Physicochemical Properties of *Melipona beecheii* Honey of the Yucatan Peninsula

Victor M. Moo-Huchin¹, Gustavo A. González-Aguilar², José D. Lira-Maas¹, Emilio Pérez-Pacheco¹, Raciel Estrada-León¹, Mariela I. Moo-Huchin³ & Enrique Sauri-Duch⁴

¹Cuerpo Académico Bioprocesos. Instituto Tecnológico Superior de Calkiní, Av. Ah-Canul, C.P. 24900, Calkiní, Campeche, México

² Research Center for Food & Development (CIAD), AC., Carretera a la Victoria Km 0.6, Hermosillo (83000), Sonora, México

³ Universidad Tecnológica del Poniente, Calle 29 Las Tres Cruces, C. P. 97800, Maxcanú, Mérida, México

⁴ Instituto Tecnológico de Mérida, C.P. 97118, km 5 Mérida-Progreso, Mérida, Yucatán, México

Correspondence: Victor M. Moo-Huchin, Instituto Tecnológico Superior de Calkiní, Av. Ah Canul, C.P. 24900, Calkiní, Campeche, Mexico. Fax: 52-996-813-4870 Ext. 2000, Tel: 52-996-813-4870 Ext. 1002. E-mail: vmoo@itescam.edu.mx

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Abstract

The knowledge regarding the physicochemical characteristics of the honey produced by stingless bees is still limited, mainly due to the high diversity of the floral resources and the low production that is inherent to these species. This manuscript describes the physicochemical characterization of 27 honey samples produced by *Melipona beecheii*, from the Yucatan Peninsula, Mexico. The objective of this study was to contribute to the establishment of standards for quality control. The following parameters were evaluated in the honey samples: reducing sugars, moisture content, acidity, pH, hydroxymethylfurfural (HMF), ash, soluble solids, formol index, proline and color. Most of physico-chemical parameters fulfilled the quality criteria established by the International Legislation for Apis honey, with the exception of moisture content, which presented higher values; for that, the results indicate that the international standard procedures are not completing adequate for all the parameters analyzed on *Apis mellifera* honey and therefore is need establish a suitable standard of quality control for honey from *Melipona*.

Keywords: honey, Melipona beecheii, physicochemical characteristics, stingless bee

1. Introduction

Honey is the nectar collected from many plants and processed by bees. Honey from different locations possesses a unique combination of components and properties due to the variety of flora, climatic conditions located in its geographical region of production, and finally by the different processing technologies and storage conditions (Turhan, Tetik, Karhan, Gurel, & Tavukcuoglu, 2008). In both ancient and modern civilizations, honey has been seen as a natural product of great importance, with many functional applications. It is a rich source of readily available sugars, organic acids, various amino acids, and a good source of many biologically active compounds (Anupama, Bhat, & Sapna, 2003; Saxena, Gautam, & Sharma, 2010). All these nutritional, therapeutic and social roles have been noted in different cultures even though no distinctions have been observed between *Apis mellifera* and stingless bee honey.

The stingless bees are one of the most diverse, attractive, fascinating, conspicuous, and useful of all the insect groups of the tropical world. The keeping of stingless bees (Apidae, Meliponini), or meliponiculture, is carried out in a rustic and traditional way in tropical America by a variety of ethnic groups and rural populations. Within the stingless bee tribe there are about 500 species, the majority of them present in South America, and some in Australia, Asia and Africa (Vit, Bogdanov, & Kilchenmann, 1994). The distribution of stingless bee honeys in the world market is limited in comparison with honeys from *A. mellifera*. This is consequence of their limited industrial production, shorter shelf life and the lack of international quality standard, which in turn is due to a relatively limited knowledge of the product such as composition of bioactive compounds and some nutritional

M. beecheii is the most important stingless bee species in the Yucatan Peninsula of Mexico due to high honey yield and its reported medicinal properties. In the Yucatan peninsula, the bee *Melipona beecheii* was named "cab" or "kab" in the Mayan language. It was considered of such importance by the Mayan people that, after a long process of appropriation, the bees were deified and named "xunan cab," or "xunan kab" (Ocampo-Rosales, 2013). A wide range of attributes may suggest that *M. beecheii* honey enhances several systems to control digestive disorders, eye diseases, and respiratory infections, wound healing, post-birth recovery, fatigue, casts for fractures and skin ulcers (Vit et al., 2004). These attributes afford stingless bee honey greater value in comparison with *A. mellifera* honey.

Due to the lack of information regarding stingless bee honey, it is not included in the international standards for honey (Codex Alimentarius, 2001) and is not specified by the food control authorities. Vit et al. (2004) have proposed values for the physicochemical parameters for stingless bee honey; however, in the Yucatan Peninsula, Mexico like in other countries, the composition and bioactivity of these honeys is still unknown. Thus, in order to increase the knowledge about shed light quality of honey from stingless bees, the aim of this study was to determine the physicochemical characteristics of *M. beecheii* honey produced in different location of the Yucatan Peninsula, Mexico.

2. Materials and Methods

2.1 Honey Samples

For this study, 27 *Melipona beecheii* honey samples from the Yucatan Peninsula, Mexico were analyzed (Table 1). Samples of honey were collected from traditional hives in tree trunks (Figure 1) at different locations in the states of Yucatan, Quintana Roo and Campeche from March to April, 2010 (Figure 2). The honey from the tree trunk hives was obtained by breaking the pots and decanting it. All samples were filtered and stored in holders and transferred to the laboratory, where they were kept at 4 °C until analysis. The floral origin of *Melipona* honey samples is multi-flora (Chan-Rodríguez et al., 2012).

Sample Code	Location	Month and year of harvesting				
H1	Temozón Norte, Yucatán	March 2010				
H2	Yaxcabá, Yucatán	March 2010				
H3	Concepción, Calkiní, Campeche	October 2010				
H4	Calkiní, Campeche	October 2010				
Н5	Mérida, Yucatán	January 2010				
H6	San Antonio Sahcabchén, Calkiní, Campeche	May 2010				
H7	Mérida, Yucatán	December 2010				
H8	Celestún, Yucatán	December 2010				
Н9	Celestún, Yucatán	December 2010				
H10	Celestún, Yucatán	December 2010				
H11	Quintana Roo	October 2010				
H12	Quintana Roo	October 2010				
H13	Yaxcabá, Yucatán	March 2010				
H14	Santa Cruz Pueblo, Calkiní, Campeche	March 2010				
H15	Maní, Yucatán	April 2010				
H16	Valladolid, Yucatán	April 2010				
H17	Maní, Yucatán	April 2010				
H18	Maní, Yucatán	April 2010				
H19	Maní, Yucatán	April 2010				
H20	Yaxcabá, Yucatán	April 2010				
H21	Yaxcabá, Yucatán	April 2010				
H22	Yaxcabá, Yucatán	April 2010				
H23	Yaxcabá, Yucatán	April 2010				
H24	Yaxcabá, Yucatán	April 2010				
H25	Yaxcabá, Yucatán	April 2010				
H26	Yaxcabá, Yucatán	April 2010				
H27	Yaxcabá, Yucatán	May 2010				

Table 1. Samples of *M. beecheii* honey, location and date of harvesting from the Yucatan Peninsula, Mexico



Figure 1. Harvesting Melipona beecheii honey



Figure 2. Honey sampling locations in the Peninsula of Yucatan

2.2 Physicochemical Parameters

Physicochemical parameters of *Melipona* honey samples were determined according to the official method of the Association of Official Analytical Chemists (AOAC, 1990) and those reported by Viuda-Martos et al. (2010). The evaluated parameters were: reducing sugars (g/100 g), moisture content (g/100 g), free acidity (meq/kg), pH, hydroxymethylfurfural content (mg/kg), ash (g/100 g), soluble solids (°Brix), formol index (mL/kg), proline (mg/kg). In the determination of the honey's color, it was used a photometer with direct readout in mmPfund. Honey color is measured in millimeters on the Pfund scale compared to an analytical standard scale of reference on the graduation of glycerin (Almeida-Muradian et al., 2013). The use of this meter removes all the guesswork commonly associated with honey color measurement, providing accurate and repeatable results.

2.3 Statistical Analysis

All analyzes were carried out in triplicate and the data were expressed as means±standard deviations (SD), which were calculated using Excel (Microsoft Office, Version 2003).

3. Results and Discussion

Honey is a very complex matrix endowed with very specific physico-chemical properties that make it unique from other viscous solutions. Stingless bee honeys are gaining the attention of researchers given their importance as foodstuffs and traditional remedies in folk medicine of countries where *Melipona* spp. is endemic (Vit et al., 1994). Curiously, although the ethnopharmacobotanical tradition linked to these honeys, there is a lack of specific parameters for quality control stablished by institutional food control authorities comparable to that of *A. mellifera* honeys that allow to marketing in local and external areas.

Table 2 shows the physico-chemical properties of the 27 Melipona honey samples from the Yucatan Peninsula in Mexico. Moisture is a physico-chemical parameter that is related to the climatic conditions and degree of maturity of honey (Baroni et al., 2009). Moisture values of samples between 21 and 25.3 g/100 g honey were obtained and excluded in the water range limits (< 20 g/100 g) approved by the Codex Alimentarius Commision standard for honey of Apis mellifera (Codex Alimentarius, 2001). However, these moisture values obtained for these honey samples were below the maximum amount of water (30 g/100 g) suggested for Melipona honey (Vit et al., 2004). Also, the values of moisture obtained for Melipona honey of this study were similar to those reported for stingless bee honeys (Vit, 2009). The moisture content of honey is an important factor contributing to its stability and shelf-life. Higher moisture content could lead to undesirable honey fermentation during storage, commonly caused by the action of osmotolerant yeasts, resulting in the formation of ethyl alcohol and carbon dioxide (Saxena et al., 2010). The ethanol may break down into acetic acid and water, giving the honey a distinctly sour or "off-taste" and a runny texture with small bubbles, surface heaving or foaming. However, from the perspective of traditional medicinal use, it must be stressed that fermented honeys – or honeys particularly rich in water – are considered by natives to be particularly indicated in the treatment of respiratory disorders. This ethnomedical aspect is consistent with other studies conducted on stingless bee honeys from other South American regions (Vit et al., 2004).

The honey samples analyzed showed ash values ranging from 0.01 to 0.6% with a mean of $0.16 \pm 0.12\%$. Similar results were detected by Souza, Marchini, Oda-Souza, Carvalho and Alves (2009) for *Melipona* honey from the northeast area of Brazil. Except for sample H2, all other *Melipona* honeys had ash contents that are in accordance with the Codex Alimentarius Commision standard for honey of *Apis mellifera* (Codex Alimentarius, 2001), which stipulates a maximum of ash value of 0.5%. The variability in the ash content of honeys could be due to the differences in soil, atmospheric conditions, and in the material collected by the bees during foraging on the flora (Finola, Lasagno, & Marioli, 2007). Certain nitrogen compounds, minerals, vitamins, pigments and aromatic substances contribute to the ash content of honey (Mairaj et al., 2008) which is considered a quality criterion indicating the possible botanical origin of honey.

Soluble solids content expressed as °Brix of the *Melipona* honey samples ranged from 72.8 to 77.3, which are lower than those of other honey samples of *Apis mellifera* from other regions (Silva, Videira, Monteiro, Valentão, & Andrade, 2009; Viuda-Martos et al., 2010). Values of soluble solids obtained from the *Melipona* honey coincide with those reported by Lage et al. (2012) for three species of the Brazilian *Melipona*. Moreover, according to Anupama et al. (2003), there was a high negative correlation between °Brix and moisture content in *Apis* honey. Therefore, the lower content of soluble solids from *Melipona* honey may be related to their higher moisture content.

The acidity of the honey contributes to its flavor, improves antioxidant activity and is effective against the action of microorganisms (Cavia, Fernández-Muiño, Alonso-Torre, Huidobro, & Sancho, 2007). Variations in acidity have been attributed to the floral source and harvest season (Ojeda de Rodríguez et al., 2004). Free acidity is due to the presence of organic acids, particularly gluconic acid, which is in equilibrium with the corresponding lactones and some inorganic ions such as phosphate or sulfate. The free acidity of the *Melipona* honey samples varied from 13.0 to 71.3 meq/kg honey, with a mean of 35.0 ± 12.8 meq/kg honey, which is similar to the results published by Souza, Marchini, Oda-Souza, Carvalho and Alves (2009). International regulations specify a free acidity not higher to 50 meq/kg honey of *Apis mellifera* (Codex Alimentarius, 2001; European Union, 2002). Except for samples H4, H21 and H3, all other *Melipona* honeys had free acidity contents in accordance with these specifications.

In this work, the pH values of *Melipona* honey varied from 2.6 to 3.3 with a mean of 3.07 ± 0.2 . This mean value of pH was previously reported by Alves, Carvalho, Souza, Sodré and Marchini (2005) for honey samples of the *Melipona* collected in Brazil (pH 3.2).

Sample	Free acidity	Soluble solids (°Brix)	Moisture	Reducing Sugar	HMF	Ash	Formol index	pН	Proline	Color	
	(meq/kg)		(g/100 g)	(g/100 g)	(mg/kg)	(g/100 g)	(mL/kg)		(mg/kg)	(mm Pfund)	
HI	28.3±1.1	75.0±1.4	22.8±1.5	74.2±1.8	16.9±1.2	0.01±0.0	2.5±0.1	3.3±0.2	264.5±1.9	34.0±1.2	
H2	46.0±3.0	74.3±1.1	23.9±2.0	73.6±1.9	10.7±0.9	0.60±0.02	2.5±0.2	3.1±0.3	959.1±5.0	83.0±1.6	
H3	54.3±4.2	73.9±0.8	24.5±1.0	72.1±2.0	23.3±0.7	0.21±0.02	3.1±0.1	3.1±0.1	883.2±9.9	113.0±1.8	
H4	71.3±4.1	72.8±0.9	25.3±1.1	65.6±1.1	15.4±0.7	0.19±0.02	2.0±0.1	2.9±0.1	722.2±6.9	53.0±1.0	
H5	46.3±4.0	74.7±1.2	23.5±1.1	66.1±2.1	4.0±0.8	0.27±0.01	2.6±0.2	3.2±0.1	1182±10	67.0±1.2	
H6	42.3±2.3	73.9±1.1	24.4±1.0	70.1±1.1	22.3±0.9	0.22±0.01	2.0±0.1	3.2±0.1	552±9.8	60.0±1.2	
H7	40.3±2.0	75.4±1.1	22.8±1.1	62.6±1.1	45.5±0.4	0.26±0.01	1.7±0.1	3.1±0.2	542.8±9.9	98.5±1.2	
H8	43.0±2.1	74.7±1.6	23.6±1.3	68.2±1.3	40.4±1.1	0.01±0.0	2.0±0.1	3.2±0.2	568.1±9.7	81.0±1.3	
Н9	42.3±2.1	74.6±1.1	23.8±1.3	71.6±1.3	42.7±0.8	0.17±0.01	2.1±0.1	2.9±0.1	568.1±8.9	78.0±1.6	
H10	31.3±1.1	73.3±1.5	24.8±1.3	70.2±1.3	10.2±0.3	0.03±0.0	2.0±0.1	3.1±0.1	365.7±8.0	59.0±1.2	
H11	30.7±1.2	76.0±1.1	22.4±1.3	67.8±1.3	21.6±0.3	0.17±0.01	2.0±0.2	2.9±0.2	322.0±9.9	50.0±2.0	
H12	20.7±1.0	74.8±1.2	23.4±1.1	69.8±1.1	15.7±1.2	0.23±0.02	2.8±0.2	3.1±0.1	308.2±7.9	74.5±1.3	
H13	25.0±0.5	73.1±1.2	25.3±1.2	72.8±1.2	16.4±1.1	0.09±0.0	2.5±0.2	3.3±0.1	379.5±5.5	54.0±1.1	
H14	20.0±1.1	75.3±1.3	23.1±0.8	74.0±1.2	13.7±0.9	0.15±0.01	2.0±0.2	3.2±0.1	423.2±7.5	121.5±1.1	
H15	13.0±1.1	76.0±1.3	22.4±0.9	71.0±1.2	9.5±0.1	0.17 ± 0.01	2.4±0.1	3.3±0.1	784.3±9.6	39.0±1.0	
H16	25.0±0.5	75.0±1.0	23.0±0.7	70.6±1.2	29.6±0.5	$0.40{\pm}0.02$	2.0±0.1	3.2±0.2	1193.7±9.0	90.0±1.7	
H17	28.0±0.9	77.3±0.8	21.0±0.5	57.1±1.2	11.1±0.6	0.09±0.0	2.0±0.1	3.0±0.2	349.6±7.5	37.5±1.3	
H18	30.5±1.1	76.4±1.2	23.3±1.1	59.7±1.3	7.1±0.3	0.07 ± 0.0	1.6±0.1	2.6±0.2	483.0±9.9	19.0±1.5	
H19	24.0±1.2	75.1±1.1	21.9±1.2	65.3±2.0	15.6±0.4	$0.12{\pm}0.01$	2.3±0.2	3.0±0.2	342.7±9.8	34.0±1.7	
H20	30.5±1.1	76.5±1.1	21.9±1.1	65.3±1.1	17.8±0.7	$0.12{\pm}0.01$	1.9±0.1	3.1±0.2	351.9±9.8	33.0±1.8	
H21	60.5±2.1	74.9±1.4	23.5±1.2	66.7±1.5	8.6±0.5	$0.10{\pm}0.01$	3.0±0.3	2.9±0.2	1053.4±9.4	105.0±1.9	
H22	41.0±1.1	75.8±1.3	22.7±1.2	67.0±1.5	27±0.6	0.13±0.01	2.0±0.1	2.8±0.2	489.9±9.6	45.0±1.8	
H23	32.5±1.2	75.5±1.2	23.0±0.7	60.3±1.4	5.3±0.8	0.09±0.0	1.5±0.1	2.9±0.1	522.1±9.7	37.5±1.7	
H24	33.0±0.9	75.6±1.2	22.9±0.7	68.0±1.3	21.3±0.9	0.11 ± 1.1	2.5±0.2	3.1±0.1	653.2±8.7	40.0±1.6	
H25	35.5±0.9	76.0±1.3	22.4±0.6	67.0±1.2	4.2±0.9	0.11 ± 0.01	2.5±0.1	3.1±0.2	522.1±9.7	38.0±1.9	
H26	25.5±0.8	76.5±0.5	22.0±0.5	72.5±1.6	5.6±0.8	$0.10{\pm}0.01$	2.0±0.1	3.1±0.2	809.6±9.7	39.0±1.6	
H27	25.0±0.5	75.7±1.2	22.8±0.5	60.8±1.5	24±0.3	$0.12{\pm}0.01$	2.0±0.1	3.2±0.2	476.1±9.6	60.0±2.0	
Average±SD	35.0±12.8	75.1±1.0	23.2±1.0	67.7±4.6	17.9±11.1	$0.16{\pm}0.12$	2.2±0.4	3.07 ± 0.2	595.2±263	60.9±26.8	
Minimun value	13	72.8	21	57.1	4	0.01	1.5	2.6	264.5	19	
Maximum vaue	71.3	77.3	25.3	74.2	45.5	0.6	3.1	3.3	1193.7	121.5	
Coefficient of variation (%)	36.5	1.3	4.3	6.7	62	75	18.1	6.5	44.1	44	

Table 2.	Physicoc	hemical	properties	of I	Iel	lipona l	beeche	<i>ii</i> h	oney	from	of the	Yucata	n Peninsul	a, 1	Mexico
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On the other hand, the reducing sugars ranged from 57.1 to 74.2 g/100 g *Melipona* honey. All the samples studied attained the minimum amount of reducing sugars (50 g/100 g) established by Vit et al. (2004) as the standard of quality for *Melipona* honey. However, samples H7, H27, H23, H18 and H17 did not reach the minimum amount of reducing sugars, established by the Codex Alimentarius Commision standard for honey of *Apis mellifera* (65 g/100 g) (Codex Alimentarius, 2001). In addition, the values of reducing sugars reported in this study are similar to those found by Vit et al. (2004) for the honeys of stingless bees in Venezuela.

The HMF content of honey is an indicator of freshness, because this compound is not generally present in a fresh honey. The honey samples analyzed in this study showed HMF content values between 4.0 and 45.5 mg/kg *Melipona* honey. Honey samples analyzed in this experiment (Table 1) did not exceed the international regulation and suggested standards for *Melipona* honey, which stablish a maximum HMF of 40 mg/ kg for *Apis Mellifera* honey (Vit et al., 2004; Codex Alimentarius, 2001; European Union, 2002), except three samples (H7, H8 and H9) (40.4-45.5 mg/kg honey) which were not in accordance with the international regulation. This is probably due to overheating, a contributing factor for HMF formation. The heating may have been caused by mishandling during honey harvesting, with the hive exposed to the sun.

Honey commonly have different color spectrum, from straw yellow to amber, and from dark amber to almost black with a hint of red. This property apparently is related to the mineral content, pollen and phenolic compounds present in the honey which varies according to the geographical origin and botanical varieties visited by the bees (Ramalhosa, Gomes, Pereira, Dias, & Estevinho, 2011). The method of production and agricultural practices and extraction procedure also influence the color of honey. Some changes also occur during storage; browning/darkening of honey attributed mainly to Maillard reactions, caramelisation of fructose and

polyphenolic reactions, depending on storage temperature and/or duration influence this negative change (Bertoncelj, Dobersek, Jamnik, & Golob, 2007). In our study, the mean value of color was found at 60.9 mmPfund with a range of 19 to 121.5. According to Color Standards Designations for extracted Honey (USDA, 1985); the color of the *Melipona* honey studied was classified as: white (14.8%), extra light amber (26%), light amber (37%), amber (18.5%) and dark amber (3.7%). However, results obtained for different honeys produced in other areas reveal a significant or similar values to those obtained in this study (Alves et al., 2005; Almeida-Muradian et al., 2013) for *Melipona* honey. The content of proline is an indication of quality of the honey and is also commonly used as an indicator of adulteration, when proline content is below of 183 mg/kg (Bogdanov et al., 1995; Bogdanov, 1999). According to table 2, all the *Melipona* honey samples we studied had good proline levels of up to 183 mg/kg, indicating that all samples obtained in Yucatan, Mexico were free of adulteration. Proline is the most abundant amino acid in honey and is used as a standard to quantify amino acid content.

Formol index is an important parameter and represents a global measurement of amino compounds which facilitate the evaluation of protein and aminoacid content of honey (Alves et al., 2005). The formol index of *Melipona* honey was found to be in the range of 1.5-3.1 mL/kg, which is lower than that reported by Souza et al. (2009) for *Melipona* honey (*M. quadrifasciata anthidioides*) (3.06-9.01 mL/kg). This difference can be attributed to the content of amino acids and proteins of honey from the nectar of flowers of each region.

It should be noted that the values of acidity, HMF, ash, proline and color reported in this work presented a high variability among the samples of *Melipona* honey, as evidenced by the high values of the coefficient of variation (36.5%, 62%, 75%, 44.1% and 44%, respectively). In order to reduce these variations of evaluated parameters, it is necessary to increase the sample number and location which in turn will give us a better idea of quality and compare more properly with other honeys from other locations and production areas with similar environmental conditions.

4. Conclusions

According to our knowledge, this is the first time that is reported that physicochemical parameters of *Melipona* honey produced in the Yucatan Peninsula, Mexico. The results revealed many similarities to other *Melipona* honeys produced in other areas.

A significant number of analyzed honey samples meet the specifications of the International Legislation for physico-chemical characteristics, with the exception of moisture. Therefore, these parameters of honey produced in the Yucatan Peninsula allow to compete with great perspective with similar products in internal and international markets.

The high moisture value of the honey reported in this work could be a limiting factor to consider, due that can affect significantly shelf-life, due to the fermentation processes that are enhanced by the presence of free water.

According to literature and the findings in this paper a maximum moisture content of 35 g /100 g for honey of Melipona is proposed.

The results show that the current legislation regarding *Apis mellifera* honey is not adequate for all the characteristics analyzed, emphasizing the need for a suitable standard for stingless bee honeys in order to prevent adulterations and to allow its commercialization on a formal market.

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