



# Quality of *Apis mellifera* honey after being used in the feeding of jandaira stingless bees (*Melipona subnitida*)

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**ABSTRACT.** The aim of this study was to evaluate the physicochemical quality and bioactive compounds of *Apis mellifera* honey as well as the alterations in the quality of *A. mellifera* honey after being used in the feeding of *Melipona subnitida* colonies. *A. mellifera* honeys were collected in apiaries, homogenised and used as feed for *M. subnitida* bees for 30 days. Every five days, honey samples were collected and evaluated for physicochemical characteristics and bioactive compounds. The treatments consisted of natural honeys of *A. mellifera* and *M. subnitida* and honey of *M. subnitida* bee after being fed with *A. mellifera* honey (modified honey). *M. subnitida* bees, when fed with honey from *A. mellifera*, modified some of its characteristics, such as moisture, reducing sugars, diastase activity, colour and flavonoid content. Natural and modified honeys of *A. mellifera* were similar to each other and different from *M. subnitida* honey in terms of minerals, free acidity, electrical conductivity, phenolic content and antioxidant activity. Treatments were similar in terms of sucrose, insoluble matter, hydroxymethylfurfural and water activity. In general, the quality attributes of the modified honey were closer to the honey of *A. mellifera* than to the natural *M. subnitida* honey.

**Keywords:** antioxidant activity; bioactive compounds; meliponiculture; physicochemical characteristics; quality control; semi-arid climate.

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## Introduction

Honey is a sweet, viscous and aromatic product, synthesised by bees after their feeding with floral nectar or other parts of plants and excretions of sucking insects, that bees collect and store in honeycombs for maturation (Belay et al., 2017; Aljohar et al., 2018; Khan et al., 2018). Brazil stands out as one of the world's largest producers of honey (Requier et al., 2018). Most of Brazilian honey production is made by European honey bees (*Apis mellifera*) (with sting). However, on a small scale, meliponines are also responsible for honey production in Brazil, which include species of the genera *Melipona*, *Trigona* and *Tetragona*, also known as melipona, indigenous or stingless bees (Alves, Carvalho, Souza, Sodr e, & Marchini, 2005; Oliveira et al., 2012; Pedro, 2014; Jaff e et al., 2015).

Despite the traditional and low-tech breeding system and lower productivity in relation to other types of honey, melipona bees' honey has a high price, mainly due to its high medicinal value (Vit, Medina, & Enr iquez, 2004; Jaff e et al., 2015; Alvarez-Suarez et al., 2018). In addition, the creation and economic exploitation of stingless bees, called meliponiculture, is a highly sustainable activity that contributes to the conservation of bees and to the preservation of native plants (Vale, Gomes, Santos, & Ferreira, 2018).

Honey production is highly dependent on plant flowering, because the nectar and the pollen of the flowers provide energy and protein, respectively, which are required to feed the bees and their offspring. However, in some seasons there is not enough food in nature, either due to destruction of native forests or due to environmental conditions that disadvantage the flowering, such as water scarcity. With this, the use of maintenance artificial feeding becomes essential, in order to guarantee the expansion of the honey production due to the increase in oviposition and in the population of the colonies.

In order to evaluate honey quality, several physicochemical analyses are performed, which can be used as tools to understand honey synthesis as a result of supplementary feeding (Solayman et al., 2016). Thus, it has been determined that melipona bees fed with *A. mellifera* honey can bring forth honeys with physicochemical characteristics distinct from *A. mellifera* honeys.

The objective of this study was to evaluate the physicochemical quality and bioactive compounds of *A. mellifera* honey collected in apiaries in the midwestern region of Rio Grande do Norte state, in Brazil, and to evaluate the changes in the quality attributes of the *A. mellifera* honey after being used in melipona bee (*M. subnitida*) feeding.

## Material and methods

### Sample collection and treatments

Firstly, *A. mellifera* honey samples of 500 g each were collected in 12 apiaries in the State of Rio Grande do Norte, Brazil, and transported to the Food Engineering Laboratory of Federal Rural University of the Semi-arid Region. All 12 samples were combined and homogenised together. A part of the samples was taken for the quality analyses, whilst the other part was used for the feeding of *M. subnitida* bees. The artificial feeding of bees *M. subnitida* was carried out with *A. mellifera* honey, in a meliponary in Mossoró, Rio Grande do Norte state (05° 12' 13" S and 37° 19' 44" W).

The experiment was conducted in two years (2016 and 2017), using a randomised complete block design, with three replicates, each one corresponding to one bee colony. The treatments consisted of (1) natural *M. subnitida* honey, without artificial supply of *A. mellifera* honey; (2) modified *A. mellifera* honey, that is, honey from *M. subnitida* bees fed exclusively with *A. mellifera* honey; and (3) natural *A. mellifera* honey previously collected.

For *M. subnitida* colonies (treatments 1 and 2), pollen and wax were placed for construction of new honey storage pots by the bees. Five days later, the food induction of treatment 2 was performed, placing in each colony a plastic pot of *A. mellifera* honey. In treatment 1, bees were fed only by forage (nectar and pollen from blooming wild plants). Five days after feeding, the collections were started, performed within this interval up to 30 days. On the day of collection, food was replenished for treatment 2. This interval of five days was chosen according to the amount of time required for complete consumption of the quantity of food provided at each feeding by the *M. subnitida* bee, defined in a preliminary feeding test. After collection, the honey samples were transported to the laboratory for analysis. All samples were evaluated in duplicate.

### Determination of physicochemical attributes

#### Moisture

Moisture of the honey samples was measured by the refractometric method, according to the method of the International Honey Commission (Bogdanov, 2009). The results were expressed in % (w w<sup>-1</sup>).

#### Reducing sugars and sucrose content

Reducing sugars (RS) were determined by the Lane–Eynon method, involving the reduction of Fehling's solution by titration at the boiling point against a diluted honey solution (1%, w v<sup>-1</sup>) with methylene blue (0.2%) as an internal indicator (Bogdanov, 2009). The results were expressed in % (w v<sup>-1</sup>).

Sucrose content was determined after inversion of honey solution (1%) with hydrochloric acid, water-bath heating at 80°C and neutralisation with sodium hydroxide. Results were expressed in % (w v<sup>-1</sup>).

#### Insoluble matter

The insoluble matter content was determined by gravimetry, which is based on the insolubility of wax, pollen grains and other components of honey sediment (Codex Alimentarius, 2001).

#### Mineral content

Mineral content (ash) was determined by the calcination method in a muffle at 550°C until reaching a constant weight, according to the method no. 942.05 of Association of Official Analytical Chemists (AOAC, 2012).

#### Free acidity

Free acidity of honey was determined by the titrimetric method no. 962.19 of AOAC (2012). The neutralisation of the honey solution was carried out using sodium hydroxide solution, with values expressed in meq kg<sup>-1</sup>.

### Diastase activity

Diastase activity was determined by the Schade procedure (Schade, Marsh, & Eckert, 1958), based on the principle that the enzyme in the honey samples under standard conditions acts upon a standard starch solution and is capable of developing, with iodine, a colour in a defined range of intensity (Sajid, Yasmin, Asad, & Qamer, 2019). This colouration was measured spectrophotometrically at 660 nm at defined time intervals and the results were expressed in Goethe units (Sakač & Sak-Bosnar, 2012).

### Hydroxymethylfurfural

The hydroxymethylfurfural (HMF) content was determined after clarifying samples with Carrez reagents and adding sodium bisulphate, according to method no. 980.23 of AOAC (2012). The absorbance was measured spectrophotometrically at 284 and 336 nm, and results were expressed in mg kg<sup>-1</sup>.

### Water activity, electrical conductivity and colour

Water activity ( $A_w$ ) was measured using an ITK Wuxi Hake Apparatus (model HD-3A) previously calibrated with a sodium chloride solution. The measurement was made in a honey sample of 7.5 g, and results were expressed on a scale of 0–1.

Electrical conductivity was measured with a Tecnopon conductometer (model mCA 150). Results were expressed in  $\mu\text{S cm}^{-1}$ .

The colour of the honey samples was determined by spectrophotometry and expressed on the Pfund scale, which ranks the honey in seven colours, ranging from white to dark amber.

### Determination of bioactive compounds and antioxidant activity

Bioactive compounds were evaluated according to the methodologies described by Meda, Lamien, Romito, Millogo and Nacoulma (2005): total phenolic content, with results expressed in mg of gallic acid equivalents (GAE) per 100 g of honey; total flavonoid content, with the flavonoid content expressed as mg of quercetin equivalents (QE) per 100 g of honey; and antioxidant activity of honey samples, tested against the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical, with results expressed as IC<sub>50</sub>, which corresponds to the antioxidant concentration required to scavenge DPPH free-radical by 50%.

Since the data obtained for all variables were consistent over the two-year period, only the average values of all harvests were subjected to the analysis of variance, and the averages of treatments were compared by Tukey's test, at 5% probability. Also, values of physicochemical analysis were compared with those indicated in Brazilian (Instrução Normativa n. 11, 2000) and international (Codex Alimentarius, 2001) legislations for quality control of *A. mellifera* honey. For *M. subnitida* honeys, however, there is no legislation for quality identification. Therefore, their physicochemical attributes were compared with parameters proposed in Brazil for stingless bee' honey, suggested by Villas-Bôas and Malaspina (2005) and Camargo, Oliveira and Berto (2017), and for melipona bees' honeys in Guatemala, Mexico and Venezuela (Vit et al., 2004) (Table 1).

**Table 1.** Physicochemical analyses of honeys and limits of their results established by Brazilian and international legislations for *A. mellifera* honey and by two proposals of regulation for stingless bees' honey.

Physicochemical parameter	<i>A. mellifera</i> honey		Stingless bees honey		
	Brazilian legislation <sup>1</sup>	International legislation <sup>2</sup>	Regulation proposal A <sup>3</sup>	Regulation proposal B <sup>4</sup>	Regulation proposal C <sup>5</sup>
Moisture (max. %)	20	20	35	40	30
HMF (max. mg kg <sup>-1</sup> )	60	80	40	20	40
RS (min. %)	65	60	50	60	50
Sucrose (max. %)	6	5	6	6	6
Minerals (max. %)	0.6	-	0.6	0.6	0.6
IM (max. %)	0.1	0.1	0.4	0.1	-
FA (max. meq kg <sup>-1</sup> )	50	50	85	50	70
DA (min. GS)	8 or 3*	3	3	-	3
EC (max. $\mu\text{S cm}^{-1}$ )	-	800	-	-	-
$A_w$	-	-	-	0.52-0.80	-

HMF: Hydroxymethylfurfural; RS: Reducing sugars; IM: Insoluble matter; FA: Free acidity; DA: Diastase activity; GS: Goethe scale; EC: Electrical conductivity. \*8 if HMF is higher than 15 mg kg<sup>-1</sup> or 3 if HMF is less than or equal to 15 mg kg<sup>-1</sup>.

Source: <sup>1</sup>Instrução Normativa n. 11 (2000); <sup>2</sup>Codex Alimentarius (2001) <sup>3</sup>Villas-Bôas and Malaspina (2005); <sup>4</sup>Camargo et al. (2017); <sup>5</sup>Vit et al. (2004).

## Results and discussion

Honey moisture content statistically differed ( $p < 0.05$ ) among the treatments, with the highest moisture in *M. subnitida* honey (26.11%), lowest in natural *A. mellifera* honey (17.59%) and intermediate moisture in modified *A. mellifera* honey (22.89%) (Table 2). This effect is to be expected because *Apis mellifera* honey is denser than that of melipona, so when ingested by stingless bees, it tends to become diluted. High moisture is unfavourable to the quality of *Apis mellifera* honey by increasing its fermentation and deterioration processes. In Brazil, Villas-Bôas and Malaspina (2005) suggest that moisture of stingless bee honey must be less than 35%. However, Vit et al. (2004) suggest that the moisture of honey from Guatemala, México and Venezuela may be up to 30%. The moisture content in samples of *A. mellifera* monofloral honey varied from 14.1 to 20.5% (El Sohaimy, Masry, & Shehata, 2015; Belay et al., 2017). However, Silva et al. (2013) noticed that *M. subnitida* honey contains more moisture (22.2 to 24.4%) when compared to that of *Apis mellifera*.

**Table 2.** Moisture, reducing sugars, sucrose, insoluble matter and mineral content of natural honey samples of *M. subnitida* and *A. mellifera* and modified honey of *A. mellifera*.

Honey	Moisture	RS	Sucrose	IM	Minerals
	%				
<i>M. subnitida</i> (natural)	26.11 a (24.00 - 31.00)	74.24 a (66.82 - 78.66)	3.12 a (0.33 - 10.55)	0.11 a (0.01 - 0.42)	0.02 b (0.00 - 0.05)
<i>A. mellifera</i> (modified)	22.89 b (20.00 - 28.00)	60.57 b (37.39 - 66.22)	7.54 a (1.12 - 35.26)	0.20 a (0.01 - 0.50)	0.13 a (0.00 - 0.22)
<i>A. mellifera</i> (natural)	17.59 c (16.50 - 18.40)	73.41 a (69.39 - 80.90)	3.66 a (0.19 - 9.40)	0.16 a (0.01 - 0.85)	0.22 a (0.01 - 0.45)

RS: Reducing sugars; IM: Insoluble matter. Minimum and maximum sample values are shown in parentheses. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Modified *A. mellifera* honey had statistically lower ( $p < 0.05$ ) reducing sugar (RS) content (60.57%) than natural *A. mellifera* (73.41%) and *M. subnitida* (74.24%) honeys, whilst these two were statistically similar ( $p > 0.05$ ) (Table 2). Thus, it is found that melipona bees may have reduced the RS content of the *A. mellifera* honey. The RS content is a physicochemical parameter that evaluates the maturity of honey, which has a minimum content of 65% recommended by Brazilian legislation and 60% by international legislation for *A. mellifera* honeys. For stingless bees' honeys, a minimum content of 60% is indicated by Camargo et al. (2017) and 50% by Villas-Bôas and Malaspina (2005). Vit et al. (2004) also recommend a minimum 50% value of RS for stingless bees' honeys. Generally, stingless bee' honeys have lower RS contents when compared to *A. mellifera* honey (Alves et al., 2005). According to Khan et al. (2018), the average concentration of RS of *A. mellifera* honeys is 76.6%. RS values ranged between 50.5 and 75.5% in melipona honeys (Souza, Marchine, Oda-Souza, Carvalho, & Alves, 2009; Silva et al., 2013). Values of RS below 60% may be indicative of unripened *Melipona* honey.

Sucrose content percentage did not differ between the treatments ( $p > 0.05$ ) (Table 2). Modified *A. mellifera* honey (7.54%) was the only one that presented values outside the 6.0% maximum established by national legislation and 5.0% by international legislation. The high content of sucrose may result from a premature harvest, when sucrose in honey has not yet been fully converted into glucose and fructose by invertase enzyme, which is secreted by bees (Singh & Singh, 2018). Besides that, high sucrose contents can indicate honey adulteration by the addition of sucrose syrups (Aljohar et al., 2018). Natural honeys of *M. subnitida* (3.12%) and *A. mellifera* (3.66%) met standard requirements for sucrose content. Thus, when used to feed melipona bees, *A. mellifera* honey may have been added to the nectar from forage plants. Due to the short time between bees feeding and honey harvesting, the action of invertase enzyme may have been insufficient for total sucrose hydrolysis, causing it to be elevated in the modified honey. This effect may have also occurred in some samples of natural *M. subnitida* honey, however in smaller proportions (Mendes, Silva, Mesquita, & Maracajá, 2009). In *A. mellifera* honey samples evaluated by El Sohaimy et al. (2015), sucrose contents ranged from 1.34 to 3.59%. Souza et al. (2009) observed a variation of sucrose from 0.2 to 9.0% in melipona bee honeys in the state of Bahia.

There was no significant difference in the insoluble matter (IM) content among the three types of honey evaluated ( $p > 0.05$ ) (Table 2). For this physicochemical characteristic, natural (0.16% IM) and modified (0.20% IM) *A. mellifera* honeys are outside the standards established by national and international legislation, whose maximum IM content limit is 0.10%. Meanwhile, honey from *M. subnitida* (0.11% IM) meets standard

requirements suggested by Villas-Bôas and Malapsino (2005) (maximum of 0.40% IM), but not by Camargo et al. (2017), which indicate a maximum IM value of 0.10%. IM content of honey is indicative of impurities present, such as bees' body parts (legs and wings), wax, dust and wood chips, which are undesirable (Rogers, Somerton, Rogers, & Cox, 2010). When evaluating 58 samples of *A. mellifera* honeys, Al-Farsi et al. (2018) found values between 0.01 and 0.337% for IM. For *M. subnitida* honey produced in the western region of Rio Grande do Norte state, Aroucha et al. (2019) reported an average IM value of 1.05%.

Regarding the mineral content, there were no differences between natural (0.22%) and modified (0.13%) *A. mellifera* honeys and *M. subnitida* honey (0.02%) ( $p > 0.05$ ) (Table 2). All honeys comply with Brazilian legislation for *A. mellifera* honey and three proposals of regulation for stingless bee honey, which require mineral contents below 0.60%. Mineral content of honey is associated with the mineral nutrition of the soil, the environmental conditions and the botanical and geographical origin of the vegetables that the bees visited in search of nectar (Souza et al., 2009; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). In eight *A. mellifera* honey samples from Morocco, El-Haskoury, Kriaa, Lyoussi and Makni (2018) found a variation from 0.13 to 0.69% in their mineral content.

Free acidity did not differ between the different types of honey ( $p > 0.05$ ) (Table 3). Both natural (47.47 meq kg<sup>-1</sup>) and modified (41.01 meq kg<sup>-1</sup>) *A. mellifera* honey and *M. subnitida* honey (22.88 meq kg<sup>-1</sup>) were within the maximum limit of 50 meq kg<sup>-1</sup> established by Brazilian and international legislation and legislative proposal for stingless bees' honey by Camargo et al. (2017). Silva et al. (2013) found variations between 24.66 and 58.33 meq kg<sup>-1</sup> in nine *M. subnitida* honey samples. Free acidity is an important parameter for evaluation of honey quality, whose high values indicate possible deterioration of honey and the fermentation of sugars into organic acids (Silva, Gauche, Gonzaga, Costa, & Fett, 2016).

**Table 3.** Free acidity, diastase activity, hydroxymethylfurfural, electrical conductivity, water activity and pH of natural honey samples of *M. subnitida* and *A. mellifera* and modified honey of *A. mellifera*.

Honey	Free acidity (meq kg <sup>-1</sup> )	DA (Un. EG)	HMF (mg kg <sup>-1</sup> )	EC (μS cm <sup>-1</sup> )	A <sub>w</sub>
<i>M. subnitida</i> (natural)	22.88 b (15.84 - 39.60)	10.44 a (0.01 - 38.67)	11.05 a (0.15 - 39.22)	77.49 b (64.48 - 115.20)	0.53 a (0.41 - 0.61)
<i>A. mellifera</i> (modified)	41.01 a (31.68 - 50.29)	6.48 b (0.01 - 13.58)	27.49 a (0.73 - 83.23)	511.90 a (206.80 - 591.50)	0.54 a (0.42 - 0.57)
<i>A. mellifera</i> (natural)	47.47 a (33.16 - 66.30)	15.05 a (9.29 - 21.94)	26.92 a (4.12 - 72.50)	469.7 a (291.70 - 579.00)	0.63 a (0.39 - 0.80)

DA: Diastase activity; HMF: Hydroxymethylfurfural; EC: Electrical conductivity; A<sub>w</sub>: Water activity. Minimum and maximum sample values are shown in parentheses. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Natural *A. mellifera* (15.05 Goethe units) and *M. subnitida* (10.44 DN) honeys presented similar diastase activity ( $p > 0.05$ ), which is larger than that of modified *A. mellifera* honey (6.48 Goethe units) ( $p < 0.05$ ) (Table 3). Thus, modified honey would not be approved by Brazilian legislation in this parameter, in view of the minimum requirement of 8 Goethe units. In contrast, international legislation is less stringent for this parameter, requiring a minimum value of 3 Goethe units, a value reached by all honeys. Villas-Bôas and Malaspina (2005) suggest for Brazilian stingless bees' honey the same minimum diastase activity of 3 Goethe units, the same that Vit et al. (2004) recommend for honey from Guatemala, Mexico and Venezuela.

The diastase enzyme, also known as  $\alpha$ -amylase, is responsible for starch hydrolysis, being related to the digestion of pollen (Silva et al., 2016). This enzyme presents a high degree of instability when subjected to high temperatures, being an important characteristic in the determination of honey freshness, since it indicates whether it was heated or adulterated (Nordin, Sainik, Chowdhury, Saim, & Idrus, 2018).

Differing from the high values of diastase activity observed in this study, several authors reported low or non-existent diastase activity in stingless bee honey (Souza et al., 2009; Chuttong, Chanbang, Sringarm, & Burgett, 2016). Therefore, low diastase activity in stingless bee honey can be an inherent characteristic of these species, and not an indicator of low quality. In *A. mellifera* honey samples from Pakistan, Sajid et al. (2019) detected values between 26.97 and 43.47 Goethe units. The use of diastase activity as an indicator of honey quality is questioned by White (1994), who points out great variation in the amount of diastase in freshly collected and unheated *A. mellifera* honey samples.

In relation to HMF content, there was no statistical difference between natural *A. mellifera* (26.92 mg kg<sup>-1</sup>) and *M. subnitida* (11.03 mg kg<sup>-1</sup>) honeys and modified *A. mellifera* honey (27.49 mg kg<sup>-1</sup>) (Table 3). Thus, for this characteristic, *M. subnitida* bee did not affect the *A. mellifera* honey. All honeys comply with

Brazilian (maximum value of 60 mg kg<sup>-1</sup>) and international (maximum value of 80 mg kg<sup>-1</sup>) legislations. For stingless bee honey, Camargo et al. (2017) and Vit et al. (2004) propose maximum limits of 20 mg kg<sup>-1</sup>, whilst Villas-Bôas and Malaspina (2005) indicate a maximum HMF content of 40 mg kg<sup>-1</sup>.

HMF content in honey can be affected by several factors, such as acidity, water content and minerals, and its concentration is increased by inadequate storage conditions and excessive heat treatment, being indicative of honey deterioration (Önür et al., 2018). Al-Farsi et al. (2018) reported a large variation in the HMF levels of 58 samples of *A. mellifera* honey produced in Oman, ranging from 0 to 1062 mg kg<sup>-1</sup>. Finola, Lasagno and Marioli (2007) detected in *A. mellifera* honeys from Argentina HMF levels between 1.1 and 44.8 mg kg<sup>-1</sup>, values close to those detected in *A. mellifera* honey in the present study. In honeys produced by five species of the genus *Melipona*, Souza et al. (2009) found variation between 0.0 and 60.2 mg kg<sup>-1</sup> in HMF content.

Electrical conductivity (EC) of natural (469.7 µS cm<sup>-1</sup>) and modified (511.90 µS cm<sup>-1</sup>) *A. mellifera* honeys was similar ( $p > 0.05$ ), and both were larger than that of *M. subnitida* honey (77.49 µS cm<sup>-1</sup>) ( $p < 0.05$ ) (Table 3). This behaviour indicates that the characteristics of natural *A. mellifera* were preserved in the modified honey. Brazilian legislation does not present reference values for this characteristic, whilst the international legislation establishes the maximum value of 800 µS cm<sup>-1</sup>, a requirement met by all honeys. The EC of honey is usually positively correlated with its mineral content (Karabagias et al., 2014). Thus, the results found in our study correlate with those of Silva et al. (2016), who indicate that the higher mineral content, the higher the resulting conductivity.

Colour, mineral content and EC are related parameters, thus darker honeys usually have higher mineral content, which results in higher conductivity (Juan-Borrás, Domenech, Hellebrandova, & Escriche, 2014). When evaluating physicochemical characteristics of honeys produced in the state of Rio Grande do Norte, Aroucha et al. (2019) verified an average EC of 299.62 and 238.58 µS cm<sup>-1</sup> in *M. subnitida* and *A. mellifera* honeys, respectively.

For the water activity ( $A_w$ ), there was no significant difference between the honeys (Table 3).  $A_w$  values of *M. subnitida* honey and natural and modified *A. mellifera* honeys were, respectively, 0.53, 0.63 and 0.54. Both Brazilian and international legislations for *A. mellifera* honey and the proposals of legislation for stingless bee honey by Villas-Bôas and Malaspina (2005) and Vit et al. (2004) do not present critical values of  $A_w$  for their respective types of honey. In contrast, Camargo et al. (2017) suggest that this characteristic should be considered, and its value must be between 0.52 and 0.80. Thus, honey from *M. subnitida* is within the scope recommended in this regulation proposal for stingless bees.

$A_w$  evaluates honey deterioration, indicating its propensity for microbiological activity.  $A_w$  value is reduced proportionally to the increase in the sugar concentration of the honeys, since a great part of the water molecules is linked to the sugars. The  $A_w$  needed for the growth and development of microorganisms depends on their class, being around 0.8 for yeasts and 0.9 for bacteria (Ávila, Beux, Ribani, & Zambiasi, 2018).

The predominance of colours varied between natural *A. mellifera* honey (predominant light amber colour), modified *A. mellifera* honey (predominant dark amber colour) and natural *M. subnitida* honey (predominant white colour) (Table 4). The samples analysed are within the standards required by Brazilian legislation, which considers acceptable variations to be from water white to dark amber for *A. mellifera* honey, the same as recommended for stingless bee honey by Camargo et al. (2017).

**Table 4.** Colour, total phenolic content, total flavonoid content and antioxidant activity of natural honey samples of *M. subnitida* and *A. mellifera* and modified honey of *A. mellifera*.

Honey	Colour (Pfund scale)	TPC (mg GAE 100g <sup>-1</sup> )	TFC (mg QE 100g <sup>-1</sup> )	AA (IC <sub>50</sub> ) <sup>1</sup>
<i>M. subnitida</i> (natural)	67% W, 22% WW, 11% A	16.09 b (16.94 – 19.72)	1.16 b (0.58 – 1.65)	610.31 b (478.23 – 711.14)
<i>A. mellifera</i> (modified)	78% DA, 11% A, 11% LA	80.36 a (33.39 – 97.83)	3.29 a (1.44 – 9.71)	59.77 a (37.95 – 80.82)
<i>A. mellifera</i> (natural)	50% LA, 25% A, 25% DA	92.90 a (61.44 – 118.00)	1.62 b (0.21 – 3.12)	59.01 a (36.09 – 90.54)

TPC: Total phenolic content; TFC: Total flavonoid content; AA: Antioxidant activity; WW: Water White; W: White; LA: Light Amber; A: Amber; DA: Dark Amber. <sup>1</sup>Required antioxidant concentration to scavenge DPPH free-radical by 50%; expressed in mg mL<sup>-1</sup>. Minimum and maximum sample values are shown in parentheses. Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

Results showed that melipona bees modified the predominant colour of *A. mellifera* honey. Honey colour is an important parameter for consumers (Sajid et al., 2019), being related to several factors, such as mineral and flavonoid contents (Finola et al., 2007). The colour of honey may vary according to its floral origin, bee species, age and storage conditions (Aroucha et al., 2019), besides the beekeeper's interventions (El Sohaimy et al., 2015). Thus, the variation observed in the colour of *A. mellifera* honey from light amber to dark amber may be related to the origin of honey samples, which were collected at several sites with different flowerings. In 49 samples of *A. mellifera* honeys from Rio Grande do Sul state, Nascimento et al. (2018) reported a diversity of colour, from extra white to amber. In nine *M. subnitida* honeys evaluated by Silva et al. (2013), one had yellow-brownish colour, whilst the others had a bright yellow colour.

Total phenolic content of natural and modified *A. mellifera* honey was similar (92.90 and 80.36 mg GAE per 100 g, respectively) ( $p > 0.05$ ) and higher than *M. subnitida* honey (16.09 mg GAE per 100 g) ( $p < 0.05$ ) (Table 4). Thus was observed a beneficial effect of melipona bees feeding with *A. mellifera* honey, since this characteristic was maintained. Concentration and type of phenolic compounds depend on the floral origin of the honey and are the main factors responsible for its biological action, including its antioxidant, antimicrobial, antiviral and anticarcinogenic activity (Kuçuk et al., 2007). Estevinho, Pereira, Moreira, Dias and Pereira (2008) observed that the phenolic compound content of light honeys is lower than that of dark honeys, a characteristic also observed in this study.

Total flavonoids content of the modified *A. mellifera* honey was statistically higher (3.29 mg QE per 100 g) ( $p < 0.05$ ) than that of *A. mellifera* and *M. subnitida*, whose contents were statistically equal (1.62 and 1.16 mg QE per 100 g, respectively) ( $p > 0.05$ ) (Table 4). In addition to using *A. mellifera* honey as food, melipona bees also foraged for nectar, mixing the food served with collected nectar. Such behaviour may explain the high flavonoid increase in the modified honey (Decourtye, Mader, & Desneux, 2010). Flavonoids are a class of polyphenols that are present in plants and serve as self-defence. Its concentration in nectar and consequently in honey is influenced by floral origin and climatic characteristics of the honey producer region. Flavonoids are some of the compounds responsible for antioxidant activity of honey (Nayik & Nanda, 2016). Values of flavonoids found in this study are similar to those reported by Nascimento et al. (2018) in *A. mellifera* honeys (0–2.60 mg QE per 100 g). In *A. mellifera* and *Melipona beecheii* honeys from Cuba, Alvarez-Suarez et al. (2018) reported average values of 2.68 and 4.19 mg QE per 100 g for flavonoids content, respectively.

Antioxidant activity of natural and modified *A. mellifera* honeys was statistically equal ( $IC_{50} = 59.01$  and  $59.77$  mg mL<sup>-1</sup>, respectively) ( $p > 0.05$ ) and higher than that of *M. subnitida* honey ( $IC_{50} = 610.31$  mg mL<sup>-1</sup>) ( $p < 0.05$ ) (Table 4). In this study, the results were expressed in  $IC_{50}$ , which represents necessary antioxidant concentration to reduce the DPPH free-radical by 50%. Thus, low  $IC_{50}$  indicates high antioxidant activity of honey samples. In this way, a beneficial effect of stingless bee feeding with *A. mellifera* honey was observed, that of preserving its high antioxidant activity. Oliveira et al. (2012) found a significant strong positive correlation ( $r = 0.87$ ) between  $IC_{50}$  and total phenolic content, then honeys with higher total phenolic content and darker colouring have high antioxidant activity.

Similar results to those of this work were observed by Oliveira et al. (2012), in honeys from different Amazonian locations, in which  $IC_{50}$  values ranged from 8.87 to 41.76 mg mL<sup>-1</sup> for *A. mellifera* honeys. In contrast, the same authors observed high antioxidant activity in *M. subnitida* honey, with  $IC_{50}$  of 6.85 mg mL<sup>-1</sup>. Estevinho et al. (2008) verified higher antioxidant activity in dark honey than in light honey.

## Conclusion

When fed with *A. mellifera* honey, *M. subnitida* bee modified some physicochemical characteristics of this honey, such as moisture, reducing sugars, diastase activity and colour, in addition to the total flavonoid content. However, the final quality attributes of the modified honey were closer to the *A. mellifera* honey than to the natural *M. subnitida* honey.

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