A Interface do Conhecimento sobre Abelhas

Alexandre Igor Azevedo Pereira
(Organizador)

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APRESENTAÇÃO

A polinização de pomares de frutas, bem como lavouras de legumes e grãos, e diversas outras espécies vegetais angiospermas, muito se deve à vida das abelhas que é, portanto, crucial para o planeta e para o equilíbrio dos ecossistemas terrestres. Pode-se afirmar que sem os serviços ecológicos ofertados pelas abelhas, a grande maioria das plantas não se reproduziriam. Aproximadamente dois terços dos alimentos que ingerimos são produzidos com a ajuda da polinização das abelhas. Apenas com esse argumento preliminar, podemos apontar, convictos, que esses insetos da ordem Hymenoptera afetam a nossa vida cotidiana, sem que nós sequer nos apercebamos disso. Dessa forma, sem as abelhas, a segurança alimentar da humanidade estaria fortemente ameaçada.

Não obstante, a sociedade civil, bem como diversos outros ramos representativos da população brasileira como os estratos envolvidos com políticas públicas de preservação e mitigação ambiental, bem como a comunidade científica, acadêmica e demais atores envolvidos com o meio ambiente de maneira direta - ou indireta - precisam ser abastecidos continuamente de informações que possam valorizar o papel das abelhas ao planeta, bem como dos produtos por elas derivados.

A presente obra "*A Interface do Conhecimento sobre Abelhas*" é a mais recente iniciativa da Editora Atena no sentido de difusão de conhecimento, demonstração de aprimoramentos e divulgação de ideias, em forma de e-book, na área de Apicultura. A importância prática da própolis, subproduto oriundo das atividades comportamentais das abelhas, bem como a compreensão dos requerimentos nutricionais desses insetos; a composição físico-química, incluindo aminoácidos e minerais, além de análises qualitativas de amostras de méis oriundas da região Norte e Nordeste do Brasil com foco em abelhas sem ferrão são temas de caráter prático e aplicado abordados na presente obra. Além disso, estudos sobre a diversidade de espécies e o número total de indivíduos em áreas restauradas do bioma Cerrado, com ênfase na conservação e restabelecimento das populações de abelhas em paisagens agrícolas, incluindo a diversidade de análises polínicas de espécies florais polinizadas pela espécie *Bombus morio* são apresentadas. Por fim, um estudo sobre a influência de fatores ambientais no fluxo de entrada de grãos de pólen e sua coloração em colmeias de abelhas do gênero *Apis mellifera* finaliza a presente obra tratando de contribuições sobre o entendimento da complexa relação entre o meio ambiente e as atividades forrageadoras das abelhas.

Esperamos que o presente e-book, de publicação da Atena Editora, possa representar como legado, a oferta de conhecimento para capacitação de mão-de-obra através da aquisição de conhecimentos técnico-científicos de vanguarda praticados por diversas instituições em âmbito nacional; instigando professores, pesquisadores, estudantes, profissionais (envolvidos direta e indiretamente) com atividades apícolas frente ao acúmulo constante de conhecimento com potencial de

transpor o conhecimento atual acerca dos processos envolvidos com a produção mel, atrelada à conservação das atividades ecológicas das abelhas: seres vivos de relevante importância a diversos sistemas naturais, bem como agroecossistemas terrestres.

Alexandre Igor de Azevedo Pereira

SUMÁRIO

CAPÍTULO 4

CHEMICAL COMPOSITION AND FREE RADICAL SCAVENGING ACTIVITY OF HONEY FROM STINGLESS *Melipona mandacaia* BEES

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ABSTRACT: *Melipona mandacaia* is a stingless bee species popularly known as 'mandaçaia' that is native to northeastern Brazil. In this study, we conducted both melissopalynological and physicochemical analyses to investigate the minerals and amino acids of four sample of mandaçaia honey. In addition, the major phenolic constituents of the honey samples were extracted and analyzed using high-performance liquid chromatography using a diode-array detector (HPLC-DAD). *Mimosa arenosa* (Fabaceae/Mimosoideae) was the predominant pollen type in the four honeys, and it represents a minimum of 44.4% to a maximum of 61.7% of the total pollen. All of the identified compounds, i.e., quercetin, luteolin, kaempferol and 3,4-dihydroxybenzoic, 1,2-dihydroxybenzoic caffeic, cinnamic, ferulic and sinapic acids, were quantified by HPLC-DAD. All of the samples of honey exhibited the presence of essential amino acids: proline, alanine, serine and threonine. The highest mineral contents consisted of calcium followed by potassium. All honey samples exhibited free-radical-scavenging activity.
KEYWORDS: Honey, *Melipona mandacaia*, analysis.

COMPOSIÇÃO QUÍMICA E ATIVIDADE ANTIRRADICALAR DOS MÉIS DAS ABELHAS SEM FERRÃO *Melipona mandacaia*

RESUMO: A espécie de abelha sem ferrão *Melipona mandacaia* é conhecida popularmente como mandaçaia e é nativa do Nordeste brasileiro. Neste estudo foram realizadas análises palinológicas, físico-químicas, aminoácidos e minerais de quatro amostras de mel da mandaçaia. Os principais

constituintes fenólicos foram extraídos e analisados por cromatografia líquida de alta eficiência acoplado ao detector de arranjo de diiodo (CLAE-DAD). A análise palinológica mostrou que o pólen predominante nas amostras de mel foi da espécie vegetal *Mimosa arenosa* (Fabaceae/Mimosoideae), variando de 44.4% a 61.7%. Os flavonóides identificados quercetina, luteolina, kanferol e os derivados de ácido: 3,4-dihidroxibenzoico, 1,2-dihidroxibenzoico, cafeico, cinâmico ferúlico e sinápico foram quantificados. Todas as amostras de mel apresentaram os aminoácidos prolina, alanina, serina e treonina. Os minerais predominantes foram o cálcio e potássio. Todos os méis apresentaram atividade sequestradora de radical livre.
PALAVRAS-CHAVE: Mel, *Melipona mandacaia*, análises

1 I INTRODUCTION

Stingless bees are highly diverse in the neotropics, with approximately 43 genera and approximately 350 species being identified (Michener 2000). These bees play an important role in Caatinga, acting as specific pollinators for this biome (HEARD, 1999).

Melipona mandacaia is a stingless bee popularly known as 'mandaçaia' that is native to Northeastern Brazil, where it faces extinction due to habitat loss. This species is endemic to Caatinga and is widespread in the states of Piauí, Ceará, Bahia, Paraíba and Pernambuco, usually found close to the São Francisco River (BATALHA-FILHO et al., 2011).

The honey from the Meliponas species has several unique features the differentiate its composition from other honeys, especially the water content (moisture), which makes it less dense than the honey of *Apis* bees. The color ranges from nearly transparent to dark amber, and the taste and sugar levels depend upon the species, region and, especially, the vegetal species. In addition to the sugars in the solution, the honey of Meliponas also contains organic acids, flavonoids and a wide variety of other organic compounds that contribute to its color, odor and flavor (SILVA et al., 2013, ALMEIDA-SILVA et al,. 2013). The demand for this product has increased recently, raising its commercial value higher than that of *Apis melifera* honey. However, there have not been any studies to quantify the amino acids and minerals of *Melipona mandacaia* honey.

The phenolic profile of honeys, and consequently their antioxidant capacity, depend on the floral sources used to produce the honey. The predominance of a particular floral source in honey is primarily influenced by geographical, seasonal and environmental factors (ANDRADE et al., 1997; SILVA et al., 2013; SILVA et al., 2014). Therefore, different properties of honeys are expected because the composition of the active compounds in honey from different locations is likely to be different.

In this study, we conducted both melissopalynological and physicochemical analyses to investigate the minerals and amino acids of mandaçaia honey. In addition, the major phenolic constituents of the honey samples were extracted and analyzed

using high-performance liquid chromatography with a diode-array detector (HPLC-DAD). The identified phenolics were quantified. The total phenolic contents were determined using the Folin-Ciocalteu test. The radical activities of the honey and the extracts were also studied by testing their scavenging effect on the molecules DPPH (1,1-diphenyl-2-picryl hydrazyl) and ABTS (2,2 azinobis 3-ethylbenzothiozoline-6-sulfonic acid).

2 I MATERIALS AND METHODS

2.1 Honey Samples

Four samples of *M. mandacaia* "mandaçaia" honey were collected in the semiarid region in the state of Bahia, Brazil. The honey was collected into small storage bottles from the hives and was refrigerated at 4 °C until it was analyzed.

2.2 Reagents and Standards

Apigenin, isorhamnetin, kaempferol, 8-methoxykaempferol, luteolin, myricetin quercetin, tricetin, dihydromyricetin, taxifolin and naringenin had been previously isolated and identified from the pollen loads (SILVA et al., 2006; 2009, FREIRE et al., 2012). Ferulic acid, 3-hydroxy-4-methoxycinnamic acid, caffeic acid, p-coumaric acid, cinnamic acid, sinapic acid, 4-methoxycinnamic acid, chlorogenic acid, 3,4,5-trihydroxybenzoic acid, 1,2-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid and syringic acid were obtained from Sigma-Aldrich (Hamburg, Germany); gallic and vanillic acids were obtained from Fluka Chemie AG (Buchs, Switzerland). All reagents used were of analytical grade. Folin-Ciocalteu's phenol reagent, DPPH (1,1-diphenyl-2-picryl hydrazyl), potassium persulfate and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were supplied by Acros Organics (Belgium). ABTS (2,2 azinobis 3-ethylbenzothiozoline-6-sulfonic acid) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Ascorbic acid and formic acid were purchased from Vetec (Brazil). Methanol (Tedia, Brazil) were of analytical grade.

2.3 Melissopalynological Analysis

The honey samples were treated using the typical melissopalynological methods (LOUVEAUX et al., 1978). The pollen sediment was acetolyzed (ERDTMAN, 1960), mounted on glycerin jelly and sealed with paraffin. To determine the frequency classes, 500 pollen grains were counted from each sample. The pollen types were placed into four percentage classes, as determined by Louveaux et al. (1978): predominant pollen (>45%); secondary pollen (≤45% to >15%); important minor pollen (≤15% to ≥3%); and minor pollen (<3%). The pollen slides from the Palynothecae of the Universidade Estadual de Feira de Santana (Bahia, Brazil) and pollen catalogs were used to identify the botanical affinities of the pollen types.

2.4 Physicochemical Analysis

The physicochemical analysis of the honey samples consisted of the following basic determinations, which were performed in triplicate: the electrical conductivity, pH and free acidity and the hydroxymethylfurfural, water, ash, reducing-sugar and moisture contents.

2.5 Extraction of the Phenolic Compounds from the Honey

The extraction was performed using previously described methods (HADJMOHAMMADI et al., 2009) with the following modification: 100 g of honey was dissolved into 200 mL of distilled water, and the solution was adjusted to pH 2.0 by adding concentrated HCl, stirring with a magnetic stirrer at room temperature for 10 min. The fluid samples were then filtered through a Celite layer to remove the solid particles. The C18 cartridge (SEP-PAK Waters) was sequentially conditioned with 30 mL of MeOH and 60 mL of distilled deionized water without allowing the cartridge to dry. The filtrate was passed through the cartridge and rinsed with 60 mL of water to remove all sugars and other polar constituents of honey, and the phenolic compounds were eluted with 8 mL of HPLC-grade methanol. The eluate was dried under reduced pressure in a rotatory evaporator at 40 °C and dissolved in methanol, filtered through a 0.45-μm nylon syringe filter (Whatman) and injected into the HPLC system.

2.6 Hplc-Dad Analysis of the Phenolics and Free Amino Acids

All chromatographic analyses were performed using a Shimadzu Prominence LC-20AT equipped with an SPD-M20A diode-array detector (Shimadzu Corp. Kyoto, Japan). For amino acid analysis, the samples were injected into a Rheodyne 7125i injector with a 20-µl loop. Amino acid derivation with AccQ•Tag reagents was conducted according to the manufacturer's protocol. Briefly, 10 and 20 µL of a standard amino acid mixed solution or the honey (0.2 g/mL), respectively, were mixed with 60 µL AccQ•Tag borate buffer and 20 µL AccQ•Tag reagent previously dissolved in 1.0 mL of AccQ•Tag reagent diluent. The reaction was allowed to proceed for 1 h at room temperature. The separation column was a Waters AccQ•Tag (3.9 mm i.d. × 150 mm, 4.0 µm particles). The column heater was set at 37 °C, and the mobile-phase flow rate was maintained at 1.0 mL/min. Eluent A was 1% AccQ•Tag solvent A, eluent B was acetonitrile and eluent C was Milli-Q water. The separation gradient was 0-0.5 min (100-99% A), 18 min (95.0% A), 19 min (91% A), 29.5 min (83% A), 33 min (60% A and 40% C), 36 min (100% A) 65 min (60% A and 40% C) and 100 min (60% A and 40% C). Ten microliters of the sample were injected for analysis. The PDA detector was set at 254 nm.

For the flavonoids, the chromatographic separation was performed with a C-18 column (150 x 4.6 mm x 5 µm, Supelco). The flavonoids were separated using a mobile phase consisting of 1% aqueous formic acid (A) and methanol (B) at a flow rate of 1 mL/min. The mobile phase was delivered using the following solvent gradient: 0-3 min

40% B, 5-15 min 45% B, 17-25 min 45% B, 25-27 min 50% B and 35-40 min 70% B. The injection volume was 10 µL. Chromatograms were recorded at 290 nm and 340 nm. The identification of the flavonoids was based on the retention times and the UV spectra with authentic markers. The flavonoids quercetin, luteolin and kaempferol were quantified using the external-standard method based on the peak area. The analyses were performed by plotting a calibration curve. To construct the calibration curve for each flavonoid, working solutions with concentrations between 0.5 and 400 mg/mL were prepared from each stock solution by diluting appropriate volumes with methanol, which were then correlated with the measured area. For each sample, the quantitative analyses were performed in triplicate at 320 nm. For the analysis of the phenolic acids, the elution system was composed of 5% formic acid (solvent A) and MeOH (solvent B). The elution conditions were: 0.01-15 min 20-30% B, 15-20 min 30% B, 20-30 min 30-40% B and 40-50 min 100% B, at a flow rate of 1.0 mL/min. The wavelengths 254 and 290 nm were employed for monitoring.

2.7 Metal Determination From the Honey

The digestion of the honey was performed in a closed microwave acid-digestion MARS 5 system (CEM Corporation, USA). The samples (500 mg) were diluted in concentrated nitric acid (5 mL). The program used had the following features: 800 W for 5 min at 120 °C and then 160 ˚C for 20 min. Upon cooling, the solution was filtered to remove any remaining solid material. The solution was diluted with deionized water prior to analysis. The analysis of metals (Cu, Fe, K, Mn, Cd, Zn, Na and Ca) was performed with a Varian AA 240 (Victoria, Australia) by flame atomic absorption spectrometry (FASS). Under optimized parameters, the standard calibration curves for the metals were constructed by plotting the absorbance against the concentration in a fixed range for each metal, and good linearity was observed. All analyses were performed in triplicate, and the mean values were reported. All of the values obtained for the metal contents in the pollen samples were calculated as mg/kg pollen.

2.8 Determination of the Total Phenolic Content and the Dpph$^{\cdot}$ Radical-Scavenging Assay and the Abts$^{+\cdot}$ Radical Cation-Decolorization Assay

The total soluble phenolic content of the MeOH extract was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) with the modification of using gallic acid as the standard phenolic compound. The MeOH extract was analyzed using DPPH (SILVA et al., 2006) for the free-radical-scavenger activity and ABTS (RE et al., 1999) for the radical cation-decolorization assay.

2.9 Statistical Analysis

All samples were analyzed in triplicate and the results are expressed as the mean ± standard deviation. All statistical analyses were performed using the Microsoft

Excel software package (Microsoft Corp., Redmond, WA, USA).

3 I RESULTS AND DISCUSSION

All honey samples studied had a bright yellow color. The results from the physicochemical analyses of the honey samples are presented in Table 1. The moisture values ranged from 24.47 to 27.39%, which are expected values for the honey of Meliponas because the water content is commonly very high and causes the honey to be more fluid. (SILVA et al., 2013; ALMEIDA-SILVA et al., 2013). The results for the pH, free acidity, reducing sugars and moisture and water contents were similar to those found for the honey of *Melipona subnitida* (SILVA et al., 2013), but the values for ash and HMF were lower and higher, respectively. The electrical conductivity ranged from 377.2 to 418.4 μS/cm. According to Almeida-Muradian et al. (2013), who analyzed the honeys of *Melipona subnitida* (mean value of 102.77 ± 1.31 μS/cm), the fact that the electrical-conductivity values were not higher than 800 μS/cm suggests that the samples are from nectar honey. The electrical conductivity is directly related to the concentration of mineral salts, organic acids and proteins and is very useful in the determination of the floral origin (ACQUARONE et al., 2007).

	Sample			
	01	02	03	04
pH	3.0 ± 0.01	3.11 ± 0.02	3.16 ± 0.02	3.17 ± 0.02
Conductivity (μS/cm)	401.5 ± 0.10	413.6 ± 0.20	418.4 ± 0.10	377.2 ± 0.20
Free acidity (mequiv./kg honey)	48.1 ± 0.10	49.7 ± 02	50.0 ± 0.10	46.3 ± 0.1
HMF (mg/kg honey)	6.0 ± 0.04	5.87 ± 0,04	5.61 ± 0.05	6.35 ± 0.04
Ash (mg/100 g honey)	27.3 ± 0.10	28.04 ± 0.20	26.9 ± 0.20	28.9 ± 0.10
Reducing sugars (g/100 g honey)	75.04 ± 0.30	76.34 ± 0.30	76.07 ± 0.40	73.9 ± 0.30
Moisture (g/100 g honey)	24.78 ± 0.56	24.47 ± 0.83	25.79 ± 1.05	27.39 ± 0.90
Aw	0.86 ± 0.07	0.85 ± 0.06	0.85 ± 0.07	0.87 ± 0.08
Iron (mg/Kg honey)	16.73 ± 0.01	30.90 ± 0.02	17.13 ± 0.01	37.35 ± 0.02
Potassium (mg/Kg honey)	76.95 ± 0.02	70.69 ± 0.02	69.07 ± 0.02	57.42 ± 0.02
Sodium (mg/Kg honey)	27.91 ± 0.02	23.20 ± 0.02	32.39 ± 0.02	36.02 ± 0.02
calcium (mg/Kg honey)	79.17 ± 0.02	78.25 ± 0.02	80.75 ± 0.02	78.33 ± 0.02

Table 1. Physicochemical composition and metals of mandaçaia (*Melipona mandacaia*) honey samples.

The results from the qualitative pollen analysis for the mandaçaia honey samples are summarized in Table 2. All results are listed as percentages of the total pollen content in each sample. *Mimosa arenosa* (Fabaceae/Mimosoideae) was the predominant pollen type in the four honeys, and it represents a minimum of 44.4% to a maximum of 61.7% of the total pollen. *M arenosa* ('calumbi' ou 'jurema-branca') is a very common plant species in the Caatinga region, and its presence in mandaçaia honey in large amounts is expected. It is a shrub with inflorescences composed of very small,

white, sweet-scented flowers. Its flowers provide nectar and pollen for many insects, such as flies, beetles and native bees. *M. arenosa* is a species of great importance for the creation of stingless bees and is essential for the production of honey (MAIA-SILVA et al., 2013). A significant amount of *Mimosa tenuiflora* (Fabaceae/Mimosoideae) was present in two samples of the honey (21.80 and 29.49%). This pollen type had already been observed in honeys collected by *Melipona subnitida* (SILVA et al., 2013) and *Frieseomelitta doederleini* (SANTISTEBAN et al., 2019). *M. tenuiflora* is a shrub known popularly as jurema-preta. This species blooms over a long period of the year, but mostly during the dry season. Its inflorescences are formed by white, small, sweet-scented flowers and provide floral resources as pollen and nectar for many species of bees, wasps, flies and other insects. This species is very important for maintaining the biodiversity and the ecosystem (MAIA-SILVA et al., 2013). Other specific plant varieties presented levels ranging from 0.3% to 11.0% of the total pollen grains. All of these are relatively common plants in Caatinga.

Family	Pollen type	Frequency (%)			
		honey 01	honey 02	honey 03	honey 04
Anacardiaceae	*Schinus*	0.3	0.5	0.3	-
Arecaceae	*Syagrus coronata*	-	0.3	1.0	-
Caesalpiniaceae	*Copaifera martii*	-	0.8	-	-
	Chamaecrista nictitans	-	-	-	0.64
	Chamaecrista ramosa	0.3	0.5	-	-
	Chamaecrista repens	0.7	0,8	0.3	-
	Senna rizzinii	2.3	1.3	1.9	3.85
Euphorbiaceae	*Croton*	-	-	0.3	-
	Phyllanthus	-	0.3	-	-
Fabaceae	Tipo Fabaceae	-	0.3	-	-
Cyperaceae	Cyperaceae	-	-	-	0.32
Mimosaceae	*Acacia*	0.3	-	-	-
	Anadenanthera colubrina		0.8	-	-
	Mimosa adenophylla	1.7	-	3.2	0.32
	Mimosa arenosa	61.7	44.4	59.4	53.53
	Mimosa pudica/sensitiva	4.3	3.0	4.8	0.32
	Mimosa tenuiflora	9.9	21.8	5.8	29.49
	Parapiptadenia		0.3	-	-
	Piptadenia stipulacea	0.3	0.5	-	0.32
	Pithecelobium	-	0.3	-	-
	Plathymenia reticulata	-	0.3	0.6	-
	Ptyrocarpa moniliformis	-	0.8	-	-
	Tipo *Mimosa*	-	0.3		-
Myrtaceae	*Myrcia* sp1	7.9	11.0	6.7	0.32
	Myrcia sp2	-	3.8	7.0	0.32
	Psidium	5.3	0.0	3.2	-
Rutaceae	*Citrus*	-	0.3	-	-
Solanaceae	*Solanum* sp1	4.0	4.6	2.2	3.53

Solanum sp2	0.3	2.4	2.6	6.41
Indeterminate	0.7	0.8	0.6	0.64
Total	100.0	100.0	100.0	100.0

Table 2. Melissopalynological analysis of mandaçaia (*Melipona mandacaia*) honey samples.

Regarding minerals, the highest content was determined for calcium (Ca), followed by potassium (K). The iron (Fe) and sodium (Na) contents vary in the samples (Table 1). The mineral content percentage is considered as a quality criterion indicating the possible botanical origin of the honey. The variability in the mineral content of honeys can arise from harvesting processes, beekeeping techniques and the material collected by the bees while foraging on the flora (FINOLA et al., 2007). The mineral elements have already been quantified in honey from Melipona, K was the most abundant element in the honeys studied of *M. fasciculata* and *M. flavoneata*, Na was the second most abundant and Ca was the third most abundant element (SILVA et al., 2013).

The samples of honey contained seven essential amino acids (Table 3). Proline was found at the highest concentrations (184.8 mg/Kg and 232.3 mg/Kg), followed by threonine (99.4 mg/Kg and 133.9 mg/Kg). According to Bergner and Hahn, 1972, proline derives mainly from the salivary secretions of *A. melifera* during the conversion of nectar into honey. The amino acid content of the honey of meliponas has not yet been reported.

	Sample			
AA	01	02	03	04
Ser	13.46	36.72	32.81	41.89
Thr	99.38	109.68	121.27	133.85
Ala	55.61	61.67	70.07	79.48
Pro	197.90	232.29	184.79	222.93
Val	nd	nd	nd	10.81
Met	nd	15.07	33.36	nd
Phe	64.13	nd	nd	nd

Table 3. Content (mg/kg pollen) of free amino acids in mandaçaia honey.

nd, not detected.

All honey samples have been found to have six phenolic compounds: 3,4-dihydroxybenzoic, 1,2-dihydroxybenzoic, caffeic, cinnamic, ferulic and synapic acids and the three flavonoids quercetin, luteolin and kaempferol (Table 4). Cinnamic acid was detected in samples 1-3. The existence of luteolin (ALMEIDA SILVA et al., 2013) and quercetin (SILVA et al., 2013) in Melipona honey from Brazil was previously reported. Kaempferol and caffeic acid were also detected in the monofloral honey of *Mimosa scabrella* provided by *Melipona marginata* in southern Brazil (BORSATO et al., 2014). Interestingly, all of the honeys analyzed predominantly contain the vegetal

species *Mimosa arenosa*, suggesting that the flavonoid luteolin may be specific for the *Mimosa* genus; therefore, its main compound may be a possible marker for the botanical classification. Honeys produced in Amazonian Ecuador (GUERRINI et al., 2009) and Venezuela (TRUCHADO et al., 2011) by *Melipona* spp. have also been reported to have a small content of flavonoid aglycones, including kaempferol and quercetin.

	Sample			
	01	02	03	04
Quercetin	47.89	31.98	31.11	17.40
Luteolin	1348.71	770.18	1047.89	1196.60
Kaempferol	18.25	34.19	19.68	29.67
3,4-dihydrobenzoic acid	39.40	139.33	37.11	60.47
1,2- dihydrobenzoic cid	851.59	345.57	931.05	669.05
Caffeic acid	13.06	27.76	16.85	11.87
Coumaric acid	107.81	101.83	103.05	67.32
Ferulic acid	8.09	64.15	14.14	131.07
Synapic acid	18.60	74.81	21.52	24.55
Cinnamic acid	21.42	27.39	15.76	nd

Table 4. Quantitative analysis of the compounds identified in the jandaira honey (*µ*g/100 g honey).

nd, not detected.

The amount of the total phenolics estimated using the Folin-Ciocalteu reagent in the various samples ranged from 90.4 to 112.1 mg GAE/g (gallic acid equivalent by gram of extract) in the MeOH extract. These results are similar to those found for *Melipona subnitida* honey but are larger than the values for the honey of *Apis melifera* (ESCUREDO et al., 2013).

Two methods were used to determine the free-radical-scavenger activity of the mandaçaia honey. All honey samples exhibited free-radical-scavenging activity. As shown in Table 5, the EC_{50} values ranged from 49.9 to 53.2 *µ*g/mL for the MeOH extract in the DPPH radical-scavenging assay. The EC_{50} results for the ABTS test varied from 19.6 *µ*g/mL to 27.2 *µ*g/mL. These methanol extracts were more active than the methanol extracts from the honey of *Melipona subnitida* (SILVA et al., 2013). This may be related to the phenolics present in mandaçaia honey.

Honey	mgGAE/g	DPPH (CE_{50}) *µ*g/mL	ABTS (CE_{50}) *µ*g/mL
01	90.4 ± 0.4	53.2 ± 0.5	23.3 ± 0.2
02	105.7 ± 0.4	51.4 ± 0.6	27.2 ± 0.3
03	99.5 ± 0.6	49.9 ± 0.5	19.6 ± 0.3
04	112.1 ± 0.6	52.9 ± 0.7	20.0 ± 0.2

Table 5. Total phenolics and free-radical-scavenger activity of the mandaçaia honey.

4 I CONCLUSIONS

The melissopalynological analysis of *M. mandacaia* honeys from a semiarid region of Brazil exhibited the predominant pollen type of *Mimosa arenosa*. The physicochemical analysis revealed that all of the samples had a similar profile. The flavonoids quercetin, luteolin and kaempferol and the phenolics 3,4-dihydroxybenzoic, 1,2-dihydroxybenzoic caffeic, ferulic and synapic acids were common in all of the samples. Coumaric acid was detected in samples 1-3. The samples of honey showed the presence of essential amino acids: proline, alanine, serine, threonine, valine, methionine and phenylalanine. Regarding minerals, the highest content was determined for calcium, followed by potassium. All honey samples exhibited free-radical-scavenging activity.

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