

Physicochemical, Antioxidants and Sensorial Properties of *Melipona subnitida* Honey after Dehumidifying

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Abstract

Physical-chemical characteristics (moisture, water activity, electrical conductivity, pH, free acidity, reducing sugars, apparent sucrose, hydroxymethylfurfural, ash, insoluble solids and color), total phenolic content, flavonoids, antioxidant and sensorial properties (aroma, flavor, color, fluidity, acceptance and purchase intention) were evaluated in honey bee *Melipona subnitida* in natura and after dehumidification. The data were submitted to analysis of variance ($P < 0.01$), the means compared by the t-test ($p < 0.05$) and the sensorial analysis by the Friedman test ($p < 0.05$). Comparing the dehumidified honey with the in natura honey, differences in the physical and chemical characteristics of moisture, water activity, reducing sugars, sucrose, ash, insoluble solids in water and color were observed. However, there was no difference in antioxidant capacity, total phenolic content, flavonoids, flavor, color, acceptance, and intention to purchase the product. In the sensorial parameters of aroma and fluidity were evidenced alterations; however, the process of dehumidification of the honey did not impair its sensorial quality and still decreased the humidity favoring its greater conservation.

Keywords: Acceptance; Sugars; Phenolic compound

Introduction

Honey is a product of bee feeding with plant nectars, whose composition can be influenced by flowering, soil, climatic conditions, bee species [1], which reflects on different sensory characteristics. Among the main constituents of Apis honey, glucose and fructose (60%-80%), sucrose (up to 10%) and other constituents such as amino acids, organic acids, enzymes, minerals, aromatic substances, pigments and pollen grains [2].

Besides these compounds, honey is also a good source of functional compounds (phenolics, flavonoids, and other substances) that act in the combat or retardation of several types of diseases [3,4].

In 2013, world honey production was led by China, followed by Turkey and Argentina. Brazil occupied the 11th place in the world ranking, with a production of 35,600 tons (Food and Agriculture Organization), the Northeast being the second largest bee-producing region. In Brazil, bee honey production is mainly exploited by small rural producers who earn income and keep the family workforce employed.

Honey bee from the Meliponini group is produced on a small scale; this is due to the traditional breeding system [5]. Its higher price differential compensates for its lower productivity, due to the regional belief that it has higher therapeutic qualities. On the other hand, honey bee *Apis mellifera L.* has a larger annual production [6].

The honey of bees of the genera *Apis* and *Melipona* have different physicochemical and sensorial characteristics [5,7]. (While the honey of *Apis mellifera L.* moisture vary from 18% to 20.4% [8], meliponine honey vary from 17.33% to 35.4% [9]. This characteristic makes meliponine honey more susceptible to fermentation [10]. In this way, the use of some technology is necessary to prolong its conservation, such as maturation, pasteurization [11] and dehumidification [12]. However, these procedures may alter the sensorial characteristics of the product [13] and affect its commercialization.

Dehumidification researches of meliponine honey (*Tetragonisca angustula*, *Melipona scutellaris* *Melipona quadrifasciata*) evidenced

alterations in several physicochemical quality characteristics of the product [12,14]. The partial withdrawal of water from honey, in addition to providing better preservation of honey, also leads to the production of a new product, which can be classified as "partially dehumidified honey meliponin" with peculiar characteristics. However, it is important to evaluate the sensory characteristics, since in natura honey is well accepted by consumers.

It is known that honey Meliponini differs in several characteristics (physicochemical and sensorial) of bee honey of the genus *Apis* [15] and that the high humidity of these honey is a limiting factor for its life shelf, this work aimed to evaluate the physical-chemical, antioxidant and sensory properties of honey from *Melipona subnitida* bees after dehumidification.

Materials and Methods

The honey samples of the bee species *Apis mellifera L.* and *Melipona subnitida* were collected in the western region of the state of Rio Grande do Norte, located in the Western South hemisphere. Its extreme points are bounded by the parallels of 4°49'53" and 6°58'57" south latitude and the meridians 35°58'03" and 38°36'12" longitude west of Greenwich. The average annual temperature is 25.5°C, the rainfall goes from 400 to 600 mm/year with rainy season concentrating in February to May, and the relative humidity of the air presents an average annual variation of

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59% to 76%. The predominant vegetation in the state of Rio Grande do Norte is the caatinga that is composed of xerophilous plants that are mostly deciduous.

After collecting and packaging the honey in containers, they were transported to the Food Technology Laboratory of the Universidade Federal Rural do Semi-Árido (UFERSA), located in the Central Campus, in the city of Mossoró-RN, where they were stored under cooling at 6°C until analyzed.

Four honey samples were collected from each bee species, the *Melipona subnitida* honey sample was divided into two sub-samples with equal amounts and one sample was submitted to dehumidification treatment (*Melipona subnitida* honey) and the other subsamples consisted of the control sample (in natura). Honey samples from *Apis mellifera* L. were analyzed in natura for comparison.

The dehumidification process was carried out in *Melipona subnitida* honey at 40°C and 30% RH in forced air circulation oven TECNAL, model TE-394/1. With the aid of the Abbé refractometer, the moisture content of the honey was monitored before and during the dehumidification (up to 20% humidity acceptable by Brazilian legislation).

Physical-chemical analysis-moisture (%): determined with a refractometer of Abbé brand SAMMAR model RT-90ATC, pH: diluting 10 g of sample in 75 mL of distilled water, the reading made in pH meter; free acidity (mEq/kg): 10 g of honey in 75 mL of distilled water, then titrated with 0.05N sodium hydroxide in a flow rate of 5 mL per minute to pH 8.5; water activity: 7.5 mL of honey was used in the ITK Wuxi Hake Apparatus model HD-3A; reducing sugars (%) and apparent sucrose (%): determined according to National Animal Reference Laboratory of Agriculture Ministration in Brazil, using alkaline copper (Fehling) reagent to cuprous oxide. For sucrose, acid hydrolysis (HCl) was performed and determined quantitatively by the Lane and Eynon method; electrical conductivity ($\mu\text{S}/\text{cm}$): determined in a solution at 20% dry honey content, with Tecnopon model mCA 150 conductivity meter and analytical balance; ash: carried out by incineration of the sample at 550°C; hydroxymethylfurfural: determined, with 5 g of honey dissolved in 25 mL of distilled water and transferred to a volumetric flask (50 mL) and 0.5 mL of Carrez I solution (15 g of potassium ferrocyanide/100 mL of distilled water) and 0.5 mL of Carrez II solution (30 g of zinc acetate/100 mL of distilled water) were added and the volume was filled with distilled water. After filtration, 5 mL was transferred to test tubes and 5 mL of distilled water (sample solution) was added and another 5 mL of 0.2% sodium bisulfite solution (control) was added. The absorbance of the solutions was measured at 284 nm and 336 nm in a Gehaka model UV-340G spectrophotometer; water-insoluble solids (%): determined by gravimetry according to the method suggested by the Codex Alimentarius Commission; color: determined according to Vidal and Fregosi [16], using a Gehaka model UV-340G spectrophotometer, at 560 nm, using pure glycerine as white. The reading was subsequently transformed into color by the Pfund scale which ranks the color by wavelength: 0.030 nm (Water White), 0.030 nm to 0.060 nm (Extra white), 0.060 nm to 0.120 nm (White), 0.120 nm to 0.188 nm (Extra amber), 0.188 nm to 0.440 nm (Light amber), 0.440 nm to 0.945 nm (Amber) and greater 0.945 nm (Dark amber).

Antioxidant properties-total flavonoids: determined according to the methodology described by Meda et al., [4] and Ahn et al. [17], with adaptations. For this a solution of 2% aluminum chloride diluted with methanol was prepared. 5 mL of this was added to the same volume of honey solution (0.02 mg/mL). Absorbance reading was done on the Gehaka model UV-340G spectrophotometer at 415 nm, after 10 min

using methanol as white. The quercetin curve (5 to 50 mg/L) was used as the standard and the results expressed in mg QE/100 g; phenolics: determined according to Meda et al., [4] using Folin-Ciocalteu reagent. For this, 5 g of honey was diluted with 50 mL of distilled water. In the honey solution (0.1 g/mL) was added an aliquot of 0.5 mL and mixed with 2.5 mL with Folin-Ciocalteu reagent. After 5 min, 2.0 mL of sodium carbonate solution (75 g/L) was added and 2 hours after the absorbance was measured at 760 nm against a blank (methanol). Through the standard curve of gallic acid (20 to 200 mg/L) the result was expressed in mg GA/100 g; antioxidant capacity: evaluated by the use of 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH), according to Meda et al., [4] in whose presence of antioxidant the color of DPPH decreases and its absorbance is read spectrophotometrically. The DPPH radical scavenger activity was expressed as IC_{50} (minimum concentration for the antioxidant to reduce 50% of the initial DPPH concentration).

Sensory analysis: performed by 52 consumers of honey, unmanaged (students and employees of UFERSA), from 9:00 am to 11:00 am and from 3:00 p.m. to 5:00 p.m. The research was submitted to the Ethics Committee (number 761.221). For the analysis of the intensity of the attributes, an adapted plug of Grosso [18] was used, with a scale varying from 1 to 10, to which the melting, color, aroma and flavor of the honey were evaluated. In a room lit with red light were evaluated the aroma and flavor and, under white light, the color and fluidity. For the acceptance test, we used the structured hedonic scale of nine points ranging from 1 (I highly disagree) to 9 (I liked it very much). And for the intent to purchase, a five-point scale, ranging from 1 (certainly would not buy) to (5 would certainly buy).

Statistical analysis: data were submitted to analysis of variance ($p < 0.01$) using SISVAR software. The mean values of the physicochemical characteristics were analyzed by the t-test ($p < 0.05$) and the results of the sensorial analysis by the Friedman test ($p < 0.05$).

Results and Discussion

Dehumidification of *Melipona subnitida* honey altered its moisture, water activity, reducing sugars, apparent sucrose, color, ash and insoluble solids (Table 1).

The moisture content of the dehumidified samples decreased by 30%, with a value of 20% that approached the values found in *Apis* honey (maximum of 19%) (Table 1), so it was within the Brazilian honey legislation of *Apis* (Codex Alimentarius). Lower results were reported by Carvalho et al., [12] in *Meliponini* honey after dehumidification in the State of Bahia, whose humidity varied from 16% to 17%, starting from samples with humidity of 25.2% to 30.0%. In *Meliponini* honey in South America, there is a variation of 21.2% to 30.8% in moisture [19]. The high humidity of honey is a negative characteristic from the point of view of conservation; according to Villas-Bôas [13], the honey of *Melipona subnitida*, with humidity of 25% to 35%, has a shelf-life of 12 months in storage temperature of 2°C to 4°C.

The water activity (A_w) in *Melipona subnitida* in natura honey (0.77) was higher than the dehumidified honey (0.67) and this was very close to the values detected for *Apis* honey (0.65) implying a factor positive for the treatment since the decrease of A_w provides a lower risk of proliferation of osmophilic yeast [20]. A_w changes within the same genus *Meliponini*, from 0.662 to 0.851 [21].

The dehumidification of *Melipona subnitida* honey did not provide a significant difference in pH and free acidity compared to in natura honey (Table 1). In contrast to these results, dehumidification altered the concentration of the hydrogen ions present in the honey of two

species of *Melipona*, significantly increasing the acidity of the honey according to the origin [12].

The pH of *Melipona* honey was lower than that of *Apis* honey, a fact commonly observed in Meliponini honey [22]. The free acidity of *Apis* honey presented values lower than 50 mEq/ g, so it is within the maximum limits established for *Apis* honey (Codex Alimentarius). However, in *Meliponasubnitida* honey, with or without treatment, the free acidity was within the limit (≤ 70 mEq/kg) established for *Melipona* honey in South America [23]. Chuttong et al., [5] verified in honey of different Meliponini species of Thailand acidity ranging from 25 to 592 mEq/kg. The acidity of honey is associated with the presence of organic acids in equilibrium with the lactone, internal esters and some inorganic ions such as sulfates, phosphates and chlorates [24].

The sugars evaluated (reducers and apparent sucrose) differed with the treatment (Table 1). *Melipona subnitida* dehumidified honey presented content of reducing sugars (64.35%) and apparent sucrose (1.94%) higher than the values detected in natura honey (60.48% and 1.01%, respectively). Despite this, the reducing sugar content was still lower than that observed in the honey of *Apis mellifera L.* (71.15%), but within the limit established for sugars in floral honey by the Codex Alimentarius and Meliponini honey South America [23]. In honey of 11 bee species from the Meliponini group in Thailand, Chuttong et al., [5] observed values of reducing sugars of 29 ± 8.2 g/100 g and of sucrose of 1.2 ± 2.6 . In general, the composition of honey sugars is influenced by floral type, climatic conditions and geographic regions [25].

The electrical conductivity of the honey did not differ with the treatment (Table 1). The values varied from 149.29 to 350.55 $\mu\text{S}/\text{cm}$ in honey samples (*Apis mellifera L.* and *Melipona subnitida*) and therefore within the limits (<800 $\mu\text{S}/\text{cm}$) established for floral honey (Codex Alimentarius). In the literature, are reported electrical conductivity of 120 to 1100.0 $\mu\text{S}\cdot\text{cm}^{-1}$ for Meliponini honey [5,26] and 144.9 to 421.5 $\mu\text{S}\cdot\text{cm}^{-1}$ for honey of *Apis mellifera L.* of different floral predominance [27]. The electrical conductivity of honey is related to the ash content (mineral) and acidity, revealing the presence of ions, organic acids and protein [28].

The ash content of the *Melipona subnitida* honey subjected to dehumidification increased significantly (Table 1). The dehumidified honey had ash content similar to *Apis* honey (0.09%). Based on the values presented, honey is found within the maximum limit (0.6%) established for *Apis* honey and Meliponini [23]. The Codex Alimentarius does not use this characteristic to assess the quality of honey. This parameter is indicative of environmental pollution and geographic origin since it

depends on the characteristics of the soils that the plant has developed [29]. Santos et al., [27] found values for *Apis* honey from 0.02% to 0.19%, according to flowering.

There was no significant difference for the hydroxymethylfurfural (HMF) content of *Melipona subnitida* honey to the treatment (Table 1). The HMF values of the honey ranged from 8.24 to 94.03 mg/kg. For tropical or arid climates, the HMF should be ≤ 80 mg/kg (Codex Alimentarius). HMF is a parameter used to evaluate the honey quality and can be influenced by storage conditions, pH and floral origin [30]. It is formed during the acid hydrolysis of hexoses, from simple sugars such as glucose and fructose that are broken in the presence of gluconic acid and other acids of honey [31]. Habib et al. [32] found that honey from the arid region HMF (0.16 to 80.13 mg/kg) was superior to that of the non-arid region (0.91 to 37.44 mg/kg).

The insoluble solids of dehydrated *Melipona subnitida* honey (0.62%) were lower when compared to in natura honey (1.05%) of the same bee species (Table 1). In this parameter, the honey presented above the standard ($\leq 0.1\%$) established by Codex Alimentarius. Higher insoluble solids indicate poor collection, processing, filtration and/or decanting and may also be related to the low hygienic habit of bees in choosing the site for honeycomb deposition and honey storage [26].

The color of the honey differed with the treatment (Table 1). After dehumidification, the honey changed the classification, by the Pfund scale, from amber (in natura) to light (dehumidified) amber, and *Apis* honey was classified as dark amber. Sousa [7] working with honey from *Melipona subnitida* found that the color of the honey varied from white-water to dark amber. Color is an important quality parameter of honey because it influences its commercialization [30] and varies with the botanical origin, ash content, and storage temperature. The Codex Alimentarius establishes for the coloring of honey color variation from colorless to dark brown.

Dehydration of *Melipona subnitida* honey did not result in statistical differences in total phenolic content (76.66 to 92.36 mg AG/100 g), flavonoids (2.55 to 3.03 mg QE/100 g) and antioxidant capacity (141.86 to 168.60 mg/g) (Figure 1). The concentration and chemical structure of phenolics depend on the floral origin of honey and are the main factors responsible for the biological activity, including antioxidant, antimicrobial, antiviral and anticarcinogenic activity [3].

The honey of *Apis mellifera L.* presented total phenolic content (134.07 mg AG/100 g) and flavonoids (4.37 mg QE/100 g) higher than *Melipona subnitida* honey in natura. The lower IC_{50} in *Apis* honey

Table 1: Means of physicochemical characteristics of *Melipona subnitida* in natura honey and submitted to dehumidification and *Apis mellifera*.

Parameters	<i>Apis mellifera L.</i>	<i>Melipona subnitida</i>	
	In natura (n=4)	In natura (n=4)	Dehumidified (n=4)
Humidity (%)	18.0 \pm 0.91	26.0 \pm 0.47 ^a	20.0 \pm 0.0 ^b
pH	3,70 \pm 0.14	3.45 \pm 0.10 ^a	3.52 \pm 0.20 ^a
Free acidity (mEq/Kg)	35.58 \pm 9.65	66.18 \pm 34.00 ^a	64.64 \pm 38.00 ^a
Water activity	0.647 \pm 0.0	0.771 \pm 0.0 ^a	0.675 \pm 0.0 ^b
Reducing sugars (%)	71.15 \pm 2.17	60.48 \pm 2.54 ^b	64.35 \pm 0.75 ^a
Sucrose apparent (%)	1.76 \pm 0.52	1.01 \pm 0.18 ^b	1.94 \pm 0.43 ^a
Electric conductivity ($\mu\text{S}/\text{cm}$)	238.58 \pm 89.29	299.62 \pm 50.9 ^a	256.68 \pm 75.3 ^a
Ashes (%)	0.09 \pm 0.07	0.04 \pm 0.0 ^b	0.09 \pm 0.0 ^a
Hydroxymethylfurfural (mg/kg)	21.83 \pm 13.59	48.78 \pm 36.22 ^a	56.46 \pm 37.57 ^a
Insoluble solids (%)	0.44 \pm 0.59	1.05 \pm 0.34 ^a	0.62 \pm 0.14 ^b
Color (Pfund Scale)	2.57 \pm 0.25	0.60 \pm 0.10 ^a	0.27 \pm 0.15 ^b

The values in the table are averages (n=4) \pm standard deviation. Means followed by the same letter do not differ by the t test ($p < 0.05$) in the lines

expresses the superiority of this because the free radical scavenging activity DPPH expressed in terms of IC_{50} is the minimum concentration of DPPH, that is, the lower the value of IC_{50} , the higher the antioxidant capacity of the substances present in the samples [4]. The values found for phenolics and flavonoids are consistent with those reported for *Meliponini* honey by Sousa et al., [7] and *Apis mellifera* L. [4].

The dehumidification treatment of *Melipona subnitida* honey did not alter the aroma, taste, and color of the honey, but the fluidity was significantly altered (Figure 2). In spite of the slight increase in the aroma score of *Melipona subnitida* dehumidified honey, in relation to the honey in natura, both were classified as having an "unpleasant" aroma. Sodr e et al., [11] and Oliveira et al., [33] did not observe differences between the aroma of the dehumidified samples and the controls, for honey of Meliponine species. No honey sample had a grade below 5.0 (lower limit acceptable for aroma), so the samples were characterized in the range of "not pleasant" to "pleasant". The honey aroma varies with floral origin, climate, and soil and beekeeper management [34].

The taste did not differ with the dehumidification treatment; the honey had notes of "intense flavor". Also, Carvalho et al., [12] did not observe the effect of the dehumidification of meliponine kinds of honey from the State of Bahia in the honey flavor.

The dehumidification process of *Melipona subnitida* honey also did not give a difference in the color of the product in relation to the honey in natura. Sousa et al., [9] analyzing meliponine kinds of honey from Rio Grande do Norte observed a variation of white-water color to dark amber, with a predominance of light amber shade. And similar to this study, Santos et al., [35] verified predominance of the amber color in *Apis* honey.

Honey presents color, flavor, and aroma varying with flowering, as well as according to its geographical location. It is an important attribute to attract the consumer, with light-colored honey being the most preferred and with higher added value in the external market [30].

The fluidity attribute differed statistically between treatments (Figure 2). *Melipona subnitida* in natura melt was lower (3.5) than the

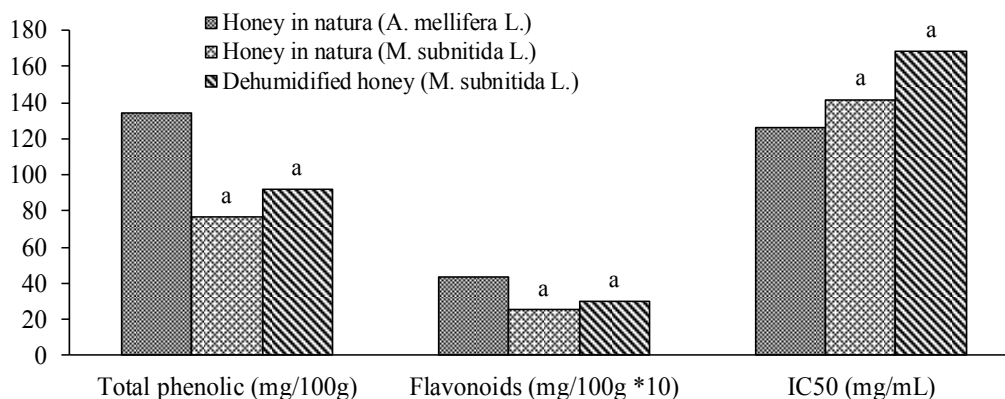


Figure 1: Averages of total phenolic content, flavonoids and antioxidant activity of *Melipona subnitida* honey (in natura and dehumidified) and *Apis mellifera* L. Equal letters do not differ by the t test ($p < 0.05$).

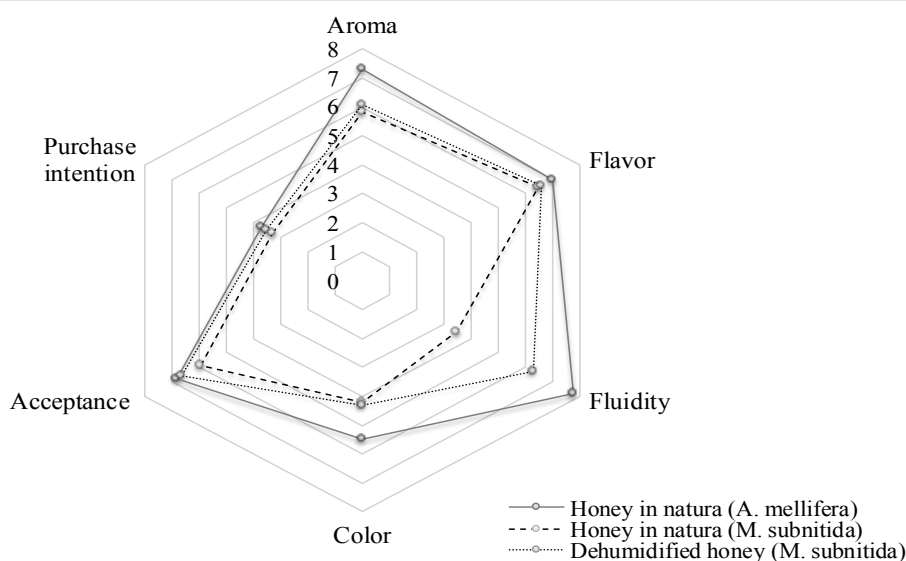


Figure 2: Sensory characteristics of *Melipona subnitida* honey (in natura and dehumidified) and *Apis mellifera* L.

Scores rated by 52 people. Aroma: 0-unpleasant; 10-pleasant. Flavor: 0-low; 10-intense. Fluidity: 0liquid; 10-very dense. Color: 0-white; 10-black. Acceptance: 1-I have greatly disliked it; 9-I liked it very much. Intent to buy: 1-willing to buy; 5-certainly would not buy.

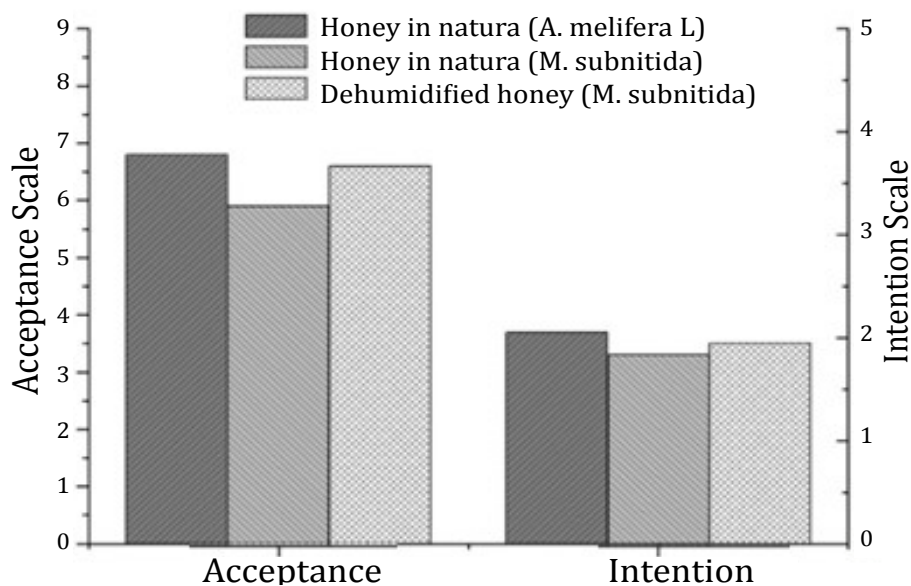


Figure 3: Acceptance and intention to buy *Melipona subnitida* honey (in natura and dehumidified) and *Apis mellifera L.* Scores evaluated by 52 people. Acceptance: 1-1 have greatly disliked it; 9-1 liked it very much. Intent to buy: 1-Willing to buy; 5-certainly would not buy.

dehumidified (6.3), and this was closer to the fluidity of the honey of *Apis mellifera L.* (7.8), whose classification was very dense. Sodr e et al., [11] and Oliveira et al., [33] observed the same behavior for meliponine dehumidified honey, with honey more fluid in natura and after dense dehumidification. The *Melipona subnitida* in natura honey is a striking feature of stingless bee honey, a factor justified by the high water content [14]. Santos et al., [27] evaluating the rheological behavior of honey from The *Melipona subnitida* in natura honey is a striking feature Rio Grande do Norte State, verified that the viscosity increases with the decrease of the moisture content, being influenced still by the temperature.

There were no statistical differences in the acceptance and intention to buy the honey with the dehumidification treatment (Figure 3). The average scores for acceptance were within the range of 5.9 to 6.8, with 6.0 being the "slightly liked" rating and the 7.0 "moderately liked" rating according to the nine-point hedonic scale. There was an increase in the note of the dehumidified honey, which was positive, since the average scores were above 5.0 (lower limit of acceptance-neither liked nor disliked), which allows affirming that, regardless of treatment, honey present characteristics suitable for commercialization.

The good acceptance of the honey by the tasters is related to the set of attributes of the same, conferring them peculiar characteristic [14]. In this way, dehumidification can be carried out without implications in the acceptance of them since they presented similar acceptance to the honey marketed in natura.

Dehumidification did not significantly influence the purchase intention of the honey (Figure 2). The average scores of *Melipona subnitida* in natura honey (3.3) increased in dehumidified honey (3.5) and was close to *Apis* honey (3.7), being in the range of "I doubt if I would buy" (grade=3.0) and "probably would buy" (grade=4.0), indicating a positive result since there was no rejection of the product ("probably would not buy" (grade=2) and "certainly would not buy" (grade=1)) by the tasters.

Sousa [9] verified in the honey of several species of Meliponineos that the intention of purchase of the tasters ranged from "certainly

would buy" to "certainly would not buy". Alves et al., [26] evaluated the purchase intentions for *Apis mellifera L.* honey from different flowering plants and observed that, depending on the blooming, the honey approved by the wine tasters were "certainly bought", and while some honey were disapproved with a note "they certainly would not buy."

Conclusion

The dehumidification process of honey gave rise to differences in the following physical and chemical characteristics: moisture, water activity, reducing sugars, sucrose, ash, insoluble solids in water and color.

Melipona subnitida dehumidified honey did not present differences in relation to honey in natura as total phenolics, flavonoids, antioxidant capacity, taste, color, acceptance and purchase intention of the product. However, in the sensorial attributes of aroma and fluidity, changes were evidenced. The dehumidification process does not affect the sensory quality of honey.

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