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ORIGINAL RESEARCH ARTICLE

Antioxidants activity and physicochemical properties of honey from social bees of the Brazilian semiarid region

Irana Paim Silva[a]*, Maiara Janine Machado Caldas[a], Cerilene Santiago Machado[a], Andreia Santos do Nascimento[a], Maurício Santana Lordêlo[b], Marivalda Figueredo Santa Bárbara[a], Norma Suely Evangelista-Barreto[a], Leticia M Estevinho[c] and Carlos Alfredo Lopes de Carvalho[a]

[a]*Centro de Ciências Agrárias, Ambientais e Biológicas, Universidade Federal do Recôncavo da Bahia, Cruz das Almas, Brazil;* [b]*Departamento de Ciências Exatas, Universidade Estadual de Feira de Santana, Feira de Santana, Brazil;* [c]*Centro de Investigação de Montanha - CIMO, Escola Superior Agrária, Instituto Politécnico de Bragança, Bragança, Portugal*

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This study compared the nutraceutical potential of *Apis mellifera* and *Melipona quadrifasciata anthidioides* honeys from the semiarid region of Bahia, Brazil, using microbiological, melissopalynological, and physicochemical techniques. Total phenols and flavonoids and the antioxidant activity were also determined. All samples had good microbiological quality and safety with an absence of coliforms, *Escherichia coli, Salmonella* spp., *Staphylococcus* coagulase positive and *Clostridium* sulphite reducing spores. Were identified 41 pollen types belonging to 23 botanical families. Myrtaceae, Anacardiaceae, and Sapindaceae were the predominant pollen types in *A. mellifera* honey, while honey Malvaceae was the most representative in the *M. q. anthidioides*. Regarding the physicochemical parameters evaluated, 75% complied with the standards established by the Brazilian and European quality legislation. The honey samples of *A. mellifera* had higher values of phenolic compounds and flavonoids (260.0 to 341.51 mgGAE.kg^{-1} and from 114.44 to 216.29 mgQE.kg^{-1}, respectively). The samples from *M. q. anthidioides* presented higher antioxidant activity. The honeys of *A. mellifera* and *M. q. anthidioides* from the semiarid regions presented distinct botanical compositions, suggesting that both species use different plant sources, which possibly influenced the parameters related to honey quality as well as the content of phenolic compounds.

Keywords: Quality; composition; social bees; phenolic compounds; antioxidant activity

Introduction

Honey is a widely appreciated natural product of plant nectar produced by bees (Almeida-Muradian et al., 2013; Pereira et al., 2009). Besides its good nutritional characteristics, honey has therapeutic properties and has been used in traditional medicine in diverse practices, such as phytotherapy (Oliveira et al., 2012; Pereira & Reis, 2015).

Honey contains more than 200 different substances in its composition, and sugars (mainly glucose and fructose) and water (Pires et al., 2015) are the most abundant. Minor components comprise minerals, proteins, vitamins, lipids, organic acids, amino acids, phenolic compounds, enzymes, phytochemicals (Silva et al., 2013), pigments, waxes, and pollen grains (Almeida-Muradian et al., 2013). However, honey composition, colour, aroma, and flavour vary according to the raw material, bee species, edaphoclimatic conditions, floral source, processing, packaging, and storage conditions (Silva et al., 2013, 2016).

Botanical origin is one of the main parameters influencing honey quality, often determining its market value (Estevinho et al., 2016). Honeys from different botanical origins have distinct sensorial characteristics and therefore meet consumer preferences distinctly (Bastos et al., 2002).

The honey physicochemical characteristics, particularly low water activity, high sugar content, and acid pH, prevent growth or even survival of some microorganisms. However, honey is not a sterile product and can be contaminated by pollen, bee material, soil, water, air, and plant nectar (Estevinho et al., 2008), as well as by sources related to processing, handling, and storage (Różańska, 2011). Therefore, identification of quality parameters is necessary to ensure safe honey consumption (Codex, 2001; Silva et al., 2016). In this context, this work compared the nutraceutical potential of *Apis mellifera* and *Melipona quadrifasciata anthidioides* honeys harvested in the semiarid region of Bahia. We assessed microbiological, melissopalynological, and physicochemical parameters and determined the total phenols and flavonoids as well as the antioxidant activity.

Materials and methods

Samples

In this study, we used 11 samples of honey (seven samples from *A. mellifera* and four from *M. q. anthidioides*.)

*Corresponding author. E-mails: anaripaim@gmail.com, anaripaim@hotmail.com

harvested in 2015 from the semiarid region of Bahia. The samples were obtained directly from beekeepers.

Characterization of samples

Microbiological evaluation

In order to analyse the microbiological quality of honey, samples were evaluated for mesophilic aerobes, moulds, and yeasts, 35 °C coliforms, *Escherichia coli, Salmonella* spp., *Staphylococcus* coagulase positive, *Clostridium* sulphite reducing spores, and *Bacillus* spp.

The enumeration of aerobic mesophilic bacteria was performed on standard count agar (PCA-Himedia) and incubated at 37 °C/48 h (Silva et al., 2010). For yeast and mould counts, 0.1 mL of each dilution on DG18 agar (Himedia) was cultured and incubated at 25 °C for 5 days (ISO, 2006). For the quantification of coliforms at 35 °C and *E. coli*, the SimPlate kit system (Biocontrol®) was used, according to the AOAC (2005) Official Method. The detection of *Salmonella* spp. was performed using the 1-2 Test immune-diffusion test (Biocontrol®), as described in the AOAC (1989) method. In the quantification of *Staphylococcus* coagulase positive, 0.1 mL of each dilution of the honey sample was plated with Baird-Parker agar (Himedia) with a solution of egg yolk tellurite and incubated at 37 °C/48 h (NP, 2002). Then, three colonies with typical characteristics were selected and submitted to tests of coagulase and catalase (Silva et al., 2010). Reducing spores of *Clostridium* sulphite were counted according to ISO (2003). For the inoculation, aliquots of the sample (0.01 mL, 0.1 mL, and 1 mL) were transferred to double-layer Petri dishes containing iron-sulphate agar and incubated at 37 °C/48 h. For *Bacillus* spp., dilutions were placed in a water bath (70 °C/1 h) for the inactivation of the sample. After this period, 0.1 mL was plated using the nutrient agar (Himedia) with 1.5% sodium chloride and incubated at 30 °C/48 h. (Filho et al., 2018).

Melissopalynological analyses

All samples were submitted to the pollen analysis by the acetolysis method (Erdtman, 1960). The pollen types were identified following the recommendations in the literature (Barth, 1989; Punt et al., 2007; Silva et al., 2016). In brief, slides were examined using a light microscope with 400× and 1000× magnification, and pollen grains were identified by comparison using as reference material from the Palinoteca of the Nucleus of Insect Study of the Federal University of the Recôncavo of Bahia. According to the relative frequency (%) the pollen types were classified as: predominant pollen ($>$ 45% of total grains), secondary pollen (16 to 45%), important minor pollen (3 to 15%), and minor pollen ($<$ 3%) (Louveaux et al., 1978).

Physicochemical analyses

The moisture content was evaluated through refractometry using an Atago pocket refractometer digital, maximum reading $\leq$ 30 (Bogdanov, 1999).

Reducing sugars and sucrose were evaluated according to the method developed by Lane and Eynon (1934) and modified by Copersucar (1987) and Marchini et al. (2004). For the analysis of reducing sugars, we weighed 2.5 g of honey sample, dissolved in 50 mL of distilled water, pipetted a 10 mL aliquot of the sample solution, and diluted to 200 mL with distilled water. This solution was titrated with 10 mL of Fehling's liquor. To determine sucrose, 10 mL was pipetted (2.5 g/50 mL) of the sample to 200 mL volumetric flask containing 20 mL of hydrochloric acid (0.75 N) and placed in a water bath (65 °C/30 min). The solution was neutralized with sodium hydroxide (0.75 N), composed the flask volume with distilled water, and was titrated this solution with 10 mL of Fehling's liquor.

Free acidity, hydroxymethylfurfural (HMF), and diastasis activity were analysed using the method of Analysis of Association of Official Analytical Chemists (AOAC, 1990). The pH was evaluated by potentiometry according to the method of Adolfo Lutz Institute (IAL, 2005). The electrical conductivity was determined by conductivity using the HI 8820 Tecnal meter (BOE, 1986) and the ash content was determined using the HI 8820 Tecnal Meter according to the manufacturer's recommendations. Colour was measured according to mmPfund colour scale, as described by Vidal and Fregosi (1984). The Fiehe test and Lugol reaction were performed according to Moraes and Teixeira (1998).

Total phenolic compounds and antioxidant activity

The content of total phenols was estimated according to the method of Folin-Ciocalteau described by Singleton et al. (1999), with adaptations. For the analysis, 0.5 mL of honey sample (1:10, w/v) was mixed with 2.5 mL of Folin-Ciocalteau (10%, w/v) and 2 mL sodium carbonate (7.5%, w/v), the reaction was kept in the dark for 30 min in a water bath (40 °C) and then absorbance was read at 760 nm using a spectrophotometer.

The flavonoid content was estimated according to Jia et al. (1999). The free radical scavenging activity - DPPH (2, 2-diphenyl-1-picryl-hydrazyl) was estimated according to Bobo-García et al. (2015). The Iron Reduction Power (FRAP) was determined by La Torre et al. (2015) and β-carotene bleaching test was based on Taga et al. (1984) and Lorenzo et al. (2014).

Data analyses

All analyses were performed in triplicate. The Shapiro-Wilk test was used to verify the normal distribution assumption. Variables were compared using the Student t test and Mann-Whitney. The Pearson correlation test

Table 1. Mean values of the microbial counts in the honey samples of *Apis mellifera* (1338, 1339, 1340, 1341, 1342, 1374, 1375) and *Melipona quadrifasciata anthidioides* (1343, 1346, 1344, 1345) of the semiarid, Brazil.

Sample	Aerobic mesophiles[a]	Coliforms 35 °C[b]	Moulds and yeasts[a]	Sulphite-reducing *Clostridium*[c]	*Bacillus* sp.[a]	*Escherichia coli*[b]	*Salmonella*[d]	*Staphylococcus* coagulase positiva
1338	<10	<1	<10	nd	<10	<1	nd	nd
1339	<10	<1	<10	nd	<10	<1	nd	nd
1340	<10	<1	<10	nd	<10	<1	nd	nd
1341	<10	<1	<10	nd	<10	<1	nd	nd
1342	<10	<1	<10	nd	4×10^1	<1	nd	nd
1374	<10	<1	<10	nd	<10	<1	nd	nd
1375	<10	<1	<10	nd	<10	<1	nd	nd
1343	<10	<1	<10	nd	<10	<1	nd	nd
1344	2×10^1	<1	<10	nd	2.4×10^4	<1	nd	nd
1346	<10	<1	1.3×10^1	nd	<10	<1	nd	nd
1345	2×10^1	<1	<10	nd	<10	<1	nd	nd

[a]Colony-forming units per gram of honey (CFU.g^{-1});
[b]Enumerated by the Most Probable Number (MPN.g^{-1});
[c](in 0.01 g);
[d](in 25 g); nd: not detected.

was used to verify possible associations between the quantitative variables evaluated. For the p-value lower than 0.05, differences were considered significant. The principal component analysis (PCA) was applied to reduce the number of variables to a smaller number of components, which adequately summarizes the data on the original variables. All statistical analyses were performed in Software R Core Team (2015).

Results

Microbiological analyses

Regarding the microbiological quality, all samples analysed to comply with the Brazilian Legislation for food and stingless bee honey (Brasil, 2001, ADAB, 2014), that is, coliform counts at 35 °C below 3.0 MPN.g^{-1}, absence of *E. coli*, *Salmonella* spp., *Staphylococcus* coagulase positive and *Clostridium* sulphite reducing spores (Table 1).

In our work, mesophilic bacteria were only detected in honey samples of *M. q. anthidioides*; however, at very low counts (2×10^1 CFU.g^{-1}) (Table 1) and did not pose a risk to consumer health.

Bacillus spp. was detected in 14% of the *A. mellifera* honey samples (4×10^1 CFU.g^{-1}) and in 25% of the *M. q. anthidioides* (2.4×10^4 CFU.g^{-1}) (Table 1). Moulds and yeasts were detected in 25% of honey samples of *M. q. anthidioides* at low scores 1.3×10^1 CFU.g^{-1} honey (Table 1).

Melissopalynological analyses

We identified 41 pollen types belonging to 23 botanical families in honey samples, distributed in different frequency classes. Only one pollen type was not identified (Table 2).

Myrtaceae (13.63%), Anacardiaceae, and Sapindaceae (4.54%) were the botanical families with the highest number of pollen types in the *A. mellifera* honey, while in *M. q. anthidioides* honey, Malvaceae (18.18%) was the most abundant family. The Fabaceae family stood out, occurring in 26.82% of the samples.

There were differences in the diversity of pollen types visited by *A. mellifera* (80.48%) and *M. q. anthidioides* (26.82%).

The *Psidium* pollen type was found in all samples of *A. mellifera*, with an average frequency of 40.89%. The *Serjania* type was the second most frequent among the honey samples of *A. mellifera*, identified in more than 50% of the samples.

Physicochemical analyses

Regarding the physicochemical parameters evaluated, 75% of samples complied with the standards established by the Brazilian and European quality Legislation (Table 3).

Of the *A. mellifera* honey samples analysed, only 28.57% (18.97 ± 1.6 to 22.40 ± 0.40) (Table 3) complied with the Brazilian and European Legislation (Brasil, 2000; Codex, 2001) for moisture. Fifty percent of the samples of *M. q. anthidioides* (28.13 ± 0.12 to > 30) complied with the values recommended by the ADAB Ordinance (ADAB, 2014) for this parameter (Table 3).

The percentage of reducing sugars observed in samples of *A. mellifera* (70.12 ± 1.92 to 83.41 ± 0.63) and *M. q. anthidioides* (60.04 ± 0.36 to 66.15 ± 0.22) (Table 3) presented values within the limits established by Brazilian and European Legislation (ADAB, 2014; Brasil, 2000; Codex, 2001). The correlation analysis showed that reducing sugars had a significant positive correlation with colour (λ), pH, diastase activity, and electrical conductivity.

For the diastase activity, all values obtained for *A. mellifera* honey samples (10.08 ± 0.10 to 16.57 ± 1.23°Göthe) (Table 3) complied with national and international standards (Brasil, 2000; Codex, 2001). *M. q. anthidioides* honey presented values of 0.07 ± 0.00 to 0.11 ± 0.00°Göthe (Table 3). The diastase activity correlated with 62.5% of the parameters evaluated,

Table 2. Pollen spectrum, percentage and frequency class of the pollen types in honeys of *Apis mellifera* (1338, 1339, 1340, 1341, 1342, 1374, 1375) and *Melipona quadrifasciata anthidioides* (1343, 1346, 1344, 1345) of the semiarid, Brazil.

		Apis mellifera							*Melipona quadrifasciata anthidioides*			
Plant family	Pollen type	1338	1339	1340	1341	1342	1374	1375	1343	1346	1344	1345
Amaranthaceae	*Amaranthus*	–	–	–	–	–	MP	–	–	–	–	–
Anacardiaceae	*Schinus*	MP	IMP	PP	MP	IMP	–	–	–	–	–	–
Anacardiaceae	*Spondias*	IMP	SP	SP	SP	IMP	–	MP	–	–	–	–
Anacardiaceae	Anacardiaceae	–	–	–	–	–	–	IMP	–	–	–	–
Arecaceae	*Cocos nucifera*	–	–	–	–	–	IMP	IMP	–	–	–	–
Asteraceae	*Bidens*	–	–	–	MP	–	–	–	–	–	–	–
Boraginaceae	*Heliotropium*	–	–	–	–	–	–	MP	IMP	–	–	MP
Convolvulaceae	*Jacquemontia*	–	–	–	–	–	IMP	–	–	–	–	–
Euphorbiaceae	*Croton* I	MP	MP	–	–	MP	MP	–	–	–	–	MP
Euphorbiaceae	*Croton* II	–	–	–	–	–	–	IMP	–	–	–	–
Fabaceae	*Acacia*	MP	MP	IMP	MP	MP	MP	IMP	–	–	–	IMP
Fabaceae	*Chamaecrista*	–	–	–	–	–	MP	–	–	–	–	–
Fabaceae	*Copaifera*	–	–	–	–	–	IMP	IMP	–	–	–	–
Fabaceae	*Leucaena leucocephala*	–	–	–	–	–	–	–	–	–	–	MP
Fabaceae	*Mimosa caesalpiniifolia*	–	–	–	–	–	MP	MP	–	IMP	–	–
Fabaceae	*Mimosa tenuiflora*	–	–	MP	–	–	MP	IMP	–	–	–	MP
Fabaceae	*Mimosa*	–	–	–	–	–	IMP	–	–	–	–	–
Fabaceae	*Piptadenia*	–	–	–	–	–	MP	IMP	–	–	–	–
Fabaceae	*Prosopis*	–	–	–	–	–	–	–	–	–	–	IMP
Fabaceae	Caesalpinoideae	–	–	–	–	–	IMP	–	–	–	–	–
Fabaceae	*Senna*	–	–	–	–	–	IMP	IMP	–	–	–	–
Lamiaceae	*Hyptis*	–	–	–	–	–	IMP	–	–	–	–	–
Loranthaceae	*Tripodanthus*	–	–	MP	MP	–	–	–	–	–	–	–
Melastomataceae	*Miconia*	–	–	–	–	–	–	MP	–	–	–	–
Meliaceae	Meliaceae	–	–	–	–	–	–	MP	–	–	–	–
Malvaceae	Malvaceae	–	–	–	–	–	–	–	–	–	–	IMP
Malvaceae	*Herissantia*	IMP	MP	–	–	MP	IMP	MP	–	–	–	–
Myrtaceae	Myrtaceae	–	–	–	MP	–	–	–	–	–	–	–
Myrtaceae	*Eucalyptus*	–	–	–	–	–	–	IMP	–	SP	–	–
Myrtaceae	*Psidium*	PP	PP	SP	PP	MP	IMP	IMP	–	MP	–	–
Poaceae	Poaceae	–	–	–	–	–	MP	MP	–	–	–	–
Polygalaceae	Polygalaceae	MP	–	–	–	–	–	–	–	–	–	–
Portulacaceae	*Portulaca*	–	–	–	–	–	MP	–	–	–	–	–
Rubiaceae	*Borreria*	–	–	–	–	–	MP		–	–	–	–
Rubiaceae	Rubiaceae I	–	–	–	–	–	IMP	–	–	–	–	–
Rubiaceae	Rubiaceae II	–	–	–	–	–	–	IMP	–	–	–	–
Rutaceae	*Citrus*	–	–	–	–	–		MP	–	–	–	–
Sapindaceae	*Serjania*	SP	–	IMP	MP	PP	–	–	–	–	–	–
Malvaceae	*Waltheria*	–	–	MP	MP	–	MP	–	PP	PP	PP	PP
Turneraceae	*Turnera*	–	–	–	–	–	IMP	MP	–	–	–	–
Verbenaceae	*Lantana*	–	IMP	MP	MP	–			–	–	–	MP
Indeterminate I	–	–	–	–	–	–		IMP	–	–	–	–
Total	–	1.079	1.059	250	1044	1111	567	234	21	193	48	519

Frequency classes: Predominant pollen (PP, >45% of total grains); Secondary pollen (SP, 16 to 45%), Important minor pollen (IMP, 3 to 15%) and Minor pollen (MP, <3%).

positive correlations were observed between the pH and the reducing sugars, colour (Λ), electrical conductivity, and ash.

The HMF content of *A. mellifera* samples ranged from 8.13 ± 5.13 to 67.61 ± 3.85 mg.kg^{-1} (Table 3) and 28% of the samples had HMF levels outside the limits recommended by the Brazilian Legislation (Brasil, 2000). All samples of *M. q. anthidioides* presented HMF values (1.25 ± 1.27 a 7.09 ± 0.75 mg.kg^{-1}) (Table 3) in compliance with the ADAB Ordinance for stingless bee (ADAB, 2014). HMF presented a negative correlation with free acidity and ash (Table 3), while the other parameters under evaluations presented low correlation.

Honey samples of *A. mellifera* and *M. q. anthidioides* had ash contents, suggesting that all honey samples are from nectar. In this study, a highly significant positive correlation was observed between electrical conductivity, pH, colour (Λ), diastase activity and free acidity, while a negative correlation was observed for HMF ($r = -0.43$) (Table 3).

The colour of *A. mellifera* honeys varied from amber (57%) to light amber (42.85%) while honey of *M. q. anthidioides* samples ranged from light amber (25%) to white (75%) (Table 3). Colour (Λ) intensity showed a positive correlation with 87% of the parameters evaluated, the electrical conductivity, diastase activity,

Table 3. Mean and standard deviation for the physical and chemical analysis of honey of *Apis mellifera* (1338, 1339, 1340, 1341, 1342, 1374, 1375) and *Melipona quadrifasciata anthidioides* (1343, 1346, 1344, 1345).

Samples	M*	Rs*$^{\Delta}$	Suc*$^{\Delta}$	Di*$^{\Delta}$	HMF*	Fa*$^{\Delta}$
1338	22.40 ± 0.40	73.88 ± 0.80	1.25 ± 1.22	15.60 ± 0.39	14.97 ± 2.17	17.83 ± 0.58
1339	21.90 ± 0.30	70.12 ± 1.92	1.51 ± 1.53	13.00 ± 0.71	10.38 ± 3.27	19.67 ± 0.58
1340	21.17 ± 0.15	75.30 ± 1.17	3.60 ± 1.86	16.57 ± 1.23	9.38 ± 3.18	18.83 ± 2.30
1341	21.30 ± 0.40	76.29 ± 0.97	2.67 ± 0.59	13.57 ± 1.16	8.13 ± 5.13	15.83 ± 1.44
1342	22.37 ± 0.25	83.41 ± 0.63	0.92 ± 0.52	15.28 ± 0.28	10.78 ± 0.94	15.00 ± 0.00
1374	19.53 ± 0.96	77.01 ± 0.72	1.97 ± 1.69	11.32 ± 0.10	61.78 ± 1.20	5.17 ± 0.29
1375	18.97 ± 1.6	77.01 ± 0.72	1.11 ± 0.82	10.08 ± 0.10	67.61 ± 3.85	5.00 ± 0.00
1343	28.50 ± 0.10	62.51 ± 1.04	2.61 ± 0.99	0.07 ± 0.00	7.09 ± 0.75	15.83 ± 1.44
1344	>30	63.86 ± 0.81	0.42 ± 2.45	0.10 ± 0.04	1.25 ± 1.27	15.50 ± 0.86
1346	>30	63.32 ± 0.36	0.95 ± 1.02	0.09 ± 0.04	2.89 ± 0.57	15.50 ± 0.86
1345	28.13 ± 0.12	66.15 ± 0.22	0.67 ± 1.31	0.11 ± 0.00	1.80 ± 2.33	18.33 ± 1.44
Correlation Pearson[a]	nd	Co (Λ) − 0.81 pH − 0.71 Di − 0.86 Ec − 0.73	ns	Co (Λ) − 0.85 pH − 0.90 Ec − 0.83 As − 0.61	Fa - (- 0.92)	As − 0.58
Student's t-Test and Mann-Whitney	0.01[c]	0.00[b]	ns[b]	0.01[c]	0.01[c]	ns[c]

Samples	**pH**	**As*$^{\Delta}$**	**EC**	**Co (Λ)**	**Co**$^{**\Delta}$	**Lr/ Fr**
1338	3.87 ± 0.6	0.404 ± 0.00	632.67 ± 7.33	0.545 ± 0.03	Am	Ne
1339	4.10 ± 0.00	0.387 ± 0.01	608.23 ± 4.77	0.528 ± 0.00	Am	Ne
1340	4.07 ± 0.06	0.188 ± 0.00	305.23 ± 0.97	0.217 ± 0.01	Lam	Ne
1341	4.27 ± 0.25	0.389 ± 0.00	615.87 ± 3.87	0.530 ± 0.01	Am	Ne
1342	3.90 ± 0.00	0.399 ± 0.00	630.20 ± 0.78	0.565 ± 0.02	Am	Ne
1374	3.64 ± 0.00	0.023 ± 0.00	394.47 ± 8.10	0.403 ± 0.00	Lam	Ne
1375	3.70 ± 0.00	0.023 ± 0.00	397.80 ± 5.77	0.413 ± 0.00	Lam	Ne
1343	3.27 ± 0.06	0.129 ± 0.00	229.57 ± 1.79	0.101 ± 0.00	Wh	Ne
1344	3.30 ± 0.00	0.086 ± 0.00	156.27 ± 0.23	0.062 ± 0.00	Wh	Ne
1346	3.33 ± 0.06	0.087 ± 0.00	160.47 ± 0.32	0.061 ± 0.00	Wh	Ne
1345	3.37 ± 0.06	0.123 ± 0.00	218.17 ± 0.84	0.130 ± 0.00	ELam	Ne
Correlation Pearson[a]	Co (Λ) − 0.80 Ec − 0.82 As − 0.72	Co (Λ) − 0.70 Ec − 0.83	Co (Λ) − 0.98	–	–	nd
Student's t-Test and Mann-Whitney	0.00[b]	ns[c]	0.01[c]	0.01[c]	–	–

M - Moisture (%); Rs - Reducing sugars (%); Suc - Sucrose (%); Di - Diastase activity (Gothe degrees); HMF - Hydroxymethylfurfural content (mg.kg^{-1}); FA - Free acidity (mEq.kg^{-1}); As - ash (%); EC - electrical conductivity (μS.cm^{-1}); Co (Λ)- Colour obtained by UV - Vis spectrophotometry; Co - colour (verified from the mmPfund scale; Am - Amber; Lam - Light amber; Wh - White; ELam - Extra light amber); Lr/Fr - Lugol reaction/Fiehe reaction (Ne - negative); nd - not detected; ns - not significant;

[a]Pearson correlation for the $p < 0.05$, the differences found were considered significant;

[b]Student's t-Test (compare means), level of significant 5% (pH, Rs and Suc);

[c]Mann-Whitney Test (compare ranks), level of significant 5%, (M, Di, HMF, As, EC, Fa and Co (Λ)); *Contemplated parameters by Brazilian (Brasil, 2001) and International (Codex, 2001) Legislation; **Contemplated parameter by Brazilian Legislation (Brasil, 2001): $^{\Delta}$Parameters according with Brazilian and International Legislation.

reducing sugars, pH and ash, having a negative correlation with free acidity (Table 3).

All samples were negative for the Lugol reaction, a negative result was also obtained for the Fiehe test.

The results obtained in this study were submitted to the PCA, which summarized the relational information between the variables in two orthogonal components that explained 82.75% of the total variation. The PC1 component explained 55.54% of the data variance, while the PC2 explained 27.21% of the variance, and the HMF content and the free acidity were the parameters responsible for its variation (Figure 1).

Total phenolic compounds and antioxidant activity

The contents of total phenolic compounds were high for *Apis mellifera* samples; however, higher antioxidant activity was observed for *M. q. anthidioides* samples. We observed a positive and significant correlation between the total phenolic (Phenols ($r = 0.01$)) and flavonoids ($r = 0.00$) ($p > 0.05$)) and the values obtained for the antioxidant activity determined by the DPPH and FRAP methods ($r = 0.00$ and $r = 0.00$, respectively, $p > 0.05$) (Table 4). The antioxidant activity is inversely proportional to the concentration used.

Discussion

Microbiological analyses

The microbiological analysis suggested adequate hygienic-sanitary conditions during processing and storage, ensuring honey safety, consumed *in natura* as food or herbal product (Table 1).

Honey quality is also associated with its physicochemical composition that determines which microorganisms can multiply (i.e. pH values between 2.9 and 4.6, and water activity from 0.54 to 0.75 for *Apis mellifera* honey and from 0.52 to 0.80 for *Melipona* spp. honey are limiting for microorganism multiplication (Franco & Landgraf, 2008; Lira et al., 2014; Camargo et al., 2017). In addition, this product has inherent antimicrobial properties, which reduce microbial survival and growth (Pires et al., 2015; Pucciarelli et al., 2014). This affects honey functional properties and consequently consumer's health (Estevinho et al., 2008). On the other hand, honey produced by stingless bees has different characteristics when compared to *Apis* honey, particularly regarding the moisture content (25.25%), making it less dense and more prone to microbial development (Lira et al., 2014; Oliveira et al., 2012).

Mesophilic bacteria were detected at low amounts in honey samples of *M. q. anthidioides* (Table 1) and thus did not represent a risk to consumer health. Although RDC n° 12 of 2001 for food does not establish a threshold for mesophylls, high counts may indicate a deficiency of raw material hygiene, inadequate processing, inappropriate incorrect hygienic manipulation, or maintenance (Brasil, 2001).

The analysis of *Bacillus* in honey is not required by the Brazilian Legislation (ADAB, 2014; Brasil, 2000); however, its presence may be a risk to consumers. Although honey is an unfavourable environment for the growth of some foodborne pathogenic bacteria, sporogenic forms may occur, due to primary and/or secondary contamination. *Bacillus* spp. can be found in honey, since there is a symbiotic relationship of these microorganisms with insects and bees (Pucciarelli et al., 2014).

Moulds and yeasts are associated to bees and occur naturally in honey. However, the presence of some filamentous fungi in food may cause serious damage to human health, due to their ability to produce mycotoxins (Pires et al., 2015).

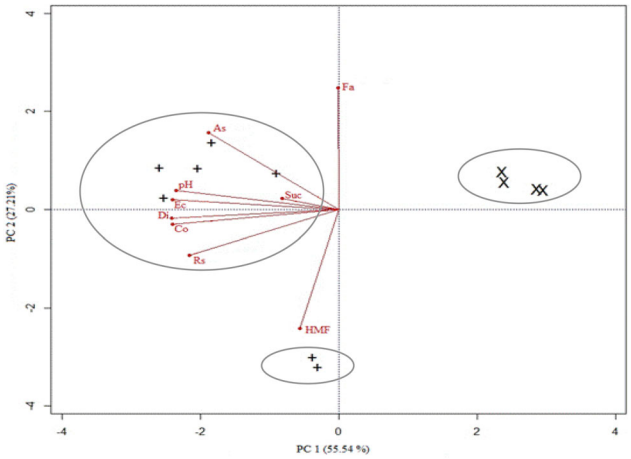

Figure 1. Principal Component Analysis (PCA) of physicochemical parameters of honey samples from *Apis mellifera* (+) and *Melipona quadrifasciata anthidioides* (x) of the semiarid, Brazil. Rs - Reducing sugars (%); Suc - Sucrose (%); Di - Diastase activity (°Göthe degrees); HMF - Hydroxymethylfurfural content (mg.kg^{-1}); Fa - Free acidity (mEq.kg^{-1}); As - ash (%); EC - electrical conductivity (μS.cm^{-1}); Co (λ) - color (Colour obtained by UV - Vis spectrophotometry).

Melissopalynological analyses

The pollen composition analysis showed that all samples, for *A. mellifera* and *M. q. anthidioides*, were classified as heterofloral honeys. The honeys of the Brazilian semiarid region are characterized by the diversity of pollen types, resulting from the floristic diversity of the region (Borges et al., 2006), which include savannas, forests, caatinga, and rupestrian fields (Couto et al., 2011), with apicultural potential (Borges et al., 2006). This diversity favours the production of honey with different characteristics.

The diversity of pollen types in the samples of *A. mellifera* could be associated to the foraging capacity of this species, which reaches an area 100 times greater than that of native species and has a diet that includes plants of the most diverse groups, reflected on the diversity of pollen types (Minussi & Alves-dos-Santos, 2007). However, social bees can change their trophic niche during the year due to the availability of floral resources

Table 4. Total phenolic values (mgGAE.kg^{-1}), flavonoids (mgQE.kg^{-1}), DPPH (μmolETEAC.L^{-1}), FRAP (μmolEFerrous sulphate.L^{-1}) e β-carotene/linoleic acid (μmolEBHA.L^{-1}) obtained for samples of honey (Mean ± Standard deviation).

Species	Sample	Phenols	Flavonoids	DPPH	FRAP	β-carotene
	1338	341.51 ± 3.85	149.63 ± 6.41	154.43 ± 1.65	232.86 ± 7.48	17.15 ± 4.21
	1339	330.03 ± 4.23	160.74 ± 12.83	151.54 ± 3.44	247.43 ± 4.94	19.71 ± 0.04
	1340	324.98 ± 5.34	216.29 ± 6.41	180.28 ± 2.55	365.35 ± 5.16	3.91 ± 0.26
	1341	333.39 ± 1.75	141.85 ± 0.641	137.23 ± 6.58	310.80 ± 2.95	3.13 ± 0.56
	1342	338.99 ± 3.03	114.44 ± 7.86	151.85 ± 0.75	341.05 ± 5.32	11.62 ± 3.27
	1374	260.00 ± 8.02	182.97 ± 6.41	152.66 ± 1.64	327.81 ± 2.91	2.45 ± 2.19
Apis mellifera	1375	273.44 ± 12.37	171.85 ± 6.42	153.47 ± 3.90	290.73 ± 5.68	5.54 ± 1.76
	1343	88.57 ± 3.361	23.70 ± 6.41	101.41 ± 0.43	147.47 ± 4.67	1.91 ± 1.59
	1346	61.96 ± 2.11	8.88 ± 11.11	101.06 ± 2.47	113.08 ± 0.27	5.95 ± 2.00
	1344	47.67 ± 5.59	14.44 ± 7.85	86.76 ± 3.40	98.43 ± 0.97	6.36 ± 2.70
Melipona quadrifasciata anthidioides	1345	89.13 ± 0.97	23.70 ± 6.41	102.81 ± 0.09	128.33 ± 1.99	9.18 ± 0.73
Student's t-Test and Mann-Whitney		0.01[a]	0.00[b]	0.00[b]	0.00[b]	ns

[a]Mann-Whitney Test (compare ranks), level of significant 5%;
[b]Student's t-Test (compare means), level of significant 5%; ns - not significant.

(pollen, nectar, and resin), climatic oscillations, distance between colony, interspecific and intraspecific competition (Silva et al., 2013).

Although both species are considered generalists in terms of nectar and pollen sources, *M. q. anthidioides* used the *Waltheria* type more frequently, whereas *A. mellifera* occasionally used this source (minor pollen). The species *Walhteria* L. are considered nectariferous and occur in the pollen spectrum of different honeys (Kwaga et al., 2016; Nascimento et al., 2015). In addition, we verified that the pollen type *Citrus* occurred between the samples of *Melipona* as minor pollen (Table 2). Generally, the *Citrus* type is under-represented in the pollen spectrum; however, species of the genus *Citrus* L. are considered nectariferous and important in honey production, especially in the production of monofloral honey (Louveaux et al., 1978; Nascimento et al., 2015).

Physicochemical analyses

As 75% of samples comply with requirements of the Brazilian and European legislation, the physicochemical quality of honey produced in the region is fit for human consumption, both as food and as a nutraceutical compound.

Moisture showed non-compliance with the legislation since this parameter can change according to climatic conditions of the region during honey sampling or storage. However, the moisture amount in stingless bees honey distinguishes this product in relation to *A. mellifera* honey (Lira et al., 2014). High moisture levels are a very important parameter for the *Melipona* honey, as it affects some characteristics, such as viscosity and flow ability. In addition, honey moisture is a useful parameter to improve its storage and conservation (Silva et al., 2013). High moisture values can accelerate crystallization and allow the multiplication of microorganisms, particularly yeasts (Gomes et al., 2010).

HMF, which indicates honey freshness, was also inconsistent with the Brazilian Legislation. HMF ingestion at high doses represents a health hazard, with risks to cytotoxic, carcinogenic, mutagenic, and genotoxic actions (Capuano & Fogliano, 2011).

HMF is formed as an intermediate in the Maillard reaction, resulting from the direct dehydration of the sugars under acidic conditions or during the prolonged storage (Lira et al., 2014; Silva et al., 2016). Codex Alimentarius determines that the HMF content should not exceed 80 $mg.kg^{-1}$ for honeys from countries or regions of tropical environments; therefore, all *A. mellifera* samples are in agreement with international standards. All samples of *M. q. anthidioides* presented HMF contents in compliance with the ADAB Ordinance for stingless bee (ADAB, 2014).

Parameters with reducing sugars and sucrose presented values within the limits established by Brazilian and European Legislation (ADAB, 2014; Brasil, 2000; Codex, 2001). The significant positive correlation in reducing sugar with colour (Λ), pH, diastase activity, and electrical conductivity could be attributed to their influence on darkening, floral origin, and enzymatic reactions. Free acidity showed a negative correlation, which is related to the degradation of sugars and the production of organic acids.

The composition of sugars in honey depends on their geographical and botanical origin and may vary according to their storage time, climate, and processing and storage techniques (Silva et al., 2016). Glucose and fructose are the main sugars found in honey. Fructose is one of the sugars responsible for sweetness and high hygroscopicity of *M. q. anthidioides*. High sucrose concentrations may occur depending on the botanical origin. In addition, high concentrations indicate premature harvest of honey, sucrose-fed bees, as well as honey adulteration (Lira et al., 2014; Silva et al., 2016).

All values obtained for *A. mellifera* honey samples comply with national and international standards for diastase activity (Brasil, 2000; Codex, 2001). Diastase, a natural honey enzyme, is very sensitive to heat and may indicate the conservation degree and product overheating (Lira et al., 2014; Silva et al., 2016). Honey of *M. q. anthidioides* complied with ADAB (2014) (Table 3). European Legislation does not establish a threshold for stingless bees honey (Codex, 2001).

The results obtained for ash suggest that all honey samples originated from nectar. The ash content reflects the chemical components of plants visited by the bees; therefore, the content of trace elements in honey depends on the soil and plant types from which the nectar was obtained (Silva et al., 2016). The high ash content also reflects possible contaminants during collection. Electrical conductivity, in turn, has been used to measure the amount of mineral elements with good electrical conduction property (Sousa et al., 2016), directly relating it to the concentration of mineral salts, organic acids, and proteins, very useful to determine the honey floral origin (Almeida-Muradian et al., 2013). Brazilian Legislation does not establish a threshold for conductivity.

According to the colour parameter, all samples comply with Brazilian and European Legislation (ADAB, 2014; Brasil, 2000; Codex, 2001). The difference in the floristic composition of the samples possibly influenced their colour. In fact, *A. mellifera* honeys presented a greater number of pollen types compared to *M. q. anthidioides*. Moreover, exposure to light, temperature, storage time, as well as enzymatic reactions, may affect the colour parameter (Silva et al., 2016).

The negative result to Fiehe and Lugol represents that the sample did not undergo sudden changes in temperature (overheating), nor received the addition of syrups and/or starch. Bera and Almeida-Muradian (2007) reported similar results for samples of commercial honey from the state of São Paulo. The Fiehe and Lugol

tests are not required by the Brazilian and International legislation; therefore, these tests are complementary to the investigation of honey quality.

The PCA performed for the physicochemical parameters revealed a formation of three clusters, two evidenced by *A. mellifera* (+) and one by *M. q. anthidioides* (x). One of the groups included the samples of *A. mellifera* (1338, 1339, 1340, 1341, and 1342) with high values for the physicochemical variables associated with PC1, while the second group presented intermediate values for these variables. Two samples of *A. mellifera* (1374 and 1375) were separated by PC2 because they presented high HMF levels and low free acidity. *M. q. anthidioides* honey samples (1343, 1346, 1344, and 1345) were placed in the positive quadrant of PC1, as opposed to the samples of *A. mellifera* honey, which were placed in the negative quadrant, because they presented low levels in the variables that define PC1.

Total phenolic compounds and antioxidant activity

Honeys of different floral origins have different amounts and classes of phenolic compounds and may be a factor for the dissimilarity between the content of these substances in *A. mellifera* and *M. q. anthidioides* honeys observed in this work (Ciulu et al., 2016; Da Silva et al., 2013). Although both honeys are multifloral, *A. mellifera* honeys showed the greater richness of plant species that may have favoured the high content of phenolic compounds (Table 4). The importance of this compound is associated with its antiviral, antibacterial, and anti-inflammatory activities (Oliveira et al., 2012).

A positive correlation ($r = 0.75$, highly significant) was observed between phenols and total flavonoids in the samples, as well as between the honey colour and phenolic compound content, indicating that dark-coloured honeys have higher contents of phenolic compounds and flavonoids than light coloured ones.

M. q. anthidioides honeys presented higher antioxidant activity in comparison with samples of *A. mellifera* honeys (Table 04). This result may be associated with the pollen composition of this honey. According to Al Muqarrabun and Ahmat (2015), the *Waltheria* pollen type, dominant in all *Melipona* samples studied, concentrates phytochemicals (alkaloids, phenylpropanoids, flavonoids, terpenoids, hydrocarbons, sugars, quinones, phenolic acids, lactones, lignans, amines, and amides), responsible for the biological properties of honey. Antioxidant compounds capture free radicals, agents involved in the pathophysiology of several diseases, namely cerebral and cardiac ischemia, Parkinson's disease, and gastrointestinal disorders (Oliveira et al., 2012).

The difference between the antioxidant activity and the content of bioactive compounds in these samples is related to the assumption that the composition of flavonoids and phenolic compounds of each sample is more important than its total content, corroborating the lack of correlation between these variables (Dias et al., 2016). In addition, different methodologies show that the antioxidant activity is also associated with the variations in the results. This can be associated with different kinetic behaviours in patterns (trolox, ferrous sulphate and BHA) and to the mechanism to eliminate free radicals (Chen et al., 2014).

Conclusions

The *Apis mellifera* and *Melipona quadrifasciata anthidioides* honeys from the semiarid region have a distinct botanical composition, suggesting that both bee species use different plant sources. The differences observed in the pollen composition of the samples possibly influenced the parameters related to honey quality and maturity, as well as the total phenolic and flavonoid content that favours their nutraceutical potential and contributes to human health. Samples of *M. q. anthidioides* showed high antioxidant activity, indicating that this honey could be promising in the fight against free radicals and diseases promoted by cellular oxidation.

Disclosure statement

The authors declare that there are no conflicts of interest.

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ORCID

Irana Paim Silva http://orcid.org/0000-0003-4448-1443
Maiara Janine Machado Caldas http://orcid.org/0000-0002-7000-5750
Cerilene Santiago Machado http://orcid.org/0000-0002-3859-6722
Andreia Santos do Nascimento http://orcid.org/0000-0001-5236-0460
Maurício Santana Lordêlo http://orcid.org/0000-0002-3791-1815
Marivalda Figueredo Santa Bárbara http://orcid.org/0000-0002-3871-2375
Norma Suely Evangelista-Barreto http://orcid.org/0000-0001-5960-0957
Leticia M Estevinho http://orcid.org/0000-0002-9249-1948
Carlos Alfredo Lopes de Carvalho http://orcid.org/0000-0002-3306-3003

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