Comparison of physico-chemical properties of honeys produced by *Melipona beecheii* bees from low deciduous forest at harvest and post-harvest seasons

INA RAMÍREZ-MIRANDA – YOLANDA MOGUEL-ORDOÑEZ – DAVID BETANCUR-ANCONA

Summary

The demand for honey produced by stingless bees such as *Melipona beecheii* has increased due to its therapeutic properties. However, its physico-chemical characterization is still limited and quality control parameters are not sufficiently established. The objective of this study was to evaluate physico-chemical characteristics of *M. beecheii* honey obtained in various production seasons in meliponaries from the low deciduous forest zone of Yucatan, Mexico. Thirty-seven samples of honey were obtained in the harvest and post-harvest seasons during 2020–2021. According to the International Honey Commission methodologies, physico-chemical analyses were carried out and statistical analysis was performed to determine differences between seasons in the parameters evaluated. There were significant differences ($p < 0.05$) between harvesting seasons in the CIE colour parameters $L^*a^*b^*$ (3.03, 0.47, 0.43 and 3.48, 1.85, 3.05, respectively), free acidity (21.3 meq·kg^{-1} and 32.4 meq·kg^{-1}), ash (2.3 g·kg^{-1} and 4.5 g·kg^{-1}), hydroxymethylfurfural content (11.8 mg·kg^{-1} and 20.4 mg·kg^{-1}, and invertase number (7.8 and 11.6). For moisture, sugars, electrical conductivity, diastase activity, insoluble matter and proline content, no differences were found ($p > 0.05$) between honeys extracted at harvest and post-harvest seasons. The differences could be related to the diversity of melliferous flora present in this ecoregion.

Keywords

stingless bee; honey; physico-chemical; hydroxymethylfurfural

The genus *Melipona* includes approximately 40 known species of stingless honey-producing bees. These are found naturally in tropical and subtropical regions, including southern Asia, northern Oceania, Africa and Latin America. In the latter, the natural distribution is from Mexico to Argentina [1].

These bees originally resided in tropical lowland rainforests, depending on the cycles and variety of forest resources. Researchers of the species found in Mexico report on the distribution that the greatest diversity of these bees occurs in the southeast, particularly in the states of Campeche, Chiapas, Oaxaca, Quintana Roo, Veracruz and Yucatan. Two species of the genus, namely, *M. beecheii* and *M. yucatanica*, inhabit the Yucatan Peninsula, Mexico. According to records, *M. beecheii* was more exploited by the Maya long before the arrival of the Spanish colonizers, stingless beekeeping being a culturally and economically important activity in that region [2]. In addition to being excellent pollinators, these bees produce honey that was used within traditional Maya medicine to treat various diseases [3].

Several studies revealed *Melipona* honey as a functional food. However, due to its characteristic flavour and aroma as well as a more fluid texture and slower crystallization in relation to *Apis mellifera* honey, this honey is appreciated by consumers worldwide, making it more commercially valuable [4]. According to Martínez-Puc [5], the sale price of *Melipona* honey is at least ten times higher than honey from *A. mellifera* bee, which represents an economically viable option

Ina Ramírez-Miranda, Institutional Postgraduate Degree in Agricultural Sciences and Management of Natural and Tropical Resources, Autonomous University of Yucatán, Merida-Xmatkuil km 15.5, 97315 Merida, Yucatan, Mexico.
Yolanda Moguel-Ordońez, Mocochá Experimental Field, National Institute of Forestry, Agricultural and Livestock Research, 97454 Mococha, Yucatan, Mexico.
David Betancur-Ancona, Food Science Laboratory, Autonomous University of Yucatan, North Peripheral km 33.5, 97203 Merida, Yucatan, Mexico.

Correspondence author:
David Betancur-Ancona, e-mail: bancona@correo.uady.mx

for rural producers. Meliponiculture has great cultural relevance for the Maya population and its rescue, conservation as well as commercialization are imperative and necessary.

Honey quality standards have been established only for *A. mellifera*, following the guidelines of the international standards of the Codex Alimentarius Commission [6]. Due to the limited knowledge of stingless bee honey, it is not included in the standards, is not regulated by food control authorities and there are no quality guarantees for consumers. A major effort has been made by the International Honey Commission (IHC) to establish quality standards for bee products other than *A. mellifera* [7]. The physico-chemical profile of honey from stingless bees has been little explored, so more complete studies are needed to generate scientific knowledge related to the particularities of honey from each species of stingless bee. These types of studies will contribute to the generation of values to establish quality standards that promote the advancement of meliponiculture. This will be of fundamental importance to increase the value of their products, especially if it is done to enhance regional aspects since it will allow honey producers to generate income effectively.

In 2014, the Agricultural Development and Protection Agency (ADAB) of Bahia in Brazil, in collaboration with the Federal University of Recôncavo Baiano (UFRB, Cruz das Almas, Bahia, Brazil), published the Technical Identity Regulations for honey produced by *Melipona* stingless bees. The regulations considered the values of the main physico-chemical quality parameters that honeys must have to avoid fraud to promote quality and to secure food safety [8].

The objective of this study was to evaluate the physico-chemical characteristics of *M. beecheii* honey obtained in various seasons in meliponaries in the low deciduous forest zone of Yucatan, Mexico.

MATERIALS AND METHODS

Samples

From February 2020 to May 2021, 37 samples of *M. beecheii* honey were collected from meliponaries located in 18 municipalities in the low deciduous forest region of Yucatan, Mexico [9]. Fourteen samples were obtained in the post-harvest season (June, July, August, September and October 2020) and 23 in the harvest season (February, March, April, May and December 2020, and January, February, March, April and May 2021) according to the apibotanical calendar of the Yucatan Peninsula. Honey extraction was done by individual stingless beekeepers. Samples were collected, transported in 500-ml polyethylene containers, protected from light in an isothermal container and kept refrigerated at 4 °C for a maximum of 7 days until analysis.

Physico-chemical analysis of samples

The physico-chemical evaluation of the honey's quality, except for the determination of proline, was done using the methods validated and standardized by the International Honey Commission [10], which are used within the scope of Codex Alimentarius and the European Union Directive. Samples were analysed in duplicate and at the same time to ensure uniform conditions and comparability.

Moisture

The moisture content of the honey samples was determined based on how the refractive index increased with solids content, using a manual refractometer with integrated thermometer Abbe 120 1T (Atago, Bellevue, Washington, USA).

Sugars

The determination of sugars (glucose, fructose, saccharose) content was based on the modified method proposed by Ciursa and Oroian [11], using an Infinity 1290 HPLC equipment (Agilent Technologies, Santa Clara, California, USA) with a refractive index detector. The separation was performed on a column Phenomenex Luna Omega 3 μm Sugar 100 Å (150 mm × 4.6 mm, Phenomenex, Torrance, California, USA). The results were expressed as grams of sugars per kilogram of honey.

Crystallinity indices glucose–water/fructose ($G–W/F$), glucose/water (G/W) and fructose/glucose (F/G) were calculated using glucose, fructose and moisture contents to evaluate the tendency to crystallize or remain liquid.

Colour

This test was performed in 8453 UV-Vis Chem Station Rev. B.04.02 (Agilent Technologies), considering ten wavelengths for each of the chromatic parameters X, Y and Z. Approximately 2 ml of honey was transferred into a cuvette with a 10 mm light passage. The cuvette was introduced into the spectrophotometer previously calibrated with an analytical grade glycerol standard reference. To determine differences between colours perceived at close wavelengths, the values of X, Y and Z were transformed to values in CIE $L^*a^*b^*$ space, where L^* is the lightness or brightness, a^* corresponds to

the red-green colour gradient and *b** corresponds to the yellow-blue gradient. The values of chroma (*C)* and hue (*h)* were also calculated.

Before the analysis of colour, the honeys were liquefied in a water bath at a temperature of approximately 40 °C to achieve transparent samples without any dilution and measurements were performed in triplicate for each sample.

pH and free acidity

For pH determination, 10 g of honey was dissolved in 75 ml of carbon dioxide-free distilled water. The pH value of this solution was determined with an Orion 370 digital potentiometer using a PerpHecT glass combination electrode (Thermo-Fisher Scientific, Waltham, Massachusetts, USA). For the determination of acidity, the honey solution was titrated with a 0.1 $mol \cdot l^{-1}$ NaOH solution to pH 8.3 within 2 min. Acidity was expressed in milliequivalents of gluconic acid per kilogram of honey.

Ash

Gravimetric analysis was used to determine ash. An amount of 2 g of sample was transferred to a previously dried and weighted crucible. Then, the sample was heated in an electric grill until carbonized and kept in a muffle at 550 °C for 1 h. Finally, it was cooled in a desiccator and weighed. The proportion of ash was expressed in grams per kilogram of honey.

Electrical conductivity

Electrical conductivity (σ) was determined with the Oakton CON 450 conductivity meter (Thermo-Fisher Scientific) in a solution of 200 g of sugars per kilogram of honey dry matter in distilled water. The conductance reading was recorded in millisiemens at a constant temperature of 20 ± 0.5 °C.

Hydroxymethylfurfural

The hydroxymethylfurfural (HMF) content of the honey samples was determined based on the UV absorbance of HMF at 284 nm [10], using an 8453 UV-Vis spectrophotometer (Agilent). The HMF content was expressed as milligrams per kilogram of honey.

Diastase

The diastase activity was determined by colorimetric titration of a reaction of starch with iodine, where the enzyme acts under established conditions [10], using 8453 UV-Vis spectrophotometer. The time was reported in minutes and calculated by dividing 300 by the time, expressing the result as diastase number in Gothe units (or Schade units) per gram of honey.

Invertase

To evaluate the invertase activity, *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was used as substrate for the determination of the saccharose or invertase number in 5 g of honey. The amount of converted substrate was determined at 400 nm using 8453 UV-Vis spectrophotometer. The results were expressed as invertase number, which indicates the amount of saccharose per gram hydrolysed in 1 h by the enzyme.

Insoluble matter

For insiluble matter determination, a solution of 10 g of honey was dissolved in 100 ml distilled water at 80 °C and filtered through dried and weighted filter paper grade 5 (Whatman, Florham Park, New Jersey, USA). The retained content was washed thrice with approximately 100 ml of hot distilled water for each wash until it was free of sugars. The filter paper was dried at 135 °C for 1 h, cooled and weighted. Insoluble solids were expressed as grams of insoluble matter per kilogram of honey.

Proline

A volume of 0.25 ml of 980 $g \cdot l^{-1}$ formic acid and 1.0 ml of 3 $g \cdot l^{-1}$ solution of ninhydrin in monomethylated ethylene glycol ether were added to a solution of 2.5 g of honey in 50 m of distilled water. The solution was kept for 15 min in a boiling water bath and then 5 ml of 2-propanol solution in distilled water (1 : 1, v/v) was added. Absorbance was determined at 510 nm against a blank prepared with distilled water instead of formic acid and nihydrin using 8453 UV-Vis spectrophotometer. The proline content was expressed as milligrams of proline per kilogram of honey [12].

Statistical analysis

The harvest and post-harvest groups were compared using Mann-Whitney U statistic. R statistical software v. 4.0.2 (Lucent Technologies, Murray Hill, New Jersey, USA) was used for data analysis.

RESULTS AND DISCUSSION

Moisture

Moisture is the second major component in honey after sugars and one of the criteria for determining its quality. In addition, moisture is one of the parameters that influence the physical

Tab. 1. Moisture, fructose, glucose and reducing sugars content of honeys extracted in harvest and post-harvest seasons.

Sample	Moisture [g·kg^{-1}]	Fructose [g·kg^{-1}]	Glucose [g·kg^{-1}]	Reducing sugars [g·kg^{-1}]
Harvest season				
01	270	398	323	721
02	280	424	309	733
03	266	402	334	736
04	260	351	393	744
05	254	390	339	729
25	258	378	378	756
27	284	298	266	564
28	298	417	315	732
29	262	386	337	723
30	232	423	394	817
31	218	442	337	779
32	270	370	401	771
33	230	436	333	769
34	226	400	336	736
35	230	365	327	692
36	246	384	321	705
37	222	463	361	824
38	242	406	350	756
39	220	421	345	766
40	222	425	339	764
41	242	411	299	710
42	238	447	299	746
43	232	428	327	755
Mean*	248 [a]	403 [a]	338 [a]	740 [a]
SD	5	7	7	10
Minimum	218	298	266	564
Maximum	298	463	401	824
Post-harvest season				
07	262	439	334	773
08	250	401	341	742
09	250	428	343	771
10	268	426	322	748
11	252	438	352	790
12	252	445	325	770
13	250	438	303	741
17	256	431	351	782
18	254	425	366	791
21	238	447	364	811
22	218	429	381	810
23	208	383	314	697
24	262	351	345	696
26	254	357	314	671
Mean*	248 [a]	417 [a]	340 [a]	757 [a]
SD	4	8	6	12
Minimum	208	351	303	671
Maximum	268	447	381	811

* – different letters in superscript indicate statistical significance difference for component and between seasons ($p < 0.05$).
SD – standard deviation.

properties of honey, such as crystallization, colour or solubility [13].

Moisture in honeys extracted in the harvest season was on average 248 g·kg^{-1} and was the same as in the post-harvest season, observing no statistical difference ($p > 0.05$) between groups (Tab. 1). Although it was been reported that *A. mellifera* honeys obtained during periods of high rainfall (rainy season) had higher moisture content than honeys produced during periods of low rainfall (drought) [14], this was not observed in the present study. This could be due to the differences in maturation processes between the two periods. Therefore, season does not seem to influence *Melipona* honeys in the way it influences *Apis* honeys. The results obtained for the extracted samples, both at harvest and post-harvest seasons, were similar to those reported for *M. beecheii* honey by Souza et al. [15] with 245 g·kg^{-1} and Moo-Huchin et al. [16] with 210–253 g·kg^{-1} in Mexico.

Honey from stingless bees is usually characterized by having a higher content of moisture compared to honey produced by *A. mellifera*, with a maximum of 200 g·kg^{-1} accepted by the Codex Alimentarius [6], which is associated with the habitat of this type of bee, lowland tropical forests, where high relative humidity is present. It is also believed that the high moisture in stingless bee honeys (> 200 g·kg^{-1}) is mainly related to the number of individuals in the hive, which in turn is associated with the nectar dehydration process during honey maturation. A hive with a lower number of individuals, up to three thousand for *M. beecheii* versus sixty thousand for *Apis*, will be less efficient and produce more watery honey [17]. Another aspect to consider is that some genera of stingless bees open or keep open honey pots to use, instead of closing them and letting the honey mature.

Sugars

de Sousa et al. [18] reported that the content of reducing sugars tends to be higher in *Melipona* honey compared to other Meliponini. The results of the content of fructose, glucose and reducing sugars in the honeys are shown in Tab. 1.

Reducing sugars values were similar to the maximum value described by Moo-Huchin et al. [16] in Mexico, with 742 g·kg^{-1} for *M. beecheii* honeys. No statistical differences ($p > 0.05$) in sugar content were found between harvest seasons of the honeys.

The differences in sugar content determined in this study and by other authors could be related to the presence of floral species native to the low deciduous forest, where bees collect nectar. Species of the Fabaceae family were reported to account for a significant part of blooming and are known

for being a good source of nectar [19] due to an explosive opening mechanism that allows them to secrete large amounts of nectar preferred by some species of the genus *Melipona* [20].

High levels of fructose, which may be responsible for the intensity of sweet taste and high hygroscopicity, keeping the honey liquid for a long time [21], are commonly found in stingless bee honeys. However, in not all honeys the sugars are precipitated and granulated at the same time. Their tendency to granulate is directly related to certain parameters (crystallinity indices), such as glucose-water/fructose ratio (*G–W/F*), glucose/water (*G/W*), fructose/glucose (*F/G*) and melezitose content [13]. In this study, the first three parameters were determined, finding *G-W/F* of 0.2 for both honeys extracted in harvest and post-harvest seasons (Tab. 2). This indicated that the honeys will remain fluid for years. Honeys with *G/W* higher than 2.1 will crystallize quickly, so the average value of 1.4 also indicated that they will remain liquid. However, *F/G* of 0.8 and glucose content of approximately 330 g·kg^{-1} indicated the opposite, since it was observed that honeys with *F/G* of 1.14 and glucose contents of 400 g·kg^{-1} will tend to crystallize [13]. Therefore, it was expected that honeys will remain liquid for a long time but may crystallize with time.

Saccharose was detected in only two of the samples, with 28 g·kg^{-1} (sample 17) and 31 g·kg^{-1} (sample 34) of honey, indicating that the honeys were not harvested prematurely, allowing the enzymatic action of invertases and the absence of adulterants.

Tab. 2. Rate of crystallization of honeys extracted in harvest and post-harvest seasons.

Sample	*G–W/F*	*G/W*	*F/G*
Harvest season			
01	0.1	1.2	0.8
02	0.1	1.1	0.7
03	0.2	1.3	0.8
04	0.4	1.5	1.1
05	0.2	1.3	0.9
25	0.3	1.5	1.0
27	0.1	0.9	0.9
28	0.0	1.1	0.8
29	0.2	1.3	0.9
30	0.4	1.7	0.9
31	0.3	1.5	0.8
32	0.4	1.5	1.1
33	0.2	1.4	0.8
34	0.3	1.5	0.8
35	0.3	1.4	0.9
36	0.2	1.3	0.8
37	0.3	1.6	0.8
38	0.3	1.4	0.9
39	0.3	1.6	0.8
40	0.3	1.5	0.8
41	0.1	1.2	0.7
42	0.1	1.3	0.7
43	0.2	1.4	0.8
Mean*	0.2 [a]	1.4 [a]	0.8 [a]
SD	0.0	0.1	0.1
Minimum	–0.1	0.9	0.7
Maximum	0.4	1.7	1.1
Post-harvest season			
07	0.2	1.3	0.8
08	0.2	1.4	0.9
09	0.2	1.4	0.8
10	0.1	1.2	0.8
11	0.2	1.4	0.8
12	0.2	1.3	0.7
13	0.1	1.2	0.7
17	0.2	1.4	0.8
18	0.3	1.4	0.9
21	0.3	1.5	0.8
22	0.4	1.7	0.9
23	0.3	1.5	0.8
24	0.2	1.3	1.0
26	0.2	1.2	0.9
Mean*	0.2 [a]	1.4 [a]	0.8 [a]
SD	0.0	0.1	0.1
Minimum	0.1	1.2	0.7
Maximum	0.4	1.7	1.0

* – different letters in superscript indicate statistical significance difference for property and between seasons ($p < 0.05$).
G–W/F – glucose-water/fructose index, G/W – glucose/water index, F/G – fructose/glucose index, *SD* – standard deviation.

Colour

Colour is the first appealing attribute of honey, making it very important for marketing since it affects consumers' acceptance and preference. Statistically significant differences were observed ($p < 0.0.5$) between chromatic parameters *X, Y* and *Z* of honeys from the harvest season (0.33, 0.34 and 0.33, respectively) and from the post-harvest season (0.41, 0.38 and 0.20, respectively). The lightest honeys were those extracted during the harvest season. For colour space, honeys analysed exhibited very low *L** values of 3.03 for the harvest season and 3.48 for the post-harvest season, which can be considered a very low lightness. From the values of *a** (0.47 and 1.85) and *b** (0.43 and 3.05) for the harvest and post-harvest season, respectively, the honeys presented tendencies towards red and yellow. The honeys from the harvest season also presented green components according to the hue angle 179° when compared to that of the post-harvest season honeys at 82°. For

chroma, which represents the amount of colour and increases with brightness, the highest value of 4.33 was observed in extra-light amber honeys extracted in the harvest season with 1.78 for post-harvest season light amber honeys.

Between the months of June and September, some of the species that bloom in the low deciduous forest of the Yucatan peninsula are leguminous plants such as box katsim (*Acacia gaumeri*), kitam che' (*Caesalpinia gaumeri*), tsalam (*Lysiloma latsiliquum*), chukum (*Havardia albicans*) or chulúul (*Apoplanesia paniculada*). From October to December, Convolvulaceae such as sak katsim (*Mimosa bahamensis*), tso'ots kàab (*Distimake aegyptia*) or ak'il xíiw (*Jacquemontia pentantha*), as well as herbaceous legumes of the genus *Senna* bloom. It was reported that both Fabaceae (legumes) and Convolvulaceae present a high content of tannins and alkaloids, which is associated with a darker colour of honeys [22].

Another aspect to consider is that three of the fourteen samples obtained during the post-harvest period were obtained from meliponaries where production was carried out in a traditional way with jobons, where the extraction technique was runoff. The darkest colour was observed in honeys harvested by the traditional method, where the exposure of honey to high environmental temperature can modify the colour by accelerating the non-enzymatic browning mechanisms together with minerals from the batumen that are released during extraction [23].

pH and free acidity

The mean pH of honeys from the harvest and post-harvest seasons was 3.90 and 3.95, respectively, with no significant statistical difference observed ($p > 0.05$; Tab. 3). These values were similar to those reported by other researchers for the same species in Central and South America [24], even though the Malaysian Standard (MS 2683:2017) established a pH range of 2.5 to 3.8 for stingless bee honeys [25].

The opposite was observed with the free acidity parameter since it was 21.4 meq·kg^{-1} for honeys from the harvest season, lower than for those from the post-harvest season with 32.4 meq·kg^{-1} (Tab. 3). Although Vit et al. [3] established a maximum value of 70 meq·kg^{-1} and the regional ADAB regulation [8] of 50 meq·kg^{-1} for free acidity of honeys from the *Melipona* genus, lower values were determined in this study.

The higher acidity value observed in honeys extracted in the post-harvest season could be mainly due to the action of the enzyme glucose oxidase secreted by the bees' salivary glands, which pro-

Tab. 3. Values of pH, free acidity, ash and electrical conductivity of honeys extracted in harvest and post-harvest seasons.

Sample	pH	Free acidity [meq·kg^{-1}]	Ash [g·kg^{-1}]	σ [mS·cm^{-1}]
Harvest season				
01	4.00	22.0	5.2	0.53
02	3.88	25.9	4.2	0.43
03	3.97	24.1	3.3	0.65
04	3.84	27.8	1.9	0.64
05	3.97	26.0	2.3	0.61
25	3.63	17.5	2.0	0.33
27	3.55	35.8	2.5	0.98
28	3.24	33.9	0.8	0.34
29	3.39	32.9	0.7	0.37
30	4.06	11.0	1.5	0.40
31	4.23	8.3	1.4	0.32
32	3.63	23.9	0.9	0.37
33	3.95	15.7	0.8	0.36
34	4.07	26.8	2.1	0.77
35	3.72	12.9	0.7	0.21
36	3.64	12.1	0.5	0.20
37	4.03	15.6	2.9	0.56
38	3.77	11.1	0.8	0.20
39	4.38	25.7	2.3	1.04
40	4.08	16.5	1.1	0.52
41	4.07	28.6	7.1	0.85
42	4.30	19.2	3.2	0.93
43	4.31	18.5	3.7	0.95
Mean*	3.90 ^{a}	21.4 ^{a}	2.3 ^{a}	0.55 ^{a}
SD	0.11	1.6	0.2	0.10
Minimum	3.24	8.3	0.5	0.20
Maximum	4.38	35.8	7.1	1.04
Post-harvest season				
07	3.89	27.6	3.0	0.26
08	3.93	34.6	4.6	0.67
09	3.89	55.3	3.7	0.60
10	3.92	30.4	2.2	0.49
11	4.03	34.8	5.1	0.70
12	3.99	21.2	4.5	0.52
13	3.95	30.2	4.7	0.60
17	3.93	30.3	3.6	0.54
18	4.18	22.1	8.0	0.64
21	4.18	29.5	4.8	1.55
22	4.13	37.7	8.9	1.63
23	3.77	35.0	2.5	0.81
24	3.88	37.7	3.7	0.69
26	3.82	26.6	3.3	1.00
Mean*	3.96 ^{a}	32.4 ^{b}	4.5 ^{b}	0.68 ^{a}
SD	0.22	2.2	0.9	0.13
Minimum	3.77	21.2	2.2	0.26
Maximum	4.18	55.3	8.9	1.63

* – different letters in superscript indicate statistical significance difference for property and between seasons ($p < 0.05$).
σ – electrical conductivity, *SD* – standard deviation.

duces gluconolactone. It was observed that this transformation is faster in fluid honeys, associated mainly with the nectar secreted by legumes and Convolvulaceae during and after the rainy season in the Yucatan Peninsula since they have a higher average moisture than the nectar used by bees in the dry season. In addition to the enzymatic action, the higher acidity of honeys collected during the post-harvest season could be associated with a fermentation process due to the action of yeasts and a high environmental temperature during the extraction of the samples, which could have accelerated the production of organic acids.

Ash

In this study, a statistically significant difference ($p < 0.05$) in ash content was observed between the harvest and post-harvest groups, with 2.3 g·kg^{-1} and 4.5 g·kg^{-1} (Tab 3), respectively. These values were higher than those reported for the same species by Souza et al. [15] with 0.2 g·kg^{-1} and Álvarez-Suárez et al. [4] with 0.46 g·kg^{-1} in Cuba, but similar to those obtained by Moo-Huchin et al. [16] with 0.1–6.0 g·kg^{-1} in Mexico. Vit et al. [3] suggested a maximum of 5 g·kg^{-1} for honeys from the *Melipona* genus, while the regional ADAB regulation of Brazil [8] establishes a maximum of 6 g·kg^{-1}.

The ash content in honey is directly associated with the concentration of minerals, which in turn are influenced by their availability and environmental pollution, i.e. the excess or shortage of certain elements in soil or water. These are reflected in the chemical composition of the plants, in their nectar and pollen.

The content of minerals in honeys may differ even when they have the same botanical origin and are collected in the same locality as well as hive but in different seasons of the year. Likewise, in 3 meliponaries where samples were obtained during the post-harvest period, the extraction technique was runoff when using jobons. The batumen, which in meliponas usually contains a large amount of mud that may be extracted along with the honey and depending on the instrument and pore size used, remain in it even after filtering. This could explain the higher content of ash in the samples.

Electrical conductivity

According to Codex Alimentarius [6], currently electrical conductivity replaces the determination of ash in routine analysis. Its values are directly proportional to the values of ash content and acidity of honey, thus revealing the presence of ions, organic acids and proteins. The mean electrical conductivity value observed was 0.55 mS·cm^{-1} for honeys extracted in the harvest season and 0.68 mS·cm^{-1} for those extracted in the post-harvest season (Tab. 3). These results agree with those reported by Álvarez-Suárez et al. [4] in Cuba, with electrical conductivity of 0.50–0.66 mS·cm^{-1} and 0.58 mS·cm^{-1} for *M. beecheii*.

Albu et al. [26] suggested that the maximum admissible value for the electrical conductivity of honeys produced by stingless bees should be 0.50 mS·cm^{-1}, lower than 0.80 mS·cm^{-1} for *A. mellifera* honeys of floral origin (nectar) according to Codex Alimentarius [6]. However, according to the results obtained in this study, the proposed parameter should be reconsidered. The mineral content and acidity in the honeys extracted during the post-harvest season might be related to the increase observed in the electrical conductivity of these samples, which in turn influences their colour.

Hydroxymethylfurfural

From harvesting to packaging, honey may be exposed to various effects that cause, to a greater or lesser extent, deterioration of its intrinsic qualities. Therefore, from the point of view of honey freshness, HMF content is the general parameter used to evaluate the quality loss of honey during storage [27]. In the honeys analysed in this study, there was a significant statistical difference ($p < 0.05$) with values of 11.8 mg·kg^{-1} and 20.4 mg·kg^{-1} between honeys extracted in the harvest and post-harvest seasons, respectively (Tab. 4). These were all high values when compared to those reported by Álvarez-Suárez et al. [4] with 9.23 mg·kg^{-1} for *M. beecheii* honeys in Cuba. The higher HMF content in the post-harvest group could be due to the adverse environmental conditions during extraction and storage. HMF formation in honey occurs naturally due to the high content of reducing sugars and the presence of proteins as well as free amino acids, in particular lysine [28]. HMF formation is accelerated if the honey is subjected to high temperatures. However, researchers report some resistance to HMF formation in honey from stingless bees due to its moisture content and acidity, which slows down the Maillard reaction [29]. That is why the ADAB regulation [8] established a maximum of 10 mg·kg^{-1} for *Melipona* honey, considering that in freshly harvested honey the HMF content is very low. However, Abu Bakar et al. [30] suggests that the maximum HMF content should be 30 mg·kg^{-1} in honey from stingless bees, while Vit et al. [3] suggest < 40 mg·kg^{-1} as established for *A. mellifera* honey after less than six months of storage. The results

Tab. 4. Hydroxymethylfurfural content, diastase and invertase numbers of honeys extracted in harvest and post-harvest seasons.

Sample	HMF [mg·kg^{-1}]	Diastase number	Invertase number
Harvest season			
01	3.5	2.4	7.1
02	8.2	1.4	4.2
03	2.8	3.1	7.8
04	2.1	0.4	8.8
05	17.0	2.6	11.0
25	15.8	2.4	3.6
27	59.7	1.2	12.6
28	0.4	1.4	2.7
29	0.9	2.0	5.1
30	22.2	1.3	2.8
31	21.3	3.8	1.9
32	9.2	2.0	6.8
33	9.5	1.2	9.0
34	19.3	3.8	5.9
35	1.7	nd	8.4
36	0.9	1.0	6.1
37	14.2	1.4	6.1
38	3.1	1.6	20.2
39	9.2	2.0	6.7
40	31.3	1.3	6.6
41	10.7	4.0	22.2
42	4.5	nd	7.1
43	4.8	nd	5.7
Mean*	11.8^{a}	2.0^{a}	7.8^{a}
SD	2.8	0.2	1.0
Minimum	0.4	0.4	1.9
Maximum	59.7	4.0	22.2
Post-harvest season			
07	16.9	2.1	7.0
08	14.8	4.2	17.1
09	18.5	nd	10.1
10	3.3	1.2	4.7
11	13.1	4.2	15.1
12	10.3	1.7	3.8
13	18.0	1.7	18.3
17	6.2	1.3	16.4
18	11.2	1.2	6.7
21	12.0	9.3	14.9
22	37.5	3.5	13.6
23	61.6	4.1	10.3
24	35.2	4.1	17.1
26	27.1	nd	7.7
Mean*	20.4^{b}	3.2^{a}	11.6^{b}
SD	4.1	1.0	1.3
Minimum	3.3	1.9	3.8
Maximum	61.6	22.2	18.3

* – different letters in superscript indicate statistical significance difference for property and between seasons ($p < 0.05$). Diastase number is expressed as Gothe units per gram of honey.
HMF – hydroxymethylfurfural, nd – not detected, *SD* – standard deviation.

obtained in this research are not in accordance with the ADAB regulation [8].

Diastase and invertase

The main proteins added by bees during the honey maturation process are enzymes [30]. Diastase (amylase), a relatively heat- and storage-stable enzyme, cleaves starch to maltose. In the honeys tested, no statistically significant difference in diastase activity ($p > 0.05$) was observed between honey from the harvest and post-harvest seasons, with diastase numbers 2.0 and 3.2, respectively (Tab. 4). Although researchers such as Álvarez-Suárez et al. [4] reported diastase number 1.30 for honeys from *M. beecheii* in Cuba, most researchers report < 3.0. That is the value proposed by Vit et al. [3], who referred that honey of *Melipona* genus presented low values but this did not necessarily mean a lack of quality. This characteristic could be due to the high acidity of these honeys and their low pH, which would denature α-amylases since they are sensitive to pH values close to 3.0 [1]. de Oliveira Alves et al. [7] mentioned that high diastase activity could be related to unintentional adulteration by prolonged feeding of *Melipona* colonies with *A. mellifera* honey.

Invertase catalyses the conversion of saccharose to glucose and fructose. However, since it is more susceptible to deterioration compared to diastase, it is not often included in quality standards. About the invertase activity, a significant difference ($p < 0.05$) between the harvest and post-harvest season groups was observed (invertase number of 7.8 and 11.6, respectively; Tab. 4). These values were well below those reported by Umaña et al. [31] for Costa Rican honeys, with invertase number of 67.5. Differences in enzyme activity related to the botanical origin of the honey have been reported [32]. It was noted there that monofloral honeys of the Burseraceae family presented low activity of both enzymes, while for honeys of the Fabaceae family they observed moderate invertase activity. In the ecoregion selected for this study, low deciduous forest, both botanical families are represented, among other nectariferous species *Bursera simaruba* in the harvest season and *Acacia gaumeri, Caesalpinia gaumeri* and *Lysiloma latsiliquum* in the post-harvest season. Certain components of the nectar from these species could be stimulating enzyme production in bees, saccharose in the case of invertase, causing the viscosity of the nectar to determine the amount of bee salivation and, therefore, enzyme secretion. However, the variation in enzyme activity observed in the samples of this study,

in addition to saccharose content and nectar flow rate, may be affected by the age of the bees and genetic differences between colonies.

Insoluble matter

The content of total solids in honey samples from the harvest season was 0.04 g·kg^{-1} and for the post-harvest season was 0.03 g·kg^{-1}, observing no significant statistical difference ($p > 0.05$; Tab. 5). The presence of insoluble matter in honey including bee pollen, remains of honeycomb, as well as bee particles and dirt are criteria of pollution of honey by the extraction process [26]. In this study, the application of good practices by the stingless beekeepers to avoid the presence of impurities was observed, since most of these honeys are marketed as therapeutics. Although only few studies analysed the content of insoluble solids in stingless bee honey, the ADAB regulation for *Melipona* honey [8] refers to a maximum permitted value of insoluble solids of 1 g·kg^{-1}. The same value is established by Codex Alimentarius for unpressed *A. mellifera* honey [6]. The honeys in this study met the established specification. Most of the published articles did not mention the honey extraction technique, which is relevant according to Živkov Baloš et al. [23], who reported a higher insoluble solids content in sunflower honey harvested through traditional methods than with modern ones, which would also apply to the difference in honey extraction techniques for stingless bees' honey.

Proline

Content of proline, the main free amino acid in honey, is a criterion for estimating the quality and antioxidant activity of honey. In the present investigation, no statistically significant difference ($p > 0.05$) was observed between honey from the harvest and post-harvest season with proline content of 148 mg·kg^{-1} and 251 mg·kg^{-1}, respectively (Tab. 5). These results were well below those reported by Moo-Huchin et al. [16] with values ranging from 264.5 mg·kg^{-1} to 1 193.7 mg·kg^{-1} for *M. beecheii* honeys in Mexico.

Content of proline is not cited in Codex Alimentarius [6], however, a value below 180 mg·kg^{-1} for A. mellifera honey indicates probable adulteration by the addition of sugars. Factors such as the degree of nectar processing by the bees (salivary and pharyngeal secretions), saccharides content and glucose oxidase activity can influence the proline content in honey, but this is more associated with geobotanical parameters [33]. Thus, the variability observed in the samples of this study could mainly be due to the nectar

Tab. 5. Insoluble matter and proline content of honeys extracted in harvest and post-harvest seasons.

Sample	Insoluble matter [g·kg^{-1}]	Proline [mg·kg^{-1}]
Harvest season		
01	0.04	213
02	0.04	83
03	0.04	236
04	0.05	222
05	0.03	133
25	0.04	194
27	0.03	266
28	0.05	73
29	0.05	162
30	0.04	44
31	0.03	33
32	0.03	197
33	0.04	33
34	0.06	162
35	0.03	95
36	0.03	81
37	0.05	72
38	0.05	52
39	0.03	315
40	0.03	71
41	0.02	187
42	0.00	259
43	0.02	225
Mean*	0.04 ^{a}	148 ^{a}
SD	0.01	18
Minimum	0.00	33
Maximum	0.06	315
Post-harvest season		
07	0.03	56
08	0.04	376
09	0.08	291
10	0.02	42
11	0.02	159
12	0.03	116
13	0.02	144
17	0.03	155
18	0.03	94
21	0.02	287
22	0.04	531
23	0.02	530
24	0.03	510
26	0.04	226
Mean*	0.03 ^{a}	251 ^{a}
SD	0.01	47
Minimum	0.02	42
Maximum	0.08	531

* – different letters in superscript indicate statistical significance difference for property and between seasons ($p < 0.05$).
SD – standard deviation.

sources and to environmental conditions to which these sources were subjected. An increase in proline content, both in pollen and in nectar, was reported in plants in response to environmental stress [33].

CONCLUSIONS

The physico-chemical parameters evaluated in 37 samples of *M. beecheii* honey obtained from meliponaries located in the low deciduous forest of Yucatan showed a statistically significant difference ($p < 0.05$) in the parameters colour, free acidity, ash, HMF content and invertase activity between the harvest and post-harvest seasons. This could mainly be associated with their different botanical origins, as well as the environmental conditions to which they were subjected, even when the samples came from the same ecosystem. Physico-chemical parameters of all honey samples complied with quality requirements of the only normative reference issued so far, the ADAB regional regulation of Bahia in Brazil [8]. This study is one of the first reports on physicochemical characteristics of *M. beecheii* honey that delimited the collection area and compared the extraction seasons.

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