Chapter

Pot-Pollen and Pot-Honey from Stingless Bees of the Alto Balsas, Michoacán, Mexico: Botanical and Physicochemical Characteristics

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Abstract

The demand for stingless bees' products (pot-honey and pot-pollen) has increased. No formal quality standards have been defined, which is very complex, because of the variety of species and types of honey specific to each region. For this reason, it is important to deepen the understanding of stingless bees' honey characteristics. From the above, the aim of this chapter is to present the advances in the characterization of botanical origin of stingless bees' honey, and the analysis of their physicochemical properties in the Alto Balsas, Michoacán, Mexico, as a way to contribute to the strengthening of new local economic strategies, generating information on the quality of the honey produced in the region.

Keywords: Meliponini, meliponiculture, botanical origin of honey, melissopalynological analysis, honey quality, analytical electrochemistry

1. Introduction

The work we present is part of a research project that has been carried out since 2014 to date, in the area that we refer to as *Alto Balsas Michoacano*. The main objective of the project has been to generate information about the human-stingless bees system in the perspective of contributing to the construction of a harmonious and sustainable relationship between the inhabitants of the study region, their surrounding ecosystems and this group of insects. This has allowed us to orient different lines of research to generate a comprehensive understanding of the relationship of humans with stingless bees in this particular region. Specifically we have directed our efforts to: (1) document the diversity of stingless bees' species; (2) document the knowledge, management practices and local uses of products obtained from these insects; (3) the implementation of alternative management practices, and (4) the analysis of the availability of plant resources for stingless bees.

At the moment we identified a total of nine stingless bees' species in the study area [1]. Our inventory included a new registry of *Plebeia fulvopilosa*, Ayala (1999) for the region [2]. Local people use all the taxa identified since they provide honey, beeswax, and pollen. In a special way, *Melipona fasciata* Latreille (1811) has the highest local preference for the quality of its honey, while *Scaptotrigona hellwegeri* Friese (1900) and *Geotrigona acapulconis* Strand (1919) have a greater preference for their wax, since according to local people this species produces the highest amount of beeswax [3].

Stingless bees have been of great economic importance for the production of honey, a product that has been used since ancient times, and particularly in the case of Mexico, have been part of the social and religious life of different cultural groups [4].

Through our experience we have observed two relevant phenomena in the context of the study region, while is true that local knowledge about use, management and ecological issues on stingless bees is persistent, is being lost intergenerationally together with the disuse of the products of these bees, accompanied by the decline in stingless bee populations [3, 5]. On the other hand, new expressions are arising which re-signify stingless bees and their ecological, economic and social importance expressing in the emergence of local initiatives that seek not only to promote the production of stingless bees' honey, but also perspectives for their conservation [1].

In this context, we have oriented efforts toward the strengthening of the productive activity focusing on the honey characteristics that is being produced in the region. Primarily, we are interested in knowing the botanical origin of honey since its properties depend on the origin of the nectar or the secretions used by bees [6, 7]. Nectar (flower honey) are classified according to the main source where the bees collect the nectar being monofloral or multifloral [8, 9]. In addition, we consider relevant this source of information to understand the implications of environmental heterogeneity on the availability of resources for bees and their foraging patterns.

In a complementary way, we have been analyzing the physicochemical properties of honey. Recently the demand for natural, organic and homeopathic products has increased, including those of stingless bees. Although there are several works that seek to characterize the physicochemical properties particularly for the stingless bees' honey [10, 11] have not yet been defined formal norms or quality standards, which is very complex because of the variety of species and types of honey specific to each region. For this reason, it is important to deepen the understanding of the physicochemical stingless bees' honey characteristics.

From these perspectives, the aim of this chapter is to present the advances in the characterization of botanical origin of stingless bees' honey, and the analysis of their physicochemical properties in the study area.

2. Study area

The Balsas River Basin covers eight states, particularly for Michoacán this basin can be divided into three sub regions, taking an altitudinal criterion: Alto Balsas, Medio Balsas, and Bajo Balsas (Tepalcatepec). The specific area of study falls inside the sub-region of Alto y Medio Balsas. The process of collecting honey samples was carried out mainly in the municipality of Madero (19° 10′, and 19° 33′ N and 100° 59′, 101° 22′ W), it borders to the north with the municipalities of Acuitzio, Morelia and Tzitzio; to the east with Tzitzio and Tiquicheo de Nicolás Romero; to the south with Carácuaro and Nocupétaro; to the west with Nocupétaro, Tacámbaro, and Acuitzio. It has an altitudinal gradient that goes from 800 to 2900 m and has a temperate humid climate with summer rains (Cw) and in the south it is warm subhumid with summer rains (Aw). It has an annual rainfall of 1654.5 mm and its temperature ranges from 7.5 to 23.9°C [12]. In the municipality, the pine-oak forests are predominant and it presents a small portion of tropical dry forest. At the moment, a total of 62 species of api-botanical interest have been recorded, of which 65% provide nectar, 22% nectar and pollen, and 13% pollen to bees [13]. The most representative families are Asteraceae, Leguminosae, and Lamiaceae. It has been described that there are two flowering peaks, one that goes from March to May corresponding to the dry season and another that goes from September to November, which corresponds to the rainy season, specifically in the fall [13].

3. Collection and analysis of pot-honey and pot-pollen samples

For the analysis of the botanical origin, samples of pot-honey and pot-pollen were collected during May 2017 (dry season) and December 2017, from four species of stingless bees: *Melipona fasciata* (H-4691; H-4690), *Nannotrigona perilampiodes* (H-4686; H-4687), *Plebeia fulvopilosa* (H-4735; H-4736) and *Scaptotrigona hellwegeri* (H-4741; H-4742). Honey samples were obtained from hives under meliponiculture management in three communities of Michoacán State: Piumo, San Pedro Piedras Gordas and Etucuaro **Table 1**. The samples were cataloged and entered into the collection of the Palynology Laboratory of the Geology Institute of the National Autonomous University of Mexico, in which the melissopalynological process was performed. In general, the samples were processed by conventional chemical methods and to know the representativeness of each taxon the percentages of each pollen type were calculated from the count of 500 pollen grains per sample, in random transects [14]. The description and identification of the pollen grains was made under the microscope. The preliminary identification of pollen grains was carried out by comparison with the help of specialized keys from the Reference Palynological Collection of the Geology Institute. Honey was characterized as "monofloral" when in its composition a botanical species with pollen percentage ≥45% and "multifloral," mixed or polifloral prevailed when two or more species presented with percentages ≥10% [14].

We analyzed also honey physicochemical properties of the most frequently used stingless bees' species: *Melipona fasciata*, *Scaptotrigona hellwegeri*, *Geotrigona acapulconis*, *Frieseomelitta nigra*, *Plebeia fulvopilosa* and *Nannotrigona perilampoides* (of wild hives and hives under meliponiculture management). The physicochemical methods used to obtain the results of **Table 2** have been previously described [15]. Quantification of HMF and fructose was carried out with differential pulse polarography (DPP) using the calibration curve method. DPP is direct, and, because no other chemical reagents are used, lowers costs and health risks. On the other hand, the potentiometric monitoring of the progression of the Fehling reaction confirmed the reaction stoichiometry and produced more accurate and reproducible results. A principal component analysis (PCA) was used to identify relationships and patterns between honey samples and physicochemical characteristics.

4. Pot-pollen and pot-honey characteristics

4.1 Botanical origin based on melissopalynological analysis

Melissopalynological study showed polylectic behavior of *M. fasciata*, *N. perilampoides*, *P. fulvopilosa* and *S. hellwegeri* to collect pollen and nectar resources. Two monofloral honey samples of *Lopezia* sp. (52.5%) and *Paullinia* sp. (78.7%) were recorded for *P. fulvopilosa* and *S. hellwegeri* respectively. Moreover, honey sample of *M. fasciata* was bifloral of both Rhamnaceae (48%) and *Quercus* sp.

	Melipona fasciata		*Nannotrigona perilampiodes*		*Plebeia fulvopilosa*		*Scaptotrigona hellwegeri*	
	Honey	**Pollen**	**Honey**	**Pollen**	**Honey**	**Pollen**	**Honey**	**Pollen**
Date of collect	**May/2017**	**May/2017**	**May/2017**	**May/2017**	**December/2017**	**December/2017**	**December/2017**	**December/2017**
Localities	**Piumo**		**San Pedro Piedras Gordas**		**Etucuaro**		**Etucuaro**	
Num. catalog	**(H-4691)**	**(H-4690)**	**(H-4686)**	**(H-4687)**	**(H-4735)**	**(H-4736)**	**(H-4741)**	**(H-4742)**
Attalea sp.						66.9		
Alnus sp.			8.4					
Anacardiaceae			1.7					
Asteraceae			22.5	2.1	4.5			
Betulaceae	2.6							
Brassica sp.			3.0					
Bursera sp.			8.4					
Cyperaceae	2.2							
Fabaceae					12.3			
Fraxinus sp.	1.5	2.7	25.8		1.1			
Lopezia sp.					52.5			
Melastomataceae		10.1						
Paullinia sp.							78.7	
Polygalaceae					3.4			
Quercus sp.	38.5		18.8	96.6				
Rhamnaceae	48.0	85.3						
Rubiaceae						24.0		
Salix sp.							9.7	98.5

	Melipona fasciata		*Nannotrigona perilampiodes*		*Plebeia fulvopilosa*		*Scaptotrigona hellwegeri*	
	Honey	**Pollen**	**Honey**	**Pollen**	**Honey**	**Pollen**	**Honey**	**Pollen**
Date of collect	**May/2017**	**May/2017**	**May/2017**	**May/2017**	**December/2017**	**December/2017**	**December/2017**	**December/2017**
Localities	**Piumo**		**San Pedro Piedras Gordas**		**Etucuaro**		**Etucuaro**	
Num. catalog	**(H-4691)**	**(H-4690)**	**(H-4686)**	**(H-4687)**	**(H-4735)**	**(H-4736)**	**(H-4741)**	**(H-4742)**
Sapindaceae			1.5					
Sicyos sp.					3.4			
Solanum sp.	2.6							
Vernonia sp.	1.8							
Others	2.8	1.9	9.9	1.3	22.8	9.1	11.6	1.5

Table 1.
Percentages of botanical taxa recovered from pot-honey and pot-pollen samples in the melissopalynological analyses.

(38.5%). Because *Quercus* sp. produces honeydew, this honey (H-4691) might be considered that was made with a mixture of nectar of flowers and honeydews. On the other hand, pot-honey sample of *N. perilampoides* was multifloral, this bee species also combined nectar of Asteraceae (22.5%) and *Fraxinus* sp. (25.8%) flowers, as well as honeydews of *Quercus* sp. (18.8%). It is important to mention that *Quercus* sp. is also considered an excellent polliniferous tree (**Table 1**).

Respect to pot-pollen samples, the pot-pollen analyses gave evidence of polliniferous preferences of only one resource in all four stingless bee species. The best polliniferous plants were Rhamnaceae (85.3%) for *M. fasciata*; *Quercus* sp. (96.6%) was exploited by *N. perilampoides*; pollen from *Attalea* sp. (66.9%) was preferred by *P. fulvopilosa* and *Salix* sp. (98.5%) was chosen by *S. hellwegeri* (**Table 1**). The results also showed alternative resources, for instance, Anacardiaceae, *Brassica* sp., Melastomataceae, and Sapindaceae among others.

4.2 Physicochemical characteristics

In relation to the chemical parameters obtained from the stingless bees' honey samples analyzed (**Table 2**), higher moisture content was observed than the levels set in the Mexican standard (or practical values) for *A. mellifera* honey (≤20.0% of humidity). The registered pH is not >4 in the analyzed samples except for *N. perilampoides*. Also, found that the samples have a lower ash content than the value reported by the Mexican standard for *A. mellifera* honey (≤0.600% of ash content). The reducing sugars content is lower than the established by the Mexican standard for *A. mellifera* honey (≤63.88% of reducing sugars) because there was high levels of humidity, except in the case of the honey sample of *M. fasciata* which showed a higher value (65.73%) than *A. mellifera*. There were low levels of fructose and saccharose in the samples analyzed with respect to the parameters established for *A. mellifera* (≤45.0% of fructose and ≤8.0% of saccharose). For the HMF parameter was found that the samples tested are below of the maximum limits established in the Mexican standard for *A. mellifera* honey (≤80.0 mg kg^{-1}), and they are even below the detection limit, which reflects their high quality and their adequate conservation and storage process.

Just as there are differences between pot-honey and the honey of *A. mellifera*, at the same time a great variation in the characteristics of the different samples of pot-honey is observed. To exemplify, the moisture percentage ranges vary from a minimum value of 25.80% (*M. fasciata*) to a maximum value of 42.99% (*S. hellwegeri*) or the variation that occurs in reducing sugars that goes from 34.50% at its minimum value (*G. acapulconis*) and 65.73% as the maximum value (*M. fasciata*) (**Table 2**).

However, it is possible to observe certain relevant patterns. As shown in **Table 3**, the first two components of the PCA explain 66% of the variation in the data. The most important variables in the first component are the percentage of ash and pH, with antagonistic effects. In the second component, reducing sugars and pH are the most relevant variables, also with antagonistic interactions. In the biplot (**Figure 1**), two groups are distinguished; the pot-honey samples that were obtained from hives managed by meliponiculture techniques (M) are separated from the pot-honey samples that were obtained by direct extraction of wild nests (E).

There is a group of three samples of honey (M) that are strongly influenced by the reducing sugars and that showed the highest values of this parameter (*M. fasciata* and *S. hellwegeri*), while there is another group of three samples of honey (E) strongly influenced by HMF and that showed the highest values of this parameter (all TDF samples) (**Figure 1**).

Species	Mt	V	Se	Humidity (%)	pH	Ash content (%)	Reducing sugars (%)	F (%)	S (%)	HMF (mg kg^{-1})
A. mellifera	n/d	n/d	n/d	20.00	4.5	0.600	63.88	45.0	8.0	80.0
M. fasciata[1]	E	TDF	R	26.50	2.9	0.010	49.00	25.6	2.1	18.8
S. hellwegeri[1]	E	TDF	R	33.00	3.2	0.010	55.50	23.1	0.3	13.4
G. acapulconis[1]	E	TDF	D	35.60	3.2	0.240	34.50	31.1	1.4	9.6
F. nigra[1]	E	TDF	R	34.00	3.6	0.010	45.70	19.1	0.6	27.6
M. fasciata[2]	M	TMF	D	26.99	3.3	0.018	62.04	11.5	0.6	LDL
M. fasciata[2]	M	TMF	R	25.80	3.9	0.007	65.73	21.9	0.3	LDL
P. fulvopilosa[2]	M	TMF	D	36.40	3.8	0.348	47.89	22.1	0.4	LDL
P. fulvopilosa[2]	M	TMF	R	31.40	3.4	0.130	49.99	28.2	2.1	LDL
S. hellwegeri[2]	M	TMF	D	42.99	3.3	0.095	48.84	21.4	0.0	LDL
S. hellwegeri[2]	M	TMF	R	27.80	3.2	0.067	59.47	17.9	0.0	LDL
N. perilampoides[2]	M	TMF	D	26.00	4.3	0.251	42.93	15.2	0.0	LDL
N. perilampoides[2]	M	TMF	R	28.00	3.9	0.199	47.00	16.0	0.0	LDL

n/d, no data; Mt, management technique; E, extraction; M, meliponiculture; V, vegetation; TDF, tropical dry forest; TMF, transitional mixed forest; Se, season; D, dry season; R, rainy season; F, fructose; S, saccharose; LDL, lower than the detection limit. Values of A. mellifera according to the Mexican norm (NMX-F-036-981, 2006); pH and fructose values for A. mellifera based on the maximum values reported by Bogdanov [16].
[1]The samples of honey were collected in 2011 in the municipality of Nocupétaro.
[2]The samples were collected in 2014 in the municipality of Madero.

Table 2.
Physico-chemical parameters of honey of six species of stingless bees, in the Alto Balsas, Michoacán, México.

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7
% of variance	36.78	29.18	13.69	12.31	4.62	2.13	1.29
% of cumulative variance	36.78	65.97	79.65	91.96	96.58	98.71	100.00
Loadings:							
Hum	0.258	0.330	0.725	0.277	0.132	0.449	0.000
pH	−0.429	0.339	−0.194	−0.348	0.639	0.352	−0.104
Ash	0.656	−0.218	−0.228	−0.151	0.662	0.000	0.000
RS	−0.304	−0.520	0.402	0.343	0.591	0.000	0.000
F	0.522	0.144	−0.183	0.284	0.614	−0.458	0.000
S	0.490	−0.105	−0.548	0.654	0.000	0.000	0.000
HMF	0.374	−0.210	0.233	−0.741	0.141	0.432	0.000

Hum, humidity; RS, reducing sugars; F, fructose; S, saccharose.

Table 3.
Importance of principal components and the relative contribution of original variables to PCA.

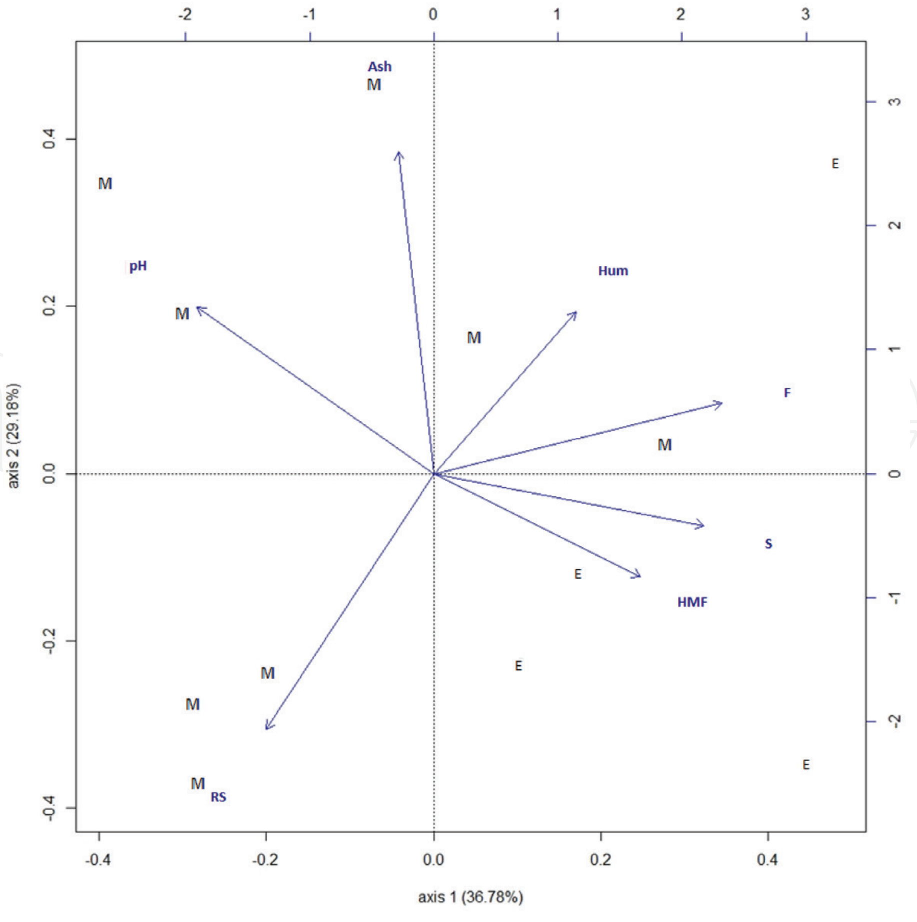

Figure 1.
Biplot for pot-honey samples.

5. Conclusions

In general terms, stingless bees from the study region use both arboreal and herbaceous species as resources, either from local vegetation or from introduced vegetation. It is interesting to note that *Quercus* sp. and *Fraxinus* sp. are not plants that require biotic pollination but nevertheless are a good source of food resources for bees. However, it is important to highlight the preliminary nature of the results obtained regarding the botanical origin of honey, this let us the goal of determining, at the level of species, the taxa presented in the melissopalynological samples of stingless bees' honey.

There is few information about physicochemical composition of stingless bee honey, and non-official quality control standards have been developed in Mexico or any country. Existing honey standards, for *A. mellifera*, in Mexico and the world cannot be applied to stingless bees' honey. In our samples, physicochemical parameters vary a lot, so which is very difficult to establish a norm. Differences in between species found in the study region is an indicator of the maturation and elaboration patterns of honey of each stingless bees species. This aspect should be considered in any attempt to promote a standardization of parameters of quality.

In a relevant way we documented in the pot-honey samples analyzed that they present an HMF content below the Mexican norm, and even below the limit of detection, which indicates that this honey is of excellent quality and had a good handling both when storing and when transporting. Regarding the moisture content, the analyzed samples presented high values not only with respect to the Mexican norm but also to other samples from other regions of Mexico. This may be due to the fact that honey was freshly collected with little storage time.

The content of total reducing sugars and fructose is higher in the honey of *A. mellifera*, which explains its greater commercialization. In the case of the pot-honey analyzed, it depends on the specie and in an important way, the vegetation type where stingless bees obtain their resources.

We consider that there is a need to generate information that allows us to establish general guidelines of quality for stingless bees' honey, and for this, it requires a wide sample of honey samples systematically generated including: diversity of species, ecosystems or vegetation types, seasonality of production and strategies of management to develop more precise statistical analyzes.

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