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Physicochemical characterization and antioxidant activity of honey samples of *Apis mellifera* and different species of Meliponinae subfamily from the Brazilian eastern Amazon region

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Abstract

In the present study, we evaluated the physicochemical characteristics (color, humidity, soluble solids, free acidity, pH, ash content, carbohydrate content, and 5-HMF) and antioxidant activity (Folin-Ciocalteu reagent and DPPH free radical scavenging) of honey samples produced by different species of Meliponinae subfamily (n = 16) and *A. mellifera* (n = 16) from the Eastern Brazilian Amazon region. Considering global averages, the stingless-bee honey had high acidity (93.45 mEq kg^{-1}), high humidity (27.43%), low content of reducing sugars (55.65%), and darker colors (Pfund scale – 162.17 mm), when compared with *A. mellifera* honey (62.96 mEq kg^{-1}, 19.35%, 65.70% and 68.43 mm for the respective parameters). Furthermore, more than 50% of samples of stingless-bee honey had values of 5-HMF above that proposed by Codex Alimentarius (max. 80 mg kg^{-1}). The two types of honey investigated showed similar results for total phenolic compounds and antioxidant activity. The principal component analysis (PCA) of the physicochemical characteristics of the honey samples, showed that the *A. mellifera* samples formed a differentiated group, while the multi-species Meliponinae samples were more scattered along the PCA axes. The distinctive characteristics of stingless-bee honey compared to *A. mellifera* honey produced in the same region, reinforces the need for specific regulations for honey produced by stingless-bees.

Keywords: Meliponinae; 5-HMF; DPPH radical; principal component analysis, bees.

Practical Application: *Apis mellifera* and Meliponinae are the main groups of bees found in Brazil. The honey produced by these groups has different physical, chemical, sensory and bioactive properties. In addition, the composition of these honeys can also be influenced by other factors, such as geographic regions. Considering also that most studies focus only on honey produced by a group of bees, having the comparison between the composition of these honeys, coming from the same geographic regions, can help in standardization and regulation of these products, in addition to the valorization in the beekeeping market.

1 Introduction

Although there are more than 20,000 species of bees in the world, only those belonging to the Apidae family produce honey (Silva et al., 2020). Apinae and Meliponinae are the most well-known subfamilies (Silva et al., 2016; Ávila et al., 2018). *Apis mellifera* (Hymenoptera: Apidae: Apinae) is the species that dominates honey production worldwide. However, Meliponinae has demonstrated growing potential in the bee market. Popularly recognized as stingless-bees (Nordin et al., 2018), this family is divided into more than 500 species which are distributed worldwide in tropical countries, mainly in South and Central America, Africa, southwest Asia, and Australia (Ávila et al., 2018).

Several species of Meliponinae subfamily (usually known as stingless-bees) differ from *A. mellifera* due to their smaller size, varied body colors, and absence of stinger. Besides, stingless-bees have short flights to collect food, prefer creeping flowers, and do not make large selections of food, that is, collect what is available and easier; as for their hive, they build a nest divided into combs for chicks and a reservoir for honey and pollen in the shape of a pot.

Honey is a natural product produced by bees that contain high content of sugars, water, and several other minor compounds, such as phenolic compounds, proteins, vitamins, free amino acids, minerals, and organic acids, which make it a nutritious and beneficial food to human health (Nordin et al., 2018). In addition, honey is a food appreciated worldwide and the investigation of this physicochemical properties is essential to its classification and characterization (Wen et al., 2017; Zhou et al., 2018; Crăciun et al., 2020). Due to the entomological characteristics,

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the floral and habitat preferences of each bee, the *A. mellifera,* and multi-species of Meliponinae subfamily produce honey samples with different characteristics. While multi-species of Meliponinae subfamily honey has a high moisture content (> 20%), acidity (> 50 meqkg^{-1}), and low sugar content (< 60% w/w) (Ávila et al., 2018), the *A. mellifera* honey samples have been usually reported with a darker color, higher carbohydrate content (70-80% w/w), and lower moisture content (< 20%) when compared with the honey obtained from different species of Meliponinae subfamily (Rizelio et al., 2020). Consequently, the sensory characteristics of both kinds of honey have also been reported as different: stingless-bee honey has a slightly acidic flavor and a fluid texture. It has been also reported that these characteristics vary when comparing different species of stingless bees (Ávila et al., 2019).

In addition, studies have reported differences in the physicochemical and sensorial characteristics of honey samples produced by different bee species; as well as the presence of minority compounds content which may also reflect on the antioxidant activity of the honey (Guerrini et al., 2009; Silva et al., 2013a; Silva et al., 2013b; Crăciun et al., 2020). The antioxidant activity is one of the main biological properties present in some foods, and it is related to the prevention of oxidative stress caused by the free radicals (Zhang, 2005; Vásquez-Espinal et al., 2019; Rizelio et al., 2020; Farias et al., 2021). In addition, this property can vary due to the phenolic compound content present in the food (Nascimento et al., 2020).

It is important to highlight that the composition of honey can also be strongly influenced by edaphoclimatic conditions, flora available for nectar collection, and the maturation period (Dourado et al., 2019; Miłek et al., 2021). Therefore, the investigation of the characteristics of honey obtained from different regions of the world is important to have a broad knowledge of their composition (Sant'ana et al., 2020).

In Brazil, especially in the Amazon region, the diversified flora combined with a hot and humid climate and the presence of high biodiversity of bee species allows the production of different types of honey with unique properties (Bandeira et al., 2018; Dourado et al., 2019). The state of Pará, located in the Eastern Amazon, has an equatorial climate (Am in the Köppen-Geiger classification), with annual thermal averages between 24 and 26 °C and high rainfall. These characteristics might impact the composition of honey and lead to undesirable chemical reactions during its production, maturation, and storage, such as the Maillard reaction which produces the contaminant 5-hydroxymethylfurfural (5-HMF) (Shapla et al., 2018).

Due to the influence of these factors, some studies have compared the honey from different geographical origins and botanical sources (Maione et al., 2019), apiary (Scholz et al., 2020), storage or processing conditions (Silva et al., 2020), aiming to assess the impact of these variables on the quality of honey, especially the physicochemical characteristics. However, most of these studies considered only *A.mellifera* honey or Meliponinae honey individually (Silva et al., 2020; Biluca et al., 2016).

Thus, analyzes of the characteristics of the honey samples produced among these two groups of bees obtained from the same region have a high relevance due to their economic and nutritional importance. The comparison of sympatric samples of *A. mellifera* and stingless-bee honey samples allows us to properly discern the species-specific differences related to the production of the honey. In this context, the present study aims to perform a comparative study of the physicochemical properties and antioxidant activity of the honey samples produced by five species of stingless-bees (Meliponinae) and *A. mellifera* produced in the state of Pará, Eastern Amazon.

2 Materials and methods

2.1 Honey samples

Sixteen samples of honey from different species of stingless-bees (Meliponinae) and sixteen *A. mellifera* honey samples were obtained directly from producers or local markets in four city cities of Pará, Brazil: Itaituba (04º16'34". 55º59'01"), Mojuí dos Campos (02º10'17". 56º44'42"), Belterra (02º32'11". 54º56'14") and Santarém (02º26'34'. 54º42'28") (Table 1 and Figure 1). The collected samples were conditioned and identified in

Table 1. Identification of species of bees and geographical description of the municipalities of origin of the samples honey.

Code*	Beespecies	City	P/M**
MCM1	*Melipona comprensipes manaosenseis*	Itaituba	M
MCM2		Itaituba	M
MCM3		Mojuí dos Campos	P
MCM4		Mojuí dos Campos	P
MCM5		Belterra	P
MCM6		Santarém	P
MCM7		Santarém	P
MCM8		Santarém	M
MCM9		Santarém	M
SS1	*Scaptotrigona* sp.	Mojuí dos Campos	P
SS2		Belterra	P
SS3		Santarém	P
TA1	*Tetragonisca angustula*	Mojuí dos Campos	P
TA2		Belterra	P
FL	*Frisiomelitta longipes*	Belterra	P
MS	*Melipona* sp.	Santarém	M
AM1	*Apis mellifera*	Itaituba	M
AM2		Itaituba	M
AM3		Itaituba	M
AM4		Mojuí dos Campos	M
AM5		Belterra	M
AM6		Santarém	M
AM7		Santarém	M
AM8		Santarém	M
AM9		Santarém	M
AM10		Santarém	M
AM11		Santarém	M
AM12		Santarém	M
AM13		Santarém	M
AM14		Santarém	M
AM15		Santarém	M
AM16		Santarém	M

*Identification code of the samples. **P = honeys collected directly by producers on rural properties; M = honeys collected at the local market.

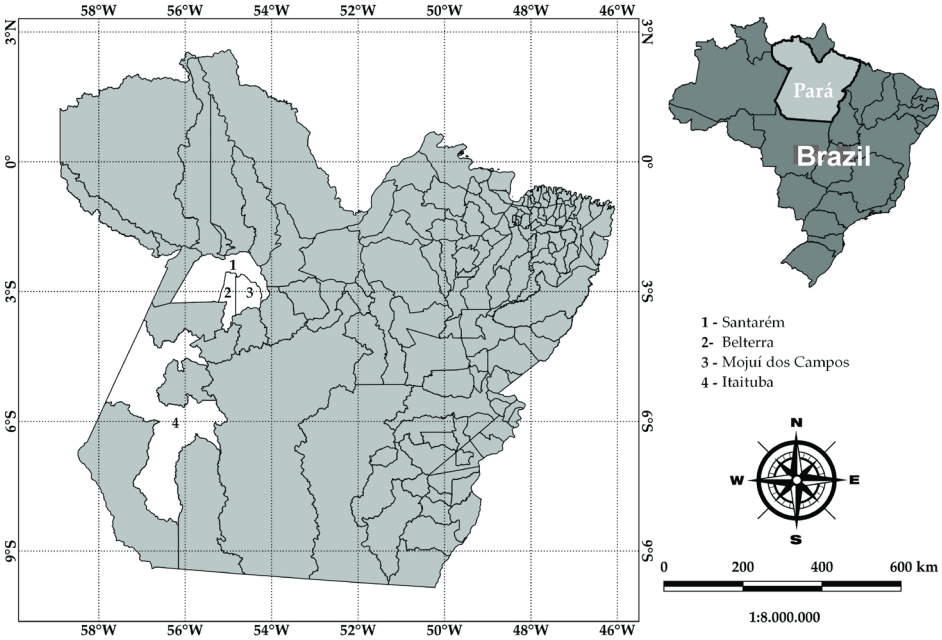

Figure 1. Map of eastern Amazon region of Brazil indicating the sampling sites of honey of *Apis mellifera* and Meliponinae bees (this figure is in color in the electronic version).

Falcon tubes. The samples were collected and transported at room temperature to the laboratory and stored at 18 ± 2 °C in a darkroom.

2.2 Reagents

All reagents were analytical grade and deionized water was purified in the Milli-Q system (Millipore, Bedford, MA, USA). 5-Hydroxymethylfurfural (5-HMF), caffeine, sodium tetraborate (STB), sodium dodecyl sulfate (SDS), sorbic acid, and cetyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich (Santa Ana. CA, USA). D-(+)-glucose monohydrate, D-fructose, and sucrose were obtained from Merck (Rio de Janeiro, RJ, Brazil). 2,2-diphenyl-1-picrylhydrazyl (DPPH), monobasic potassium phthalate, α-tocopherol, and Folin-Ciocalteu were obtained from Sigma Aldrich (Santa Ana, CA, USA). Sodium hydroxide, gallic acid, sodium carbonate, and reagent ethanol reagents from Vetec (Rio de Janeiro, RJ, Brazil).

2.3 Physicochemical parameters

Moisture and soluble solids

Moisture (% m/m) and soluble solids (ºBrix) were determined by refractometry using Abbe Tropen model I refractometer (Carl Zeiss Jena, Germany) according to protocols 969.38 and 932.12 of Association of Official Analytical Chemists (2005), respectively.

Free acidity and pH

To evaluate the pH of the honey, 10 g of each sample were diluted in 75 mL of deionized water and then the pH was measured in pHmeter model mPA-210 (MS Tecnopon, Piracicaba, Brazil). Then, the solutions were titrated with 0.101 mol L^{-1} NaOH solution (standardized with monobasic potassium biftalate) until pH 8.5 (Association of Official Analytical Chemists, 2005).

Ash content

The total ash content of the honey was measured by gravimetry, according to the method described by AOAC (Association of Official Analytical Chemists, 2005).

2.4 Analyzes in capillary electrophoresis (EC): 5-hydroxymethylfurfural (5-HMF) and carbohydrates

The contents of 5-HMFhydroxymethylfurfural and carbohydrates were determined by capillary electrophoresis (EC) (EC-Model 7100, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector and a temperature control device maintained at 25 °C. Data acquisition and processing software were provided by the manufacturer (HP ChemStation rev. B 0.4.03).

The capillary micellar electrokinetic chromatography technique was used for the quantification of 5-HMF. The running electrolyte (BGE) was composed of 5 mmol L^{-1} STB and 120 mmol L^{-1} SD at pH 9.3 and stored at room temperature. The analyzes of the samples were conducted in uncoated fused silica (Polymicro Technologies, Phoenix, AZ, USA) capillary of 32.0 cm total length (8.5 cm effective length × 50 μm ID × 375 μm OD). The separation voltage of 30 kV with positive polarity was applied at the injection end and the detection apparatus at 284 nm (Rizelio et al., 2012; Biluca et al., 2014).

The contents of fructose, glucose, and sucrose were quantified using the capillary zone electrophoresis technique, via indirect detection. For these analyzes, the running electrolyte (BGE) was composed of 20 μmolL^{-1} sorbic acid, 0.2 μmolL^{-1} CTAB, and 40 mmolL^{-1}NaOH at pH = 12.2. For the analysis of the samples, a capillary with 60 cm of total length (8.5 cm of effective length × 50 μm ID × 375 μm of OD) was applied with injection done by hydrodynamic mode using a voltage of 25 kV and indirect detection at 254 nm (peak inversion at 360 nm) (Biluca et al., 2014; Rizelio et al., 2012).

2.5 Color evaluation

For color evaluation, the honey samples were diluted in deionized water to obtain a concentration of 50% (v/v). Then, the solutions were centrifuged at 300 rpm for 10 min in a centrifuge model NT815 (Nova Técnica, Piracicaba, Brazil). These solutions were read in a UV/Vis spectrophotometer (Nova 3300, Nova Instruments, Piracicaba, Brazil) at 635 nm. Finally, the absorbances were transformed to the Pfund scale (Gomes et al., 2017).

2.6 Reducing capacity activity and free radical scavenging activity

To evaluate the reducing activity and DPPH free radical scavenging activity, the honey samples were accurately weighed (2.5 g), and dissolved in deionized water using the proportion 1 : 2 (w : v).

The total reducing activity of the samples was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965), and aliquots of 0.1 mL of an aqueous solution of honey (0.5 g mL^{-1}) were homogenized with 2 mL of deionized water, 0.5 mL of Folin-Ciocalteu, and 1.5 mL of sodium carbonate solution (20% w/v), then adjusted to 10 mL volumetric flask. After 2 h the absorbance was measured at 765 nm in spectrophotometer SB 1810-S (Spectro Visium, Brazil). Deionized water was used as a blank. The results expressed as mg gallic acid equivalent (GAE) 100 g^{-1}.

The DPPH free radical scavenging activity of the samples was determined according to a previously published protocol (Gomes et al., 2017). An aliquot of 0.4 mL of honey solution (1: 5, v/v) was diluted with1.6 mL of ethanol and 0.2 mL of 1.2 mmol L^{-1} DPPH solution. The mixtures were allowed to stand for 30 min under light and at room temperature. The absorbance was measured in UV/Vis spectrophotometer (NOVA 3300, Brazil) at 555 nm. The sequestration capacity of DPPH was expressed as a percentage. For calibration, a standard curve of α-tocopherol (vitamin E) was performed in a 0.2 mmol L^{-1} ethanolic solution, with concentrations ranging from 0 to 148 2.50 mg L^{-1}.

2.7 Statistical analysis

All analyzes were performed in triplicate with values expressed as mean ±standard deviation and submitted to analysis of variance (ANOVA). The Tukey test at the 5% probability level was applied to evaluate the difference between the analyzed samples. The principal component analysis (PCA) was used to explain the interdependence of the analyzed physicochemical properties. All statistical analyses were performed using Minitab 14 software (Minitab, Pennsylvania, USA).

3 Results

The physicochemical properties obtained for the investigated honey samples are shown in Table 2 (moisture, free acidity, 5-HMF, pH, ash, and color) and Table 3 (soluble solids, glucose, fructose, and sucrose).

Observing the results obtained for the humidity parameter, it is possible to notice that for stingless-bee honey the values vary from 18.01 to 32.64% w/w, with more than 90% of the samples showing humidity greater than 20% (value established by Codex as the maximum acceptable limit). In contrast, the values found for *A. mellifera* bee honey vary from 12.07 to 23.51% w/w, showing that 75% of the samples had values lower than 20%. Moreover, these results reinforce that the stingless-bee honey has as characteristics a higher moisture content when analyzed the average moisture values obtained for the stingless-bee honey (27.43%) versus the *A. mellifera* honey (19.35%).

The free acidity of the samples varied between 15.88 to 328.56 mEq kg^{-1} for Meliponinae honey and 23.31 to 124.53 mEq kg^{-1} for *A. mellifera* honey. For both groups of samples, it was observed that 60% of presented values were above the limits stipulated by Codex (Max. 50 mEq kg^{-1}). However, it is interesting to note that honey samples from Meliponinae subfamily, showed seven samples with values above 100 mEq kg^{-1}, and a sample with acidity above 300 mEq kg^{-1}.

The results of the pH analysis demonstrated that there was no discrepancy between the studied samples. Honey samples obtained from Meliponinae subfamily showed an average pH of 3.6 and *A. mellifera* honey samples showed an average pH of 3.9.

In the analyzes performed to identify and quantify 5- HMF in stingless-bee honey, it was observed that the values ranged from < LOQ to 195.43 mg kg^{-1} and that more than 50% of the analyzed samples were with values higher than those acceptable (Max. 80 mg kg^{-1}- (Codex Alimentarius Commission, 2001). On the other hand, the results found for *A. millifera* bee honey showed variable values from < LOQ to 113.26 mg kg^{-1}, with more than 80% of the samples within the values proposed by Codex, that is, only two samples will show disagreement. The highest values of 5-HMF found in stingless bee honey are from samples purchased directly from the local market (samples MCM1, MCM2, and MS). These results suggest that this type of honey could be commercialized under inadequate temperature conditions, favoring the formation of this compound.

For stingless-bee honey, the ash content ranged from 0.01 to 0.49%, and for bee honey *A. mellifera* the results range from 0.02 to 0.36%.

Meliponinae subfamily honey samples tended to have darker colors (Table 2, Figure 2). The samples show colors that range from extra-white to dark amber, however, the dark amber has been the predominant color of the analyzed samples (56.25%). It was also verified that all the samples of honey from *Scaptotrigona* sp. presented a dark amber color, even though they

Table 2. Phisicochemical characteristics of *Meliponinae* and *A. mellifera* honey samples.

Samples	Moisture (% w/w)	Free acidity (mEq kg^{-1})	5-HMF* (mg kg^{-1})	pH	Ash (% w/w)	Pfund scale (mm)	Color
MCM1	31.93 ± 0.20^{ab}	15.88 ± 1.00^{r}	157.50 ± 4.00^{c}	3.25 ± 0.01^{op}	0.01 ± 0.01^{o}	73.95 ± 3.00^{m}	Light amber
MCM2	30.30 ± 0.10^{ab}	17.21 ± 0.02.00^{qr}	167.00 ± 4.00^{b}	3.08 ± 0.01^{q}	0.02 ± 0.01^{mno}	173.61 ± 3.00^{f}	Dark amber
MCM3	31.27 ± 0.02^{ab}	36.68 ± 1.00^{o}	< LOQ	3.94 ± 0.1^{gh}	0.03 ± 0.02^{fgh}	39.91 ± 0.90^{n}	Extra-light amber
MCM4	29.18 ± 0.04^{bc}	37.25 ± 9.00^{no}	< LOQ	3.81 ± 0.07^{hi}	0.01 ± 0.01^{o}	39.42 ± 7.00^{l}	Extra-light amber
MCM5	32.13 ± 0.01^{ab}	132.36 ± 2.00^{d}	57.12 ± 0.40^{l}	3.16 ± 0.06^{pq}	0.06 ± 0.03^{k-o}	130.65 ± 4.00^{r}	Dark amber
MCM6	25.05 ± 0.01^{ab}	104.00 ± 3.00^{j}	83.87 ± 0.20^{f}	3.32 ± 0.02^{lmn}	0.08 ± 0.02^{k-o}	154.18 ± 1.00^{p}	Dark amber
MCM7	25.91 ± 0.01^{def}	187.2 ± 10.00^{f}	94.22 ± 1.00^{g}	3.23 ± 0.01^{nop}	0.11 ± 0.04^{j-m}	14.53 ± 0.2.00^{p}	Extra white
MCM8	30.78 ± 0.01^{de}	62.69 ± 2.00^{b}	92.14 ± 1.00^{f}	3.48 ± 0.02^{opq}	0.06 ± 0.01^{i-l}	82.50 ± 3.00^{b}	Light amber
MCM9	22.21 ± 0.06^{fgh}	34.79 ± 1.00^{o}	4.90 ± 0.60^{q}	4.16 ± 0.04^{ef}	0.31 ± 0.01^{bc}	29.64 ± 0.7.00^{a}	White
SS1	25.87 ± 0.01^{de}	159.90 ± 2.00^{c}	147.20 ± 3.00^{d}	3.78 ± 0.03^{hi}	0.08 ± 0.01^{j-m}	362.52 ± 4.00^{no}	Dark amber
SS2	29.39 ± 0.01^{bc}	122.30 ± 1.00^{e}	< LOQ	3.52 ± 0.02^{j-m}	0.25 ± 0.01^{def}	151.82 ± 6.00^{g}	Dark amber
SS3	32.64 ± 0.01^{a}	328.6 ± 6.00^{a}	20.42 ± 5.00^{o}	3.39 ± 0.06^{l-o}	0.26 ± 0.02^{cde}	414.27 ± 13.00^{g}	Dark amber
TA1	27.11 ± 0.04^{cd}	18.57 ± 2.00^{qr}	80.49 ± 0.60^{gh}	3.98 ± 0.04^{g}	0.01 ± 0.01^{o}	31.00 ± 5.00^{h}	White
TA2	26.90 ± 0.01^{cd}	122.00 ± 2.00^{e}	12.65 ± 3.00^{p}	3.51 ± 0.01^{klm}	0.21 ± 0.01^{efg}	189.83 ± 0.90^{p}	Dark amber
FL	20.21 ± 0.01^{hij}	72.54 ± 2.00^{hi}	92.68 ± 6.00^{f}	3.55 ± 0.07^{jkl}	0.22 ± 0.02^{efg}	456.24 ± 2.00^{c}	Dark amber
MS	18.01 ± 0.05^{j}	43.03 ± 4.00^{mn}	195.40 ± 6.00^{a}	4.35 ± 0.02^{cd}	0.49 ± 0.01^{a}	250.61 ± 6.00^{d}	Dark amber
Maximum	32.64	328.56	195.43	4.16	0.49	456.24	Dark amber
Minimum	18.01	15.88	< LOQ	3.08	0.01	14.53	Extra white
Mean	27.43	93.45	75.35	3.60	0.14	162.17	Dark amber
AM1	19.42 ± 0.01^{hij}	25.01 ± 3.00^{p}	< LOQ	4.75 ± 0.02^{a}	0.36 ± 0.1^{b}	105.28 ± 3.00^{j}	Amber
AM2	18.15 ± 0.10^{k}	38.84 ± 3.00^{no}	< LOQ	4.52 ± 0.02^{ab}	0.12 ± 0.03^{ijk}	75.69 ± 0.01^{m}	Light amber
AM3	19.36 ± 0.06^{hij}	38.17 ± 3.00^{no}	< LOQ	4.55 ± 0.07^{b}	0.13 ± 0.01^{hij}	93.89 ± 2.00^{k}	Amber
AM4	21.30 ± 0.08^{ghi}	54.73 ± 0.70^{k}	< LOQ	4.30 ± 0.07^{de}	0.19 ± 0.05^{fgh}	73.34 ± 1.00^{m}	Light amber
AM5	19.34 ± 0.01^{hij}	48.27 ± 2.00^{lm}	68.58 ± 0.60^{ijk}	4.58 ± 0.01^{ab}	0.08 ± 0.01^{j-m}	72.96 ± 0.60^{m}	Light amber
AM6	23.51 ± 0.02^{efg}	23.31 ± 2.00^{pq}	113.26 ± 1.00^{e}	3.90 ± 0.07^{gh}	0.02 ± 0.01^{no}	42.76 ± 6.00^{n}	Extra-light amber
AM7	19.31 ± 0.01^{hij}	46.96 ± 3.00^{m}	47.55 ± 1.00^{m}	3.45 ± 0.09^{lmn}	0.06 ± 0.03^{k-no}	30.38 ± 0.01^{p}	White
AM8	18.84 ± 0.08^{ij}	69.44 ± 0.50^{i}	36.86 ± 1.00^{n}	3.32 ± 0.06^{nop}	0.05 ± 0.02^{mno}	33.47 ± 0.20^{op}	White
AM9	19.32 ± 0.02^{hij}	53.92 ± 0.20^{kl}	70.82 ± 0.10^{ijk}	3.37 ± 0.09^{mno}	0.02 ± 0.01^{mno}	23.20 ± 0.40^{q}	White
AM10	20.66 ± 0.05^{ghij}	98.16 ± 4.00^{f}	68.50 ± 0.50^{ijk}	3.65 ± 0.06^{ijk}	0.05 ± 0.01^{l-o}	124.46 ± 0.20^{i}	Dark amber
AM11	20.00 ± 0.01^{hij}	124.53 ± 3.00^{e}	39.85 ± 0.40^{n}	3.69 ± 0.10^{ij}	0.16 ± 0.03^{ghi}	151.33 ± 0.60^{j}	Dark amber
AM12	19.19 ± 0.08^{ij}	78.42 ± 5.00^{gh}	72.16 ± 1.00^{ijk}	3.32 ± 0.20^{nop}	0.12 ± 0.01^{ijk}	43.38 ± 0.40^{n}	Extra-light amber
AM13	19.73 ± 0.04^{hij}	38.99 ± 0.30^{no}	75.21 ± 0.50^{hij}	3.48 ± 0.06^{lmn}	0.03 ± 0.01^{mno}	18.37 ± 0.80^{qr}	White
AM14	18.93 ± 0.02^{ij}	83.35 ± 8.00^{g}	77.46 ± 3.00^{ghi}	4.02 ± 0.20^{fg}	0.20 ± 0.07^{efg}	85.47 ± 0.40^{l}	Amber
AM15	19.10 ± 0.01^{ij}	81.35 ± 3.00^{g}	92.73 ± 0.70^{jk}	3.39 ± 0.08^{l-o}	0.04 ± 0.01^{mno}	33.23 ± 0.20^{p}	White
AM16	19.44 ± 0.02^{hij}	103.87 ± 1.00^{f}	65.74 ± 0.40^{k}	4.32 ± 0.40^{de}	0.29 ± 0.10^{cd}	87.70 ± 0.20^{l}	Amber
Maximum	23.51	124.53	113.26	4.75	0.36	151.33	Dark amber
Minimum	12.07	23.31	< LOQ	3.32	0.02	18.37	White
Mean	19.35	62.96	69.07	3.91	0.12	68.43	Light amber

Different letters in each column mean significant differences according to Tukey's test ($p < 0.05$); data are mean ± SD of triplicate measurements; <LOQ = Limits of quantitation. *5-HMF = 5- hydroxymethylfurfural

were collected in different regions. Regarding the A. *mellifera* honey, the samples showed the same color range as stingless-bee honey (extra-white to dark amber), but a predominant color was not observed in the analyzed samples (Figure 2). The color is an important indication of the multiflora or uniflora origin of the honey samples (Wen et al., 2017). Honey samples obtained from native flora as those collected in the present study tend to show a broad spectrum of colors that tend to the dark range. However, it is important to highlight that the inappropriate storage of honey samples could also lead to the Maillard reaction that produces the contaminant 5-hydroxymethylfurfural which is also involved with the presence of the dark color of some investigated samples (Shapla et al., 2018).

Table 3 shows the results for °Brix and sugars quantified in honey samples. In °Brix analysis, the values found in stingless-bee honey ranged from 66.10 to 80.23 °Brix. While for *A. mellifera* bee honey the values ranged from 74.70 to 80.10.

Table 3. Soluble solids and sugar content (fructose, glucose and sucrose) of Meliponinae and *A. mellifera* honey samples.

Samples	Soluble solid (°Brix)	Fructose (% w/w)	Glucose (% w/w)	Sucrose (% m/m)	G + F*	F / G**
MCM1	66.63 ± 0.20^{s}	29.30 ± 0.80^{jk}	27.17 ± 0.20^{d-g}	< LOQ	56.46 ± 0.90^{hij}	1.08 ± 0.02^{jkl}
MCM2	68.20 ± 0.01^{q}	29.21 ± 0.80^{jk}	26.83 ± 0.40^{d-g}	< LOQ	56.04 ± 1.00^{ij}	1.09 ± 0.03^{i-l}
MCM3	67.30 ± 0.01^{r}	29.13 ± 20^{jk}	27.58 ± 1.00^{a-f}	< LOQ	56.71 ± 4.00^{hij}	1.06 ± 0.02^{kl}
MCM4	69.00 ± 0.01^{p}	30.30 ± 2.00^{h-k}	27.97 ± 1.00^{cde}	6.02 ± 0.20	58.27 ± 3.00^{f-j}	1.08 ± 0.02^{jkl}
MCM5	66.50 ± 0.01^{t}	29.45 ± 0.60^{ijk}	28.25 ± 0.90^{cde}	< LOQ	57.70 ± 1.00^{f-j}	1.04 ± 0.03^{l}
MCM6	73.50 ± 0.01^{r}	29.33 ± 1.00^{h-k}	17.76 ± 1.00^{e-h}	< LOQ	47.09 ± 3.00^{g-j}	1.65 ± 0.03^{hij}
MCM7	72.70 ± 0.01^{l}	33.02 ± 2.00^{jk}	28.99 ± 2.00^{l}	< LOQ	62.01 ± 3.00^{k}	1.14 ± 0.01^{d}
MCM8	67.30 ± 0.01^{m}	30.61 ± 0.90^{e-j}	26.39 ± 0.30^{cd}	< LOQ	57.00 ± 1.00^{d-g}	1.16 ± 0.02^{h-k}
MCM9	75.80 ± 0.01^{j}	37.06 ± 2.00^{b-g}	25.71 ± 1.00^{f-i}	< LOQ	62.77 ± 4.00^{c-f}	1.44 ± 0.03^{ef}
SS1	72.70 ± 0.01^{m}	38.58 ± 1.00^{b-e}	8.93 ± 0.80^{m}	< LOQ	47.50 ± 2.00^{k}	4.34 ± 0.20^{a}
SS2	69.00 ± 0.01^{p}	31.93 ± 2.00^{f-k}	21.75 ± 1.00^{cj}	< LOQ	53.68 ± 3.00^{j}	1.47 ± 0.02^{ef}
SS3	66.10 ± 0.01^{u}	26.61 ± 0.90^{k}	20.12 ± 0.50^{jk}	< LOQ	46.73 ± 1.00^{k}	1.32 ± 0.01^{g}
TA1	71.00 ± 0.01^{n}	31.22 ± 1.00^{g-k}	28.25 ± 0.90^{cde}	4.14 ± 0.40	59.47 ± 2.00^{f-i}	1.10 ± 0.01^{h-l}
TA2	71.50 ± 0.01^{o}	35.60 ± 0.70^{b-i}	18.25 ± 0.40^{kl}	< LOQ	53.86 ± 1.00^{j}	1.95 ± 0.03^{c}
FL	77.80 ± 0.01^{g}	37.28 ± 1.00^{b-g}	16.24 ± 0.40^{l}	< LOQ	53.52 ± 1.00^{j}	2.30 ± 0.02^{b}
MS	80.23 ± 0.20^{a}	40.55 ± 0.80^{abc}	21.06 ± 0.30^{cd}	< LOQ	61.62 ± 1.00^{a}	1.93 ± 0.01^{c}
Maximum	80.23	40.55	28.99	6.02	62.77	4.34
Minimum	66.10	26.61	8.93	< LOQ	46.73	1.04
Mean	70.95	32.45	23.20	5.08	55.65	1.57
AM1	79.00 ± 0.01^{e}	40.12 ± 4.00^{a-d}	28.47 ± 3.00^{cde}	< LOQ	68.60 ± 7.00^{ab}	1.41 ± 0.01^{fg}
AM2	80.10 ± 0.01^{b}	39.96 ± 1.00^{a-d}	27.88 ± 0.60^{c-f}	< LOQ	67.83 ± 2.00^{abc}	1.43 ± 0.03^{ef}
AM3	79.00 ± 0.01^{e}	24.23 ± 2.00^{d-j}	24.15 ± 1.00^{a}	< LOQ	58.37 ± 3.00^{f-j}	1.42 ± 0.02^{ef}
AM4	77.00 ± 0.01^{h}	36.29 ± 2.00^{b-h}	24.25 ± 0.60^{hi}	< LOQ	60.54 ± 2.00^{e-i}	1.50 ± 0.03^{ef}
AM5	79.00 ± 0.01^{e}	36.27 ± 0.70^{b-h}	25.14 ± 2.00^{ghi}	< LOQ	61.41 ± 2.00^{d-h}	1.45 ± 0.08^{ef}
AM6	74.70 ± 0.01^{k}	31.78 ± 2.00^{f-k}	28.84 ± 2.00^{cd}	7.63 ± 0.40	60.62 ± 4.00^{e-i}	1.10 ± 0.02^{i-l}
AM7	79.00 ± 0.01^{e}	45.92 ± 2.00^{a}	24.56 ± 0.70^{hi}	< LOQ	70.48 ± 3.00^{a}	1.87 ± 0.03^{c}
AM8	79.50 ± 0.20^{c}	37.21 ± 0.70^{b-g}	31.24 ± 0.30^{ab}	< LOQ	68.45 ± 0.80^{ab}	1.19 ± 0.02^{h}
AM9	79.00 ± 0.01^{e}	40.69 ± 1.00^{ab}	27.09 ± 2.00^{d-g}	< LOQ	67.78 ± 2.00^{abc}	1.51 ± 0.10^{e}
AM10	77.80 ± 0.01^{g}	33.57 ± 2.00^{e-j}	31.22 ± 0.70^{ab}	< LOQ	64.79 ± 2.00^{b-e}	1.07 ± 0.03^{jkl}
AM11	78.20 ± 0.01^{f}	37.19 ± 2.00^{b-g}	33.08 ± 1.00^{a}	< LOQ	70.27 ± 3.00^{a}	1.12 ± 0.02^{h-l}
AM12	79.13 ± 0.20^{d}	37.53 ± 2.00^{b-f}	31.85 ± 1.00^{a}	< LOQ	69.38 ± 3.00^{ab}	1.18 ± 0.02^{hi}
AM13	78.20 ± 0.01^{f}	34.37 ± 0.60^{c-j}	32.00 ± 0.50^{a}	< LOQ	66.37 ± 1.00^{a-d}	1.07 ± 0.01^{jkl}
AM14	79.40 ± 0.01^{c}	34.51 ± 3.00^{b-j}	31.40 ± 2.00^{ab}	< LOQ	65.91 ± 6.00^{a-d}	1.10 ± 0.02^{i-l}
AM15	79.40 ± 0.01^{c}	36.15 ± 2.00^{b-h}	32.28 ± 1.00^{a}	< LOQ	68.43 ± 3.00^{ab}	1.12 ± 0.02^{h-j}
AM16	76.60 ± 0.01^{i}	32.47 ± 4.00^{e-k}	29.58 ± 3.00^{bc}	< LOQ	62.04 ± 7.00^{d-g}	1.10 ± 0.03^{i-l}
Maximum	80.10	45.92	33.08	7.63	70.48	1.87
Minimum	74.70	31.78	24.15	< LOQ	58.37	1.07
Mean	78.44	36.77	28.94	7.63	65.70	1.29

Different letters in each column mean significant differences according to Tukey's test. ($p < 0.05$); data are mean ± SD of triplicate measurements; <LOQ = Limits of quantitation. *G + F = the sum of the glucose and fructose contents. **F / G = the ratio between the fructose and glucose contents.

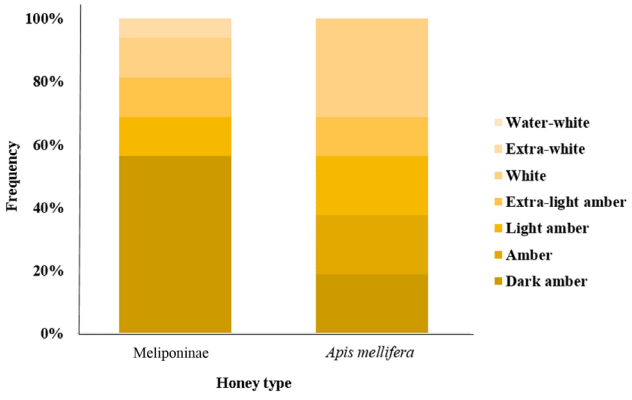

Figure 2. Percentage of honey color categories of *Apis mellifera* and Meliponinae from eastern Amazon, Brazil (this figure is in color in the electronic version).

The sugar content varied greatly among samples of both *A. mellifera* and stingless-bee samples (Table 3). Considering the stingless bee honey, the values ranged from 28.99 to 40.55% for fructose and 8.93 to 26.61% for glucose. Regarding the *A. Mellifera* samples, the values were higher, ranging from 33.08 to 45.92% for fructose and 24.15 to 31.78% for glucose. When the sum of glucose and fructose is observed, it is even clearer than the samples obtained from the species of Meliponinae subfamily honey has lower reducing sugar values. About 80% of the stingless bee honey samples have reduced sugar values (G + F), which are below the minimum values required by Codex Alimentarius (min. 60%) (Codex Alimentarius Commission, 2001). In addition, the F/G rate of the samples analyzed was 1.04 to 4.34 for stingless-bee honey and 1.7 to 1.87 for *A. mellifera* honey. Among the total samples analyzed, only three were quantified with sucrose (MCM4 = 6.02%; TA1 = 4.14% e TA1 = 7.63%).

Table 4. Folin-Ciocalteu Reagent (FCR) reducing capacity and DPPH free radical scavenging activity of Meliponinae and *A. mellifera* honey samples.

Samples	FCR (mg EAG 100 g^{-1})	DPPH (%)
MCM1	22.97 ± 1.00^{s}	30.02 ± 4.00^{n}
MCM2	27.85 ± 0.60^{qr}	35.92 ± 15.00^{mn}
MCM3	27.10 ± 1.00^{r}	20.20 ± 1.00^{o}
MCM4	102.64 ± 3.00^{t}	96.53 ± 0.50^{p}
MCM5	22.43 ± 0.30^{p}	11.17 ± 1.00^{n}
MCM6	18.00 ± 0.50^{n}	10.63 ± 2.00^{n}
MCM7	62.87 ± 0.70^{f}	60.80 ± 1.00^{e-i}
MCM8	36.90 ± 1.00^{kl}	32.43 ± 3.00^{efg}
MCM9	68.10 ± 1.00^{f}	53.60 ± 1.00^{c}
SS1	129.61 ± 5.00^{d}	88.83 ± 0.60^{a}
SS2	65.96 ± 4.00^{ij}	60.73 ± 0.40^{ef}
SS3	52.50 ± 0.40^{hi}	31.00 ± 5.00^{ef}
TA1	76.26 ± 4.00^{s}	56.13 ± 1.00^{p}
TA2	58.89 ± 1.00^{gh}	57.97 ± 2.00^{g-k}
FL	74.74 ± 4.00^{b}	78.73 ± 7.00^{b}
MS	266.96 ± 4.00^{a}	95.77 ± 0.80^{a}
Maximum	266.96	96.53
Minimum	18.00	10.63
Mean	69.61	51.28
AM1	38.19 ± 0.20^{p}	50.23 ± 4.00^{i-l}
AM2	54.95 ± 2.00^{mn}	76.50 ± 4.00^{cd}
AM3	56.16 ± 0.30^{lm}	71.39 ± 2.00^{c}
AM4	57.40 ± 0.01^{klm}	56.92 ± 3.00^{e-h}
AM5	60.56 ± 0.60^{jk}	87.57 ± 0.90^{b}
AM6	31.06 ± 2.00^{q}	14.69 ± 2.00^{op}
AM7	77.14 ± 2.00^{f}	39.86 ± 0.60^{m}
AM8	58.07 ± 0.80^{klm}	51.25 ± 1.00^{h-l}
AM9	106.50 ± 2.00^{c}	47.95 ± 3.00^{kl}
AM10	69.73 ± 2.00^{g}	51.70 ± 1.00^{g-l}
AM11	77.26 ± 1.00^{f}	61.06 ± 1.00^{e}
AM12	86.09 ± 4.00^{e}	57.05 ± 3.00^{e-h}
AM13	44.71 ± 2.00^{o}	46.52 ± 0.70^{l}
AM14	59.10 ± 2.00^{kl}	49.39 ± 4.00^{jkl}
AM15	51.49 ± 1.00^{n}	54.71 ± 1.00^{f-j}
AM16	52.15 ± 1.00^{n}	50.80 ± 8.00^{h-l}
Maximum	106.50	87.57
Minimum	31.06	14.69
Mean	61.28	54.22

Different letters in each column mean significant differences according to Tukey's test. ($p < 0.05$); data are mean ± SD of triplicate measurements.

The results for the evaluation of total phenolic contents and DPPH free radical scavenging activity honey are shown in Table 4. The values for the Folin-Ciocalteu reagent (FCR) indicate the total phenolics contents ranged from 18.00 to 266.96 mg GAE 100g^{-1} in stingless-bee honey and, 31.06 to 106.50 mg GAE 100g^{-1} in *A. mellifera* honey.

The results of the DDPH radical scavenging activity (%) varied between 10.63 and 96.53% for honey samples obtained from species of Meliponinae subfamily. In contrast, the DPPH scavenging activity (%) varied from 14.69 to 87.57% for *A. mellifera* honey. A significant difference was observed between the two kinds of honey studied, regardless of the species or collected regions. On the other hand, when analyzing the global averages, it is possible to observe that stingless bees honey and *A. mellifera* honey kinds of honey present show close percentages (51.28% and 54.22%, respectively).

Principal component analysis (PCA) was performed aiming to evaluate the data of the physicochemical, FCR, and DPPH for the discrimination of honey samples according to their bee species origin. The chemometric evaluation shows that PC1 explained up to 39.7% of the total variance, and PC2 explained 22.6%. Thus, the first two PCs explained 62.3% of the variability in the data (Figure 3).

4 Discussion

4.1 Physicochemical properties

In the present study, we noticed differences in the moisture content between the honey obtained from the species of the Meliponinae subfamily and the honey from *A. mellifera*. Some studies have also reported that honey produced by stingless bees has a high moisture content, reaching values greater than 40% (w/w) (Nordin et al., 2018; Biluca et al., 2016). These results can be attributed to different factors, such as the humidity of environments, nectar collection from undergrowth flowers, ripe fruits that are rich in water, or even different species of stingless bees (Ávila et al., 2018). It is also important to note that high moisture content in stingless-bee honey influences the sensorial

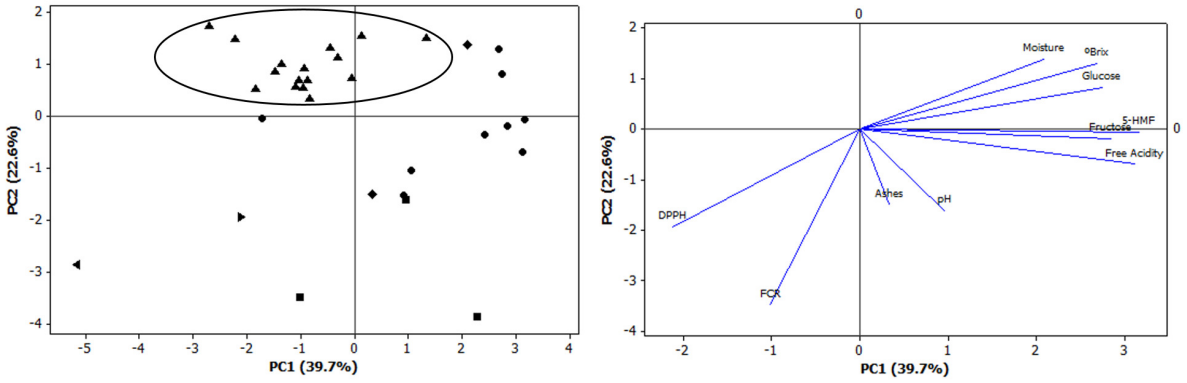

Figure 3. PCA scatter plot of honey samples and the descriptors: physicochemical and bioactive characteristics. Considering *Apis mellifera* (▲), *Melipona comprensipes manaosenseis* (●), *Scaptotrigona* sp. (▪), *Tetragonisca angustula* (♦), *Melipona* sp. (◀) and *Frisiomelitta longipes* (▶) species of bee (this figure is in color in the electronic version).

characteristics, due to its lower viscosity and reduced shelf life due to its accelerated fermentation.

Free acidity is a parameter of quality-related mainly to the presence of organic acids obtained from the nectar, derived from the fermentation process of sugars – producing acetic acid – as well as from some enzymatic mechanisms, such as the transformation of glucose into gluconic acid by glucose oxidase (Ávila et al., 2018). Interestingly, some studies carried out with stingless-bee honey have shown higher free acidity content than those reported for *A. mellifera* honey. For example, Duarte et al. (2018) reported a free acidity for stingless bee honey ranged ranging from 17 mEq kg^{-1} to 125 mEq kg^{-1}(Duarte et al., 2018). In contrast, Chuttong et al. (2016), exhibited high acidity for these honey samples, ranging from 440.0 to 592.0 mEq kg^{-1}.

The pH results corroborate with previous findings reported for honey samples from some species of Meliponinae subfamily collected from the Amazon region (Silva et al., 2013a) that ranged from 3.10 to 5.04 mEq kg^{-1}. Similarly, another study reported for *A. mellifera* honey samples showed acidity ranging from 3.86 to 4.41 mEq kg^{-1} (Silva et al., 2020).

It is worth mentioning that regions with a hot and humid climate as the Amazon region, favor the formation of 5-HMF (Bandeira et al., 2018) even during storage at room temperature (Khalil et al., 2010). Studies with Brazilian stingless bee honey from other climate conditions observed the absence of 5-HMF for fresh honey (Biluca et al., 2016). Thus, the climatic conditions used to store the *A. mellifera* and Meliponinae honey samples produced in the Amazon eastern region in general favor the formation of 5-HMF.

The ash content represents the total mineral residue present in honey and depends mainly on the composition of the collected nectar, due to the absorption of nutrients from the soil by the plant (Silva et al., 2016); Nordin et al., 2018).

Although the Codex Alimentarius (Codex Alimentarius Commission, 2001) does not provide a standard value for this parameter, studies with *A. mellifera* honey have shown the average ash content in honey is 0.17% (w/w), ranging between 0.02% and1.03% (w/w) (Chakir et al., 2011). However, Gomes et al. (2017) and Silva et al., 2013a found ash values for stingless-bee honey in the state of Pará, varying from 0.03 to 0.33% and 0.09 to 1.11%, respectively. Mineral content may have a relation to the color and flavor of honey, with a higher mineral content leading to a darker and stronger flavor, which are attractive features for its consumption (Silva et al., 2016).

The prevalence of dark color was also found in a previous study that analyzed samples of stingless-bee honey from the same studied region (Gomes et al., 2017). Similarly, another study analyzed honey samples of the species of the Meliponinae family from the Brazilian semi-arid region, and they also observed colors ranging from white to amber (Sant'ana et al., 2020). The range of colors from *A. mellifera* honey samples was also observed in a previous study that analyzed honey samples from southern Brazil, but the authors identified a predominance of dark colors (Rizelio et al., 2020). Studies have also suggested that stingless-bees from the Amazon eastern region tend to produce darker honey (Silva et al., 2013; Silva et al., 2013a; Gomes et al., 2017).

Regarding soluble solids, similar results are found in other studies, with values between 55.2 and 76.1 °Brix (Biluca et al., 2016) and 70.0 and 85.0 °Brix (Alqarni et al., 2016), for Meliponinae and *A. mellifera* honey, respectively.

Sugars are the major components in the composition of honey, where fructose and glucose are the reducing sugars present in the greatest amounts. Some studies have also reported lower reducing sugar values than those found in the *A. mellifera* samples, thus highlighting this characteristic of stingless-bee honey (Ávila et al., 2018; Biluca et al., 2016). The F / G ratio points to the time required for honey to crystallize, i.e. honey with a ratio greater than 1.3 may remain fluid for longer (Escuredo et al., 2014). Considering the honey samples analyzed in the present study, showed slow crystallization.

High values of sucrose usually indicate a premature harvest of honey, since the enzyme still invertase did not completely dissociate sucrose into glucose and fructose (Silva et al., 2016). Biluca et al. (2016) reported the absence of sucrose in thirty-three samples of stingless-bee honey (Biluca et al., 2016). However, Chuttong et al. (2016) reported an average content of 19% (w/w) of sucrose in stingless bee honey (Chuttong et al., 2016).

4.2 Reducing activity and free radical scavenging activity

The differences in the phenolic contents of different types of honey are largely related to the floral origin of the honey. Moreover, the constituents of the floral source could be influenced by the nectar and pollen, the geographical origins, and the honey-producing bee species (Shamsudin et al., 2019).

Thus, the values of radical scavenging activity found varied for both analyzed honey types. However, it is important to highlight that for stingless-bees honey, this variation has already been reported in previous studies performed in Malaysia, which showed values of 27.33 and 55.86 mg GAE $100g^{-1}$ for honey samples obtained from different regions (Shamsudin et al., 2019). In contrast, another study performed in Brazil presented values from 10.3 to 98 mg GAE $100g^{-1}$ for Brazilian honey (Biluca et al., 2016). Similarly, a previous study reported values between 18.20 and 148.62 mg GAE 100 g^{-1}, respectively, for *A. mellifera* honey samples collected from the southern region of Brazil (Rizelio et al., 2020).

Currently, different methods have been applied to determine the antioxidant activity of foods (Amorati & Valgimigli, 2015; Nascimento et al., 2020). In the present study, the antioxidant activity was determined by the DPPH radical scavenging activity. Other author shave found similar results for antioxidant activity. For honey samples of the same geographical origin, the DPPH radical scavenging activity (%) ranged from 11.98 to 70.51% for stingless-bees honey (Gomes et al., 2017) and from 49.18 to 61.62% for *A. mellifera* honey (Bandeira et al., 2018).

4.3 Chemometric analysis

The scatter plot showed that honey samples of AM were the most homogeneous in the measured analyzed parameters. According to the scatter plot, the weights of these parameters do not have relevance to separate differentiate these samples.

The diametrical opposition of pH and ashes from the quadrant of those samples shows these parameters as the most different regarding values found for TA, MCM, and SS samples. One AM sample has moisture content similar to TA and MCM samples and was grouped with these clusters.

The contents of soluble solids (ºBrix), glucose, 5-HMF, fructose, free acidity, pH, and ashes were similar between MCM samples clustering them, one TA sample was grouped with MCM samples by moisture content while another one was grouped with one MCM and one SS sample by ashes contents. One SS sample was grouped into an MCM sample by pH content, these samples coded as SS presents an outlier, and one sample was separated from others by FCR values. Other samples do not show the formation of groups. According to the scatter plot, the values of DPPH and FCR have a diametrical opposition to the majority of parameters, therefore the increase of one of these parameters is followed by the decrease of the other.

Considering these findings, we can affirm that the physicochemical composition between the samples of stingless-bee honey is variable, which impairs the correct discrimination of the samples at the species level. However, we noted that their characteristics are different when compared with *A. mellifera* honey which enables their discrimination in different clusters in the PCA plot.

5 Conclusion

In the present study, honey samples obtained from different species of Meliponinae subfamily showed a greater free acidity, high moisture content, lower content of reducing sugars, and darker color, when compared to *A. mellifera* honey samples collected from the same Brazilian Amazon Eastern region. These differences contributed to the discrimination of these honey samples through their physicochemical properties analyzed by the PCA plot. In addition, the honey samples of different species from the Meliponinae subfamily showed antioxidant activity comparable to that reported for *A. mellifera* honey samples. Thus, our results reinforce that the stingless-bee honey samples have unique characteristics, requiring a specific Brazilian regularization for them.

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