashiba[3], RO Bueno[1], PAA Bueno[1], MG Caxambu[1], MJ Sereia[1], LC Marchini[4], ACCC Moreti[5], VAA Toledo[2]

1 - Universidade Tecnológica Federal do Paraná – UTFPR, Campo Mourão-PR, Brazil
2 - Universidade Estadual de Maringá – UEM, Maringá-PR, Brazil
3 - Universidade Federal de Santa Maria – UFSM, Santa Maria-RS, Brazil
4 - Escola Superior de Agricultura Luiz de Queiroz – Esalq/USP, Piracicaba-SP, Brazil
5 - Instituto de Zootecnia, Nova Odessa-SP, Brazil

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Corresponding author
ES Sekine
Departamento de Biodiversidade e Conservação da Natureza (DABIC)
Universidade Tecnológica Federal do Paraná
Via Rosalina Maria dos Santos nº 1233, CP: 271
CEP: 87301-899 - Campo Mourão-PR, Brasil.
E-Mail: essekine@gmail.com

Abstract

Physical and chemical characteristics of honey may vary due to the diversity of flora and soil characteristics, or seasonal factors. This study was carried out in two counties, Nova Aurora and Ubiratã, located in the West and Center-West regions of the State of Paraná. The objective of the study was to verify if the physical and chemical parameters of *Apis mellifera* (L.) honey are in accordance with the national standard, as well as to verify how the 21 samples collected in the two localities are grouped, based on the physical, chemical and pollen characteristics. Honey was analyzed for sugar, ash, protein, moisture, color, electrical conductivity, formaldehyde index, diastase and viscosity, water activity and pollen content. Samples of honey containing the dominant pollen types *Glycine max* (L.) Merr. and *Eucalyptus* sp. formed groupings similar to those based on physical and chemical characteristics, however, the multivariate classification of honey samples in groups based on pollen types was not an efficient method to group samples of polyfloral honey.

Introduction

Honey is a substance made mainly from the nectar of flowers, and because of the diversity of the Brazilian flora, it may present peculiarities in its physical and chemical characteristics, depending on the type of plant that is part of its composition. Different environments may lead to variations in honey characteristics due to floral species and soil characteristics, or due to seasonal factors such as temperature and rainfall (Crane, 1990; Marchini et al., 2004a).

Depending on the number of predominant floral species, honey may be classified as monofloral, bifloral or heterofloral. The latter, also called wild honey, has quite variable properties, depending on the environmental conditions and the type of flower used, and it is not possible to generalize its characteristics (Barth, 2004). For this reason, there is a need to compile data on the physical and chemical characteristics of honeys produced in the south and southeast regions of Brazil, as there are indications that the standards established in the Brazilian legislation (Brasil, 2000) do not include all these honeys (Barth et al., 2005).

As the characteristics of honey depend on the species available for the supply of nectar, investigations aimed at identifying the plants used by bees in different regions of the country provide information that can be used in the management of hives and in the adoption of strategies for a better utilization of the flora with potential for honey production (Marchini et al., 2004a; Nogueira Couto & Couto, 2006).

Several studies have been carried out in the southern and southeastern Brazil to investigate the floral origin of honey (Bosco & Luz, 2017; Sekine et al., 2013) and its physicochemical characteristics (Mendonça et al., 2008; Sereia et al., 2011; Barth et al., 2013; Alves et al., 2011). This study aimed to analyze the floral origin, physical and chemical characteristics of samples of honey collected in two counties of Paraná state at different times of the year and to verify whether physicochemical characteristics are grouped depending on the floral species used in honey production.

Material and methods

Obtaining samples of honey

Honey samples were collected in two apiaries (Fig 1): Apiary A, located in the county of Nova Aurora, State of Paraná (24°31'50" S and 53°11'50" W) and Apiary B, located in the county of Ubiratã, State of Paraná (24°29'41" S and 53°02'43" W). These counties are located in the phytogeographical domain of submontane semidecidous tropical forest (IBGE, 2012). In both properties, beekeeping is an activity complementary to the family income, so the area surrounding the apiaries is occupied by agriculture and the properties have a forest fragment as legal reserve area of 13.65 ha and 2.39 ha for apiaries A and B, respectively

In order to obtain the honey samples, three hives were randomly marked in each apiary. All hives were located near or inside the forest fragments. In each hive, we placed every month a frame with new bee-wax foundation without combs, or (when available) a frame with formed combs to obtain the honey produced by the bees during the period. Inspection of hives was performed monthly to verify the existence of mature honey. To avoid sampling immature honey, honeycombs, with at least 75% of capped cells, were removed and centrifuged separately, in individual plastic bags, avoiding the honey mixture. After each harvest, the frames were replaced with other empty ones. The samples were transported to the laboratory in thermal boxes and kept under refrigeration for approximately six months.

A total of 21 samples were taken for the two apiaries (nine from apiary A and 12 from apiary B) between December 2008 and May 2009. In April and May there was no mature honey in apiary A.

Physical and chemical analysis

Physical and chemical analyses were performed in triplicate for each sample. Reducing sugars, total reducing sugars, sucrose, ash and protein were verified according to the methods adopted by the Adolfo Lutz Institute (Zenebon et al., 2008). The techniques used to determine the moisture content (Atago Co, 1988), color (Vidal & Fregosi, 1984), electrical conductivity (BOE, 1986); pH, acidity, formaldehyde index (Moraes & Teixeira, 1998), diastase activity (C.A.C, 1990) and viscosity were done according to the methods cited by Marchini et al. (2004a). The water activity (Aw) determination was made directly using a water activity meter Aqualab®. The analyses were carried out at the Laboratory of Useful Insects of the Department of Entomology and Acarology of the School of Agriculture, Luiz de Queiroz-Esalq/USP and at the Chemistry and Ecosystems laboratories of the Federal Technological University of Paraná - UTFPR Campo Mourão.

Pollen analysis

An aliquot of 10 g of each sample was diluted in distilled water and centrifuged for acetolysis (Erdtman, 1952). The pollen analyses were: a) qualitative, by the identification of the pollen types by comparison with the collection of pollen slides of the plants collected in the region, and specialized literature; and b) quantitative, by the average count of 300 to 500 pollen grains on microscopy slides, in triplicate. Pollen types were

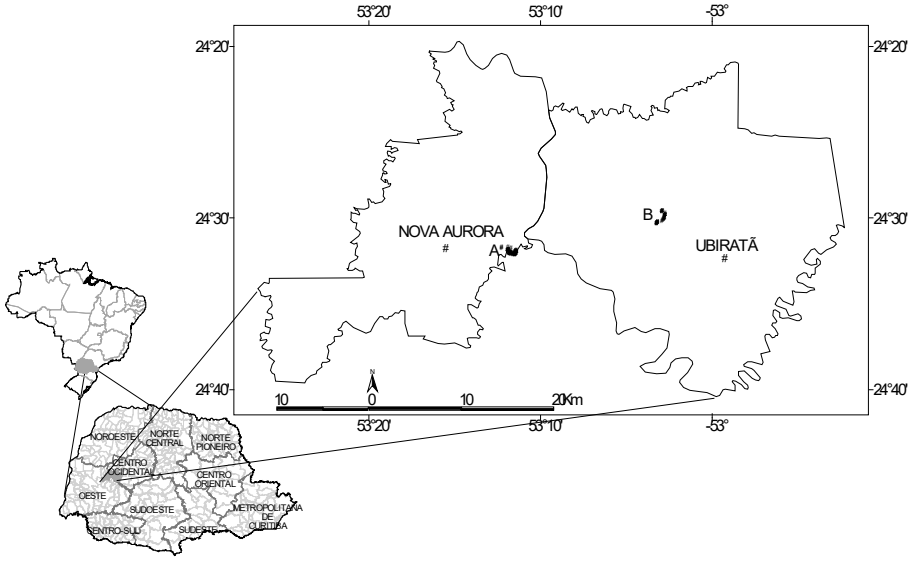

Fig 1. Location of the sampling sites. A: Apiary in Nova Aurora; B: Apiary in Ubiratã, State of Paraná.

classified into four frequency classes (Louveaux et al., 1978; Barth, 1989): Dominant pollen (frequency over 45%), Accessory pollen (from 16 to 45%), Important isolated pollen (from 3 to 15%), Occasional isolated pollen (frequency below 3%).

Data analysis

We applied qualitative and quantitative descriptive statistical analysis and multivariate statistical analysis: clustering technique for the honey samples, using the statistical software R (2016). For the pollen data of the two areas, we calculated the Sorensen Similarity Index (ISS) (Legendre & Legendre, 1984; Mueller-Dombois & Ellenberg, 1974; Pielou, 1975), which is the most commonly used to compare qualitative floristic data between communities. Mathematically, the index is defined as: $ISS = 2c/(a+b+c) \times 100$, where: a = number of species restricted to area a, b = number of species restricted to area b and c = number of species common to areas a and b.

The results of the physical and chemical analyses of honey were compared between the sampling sites by clustering analysis using the Euclidean distance by the UPGMA clustering method. In the cluster analysis, the mean Euclidean distances for the properly standardized data were adopted as dissimilarity means. The clustering based on the pollen types found in honey was made from the presence and absence of pollen types using the Jaccard coefficient by the UPGMA linkage method using the vegan package (Oksanen, 2017) using the statistical software R. Jaccard coefficient expresses the similarity between honey samples, based on the number of common pollen types.

Results

Physical and chemical characteristics

There was predominance of the extra-light amber color (50%). Light amber (27%) and white (23%) were also found in honey samples. The complete results of the physical and chemical parameters analyzed are listed in Table 1.

The parameters that have limits established by the Brazilian legislation (diastase, moisture, pH, acidity, reducing sugars, sucrose and ash) (Brasil, 2000) were met in the samples collected (Table 1). Only one of the samples contained moisture above the established values.

Pollen analysis

In the honey samples, we identified 49 pollen types belonging to 25 families (Table 2). Two pollen types were not identified at the family level. The pollen types with higher frequency belong to the families Fabaceae (34.9%), Myrtaceae (11.7%) and Piperaceae (11.4%) (Figure 2A).

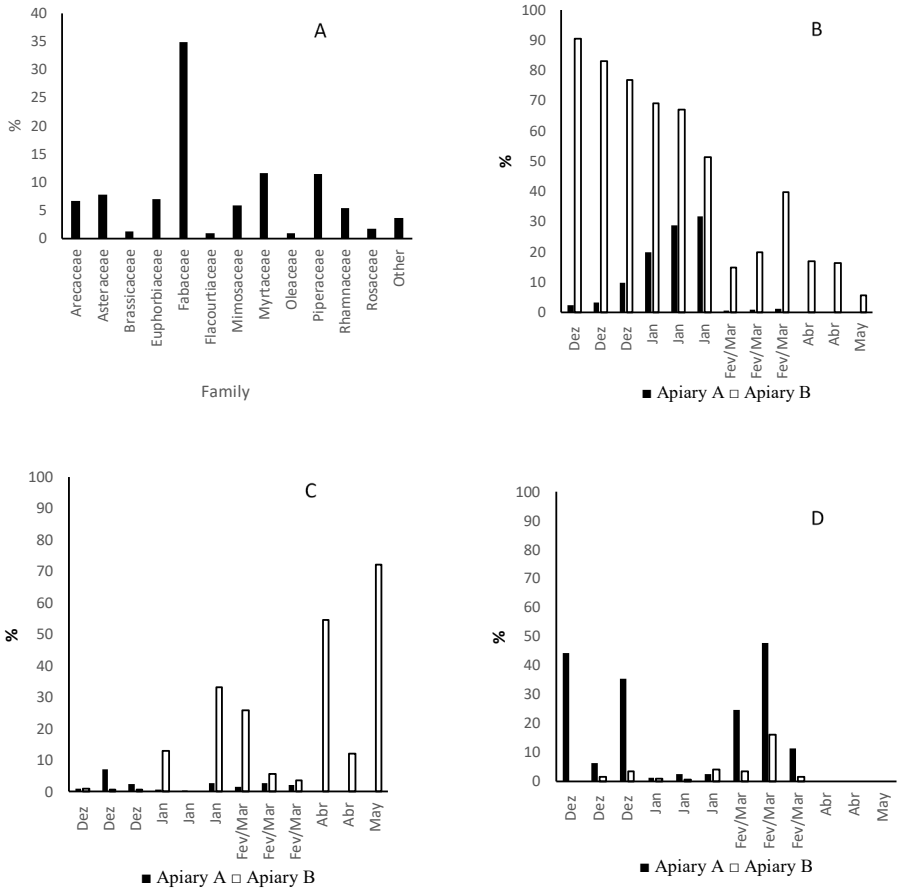

Fig 2. Pollen types frequency in honey samples from two apiaries in the counties of Ubiratã and Nova Aurora in the Paraná state, Brazil. A) Frequency of the main families found in honey in the two apiaries; B, C and D, pollen frequency of the dominant species found in the two apiaries from December 2008 to May 2009, respectively, *Glycine max*, *Eucalyptus* spp., and Piperaceae type.

Table 1. Physical and chemical parameters of 21 samples of *Apis mellifera* honey, collected from December 2008 to May 2009 in the county of Nova Aurora (Apiary A) and Ubiratã (Apiary B), State of Paraná.

Apiary	Month	Sample	Diastase (Gothe)	Condutiv ($\mu S.cm^{-1}$)	Moisture (%)	pH	Protein %	Acidity ($meq.kg^{-1}$)	FI[1] (mL.kg-1)	Ash (%)	Viscos (mPa.s)	WA[2] a_w	TRS[3] (%)	RS[4] (%)	Sucrose (%)	Color**	DP[5]
		1	24.24	387.33	18.10	3.51	0.23	27.11	10.37	0.43	2545.00	0.54	76.62	72.29	4.11	ELA	Polifloral
	Dec	2	14.05	382.00	19.20	3.59	0.20	18.74	8.70	0.07	1030.00	0.60	81.25	76.45	4.56	ELA	Polifloral
		3	24.91	381.67	18.20	3.47	0.28	23.72	9.36	0.07	1535.00	0.62	78.08	75.35	2.60	ELA	Polifloral
		4	20.29	382.33	18.40	3.44	0.34	32.97	11.05	0.09	1220.00	0.62	80.19	74.46	5.44	ELA	Polifloral
A	Jan	5	20.68	383.67	19.20	3.45	0.34	31.35	11.40	0.20	1025.00	0.65	76.86	74.96	1.80	LA	Polifloral
		6	33.48	366.67	17.10	3.49	0.36	32.17	12.73	0.20	2670.00	0.56	79.38	76.11	3.10	LA	Polifloral
		7	16.95	319.67	19.10	3.42	0.36	28.71	8.39	0.16	1130.00	0.60	77.33	76.48	0.81	ELA	Polifloral
	Mar	8	31.58	374.00	17.10	3.49	0.46	31.43	10.70	0.17	3115.00	0.58	78.71	76.90	1.73	LA	Piperaceae
		9	14.74	301.33	19.10	3.32	0.27	27.19	10.07	0.13	1040.00	0.61	78.06	74.38	3.49	ELA	Polifloral
Mean A			22.32	364.30	18.39	3.46	0.31	28.15	10.31	0.17	1701.11	0.60	78.50	75.26	3.07		
Standard Deviation			6.91	31.43	0.85	0.07	0.08	4.62	1.37	0.11	835.15	0.03	1.55	1.44	1.49		
		10	18.33	106.33	16.70	3.54	0.25	17.95	12.42	0.11	2940.00	0.57	78.64	74.09	4.33	W	*Glycine max*
	Dec	11	23.27	137.77	16.40	3.53	0.26	17.76	10.72	0.06	4115.00	0.56	77.07	75.76	1.24	W	*Glycine max*
		12	34.02	120.23	16.70	3.50	0.26	21.11	12.40	0.05	3580.00	0.62	75.44	74.85	0.56	W	*Glicine max*
		13	28.45	141.13	17.10	3.56	0.27	23.62	13.57	0.48	2290.00	0.60	71.42	69.92	1.43	W	*Glycine max*
	Jan	14	36.47	155.17	16.50	3.52	0.29	23.11	13.40	0.08	3405.00	0.56	76.34	73.02	3.16	ELA	*Glycine max*
		15	24.74	146.70	17.20	3.55	0.25	21.47	12.41	0.07	2220.00	0.63	77.69	72.38	5.05	ELA	*Glycine max*
B		16	54.02	342.00	19.00	3.47	0.22	43.21	10.37	0.45	395.00	0.64	75.42	71.02	4.18	ELA	Polifloral
	Mar	17	62.25	342.33	17.90	3.56	0.33	25.81	14.08	0.11	1855.00	0.58	77.03	72.01	4.77	ELA	Polifloral
		18	48.67	317.33	18.10	3.53	0.34	25.09	10.37	0.38	1450.00	0.59	75.68	72.93	2.61	ELA	Polifloral
	Apr	19	37.72	466.00	18.10	3.63	0.31	37.18	10.38	0.14	1380.00	0.59	78.57	72.57	5.70	LA	*Eucalyptus*
		20	48.17	348.33	21.40	3.48	0.29	31.83	12.06	0.08	465.00	0.65	76.57	71.23	5.07	LA	Polifloral
	May	21	46.18	517.00	17.30	3.56	0.36	39.26	9.71	0.46	1960.00	0.61	79.37	74.74	4.40	LA	*Eucalyptus*
Mean B			38.52	261.69	17.70	3.54	0.29	27.28	11.82	0.21	2171.25	0.60	76.60	72.88	3.54		
Standard Deviation			13.53	144.31	1.40	0.04	0.04	8.55	1.47	0.18	1177.42	0.03	2.08	1.74	1.71		
Total mean			32.31	304.97	17.94	3.51	0.29	27.60	11.31	0.19	2011.75	0.60	77.42	73.77	3.47		
Standard Deviation			13.61	123.74	1.23	0.07	0.06	7.17	1.49	0.16	1056.95	0.03	2.12	1.95	1.53		
Standard*			Min. 8	-	Max. 20	3.3-4.6	-	Max. 50	-	Max. 0.6	-	-	-	Min. 65	Max. 6		

[1]Formol index; [2]Water activity; [3]Total reducing sugar; [4]Reducing Sugar, [5]Dominant pollen (> 45%); *BRASIL (2000); **ELA = Extra Light Amber; LA = Light Amber; B = White.

The families with the highest number of pollen types represented were Asteraceae (seven), Euphorbiaceae, Mimosaceae and Myrtaceae (four types each).

Dominating pollen types (D) were found in several of the samples collected in apiary B, with *Glycine max* (soybean) in the months of December and January, with frequencies between 51.4% and 90.6%. and *Eucalyptus* spp. in the months of April (54.7%) and May (72.2%). In apiary A, the pollen of Piperaceae was dominant (47.8%) in February (Figure 2B, C, D). Soybean pollen was also found as accessory pollen in seven other samples, Piperaceae in four and *Eucalyptus* in two samples. Also found as accessory pollen, in at least one sample, pollen types of the genera *Baccharis*, *Mikania, Lonchocarpus* and *Campomanesia* and the species *Alchornea triplinervea* (Spreng.) Müll.Arg. *Parapiptadenia rigida* (Benth.) Brenan (Mimosaceae) appeared in all samples, but its contribution never reached more than 10% of the total of a sample. Samples 5 and 7, both of apiary A, had higher number of pollen types (17).

Important isolated pollen (I) totaled 80 occurrences, from 30 pollen types. Occasional isolated pollen (O) had 175 occurrences, from 50 pollen types. The similarity between the areas, measured from the pollen types present in the samples of honey, was 87%.

Cluster analysis

Cluster analysis based on the physical and chemical characteristics (Figure 3A), showed two groups, evidencing the collection sites. One group consists mainly of samples of apiary A with four samples of apiary B, and the other group consisted mainly of samples of apiary B and only three samples of apiary A. The dendrogram formed by the pollen types presented high similarity (Figure 3B), showing samples from the same site and month of collection, except for sample B19 and B21.

Discussion

The color of the honey samples indicates the presence of mineral content (Bath & Singh, 1999; Finola et al., 2007). Wild or polyfloral honey tends to exhibit a large color variation (Almeida-Anacleto & Marchini, 2004). The samples analyzed here have a light color. In general, honey with light color has higher market value (Boffo et al., 2012; Silva et al., 2013; Almeida-Muradian et al., 2014).

Samples are within the ranges established in the Brazilian legislation for all analyzed parameters. Only one exceeded the maximum allowed for moisture (21.4%). The high moisture content is verified in honey harvested when the alveoli were not completely sealed. Additionally, relative air humidity may also contribute to moisture in honey (Faria, 1993; Sereia et al., 2011). Despite the care in collecting the samples with 75% of the alveoli covered, this sample had higher humidity. Values lower than 18.5% usually indicate mature honey (Marchini et al., 2004b). Honey from partially capped cells often presents a high percentage of moisture (Sanz et al., 1995). The stability of honey with moisture between 17 and 20% depends on a low content of microorganisms (Malacalza et al., 2007).

Some parameters limited by Brazilian legislation are indicative of honey quality. Among them, the diastase index refers to alpha-amylase, the enzyme responsible for the digestion of starch. Its origin is attributed to the salivary secretion of bees and is a parameter that gives indications of overheating. However, this is not always true because some types of honey naturally have less diastase than others, once the enzyme is added by the bees during the ripening of the nectar to the thicker consistency of honey. As some types of nectar are naturally thicker than others, they require less processing by the bee and, consequently, less diastase (White, 1994). The percentage of ash in honey expresses the content of mineral material, being a parameter widely used to check honey

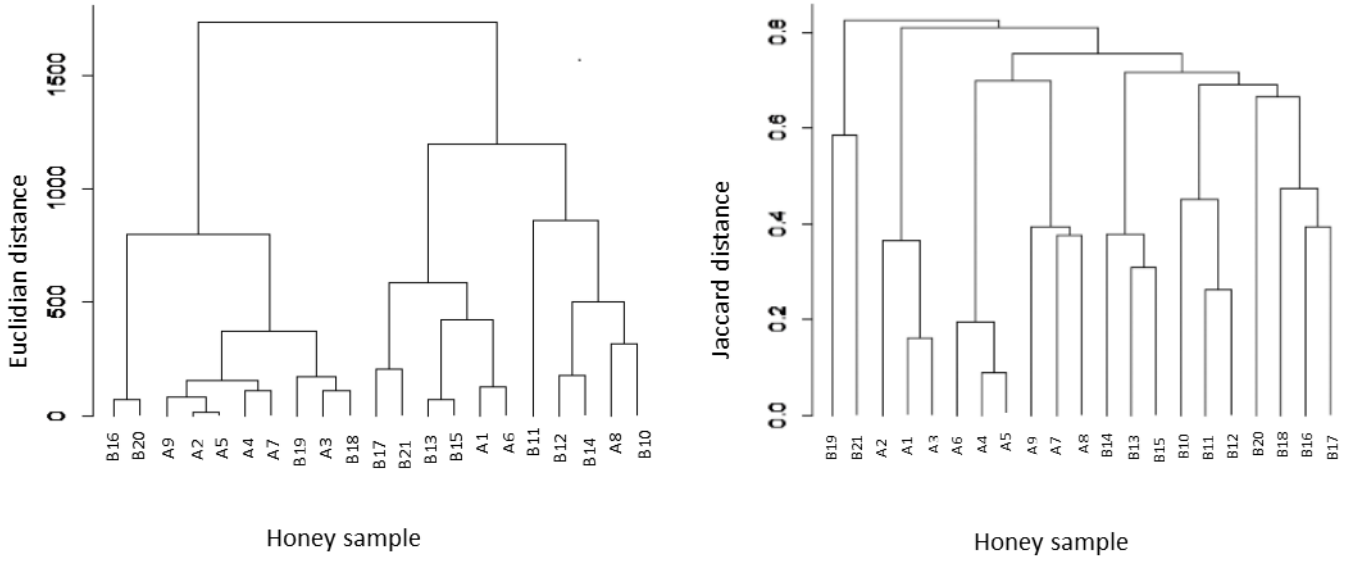

Fig 3. Clusters obtained by the UPGMA method - A) Euclidean distance from physical and chemical characteristics and B) Jaccard method from the pollen types. Analyses made on samples of *Apis mellifera* honey, collected from December 2008 to May 2009, in the counties of Nova Aurora and Ubiratã, State of Paraná.

quality (Marchini et al., 2005). Acidity is also an important component of honey, as it contributes to its stability, against the development of microorganisms (Marchini et al., 2004b).

Brazilian legislation establishes a minimum of 65% of reducing sugars and a maximum of 6% of sucrose (Brasil, 2000). In general, fructose and glucose account for about 80% of the total sugar content in honey, while sucrose and maltose represent about 10%. The balance of the different types of sugars results in differences in honey, such as viscosity, density and crystallization (White, 1975). However, the proportion of sugars may vary according to the botanical origin (Nozal et al., 2005; De-Melo et al., 2017; Manzanares et al., 2017).

With respect to the parameters not covered by the Brazilian legislation, the percentage of protein can be used to detect honey adulteration, with 0.26% being the average value of the international standard (Almeida-Anacleto & Marchini, 2004). However higher values were found in samples from different sources, reaching 1-2% (Rebane & Herodes 2008; De-Melo et al., 2017). The electrical conductivity is a parameter that can be used to determine the origin of honey (Aganin, 1971) and is related to botanical origin, mineral content, pH, acidity, proteins and other substances present in honey (White Jr, 1975; Bogdanov, 1999). The electrical conductivity ranged from 106.33 to 517 μS.cm-1 in the presente study. Higher values have been recorded in honey from different locations (Sodré et al., 2003; Sodré et al., 2007; Almeida-Anacleto & Marchini; Marchini et al., 2005; Mendonça et al., 2008; Alves et al., 2011). Similar or lower values were also found (Arruda et al., 2005; Sereia et al., 2011). The values obtained for the formalin index ranged from 8.70 to 14.08 mL.kg-1. These values are similar to other studies (Marchini et al., 2005; Mendonça et al., 2008; Sodré et al., 2003; Almeida-Anacleto & Marchini, 2004; Alves et al., 2011).

Viscosity and water activity also have no values established in Brazilian legislation. The viscosity is mainly influenced by the percentage of water and the fructose and glucose ratio, honey with higher fructose content has lower viscosity, and also depends on temperature and floral origin. (Fattori, 2004). In the present study viscosity values were between 395 and 4115, with the highest values observed in honeys with dominant pollen types *Glycine max* and Piperaceae. The values of water activity are between 0.54 and 0.65, with an average of 0.60 ± 0.03. Under aerobic conditions, the inhibitory water activity is aw = 0.86, and substrates with aw = 0.6 are assured of microbial deterioration (Almeida-Anacleto, 2004). In general, the results of the physical and chemical parameters are quite variable between samples from different regions which makes difficult to establish a pattern related to the parameters not covered by the legislation.

Considering the pollen types present in the samples, the families Asteraceae, Euphorbiaceae, Mimosaceae and Myrtaceae were also frequent in other studies in the southern and southeastern regions, with similar vegetation (Sereia et al., 2011; Araujo et al., 2013; Bosco & Luz, 2017).

The studied region is soybean producer (*G. max*) and six samples had this species as dominant pollen type. The two counties are located in agricultural areas, and the main crops are corn and soybeans. The genus *Eucalyptus* was present in many samples. The contribution of species of this genus as dominant pollen in honey samples was verified in other studies in the southeast region (Bastos et al., 2003; Sodré et al., 2003; Barth et al., 2005; Luz et al., 2007; Mendonça et al., 2008; Silveira et al., 2012; Araujo et al., 2013; Barth et al., 2013). The increase of this pollen type in honey samples can be explained by the increase of reforested areas with species of *Eucalyptus*.

In the study area, it was verified that there are four species of *Eucalyptus*, three that bloom between January and April and one that blooms in November. Soybean flowering in the region occurs in the months of November, December and March (Sekine et al., 2013). Samples with these dominant pollen types were verified only in apiary B, which has smaller forested area. In apiary A, which has a larger forest remnant, polyfloral honey samples were collected. Despite the predominance of agricultural crops in both properties, pollen analysis results reflect the greater diversity of this environment.

The high similarity of pollen types shown in the dendrogram was expected, since the two apiaries belong to the same phytogeographic region at natural restoration stage. Samples from apiary A, A1 and A3, contained *Piperaceae* spp. as accessory pollen, and sample A2, *Lonchocarpus* sp. and *Campomanesia* spp. also as accessory pollen. Samples A4, A5 and A6 had as accessory pollen *Arecaceae* spp. and *G. max*. Samples A7, A8 and A9 had in common several species, including Piperaceae as dominant, accessory or important isolated pollen and *Acacia* and *P. rigida* as important isolated pollen in the three samples. From apiary B, samples B19 and B21, had *Eucalyptus* spp. as dominant pollen. Samples B10 to B15 had in common *G. max* as dominant pollen and B17 to B20 *G. max* as accessory pollen.

Comparing the two dendrograms, it was observed that the clusters were not coincident, which shows that polyfloral honey samples are quite variable for physical and chemical characteristics. However, samples of the apiary B containing dominant pollen *Glycine max* and Piperaceae are grouped and present the highest values of viscosity and lower conductivity.

Marchini et al. (2004b) observed the influence of accessory and dominant pollen types on the physical and chemical characteristics of honey samples in the State of Tocantins. In samples from the State of São Paulo, it was also verified the grouping of physical and chemical characteristics in *Eucalyptus* honey and wild honey (Marchini et al., 2005). In another study also in São Paulo state, it was verified the grouping of monofloral samples of orange, but the *Eucalyptus* samples were distributed in several different groups (Marchini et al., 2007). It was also verified the grouping of physical and chemical characteristics in Moroccan unifloral honeys of *Eucalyptus*, *Citrus* and honeydew (Terrab et al., 2003).

Table 2. Pollen spectrum and frequency classes in 21 samples of *Apis mellifera* honey collected at two locations in the counties of Nova Aurora (Apiário A) and Ubiratã (Apiário B) from December 2008 to May 2009.

Family	Pollen type	APIARY A									APIARY B											
		Dec			Jan			Feb/Mar			Dec			Jan			Feb/Mar			Apr		May
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Anacardiaceae	*Mangifera indica*																					O
	Schinus terebinthifolius												O	I	O	O	O	I	O			
Arecaceae	Arecaceae type				**A**	**A**	**A**	I		**A**		O		O	I		I	O		O	O	O
Asteraceae	*Baccharis* spp.				**A**	I	I							O	I	O	I	**A**	O	**A**	I	I
	Bidens spp.													O	O	O						
	Calyptocarpus biaristatus																O	O				
	Chromolaena pedunculosa																					O
	Conyza bonariensis				O	O	O															
	Mikania spp.							I	O	**A**							I	I	O			
	Senecio brasiliensis											O	O									
Boraginaceae	*Cordia ecalyculata*				O	O	O															
	Cordia trichotoma																					O
Brassicaceae	Indeterminada spp.				I	I	I	O	O	O												
Caesalpinaceae	*Bauhinia forficata*								I					O	O	O	O					
Combretaceae	*Combretum fruticosum*					O	O							O		O						
Euphorbiaceae	*Alchornea triplinervea*	A	I	I				O	O	O	I	O	O		O	O	O	O				
	Bernardia pulchela				O	O	I											O	O			
	Ricinus communis	O		O									O									O
	Sebastiania brasiliensis				I	I	O	O	O					O	I	O						
Fabaceae	*Glicyne max*	O	I	I	**A**	**A**	**A**	O	O	O	**D**	**D**	**D**	**D**	**D**	**D**	I	**A**	**A**	**A**	**A**	I
	Lonchocarpus sp.	I	**A**	I	I	I	I	O	I	I	O	I	I				I	O	I			
Flacourtiaceae	*Casearia sylvestris*	O	I	O	I	I	O															
Lamiaceae	*Hyptis mutabilis*							O	O													
	Leonurus sibiricus	O	I	O																		
Malvaceae	*Wissadula subpeltata*							O							O					O		O
Meliaceae	*Melia azedarach*	O	O	O																		
Mimosaceae	*Acacia* type				O	O		I	I	I	O	O	O				I	O	O	O	I	
	Leucaena leucocephala	O	O	O	O	O	O													O		
	Mimosa sp.								O											O		
	Parapiptadenia rigida	O	I	O	O	O	O	I	I	I	O	O	O	I	O	O	I	I	O	O	I	O
Myrtaceae	*Campomanesia* spp.	O	**A**	I																		
	Eucalyptus spp.	O	I	O	O	O	O	O	O	O	O	O	O	I		**A**	**A**	I	I	**D**	I	**D**
	Hexaclamys edulis	O	I	I								O	O									
	Myrcia type																			I		

Table 2. Pollen spectrum and frequency classes in 21 samples of *Apis mellifera* honey collected at two locations in the counties of Nova Aurora (Apiário A) and Ubiratã (Apiário B) from December 2008 to May 2009. (Continuation)

Family	Pollen type	APIARY A									APIARY B											
		Dec			Jan			Feb/Mar			Dec			Jan			Feb/Mar			Apr		May
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Oleaceae	*Ligustrum* sp										O	O	I	O	I	O	O	O	O	O	O	
Piperaceae	Piperaceae type	A	I	**A**	O	O	O	**A**	**D**	I		O	I	O	O	I	I	**A**	O			
Rhamnaceae	*Hovenia dulcis*	O		I							O											
	Rhamnaceae type							**A**	I	O							I	O	**A**	O	**A**	
Rosaceae	*Eriobotrya japonica*				O	O	O	I	O	I												
	Prunus sellowii							O	O	O												
	Rosaceae type	O	O	O																		
Rubiaceae	*Borreria* sp.																			O		O
Rutaceae	*Zanthoxylum* sp.		I	I																	I	
Sapindaceae	*Serjania* spp.																			O		O
Solanaceae	Solanaceae type																			O	O	
	Solanum mauritianum																			O		O
	Solanum sanctae-catharinae										O	O	O									
Tiliaceae	*Luehea divaricata*							O														
NI 1																	I	O	I			

*D = dominant pollen (> 45,0%); A = accessory pollen (16,0 a 45,0%); I = important isolated pollen (3,0 a 15,0%);
O = occasional isolated pollen (< 3,0%). NI = Not identified.

The geographic origin of the honey, collected in different localities with different types of vegetation can also influence the groupings of physical and chemical characteristics (Santos et al., 2008) which was not verified in the present study.

The similarities occurring in honey samples can be caused by the variability of floral species used in honey composition or due to the abiotic characteristics of the collection sites, which may influence the characteristics of the honey (Crane, 1990; Marchini et al., 2004b; Barth, 2004). The samples form clusters of physical-chemical and pollen similarities related to the collection sites. Samples of honey containing the dominant pollen *G. max* and *Eucalyptus* were grouped according to physical and chemical characteristics, which shows the influence of the pollen type on the characteristics of honey, when the floral species present high representativeness. However, multivariate classification in groups based on pollen types formed no clusters in samples of polyfloral honey.

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