Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v45i1.59799

Physicochemical and bioactive properties of *Apis* and stingless bee (Meliponini) honey from Brazilian Caatinga

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ABSTRACT. Since the composition of honey varies with the species of bee as well as flowering and geographical aspects, this study aimed to evaluate the physicochemical and bioactive properties of *Apis*and stingless bees'honey from the Brazilian Caatinga. Samples of different species of *Apis mellifera L.Meliponini* (*Melipona subnitida*, *Frieseomellita varia*, *Melipona mandacaia*, *Plebeia* sp.) and *Apis mellifera* L.werecollected from honey producersin the state of Rio Grande do Norte. Honey from *A. mellifera* and stingless bees showed physicochemical differences in some parameters, especially in moisture, free acidity, HMF, water activity, sugars and electric conductivity. There were no differences in color between honeys from *A. mellifera* and stingless bees. Honeys from*Plebeia* sp., *F. varia* and *A. mellifera* showed higher antioxidant capacity followed by honeys from*M. mandacaia* and *M. subnitida.* Flavonoids had little influence on the differentiation of antioxidant activities of stingless bees, while the opposite occurred with the phenolic content, where honeys with the highest levels of phenolic also showed higher antioxidant capacity.

Keywords:*Apis mellifera* L.;hydroxymethylfurfural; flavonoids;meliponines.

Received on June 23, 2021.
Accepted on April 13, 2022.

Introduction

Honey is a natural sweet substance produced by bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Wilczynska, 2014). The major component in honey is sugars and small amounts of interesting compounds, such as phenolics, whichhave potential beneficial properties (Osés, Pascual-Maté, Fernández-Muino, López, & Sancho, 2016).

Honey can originate from single or multiple plant species, and its biochemical composition is affected by the floral source. Composition, color, aroma and flavor of honey depend mainly on the species of flower, geographical regions, climate and honeybee species (Sousa et al., 2016) involved in its production, and are also affected by weather conditions, processing, manipulation, packaging and storage time (Tornuket al., 2013).

Stingless bees can be found in most tropical and subtropical regions of the world. There are over 500 described species in 32 genera, which produce and store much less honey on a per hive basis when compared to the western honey bee *Apis mellifera*, and due to insufficient knowledge about the product, stingless bee honey is not included in international standards for honey (Food and Agriculture Organization [FAO], 2019) and is not regulated by food control authorities (Chuttong, Chanbang, Sringarm, & Burgett, 2016).

Since honey types differ from one country to another and in different regions in the same country due to floral origin, soil composition and other factors, consequently, quality criteria differ from one honey type to another, i.e. blossom honey is greatly different than honeydew (Alqarni, Owayss, & Mahmoud, 2016). There are few studies in the literature about stingless bee species mainly originating from the native flora of the Caatinga.

Caatinga is a biome of exclusive occurrence in the Northeastern Region of Brazil and covers approximately 10% Brazilian territory. Thus, the Caatinga scrub is the largest dry forest region in South America, characterized by a semiarid climate, low and irregular rainfall, fertile soil and apparently dry vegetation. The

climate of the Caatinga is strongly seasonal and severe droughts are relatively frequent. Rainfall is more intensive in February, March and April. Occasional rain occurs in June and July, whereas the dry season extends from August to January. In addition, plants growing under stress conditions (biotic and abiotic) have great secondary metabolism that provides more defense compounds, such as phenolics (Borges & Amorim, 2020). Honey from multiple stingless bee species exhibit a great potential for human consumption and commercialization due to the higher content of polyphenolic compounds, flavonoids, and antioxidants, as compared to *Apis mellifera*honey (Khongkwanmueang, Nuyu, Straub, & Maitip, 2020).

The aim of this study was to evaluate the physicochemical and bioactive characteristics of *Apis mellifera* L. honeys and stingless bee species honeys produced in the Brazilian caatinga.

Material and methods

Forty-five honey samples were collected from May to September 2013 from different producers in various municipalities of the state of Rio Grande do Norte, Brazil (Table 1). Samples were placed in sealed containers and stored under refrigeration at 6˚C for three months until analysis.

Table 1. Classification of honey and regional sources.

Botanical name	Common name	Type of honey	Geographic Coordinate	Color*
Melipona subnitida	Jandaíra	Heterofloral	05° 39’ 51”S37°47’56”W	Light amber
Melipona mandacaia	Mandaçaia	Heterofloral	05° 47’ 23” S 37°57’18”W	Dark amber
Plebeia sp.	Mosquito	Heterofloral	04° 56’ 52”S37° 7’ 28”W	Amber
Frieseomelitta varia	Moça Branca	Heterofloral	05° 27’ 34”S37°31’16”W	Amber
Apis mellifera L.	Europeanbee	Heterofloral	05° 39’ 51”S 37°47’56”W	Dark amber

*Pfund color scaleaccording toMarchini, Sodré, and Moreti (2004).

Determination of physicochemical properties

Color

Color was determined as described by Vidal and Fregosi (1984), using a spectrophotometer UV-340G Gehaka model 560nm withpureglycerin as ablank. The reading wassubsequently converted into thePfund color scale,which classifies color by wavelength: 0.030nm (white), 0.030 to 0.060nm (extra white), 0.120 to 0.188nm (extra light amber), 0.188 to 0.440nm (light amber), 0.440 to 0.945nm (amber) and more than 0.945nm (dark amber).

Moisture content

Moisture content was determined using a refractometer (Abbe Sammar RT-90ATC), according to Association of Official Analytical Chemists (AOAC, 2019), at 20ºC. Results were expressed in percentage.

Acidity

Acidity was determined according to the AOAC method (2019) by diluting 10 g honey in 75 mL distilled water, which was then titrated with 0.05N sodium hydroxide at a flow rate of 5 mL per minute to pH 8.5.

Hydroxymethylfurfural

Hydroxymethylfurfural was determined according to the AOAC method (2019), in which 5 g honey were dissolved in 25 mL distilled water, transferred to a volumetric flask (50 mL), added with 0.5 mL Carrez solution (15 g potassium ferrocyanide 100 mL distilled water^{-1}) and 0.5 mL Carrez II solution (30 g zinc acetate 100 mL distilled water^{-1}), and distilled water to complete the volume. After transferring the filtration, 5 mL aliquots of the honey solution were added with 5 mL distilled water (sample solution), and another 5 mL 0.2% sodium bisulfite solution (control) were placed in test tubes.Samples were read at 284 and 336nm in a Gehaka UV-340G spectrophotometer.

Reducing sugars and apparent sucrose

Reducing sugars was determined according to Lane and Eynon (1934), using the Fehling’s alkaline copperreagent. The end point is indicated by reduced methylene blue. Initially, sucrose was executed in acid hydrolysis (HCl) and quantitatively determined by the abovementioned method.

Ash content and electrical conductivity

Ash contentwas determined by the method suggested by Pregnolato and Pregnolato (1985) with sample incineration at 550ºC and applying a mass balance. Electrical conductivity was determined on a 20% dry matter honey, using Conductivity TecnoponmCA model 150 and an analytical balance.

Determination of the total flavonoids content

Total flavonoids content was determined according to the methodology described by Meda, Lamien, Romito, Millogo, and Nacoulma(2005), with adaptations. Initially, a 2% aluminum chloride solution in methanol was prepared; 5 mL of this solution was mixed with the same volume of a honey solution (0.02 $mgmL^{-1}$). Absorbance was read in a UV- 340G Gehaka spectrophotometer, in a wavelength of 415 nm after 10 minutes using methanol as blank. A quercetin curve (5 to 50 mg L^{-1}) was used as standard. The flavonoid content was expressed in cg equivalent of quercetin (EQ)100 g of $honey^{-1}$.

Determination of the total phenolic content

Total phenolic content was determined according to the method described by Meda et al. (2005) using the Folin-Ciocalteu reagent (Singleton & Rossi, 1965). For this, 5 g honey were diluted in 50 mL distilled water. From the honey solution (0.1 g mL^{-1}), an aliquot of 0.5 mL was mixed with 2.5 mL Folin-Ciocalteu reagent. After 5 minutes, 2 mL sodium carbonate (75 g L^{-1}) was added. After 2 hours, the absorbance was read in a spectrophotometer (UV- 340G Gehaka) at 760 nm against a blank (methanol). For calculation of the total phenolic content, a standard curve of gallic acid (20 to 200 mg L^{-1}) was used. Results were expressed in mg gallic acid (GA)100 g $honey^{-1}$.

Antioxidant capacity and antioxidant content

The antioxidant capacity of honeys was quantified with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical, according to Medaet al. (2005). In presence of an antioxidant, purple color of the DPPH decays and the change in absorbance can be read spectrophotometrically. The free radical scavenging activity of DPPH was expressed as IC_{50} (minimum concentration for the antioxidant to reduce the initial DPPH concentration by 50%). The antioxidant content was assessed as described by Meda et al. (2005) with adaptations. Samples were dissolved in methanol (100 mg mL^{-1}) and 0.75 mL of each sample was mixed with 1.5 mL DPPH solution (0.02 mg mL^{-1}) diluted in methanol. The mixtures were keptat room temperature in the dark for 15 minutes. Absorbances was read using a UV- 340G Gehaka spectrophotometer at a wavelength of 517 nm. The blank consisted of 0.75 mL methanol and 1.5 mL DPPH solution. The antioxidant content was determined using a standard curve for ascorbic acid (0-10 g mL^{-1}) and quercetin (0 to 6.25 µg mL^{-1}). The mean of the values obtained in triplicate is expressed in mg equivalent of ascorbic acid (EAA) per 100 g honey and mg equivalent of quercetin (EQ) per 100 g sample.

Statistical analysis

Analyses were performed in triplicate. Results were tested by analysis of variance (ANOVA) and means were compared by Tukey's test, with a significance level of 95% ($p < 0.05$). Graphics were constructed inMicrosoft Excel 2007 software package (Microsoft Corp., Redmond, USA).

Results and discussion

Physicochemical properties

Physicochemical composition of honeys from*A. mellifera* and stingless bees from the Brazilian Caatinga is presented in Table 2.

Color

Color of honeys was classified by the Pfund scale, ranging from light amber (0.188-0.440) to dark amber (> 0.945) (Table 2), showing that there was no statistical difference between the values obtained. In general, honeys from *Meliponini* bees are lighter in color than honeys from Apis, and in this study, it became clear the variation from clear amber to dark amber; honey color is strongly influenced by the bloom that originated the honeys (Silva, Gauche, Gonzaga, Costa, & Fett, 2016). In this study, *A. mellifera* honey color was dark amber

by the Pfund scale.It is a characteristic that influences honey marketing, being established by the Codex Alimentarius a variation in honey color, from colorless to dark brown.

Table 2. Physicochemical composition of honeys from *Apis mellifera* and stingless bees from the Brazilian Caatinga.

	M. subnitida (n= 18)	*M. mandacaia* (n= 9)	*Plebeia* sp. (n=3)	*F. varia* (n=3)	*A. mellifera* (n= 12)
Color	0.26±0.08^{a}	1.04±0.84^{a}	0.86±0.07^{a}	0.91±0.15^{a}	1.41±1.15^{a}
Moisture content (%)	26.02±1.2^{a}	26.04±0.7^{a}	26.00±0.1^{a}	23.20±0.1^{b}	17.60±0.7^{c}
Acidity (mEqkg^{-1})	30.1±12.7^{b}	81.8±18.0^{a}	114.2±0.5^{a}	113.3±1.3^{a}	42.1±18.2^{b}
HMF (mgkg^{-1})	30.9±25.5^{b}	21.0±16.1^{b}	12.0±1.0^{b}	18.1±0.6^{b}	57.6±13.2^{a}
Reducing sugars (%)	67.0±5.8^{a}	67.6±4.5^{a}	42.0±0.6^{b}	51.1±1.0^{b}	72.8±5.9^{a}
Sucrose (%)	6.5±3.4^{a}	3.1±2.5^{ab}	2.1±1.0^{ab}	1.4±0.5^{b}	4.8±1.1^{ab}
Ash (%)	0.23±0.2^{c}	0.54±0.21^{ab}	0.44±0.01^{abc}	0.63±0.04^{a}	0.28±0.13^{bc}
EC (µScm^{-1})	297.8±110.1^{d}	1206.3±138.4^{c}	2445.0±7.1^{a}	1443.0±15.6^{b}	469.0±81.9^{d}

Values are presented as mean ± standard deviation. $^{a-d}$Means followed by different letters, in the same row, are significantly different by Tukey's test at 5% probability. HMF: hydroxymethylfurfural; EC: electric conductivity.

Moisture content

The moisture content of the samples ranged from 17.60 to 26.04% (Table 2). *Apis'* honey had significantly lower moisture compered to honeys from*Meliponini*. This distinguishes honeys from the genus *Apisto Meliponini*. The moisture content of *A. mellifera* honey is within the range specified by the 19), which establishes a maximum of 20%. Similar results were reported by Habib, Meqbali, Kamal, Souka, and Ibrahim (2014), in *Apis*honey from the United Arab Emirates (13.6 to 20.6%). On the other hand, *Meliponini* honey from the species *Frieseomellita varia* presented moisture content 11.4% lower than honeys from *Melipona subnitida*, *Melipona mandacaia* and *Plebeia* sp. Also, Vit (2013) found in honey from *Meliponini* in South America a range from 21.2 to 30.8%. Chuttonget al. (2016) evaluated honey from 11 species of stingless bees in Thailand and reported a variation in moisture from 25.2 to 47.4%. The moisture content of honey is an important factor influencing their shelf life, since the high water content favors the activity of osmophilic fungi that cause fermentation and spoilage (Gleiter, Corno, & Isengard, 2006). All of stingless bees honeys are in accordance with Decree 30860 of the state of Rio Grande do Norte (Brasil, 2021), which establishes a maximum of 40%.

Free acidity

The free acidity of honey samples ranged from 30.1 to 114.2 mEq kg^{-1} (Table2). Mean values of free acidity of honeys from *A. mellifera* and *M. subnitida* were less than 50 mEq kg^{-1},therefore within the maximum limits for *Apis* honey (FAO, 2019) and *Meliponini* honey from Decree 30860 of the state of Rio Grande do Norte (Brasil, 2021). In their review, Silvaet al. (2016) argued that*Apis*honeys from different continents have acidity ranging from 3.86 to 45.5 mEq kg^{-1}. The free acidity of honeys from *M. mandacaia*, *Plebeia* sp. and *F. varia*was greater than 70 mEq kg^{-1}. Chuttonget al. (2016) analyzed 28 honey samples from different species of *Meliponini* from Thailand, and the total acidity varied between 25 and 592 mEq kg^{-1}.

Hydroxymethylfurfural

The hydroxymethylfurfural (HMF) content of honey samples ranged from 12.0 to 57.6 mg kg^{-1}, with lower values for*Meliponini* (Table 2). For tropical or arid regions, values detected in this study met the standard (<80 mg kg^{-1}) set by the FAO (2019) and Brazilian standard (<60 mg kg^{-1}) (Brasil, 2000). On the other hand, according to Decree 30860 of the state of Rio Grande do Norte for stingless bee honey (Brasil, 2021), only *Plebeia* sp. and *F. varia* honeys met the standard (<20 mg kg^{-1}). The HMF content is a parameter used to evaluate the quality of honey, which can be influenced by storage conditions, pH and floral origin (Salazar, Freitas, Luz, Bersch, & Salazar, 2017). In honeys from aridregions, HMF (0.16 to 80.13 mg kg^{-1}) was higher than in non-arid region (0.91 to 37.44 mg kg^{-1}) (Habib et al., 2014).

Sugars

Reducing sugars content ranged from 42.0 to 72.8% according to the species of bee studied (Table 2). Mean valuesof reducing sugars in honeys from *A. mellifera*, *M. subnitida* and *M. mandacaia* were above 60%, within the minimum limits for honey by the FAO (2019) and Decree 30860 of the state of Rio Grande do Norte (Brasil, 2021). Unlike the *Plebeia*sp. honey (42.0 ± 0.6%), *F. varia*honey (51.1 ± 1.0%) had reducing sugar content within the

minimum (50%) established for *Meliponini*honeys from South America (Vit, Medina, & Enriquez, 2004). In honeys from 11 species of *Meliponini* from Thailand, Chuttong et al. (2016) observed lower values (29.0 ± 8.2 g 100 g^{-1}) than those observed herein. According to Gleiteret al. (2006), the reducing sugars ratio (fructose/glucose) influences free water content present in honey, and the highest fructose/glucose ratio favors the increase in water activity. On the other hand, sucrose concentration ranged from 1.4 to 6.5% depending on the species of bee (Table 2). The honey bee *M.subnitida*contained sucrose content (6.5 ± 3.4%) higher than 5%, therefore, above the maximum value established for floral honey (FAO, 2019) and Meliponini's honey (6%) in South America (Vit et al., 2004) and Decree 30860 of the state of Rio Grande do Norte (Brasil, 2021). In general, composition of honey sugars is influenced by floral type, climatic conditions and geographical regions (Torres et al., 2020).

Ash content

Ash content of the samples analyzed ranged from 0.23 to 0.63% (Table 2). The Codex Alimentarius does not use this feature to evaluate quality of floral honey, however, in South America, the maximum value established for Meliponini honey is 0.5% (Vit et al., 2004) and for stingless bee honey is 0.6% by Decree 30860 of the state of Rio Grande do Norte (Brasil, 2021). Honeys from *M. mandacaia* and *F. varia* showed levels over 0.5%. Ash content is a measure of quality that evaluates the mineral content present in honey. The mineral content may be asign of environmental pollution and geographical origin, because the content depends on the type of soil used for flowers from which the nectar was collected (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). Thus, Santos et al. (2014) detected in Apis honey from different blooms in the Caatinga region of Brazil, ash content between 0.02 and 0.19%.

Electrical conductivity

The electrical conductivity of honey samples ranged between 469 and 2445.0 µS cm^{-1} (Table 2). Mean valuesof electrical conductivity of *A. mellifera* and *M. subnitida*honeyswere lower than 800 µS cm^{-1}, therefore within the maximum limits established by FAO (2019) for floral honey. For the otherhoneys from*Meliponini*, the electrical conductivity was higher than set forth by the international standard. Mean electrical conductivity values of *Meliponini* honey from Thailand were 1100 ± 78 µS cm^{-1} (Chuttong et al., 2016), but for *A. mellifera* L and *M. subnitida*honeys from the Brazilian Caatinga, EC values were 469.7 and 77.49 µS cm^{-1}, respectively (Tôrres et al., 2021).

Total phenolics, flavonoids and antioxidant capacity

Total phenolics, flavonoids and antioxidant capacity (IC_{50}) of honeys from Meliponini and Apiscan be seen in Figure 1.

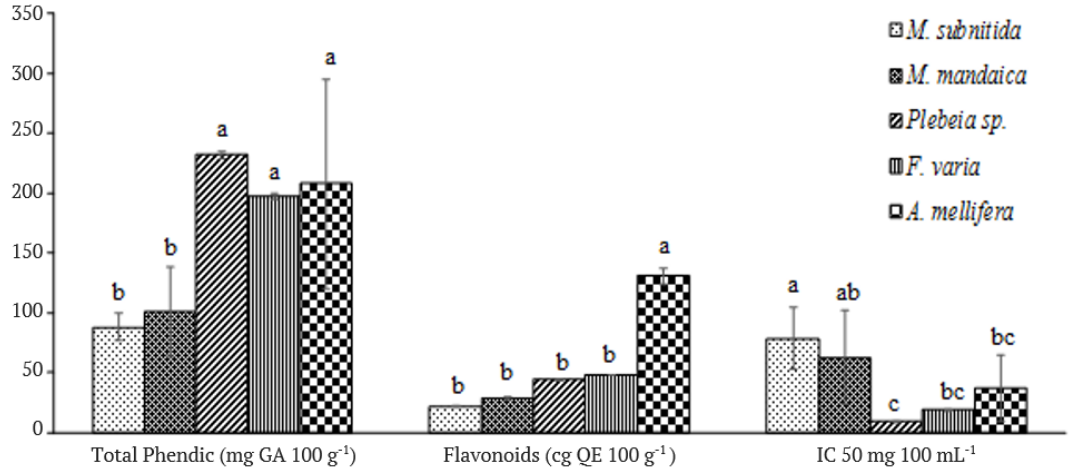

Figure 1. Total phenolics, flavonoids and antioxidant capacity (IC_{50}) of honey samples from Meliponini and Apis. Vertical bars indicate standard deviation of the means.

Total phenolics from the studied honeys ranged from 88.3 to 231.6 mg GA 100 g^{-1} (Figure 1). For *M. subnitida*and *M.mandacaia,* values ranged from 78.7 to 231.6 mg GA 100 g^{-1}, respectively. While for honeys from *A. mellifera*, *Plebeia* sp. and *F. varia*,valuesranged from 197.7 to 231.6 mg GA 100 g^{-1}. Can et al. (2015) found total phenolic

content from 98.26 to 105.46 mg GA 100 g^{-1} in chesnut and heather honey, respectively. The total phenolic content in honey is related to the honey floral source, because phenolic compounds are related to the botanical origin of nectar, pollen, and to the species of honey-producing bee (Sousa et al., 2016).

The flavonoid content of honeys from *Meliponini* ranged from 2.2 to 4.8 mg QE 100 g^{-1} (Figure 1) and were lower than found for honey from *Apis* (13.1 ± 6.6). It was observed that flavonoid content was low and thus contributed little to the total content of phenolics. According to Gheldof, Wang, and Engeseth (2002), in honey, the antioxidant capacity is the result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, Maillard reaction products and possibly other minor components. Sousa et al. (2016) verified a flavonoid content from 1.8 to 4.8 mg QE 100 g^{-1} in honeys produced in the Brazilian semiarid. Meda et al. (2005) reported a variation in flavonoid content between 0.17 and 8.35 mg QE 100 g^{-1} in *A. mellifera* honey from South Africa.

Environmental and climatic conditions where plants grow define their metabolism and nectar composition. In this way, sun-exposed plants may contain much more total phenolics than the same varieties or others when grown in the shade (Tenore, Ritieni, Campiglia, & Novellino, 2012).

Results of DPPH antioxidant activity for different honeys are illustrated in Figure 1. The IC_{50} value ranged between 9.5 and 78.7 mg mL^{-1}. The lowest IC_{50} was found in honey from *Plebeia* sp. and the highest, from *M. subnitida*. The scavenging activity of the DPPH free radical, expressed in terms of IC_{50},indicates the minimum concentration for the antioxidant to reduce the initial DPPH concentration by 50%, in other words, the lower the IC_{50} value, the greater antioxidant power of the substance present in the sample (Meda et al., 2005).

Honeys from *Plebeia* sp. and *F. varia* showed higher antioxidant capacity (Table 3).

Table 3. Antioxidant capacity of Meliponini and *Apis mellifera*' honey samples.

Properties	*M. subnitida* (n=18)	*M. mandacaia* (n=9)	*Plebeia* sp. (n=3)	*F. varia* (n=3)	*A. mellifera* (n=12)
Antioxidant content (mg EQ 100 g^{-1})	6.2±2.0^{c}	9.1±4.5^{bc}	14.5±1.2^{b}	24.5±0.5^{a}	10.7±4.6^{b}
Antioxidant content (mg EAA 100 g^{-1})	9.5±3.0^{c}	13.9±6.8^{bc}	22.2±1.9^{b}	37.5±0.8^{a}	16.3±7.1^{b}

Values are presented as mean ± standard deviation. $^{a-c}$Means followed by different letters, in the same row, are significantly different by Tukey's test at 5% probability.

Sousa et al. (2016) found that honey from *Melipona subnitida* Ducke (jandaira) of jujube and white canopy flowering showed a higher inhibition when compared to the same flowering in *M. scrutellaris* Latrelle (uruçu). In honeys from *Apis* analyzed in South Africa, values of IC_{50} ranged from 1.63 to 29.13 mg mL^{-1} (Medaet al., 2005).

The antioxidant capacity measured in quercetin was much lower than measured in ascorbic acid (Table 3), similar to that reported for *Meliponini* honey by Sousa et al. (2016) and *Apis* honey by Meda et al. (2005), with a ratio of antioxidant content in mg ascorbic acid and 1.53 mg quercetin samples for all honeys.

Conclusion

The physicochemical profile of honeys from stingless bees and Apis from the Brazilian semiarid region differed in one or more quality characteristic. Regardless of the honeybee, some quality characteristics of honey differed from international standards. *M. subnitida* honey presented higher moisture content than *A. mellifera* L. honey, but *F. varia* honey presented moisture, acidity, reductors sugars, ash and EC intermediate. Honeys from stingless bees showed significant differences in moisture, acidity, reducing sugars, sucrose, ash and EC according to the Melliponini species. Honey with the highest phenolics content had high antioxidant capacity. Honeys from*Plebeia* sp., *F. varia* and *A. mellifera* L. showed the highest antioxidant capacity.

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