Physicochemical properties of *Scaptotrigona mexicana* honey from the Highlands of Veracruz, Mexico

Propiedades fisicoquímicas de miel de *Scaptotrigona mexicana* de la Región Montañosa de Veracruz, México

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ABSTRACT. Stingless bees play an important role in maintaining the balance of the ecosystems they inhabit by actively participating in the pollination of most flowering plants. The objective of this study was to characterize physicochemically honey from the stingless bee species *Scaptotrigona mexicana* from Fortín de las Flores, Cañada Blanca, and Manuel León, in Veracruz, Mexico, collected in 2017 and 2018. The experimental design was completely randomized, with two factors: location and year of sampling. The analysis of variance showed that the interaction of factors affected water activity, but not hydroxymethylfurfural content. In honey from Fortín de las Flores, greater values for color, electrical conductivity, pH, lactone acidity, and total acidity were observed. The year with the highest recorded values of the measured parameters was 2017. The data obtained indicate good quality of young honey and that the highest values of the measured variables were observed in Fortín de las Flores.
Key words: Color, electrical conductivity, hydroxymethylfurfural, Meliponini, water activity.

RESUMEN. Las abejas sin aguijón juegan un papel importante para mantener el equilibrio de los ecosistemas en los que habitan al participar activamente en la polinización de la mayoría de las plantas con flores. El objetivo de este estudio fue caracterizar fisicoquímicamente la miel de *Scaptotrigona mexicana* de Fortín de las Flores, Cañada Blanca y Manuel León, en Veracruz, México, recolectada en 2017 y 2018. El experimento tuvo una distribución completamente al azar con diseño factorial, con los factores lugar y año de muestreo. El análisis de varianza mostró que la interacción de los factores afectó la actividad de agua, pero no el contenido de hidroximetilfurfural. En la miel de Fortín de las Flores se observaron mayores valores de color, conductividad eléctrica, pH, acidez de lactona y acidez total. El año con los valores más altos registrados de los parámetros medidos fue el 2017. Los datos indican buena calidad de miel joven y que los valores más altos de las variables medidas se observaron en Fortín de las Flores.
Palabras clave: Color, conductividad eléctrica, hidroximetilfurfural, meliponini, actividad de agua.

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INTRODUCTION

In addition to its significant nutritional characteristics, honey from stingless bees (family Apidae, tribe Meliponini) is widely used in traditional medicine in countries such as Mexico, Guatemala, Costa Rica, Colombia, and Brazil. Due to its properties, this honey has shown effectiveness in controlling eye, respiratory, digestive, gynecological, and dermatological ailments, placing the product in great demand in health food stores and pharmacies (Grajales-Conesa *et al.* 2018).

The physical and chemical parameters of natural honey, such as the concentration of hydroxymethylfurfural (HMF), color, acidity, and electrical conductivity, are strictly defined and constitute quality indicators that characterize individual varieties of honey. Measurement of these parameters is relatively simple, and the information provided is invaluable (May-Canché *et al.* 2022) and has allowed several laboratories to conduct in-depth studies on the subject (Hossain *et al.* 2022).

The parameter used for measuring the aging of honey is HMF concentration, which is the most significant intermediate product of two reactions: degradation of hexose and decomposition of 3-deoxyosone in the Maillard reaction (Salis *et al.* 2021). The color depends on several factors, although it is primarily related to the botanical origin and composition of the nectar, collection process, temperature, and storage time (Tkáč *et al.* 2022). Water activity (a_w) is a significant factor in preventing or restricting microbial growth and, in many cases, is the key parameter responsible for the stability of food, modulation of the microbial response, and determination of the type of microorganisms found in foods (Ikhsan *et al.* 2022).

The pH of honey is low which inhibits the presence and growth of microorganisms and allows the compatibility of honey with many food products in terms of pH and acidity (Islam *et al.* 2022). Stingless bees or meliponines represent “environmental health” for the ecosystems that they inhabit, as well as balance, to the extent that they actively participate in pollination of most flowering plants. In addition, these stingless bees are the backbone of the food chain that gives meaning to the complex and fragile balance of life in jungles and tropical and subtropical forests (de Matos Barbosa *et al.* 2022). Meliponiculture is an activity that is still in an early stage of development and requires a greater research effort. In addition to their biology, distribution, and classification, more information about meliponines is needed regarding their properties, uses, production, and transformation. Moreover, more information is needed about marketing of products from meliponines and especially regarding their relationship with native and cultivated plants within agro-ecosystems (Bratman 2020, May-Itzá *et al.* 2022, Simms and Porter-Bolland 2022). Despite the fact that several species of these stingless bees are distributed in Mexico, their study has been limited. This study aimed to physically and chemically characterize honey from these stingless bees in the locations Cañada Blanca and Manuel León of the municipalities Amatlán de los Reyes and Fortín de las Flores, Veracruz, Mexico, in a completely randomized experiment with a factorial design, being the two study factors: location of sampling (L) and year of sampling (Y).

MATERIALS AND METHODS

Honey

Samples of multifloral honey from the stingless bee species *Scaptotrigona mexicana* were collected in three different locations of the state of Veracruz, Mexico, during the spring of 2017 and 2018, specifically in the month of March. The sampling sites were Cañada Blanca (18° 57’ 10.5” LN, 96° 51’ 40.4” LO, 787 m), Manuel León (18° 54’ 27.8” LN, 96° 57’ 38.4” LO, 650 m), and Fortín de las Flores (18° 57’ 10.4” LN, 96° 55’ 40.4” LO, 884 m).

In Cañada Blanca, the most abundant flora is *Heliocarpus* (Malvaceae, Grewioideae), followed by the predominance of *Bursera simaruba* (Burseraceae). In Manuel León, the flora with the greatest presence are *Chamaecrista* (Fabaceae, Caesalpinioideae) and *Parthenium fruticosum* (Asteraceae). Finally, Fortín de las Flores has an abundant natural flora, including *Verbesina* (Asteraceae) and *Solanum* (Solanaceae), as well as

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the practice of growing flowers such as *Anthurium*, Orchidaceae, Arecaceae, and Tracheophyta. The location of honey collection site (L) and the year of sampling (Y) were included as study factors in a completely randomized factorial experiment.

Samples were collected in accordance with Codex Stan 12 (2001). Samples of 1 L were collected from each site, which were stored in amber glass vials at 4 °C for further analysis. Sample analysis was performed within 2 months of sample harvest.

Water activity (a_w)

This variable was determined using a Pawkit portable instrument (Aqualab Nelson; Pullman, WA, USA) with an accuracy of $\pm$ 0.02. For measurement, a honey sample was added to the capsule with the sensor placed above, and the reading was obtained over 5 min.

Color

This test was conducted with a spectrophotometer (HANNA HI96785; Woonsocket, RI, USA) with direct readings in mm Pfund in the range of 0-150 mm Pfund and an accuracy of $\pm$ 2 to 80 mm Pfund at 25 °C. Color classes are expressed in millimeters of the Pfund range and compared to a reference standard analytical range graduated with glycerin. In this case, honey was placed in the cell, and once the equipment was calibrated, the cell was inserted to perform the reading.

Electrical conductivity (EC)

A conductometer was used (HANNA DisT 3 HI98303; Woonsocket, RI, USA) with a measurement range of 1.999 dS m^{-1}, resolution of 0.001 dS m^{-1}, and accuracy of $\pm$ 2%.

Hydroxymethylfurfural (HMF)

This compound was determined by a reflectometer (Merck RQflex 10; Darmstadt, Germany) using Reflectoquant® test strips (Merck Millipore; Darmstadt, Germany), for which 5 mL of honey was diluted in 20 mL of distilled water and stirred. The strip was inserted and then read by the instrument.

Soluble solids

A digital refractometer was used (HANNA HI96813; Woonsocket, RI, USA) in which a drop of honey was placed on a lens that was traversed by a beam of light to indicate the content of soluble solids.

pH, free acidity, lactone, and total acidity

The pH value was measured with a potentiometer (Oakton CyberScan pH 2100 series; Vernon Hills, IL, USA) at a temperature between 25 and 28 °C. The analysis method is based on the neutralization of acids present in honey by titration according to method 962.19 of the AOAC manual (1995). For this procedure, 10 g of honey was dissolved in 75 mL of distilled water, which was stirred and titrated with 0.05 N NaOH at pH 8.5 (free acid). Then, the addition was stopped, and 10 mL of 0.05 N NaOH was immediately added for back titration with 0.05 N HCl at pH 8.3 (lactone acid). The total acidity is the sum of the free and lactone acid. The results are expressed in meq kg^{-1}.

Statistical analysis

Analysis of variance and Tukey's comparison of means tests ($p \leq$ 0.05) were conducted with the results obtained for the analyzed variables. The experiment had a completely randomized design with a factorial arrangement, with location of sampling (L) and year of sampling (Y) as the main factors to be evaluated. The statistical model is described as follows:

$$y_{ijk} = \mu + L_i + Y_j + (L \times Y)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the response variable at the location i(i=1,2,3), sampling year j(j=1,2), and replicate k(k=1,2,3), μ is the overall mean, L_i is the fixed effect due to location, M_j is the fixed effect due to sampling year, $(L \times Y)_{ij}$ is the interaction effect between location and sampling year, and ε_{ijk} is the experimental error assuming that each ε_{ijk} has a normal distribution with mean zero and constant variance σ^2.

RESULTS AND DISCUSSION

In Table 1, the main effect of the sampling site is observed, in which the sample from Fortín de las Flores had the highest average with a value of 4.81 $\pm$ 0.01 (n = 6). With respect to sampling year, two statistical groups were found, in which the 2018 harvest exhibited the highest pH (p < 0.001). For the interaction of both study factors, three statistical groups were obtained (p < 0.001), in which the Fortín de las Flores sample from both harvest years had a higher pH (4.81).

Physical and chemical characterization is usually performed to determine the qualities of honey, especially for marketing purposes. To analyze the composition of the honey, established parameters for assessing honey from *Apis mellifera* and *S. mexicana* were used. For the latter, Vit *et al.* (2004) have proposed some reference values. Free water is measured as the a_w and is considered an indicator of purity and the degree of maturity and stability of honey during storage (likelihood of decomposition by fermentation) (Da Silva *et al.* 2016). The average values reported in honey range from 0.77 to 0.91 (Ávila *et al.* 2019). The a_w values obtained in this study are slightly lower than those reported previously.

Many species of bacteria will grow if the a_w is between 0.94 and 0.99, and the a_w of ripened honey does not support the growth of yeast. For this reason, diluted honey with a higher a_w will not be effective against those species of bacteria that grow most rapidly at an a_w of 0.99 (Saranraj *et al.* 2016).

Assessment of HMF is used to determine the quality of honey. In fresh honey, this compound is usually not present. High concentrations of HMF in honey indicate overheating, poor storage conditions, and aging of the honey (Tadese *et al.* 2020). The Codex Alimentarius Commission and the European Union established that the allowable concentration of HMF in honey must not exceed 80 and 40 mg kg^{-1}, respectively. However, the European Union provides for some exceptions, for example, the allowable value in honey from countries or regions with tropical climates is up to 80 mg kg^{-1}, as is the case of honey tested in our country (Codex Alimentarius Alinorm 2000). In this study, the recorded values of HMF in all places and years of sampling are low, which is indicative of young, high-quality honey.

The color of liquid honey ranges from clear and colorless to dark amber or black and depends on its botanical origin, age, and storage conditions. The color values obtained in this study were generally above 60 mm Pfund, indicating dark honey. When analyzing the color of honey samples produced in the provinces of Chaco, Argentina, Salgado and Maidana (2014) reported differences depending on the vegetation at the collection site. They observed that dark honey samples were obtained from areas where the native forests still prevail and forest species are the primary source of nectar, while clear honey samples were collected in agricultural areas of the province, where there is little crop diversity. Likewise, in this study, the honey that had the highest color value was obtained from Fortín de las Flores, an area with greater variability of flora and fewer disturbances, given the distance that stingless bees travel to find meliponaries. While the Cañada Blanca location also features little disturbance, its landscape is dominated by coffee and citrus orchards.

Similar to color, the EC is a good criterion for the determining the botanical origin of honey and is therefore a variable often used in the control of quality and purity. Honey contains organic acids and mineral salts, compounds that are chemically "ionizable" when in solution and can conduct electricity (Yadata 2014). In the case of honey collected in Fortín de las Flores, there was a positive relationship between color and EC, which is consistent with the results reported by Salgado and Maidana (2014). Furthermore, the EC values obtained, particularly in honey from Fortín de las Flores, greatly exceed the value established by Codex Alimentarius for floral honey, which is 0.8 dS m^{-1} (Codex Alimentarius Alinorm 2000). These high EC values could indicate the presence of honeydew in the honey (honeydew honey), which is also associated with darker honey, as in this case (Escudero *et al.* 2012).

The soluble solids content for honey is 75 °Bx according to AOAC-932.12. Pattamayutanon *et al.* (2015) reported an average of 81.18 °Bx in a study

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Table 1. Main effects of study factors (location and year of sampling) and their interactions on the measured variables in stingless bee honey.

Study factor	Level of factor	Water activity (a_w)	HMF (mg kg^{-1})	Free acidity (meq kg^{-1})	Lactone acidity (meq kg^{-1})	Total acidity (meq kg^{-1})
Location of sampling (L)	Cañada Blanca	0.732 ± 0.010 (n = 6)^a	2.88 ± 0.70 (n = 6)^a	1.027 ± 0.098 (n = 6)^a	39.67 ± 05.05 (n = 6)^{ab}	40.69 ± 05.13 (n = 6)^{ab}
	Manuel León	0.723 ± 0.012 (n = 6)^a	3.45 ± 0.35 (n = 6)^a	0.953 ± 0.308 (n = 6)^b	37.33 ± 01.97 (n = 6)^b	38.52 ± 01.96 (n = 6)^b
	Fortín de la Flores	0.732 ± 0.012 (n = 6)^a	2.81 ± 0.59 (n = 6)^a	1.010 ± 0.156 (n = 6)^a	42.17 ± 02.48 (n = 6)^a	43.18 ± 02.61 (n = 6)^a
Year of sampling (Y)	2017	0.730 ± 0.014 (n = 9)^a	3.17 ± 0.48 (n = 9)^a	1.016 ± 0.170 (n = 9)^a	37.67 ± 02.92 (n = 9)^b	38.68 ± 02.87 (n = 9)^b
	2018	0.728 ± 0.008 (n = 9)^a	2.92 ± 0.63 (n = 9)^a	0.978 ± 0.231 (n = 9)^b	41.78 ± 03.60 (n = 9)^a	42.91 ± 03.60 (n = 9)^a
Location of sampling (L)	**Year of sampling (Y)**	**Water activity (a_w)**	**HMF (mg kg^{-1})**	**Free acidity (meq kg^{-1})**	**Lactone acidity (meq kg^{-1})**	**Total acidity (meq kg^{-1})**
Cañada Blanca	2017	0.740 ± 0.000 (n = 3)^a	2.10 ± 0.90 (n = 3)^a	0.940 ± 0.035 (n = 3)^c	35.33 ± 00.58 (n = 3)^b	36.27 ± 00.56 (n = 3)^b
	2018	0.723 ± 0.006 (n = 3)^{ab}	3.67 ± 0.76 (n = 3)^a	1.113 ± 0.012 (n = 3)^b	44.00 ± 02.65 (n = 3)^a	45.11 ± 02.64 (n = 3)^a
Manuel León	2017	0.713 ± 0.006 (n = 3)^b	3.57 ± 0.29 (n = 3)^a	1.233 ± 0.031 (n = 3)^a	37.00 ± 02.65 (n = 3)^b	38.23 ± 02.62 (n = 3)^b
	2018	0.733 ± 0.006 (n = 3)^{ab}	3.33 ± 0.47 (n = 3)^a	0.673 ± 0.023 (n = 3)^d	37.67 ± 01.53 (n = 3)^b	38.81 ± 01.53 (n = 3)^b
Fortín de las Flores	2017	0.737 ± 0.012 (n = 3)^a	3.87 ± 0.08 (n = 3)^a	0.873 ± 0.046 (n = 3)^c	40.67 ± 02.08 (n = 3)^{ab}	41.54 ± 02.11 (n = 3)^{ab}
	2018	0.727 ± 0.012 (n = 3)^{ab}	1.77 ± 0.20 (n = 3)^a	1.147 ± 0.050 (n = 3)^{ab}	43.67 ± 02.08 (n = 3)^a	44.81 ± 02.12 (n = 3)^a

Table 1. Continuation.

Study factor	Level of factor	Color (mm Pfund)	Electrical conductivity (dS m^{-1})	Soluble solids (°Bx)	pH
Location of sampling (L)	Cañada Blanca	91.33 ± 55.35 (n = 6)^b	0.550 ± 0.100 (n = 6)^c	71.5 ± 1.1 (n = 6)^a	4.65 ± 0.07 (n = 6)^b
	Manuel León	77.50 ± 4.55 (n = 6)^c	0.817 ± 0.090 (n = 6)^b	71.6 ± 0.4 (n = 6)^a	4.65 ± 0.07 (n = 6)^b
	Fortín de la Flores	128.33 ± 23.88 (n = 6)^a	1.233 ± 0.200 (n = 6)^a	71.7 ± 0.7 (n = 6)^a	4.81 ± 0.01 (n = 6)^a
Year of sampling (Y)	2017	124.00 ± 33.26 (n = 9)^a	0.933 ± 0.370 (n = 9)^a	71.2 ± 0.6 (n = 9)^b	4.66 ± 0.12 (n = 9)^b
	2018	74.11 ± 28.52 (n = 9)^b	0.800 ± 0.260 (n = 9)^b	72.0 ± 0.6 (n = 9)^a	4.75 ± 0.05 (n = 9)^a
Location of sampling (L)	**Year of sampling (Y)**	**Color (mm Pfund)**	**Electrical conductivity (dS m^{-1})**	**Soluble solids (°Bx)**	**pH**
Cañada Blanca	2017	141.67 ± 7.64 (n = 3)^a	0.633 ± 0.058 (n = 3)^{de}	70.5 ± 0.3 (n = 3)^d	4.58 ± 0.01 (n = 3) ^{c}
	2018	41.00 ± 1.00 (n = 3)^d	0.467 ± 0.058 (n =3)^e	72.5 ± 0.4 (n = 3)^a	4.71 ± 0.01 (n = 3)^b
Manuel León	2017	80.33 ± 5.33 (n = 3) ^{c}	0.767 ± 0.116 (n = 3)^{cd}	71.9 ± 0.2 (n = 3)^{ab}	4.58 ± 0.02 (n = 3)^c
	2018	74.67 ± 1.52 (n = 3) ^{c}	0.867 ± 0.058 (n = 3) ^{c}	71.2 ± 0.2 (n = 3)^{bc}	4.71 ± 0.01 (n = 3)^b
Fortín de las Flores	2017	150.00 ± 0.00 (n = 3)^a	1.400 ± 0.058 (n = 3)^a	71.1 ± 0.2 (n = 3)^{cd}	4.81 ± 0.02 (n = 3)^a
	2018	106.67 ± 4.16 (n = 3)^b	1.067 ± 0.058 (n = 3)^b	72.3 ± 0.2 (n = 3)^a	4.81 ± 0.02 (n = 3)^a

Means ± SD with different superscript letters for each study factor or interaction for each variable indicate statistically significant differences (Tukey's test, $p \leq 0.05$).

conducted on honey samples of *A. mellifera* in Thailand. These values are higher than those recorded in this study, and there was no statistically significant difference by location of sampling sites. The low pH of honey inhibits the presence and growth of microorganisms and allows compatibility of honey with many food products in terms of pH and acidity (Silva *et al.* 2013). According to a study conducted on *A. mellifera* honey samples in Cuba, the pH value was 4.76 (Alvarez-Suarez *et al.* 2018). In honey of the species *S. mexicana*, the pH was 3.50 to 3.96 (Jimenez *et al.* 2016). In this study, higher pH values were obtained.

The most prevalent acid in honey is gluconic acid (AOAC 1995), which results from the action of the enzyme glucose oxidase on a glucose molecule. According to Jimenez *et al.* (2016), the value for acidity in honey from the species *S. mexicana* is 32.90 to 35.10 meq kg^{-1}. The allowable value for acidity in honey from the species *Scaptotrigona* is 85.0 meq kg^{-1}, thus the samples tested are below the maximum allowable value, indicating that they are not very susceptible to fermentation (Vit *et al.* 2013). In addition, it has been noted that the acidity of honey is important for preventing the growth of microorganisms (Lage *et al.* 2012). The lactic acidity (lactone) values in this study were greater than those reported by Bergamo *et al.* (2019) in honey of *A. mellifera* (2.18 and 13.61 meq kg^{-1}). In *Heterotrigona itama* honey from Malaysia, values between 129.2 and 144.4 meq kg^{-1} have been reported for this indicator (Kek *et al.* 2018). Comparatively, these values are higher than those recorded in this study.

The honeys analyzed from the different municipalities have important physicochemical differences, depending on the place and the year of sampling, and these results can be compared with other studies to contribute to the development of a specific standard for determining the quality of this honey.

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