Chapter 25
Melipona favosa Pot-Honey from Venezuela

Patricia Vit

To the memory of Father Santiago López Palacios of Universidad de Los Andes, Venezuela, for his inspiration to investigate bee botany and honey.

To the retired Dr. Livia Persano Oddo and Dr. Stefan Bogdanov for their truthful interest and timely scientific collaboration to study this unknown honey in Europe.

To the memory of Professor João MF Camargo for identifying stingless bee species to name pot-honey beyond expectations.

25.1 Introduction

During his visit to Venezuela in 2008, Prof. JMF Camargo could not observe the *Melipona favosa* (Fabricius 1798) that he kindly identified, in their cactus wild nests (see Fig. 25.1). However, he informed us that this was the first species of Meliponini accurately described, probably with a specimen from French Guiana. Prof. Camargo also authored and anchored the idea of pot-honey as the first honey on planet Earth, dating back to the late Cretaceous, before comb honey was produced by *Apis mellifera*. He had studied the oldest bee fossil, *Cretotrigona prisca*, preserved in amber from New Jersey (Michener and Grimaldi 1988a, b), and knew that dinosaurs and stingless bees shared landscapes 97–74 million years before present. This bee from the Paraguaná Peninsula (Falcón state, Venezuela) was undisturbed by *Apis mellifera*, until honey bee swarms were seen after the floods caused by el Niño at the end of 1999. But the Africanized honey bee colonized Venezuela since 1975 in southern Amazon state, and 1976 in Santa Elena de Uairén, Bolívar state (Gómez Rodríguez 1986).

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Fig. 25.1 Entrance of *Melipona favosa* nest in a columnar cactus "cardón" *Stenocereus griseus*, Paraguaná Peninsula, Falcón state, Venezuela Photo: P. Vit

M. favosa is mostly known as "erica" but is also named "maba" in a few places. It is a smaller bee than other Venezuelan *Melipona* such as *M. compressipes* and *M. trinitatis*, named "guanota." However, it is bigger than stingless bees from other genera different from *Melipona*, like *Tetragonisca* and *Scaptotrigona*. The honey pots also have an intermediate size. This honey is reported in the classic novel "Doña Bárbara" (Gallegos 1973), the Venezuelan book on creole bees (Rivero Oramas 1972), and the chapter on Meliponini in the Catalogue of Bees in the Neotropical Region (Camargo and Pedro 2007), but is not considered in Venezuelan honey standards (Vit 2008a). It was available during field work in the plains and coastal regions of Venezuela. The honey harvest is traditionally made by removing sealed pots from the storage area in the hive, on a dish. The honey pots are compressed with forks or hands, and honey is decanted, and bottled, as learned from Venezuelan stingless bee-keepers, also known as "meliponicultors" (Vit 1994a, b).

Our analytical pot-honey harvests were done by extraction with rubber tube adapted to a syringe, after piercing sealed honey pots, to avoid contamination from pollen pots. However, in a preliminary sensory trial in the Food Science Department at Universidad de Los Andes held in 2007, the additional sour taste, derived from fermented pollen in honey extracted by compression (by hand, with honey and pollen pot contents admixed), was highly appreciated (Vit et al. 2010b). Currently, suction pumps are used for meliponine honey extraction in Brazil (see Alves chapter in this book), while piercing and decantation are used in Australia (TA Heard and M Halcroft, personal communication).

Comb honey from *A. mellifera* is different from pot-honey of *Melipona*. However, both honey types have practical applications as sweeteners, and prototypical medicinal uses conferred by the high osmotic pressure, and the action of minor components of botanical (see Tomás-Barberán chapter in this book) and bee origin. The enormous biodiversity of Meliponini, and their associated microorganisms (see chapters by Menezes et al., and Morais et al., this book), may add further functional properties to pot-honey, unknown for comb honey. Here I analyze the *M. favosa* honey composition in a collection of five samples from the Paraguaná Peninsula and review a database of 40 *M. favosa* pot-honeys from Venezuela, including their bioactive and sensory properties.

25.2 A Peculiar Honey, with Similarities to and Differences from *Apis mellifera*

Since 1985, the collection of *M. favosa* honey has steadily increased. Only recently, a false *M. favosa* honey invaded the Venezuelan market (Vit et al. 2011). This fact should be of interest for Venezuelan sanitary authorities, and not ignored, as is often the case for fraudulent or adulterated honey of *A. mellifera*. It remains the responsibility of the consumer to determine the authenticity of honey, when needed for medicinal use. Venezuelan norms for honey created in 1984 (Comisión Venezolana de Normas Industriales 1984a, b) have not been revised, in contrast to the recent assessment of Colombian norms, in which honey produced by native bees was included for the first time in a honey regulation (ICONTEC 2007). This is a promising example for other countries to join the quest of setting standards for the honey produced by Meliponini, instead of searching for a new word such as "divine elixir" (Vit et al. 1998b). The word honey is not a trademark for that made by bees in combs and can be used for both the honey produced in pots and in combs (Vit 2010a).

A number of collaborators were attracted by this honey processed in pots, and from that of other stingless bee species (their ability of transporting and storing the energy of the sun—as watery sugars—in flexible pots built up with cerumen, able to expand and reduce volumes during fermentive process) (see Fig. 25.2).

Fig. 25.2 *Melipona favosa* (**a**) on a bottle of fermenting honey harvested from sealed honey pots and (**b**) her storage pots in the nest Photo: P. Vit

This sour–sweet honey, with its own sugar spectra (Bogdanov et al. 1996; Vit et al. 1998a), has very low diastase activity, higher moisture and higher free acidity than that of *A. mellifera*, but similar ash, sucrose, and nitrogen content (Vit et al. 1994, 1998b). Possibly, it is a honey finished and stored with lower processing of nectar, causing nose perceptions resembling those of the foraged flowers, from a more diluted sugar matrix less transformed than *A. mellifera* comb honey, which is permitted by resin, which kills the bacteria, and by mutualistic microbes in the gut—just like *Apis*—which kill pathogenic microbes that would otherwise ruin both the honey and pollen (DW Roubik, personal communication).

25.3 Composition of *Melipona favosa* Honey Collected in Rational Hives

Honey pots of *M. favosa* from Paraguaná Peninsula, Falcón state, Venezuela, were pierced to collect the honey by syringe extraction. Honey samples were harvested from five *M. favosa* hives, the same day. Physicochemical parameters were analyzed in duplicate according to the methods recommended by the Venezuelan regulations (COVENIN 1984a). Parameters measured included ash (gravimetric method), water content (refractometric method), reducing sugars and sucrose (titrimetric method), pH, free acidity (titrimetric method). Color was measured by optical comparison (instrumental method). Nitrogen was determined by a standard micro Kjeldahl method (AOAC 1984). The analytical results on chemical composition of the five samples of *M. favosa* honey are shown in Table 25.1.

The honey produced by *M. favosa* is light in color. In the five samples analyzed here, the color varied between 20 and 27 mm Pfund. The moisture content varied between 29.7 and 30.2 g water/100 g honey, which is higher than the honey standard for *A. mellifera*, and typical for the values in meliponine honeys reported since Gonnet et al. (1964). The ash content varied between 0.07 and 0.14 g ash/100 g honey, falling below the maximum 0.5 g/100 g *A. mellifera* honey standard. The pH

Table 25.1 Composition of *Melipona favosa* pot-honey from the Paraguaná Peninsula of Venezuela, $n=5$

Physicochemical parameters	Mean ± SD	Min	Max
Color (mm Pfund)	23.2 ± 2.7	20	27
Moisture (g/100 g honey)	30.0 ± 0.2	29.7	30.2
Ash (g/100 g honey)	0.10 ± 0.02	0.07	0.14
pH	3.7 ± 0.2	3.5	3.9
Free acidity (milliequivalents/kg honey)	50.6 ± 18.3	34.2	85.2
Nitrogen (mg/100 g honey)	41.7 ± 8.1	30.0	53.4
Sugars (g/100 g honey)			
Reducing sugars	64.6 ± 2.3	61.4	69.0
Apparent sucrose	1.3 ± 0.5	0.7	2.0

values are in the same range of *A. mellifera* honey, whereas the average free acidity (50.6 meq/kg honey) is higher than the maximum 40 meq/kg *A. mellifera* honey standard (COVENIN 1984b). This indicates the presence of higher amounts of weak acids, such as organic acids with low ionization.

The nitrogen content varied between 30.0 and 53.4 mg N/100 g honey with an average of 41.7, similar to 40.66 mg N/100 g reported for *M. favosa* honey in a previous work, and slightly lower than 57.1 mgN/100 g found in *A. mellifera* honey from Venezuela (Vit et al. 1994). The average concentration of reducing sugars is into the limit of the minimum 65 g/100 g and of the maximum 5 g/100 g prescribed by the *A. mellifera* honey standards (COVENIN, 1984b). This means that some *M. favosa* honey samples do not fulfill this parameter due to a slightly lower concentration of reducing sugars, which is consistent with previous results (Vit et al. 1998b).

25.4 Sensory Attributes of *Melipona favosa* Honey

A honey tasting sensory assay was initiated with the system used for *A. mellifera*. Sensations in the nose are called “odor,” whereas the multimodal sensations in the mouth—differing from taste and trigeminal sensations, are called “aroma.” Seven families of sensory attributes in the odor-aroma wheel (Piana et al., 2004) were adapted to eight sensory odor-aroma families in a table for stingless bees: (1) Floral-fruity. (2) Vegetable. (3) Fermented. (4) Wood. (5) Bee hive. (6) Mellow. (7) Primitive. (8) Industrial chemicals (Vit et al. 2007). This is a cognitive construct to facilitate the perception of pot-honey in this system. A histogram of odor-aroma families perceived in one sample of *M. favosa* honey by eight assessors is shown in Fig. 25.3. The highest count for odor was halved with a dotted line, and for aromas with a straight line. Bars above the lines are considered primary odors and aromas, respectively, and below the lines are considered secondary odors and aromas.

The family floral-fruity described both primary odor and aroma. The peculiar smell of the *M. favosa* nest is a primary attribute more frequent than woody, mellow, and primitive odors. Fermented, vegetable, and primitive secondary aromas are more frequent than woody, nest, and mellow. Overall, this *M. favosa* is a floral-fruity and fermented honey with the bouquet of the hive (given by the bees, collected substances and products). Other secondary odors and aromas were less frequent.

The fermenting honey, noted as a sensory attribute, is interpreted as an indication that Meliponini process their food with microorganisms, possibly as evolutionary ability. The sensory concept, that fermented meliponine honey is not spoiled, was recently assessed during the 8th Pangborn Sensory Science Symposium (Vit et al. 2009b). In fact, meliponine honey is not to be considered a spoiled honey, even if it may ferment in the storage pots inside the hive and after harvest, due to the high water content and associated microorganisms. On the contrary, fermentation contributes to the typical sensory profile of this honey and also increased the antioxidant activity of *T. angustula* honey (Pérez-Pérez et al. 2007).

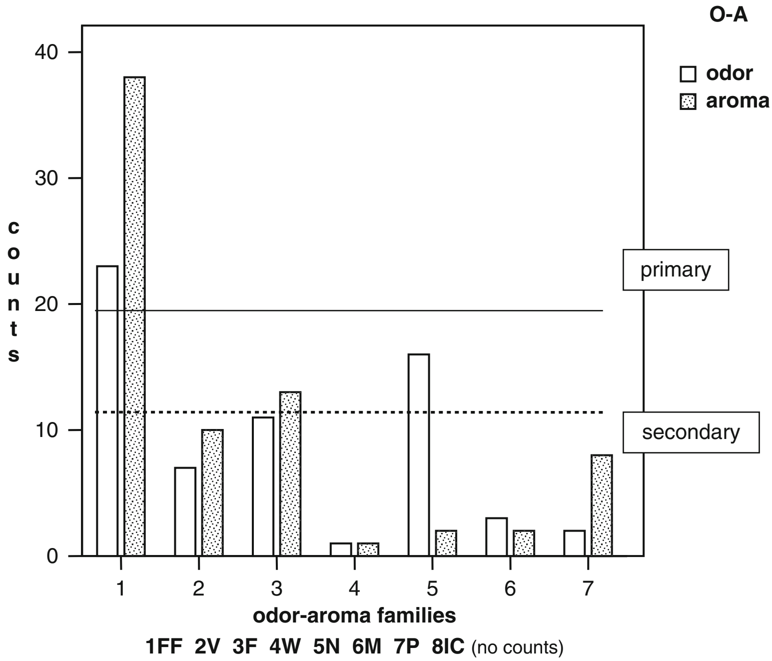

Fig. 25.3 Sensory profile of *Melipona favosa* honey with a trained panel. *FF* floral-fruity, *V* vegetable, *F* fermented, *W* woody, *N* bee hive, *M* mellow, *P* primitive. The highest count for odor was halved with a *dotted line*, and for aromas with a *straight line*. *Bars* above the lines are considered primary odors and aromas, respectively, and below the lines are considered secondary odors and aromas. No counts for the industrial chemical family 8IC Modified from: Vit (2008b). Permission granted by Revista de la Facultad de Farmacia

25.5 Database of *Melipona favosa* Honey from Venezuela

Settings of honey standards were suggested for the most studied stingless bees, four species of *Melipona* (*M. asilvai*, *M. compressipes*, *M. favosa*, *M. mandacaia*) and *Tetragonisca angustula*. The averages values found for 20 samples of *M. favosa* honey in a previous review were free acidity 49.9 meq/kg, 0.22 g ash/100 g, 55.8 mg nitrogen/100 g, 71.2 g reducing sugars/100 g, 1.7 g apparent sucrose/100 g, and 24.8 g water/100 g (Souza et al. 2006).

The seven physicochemical standards in the Venezuelan norm COVENIN 2191–84 are set for *A. mellifera* but not for Meliponini pot-honey: (1) Moisture (Max 20%), (2) Reducing sugars (min 65%), (3) Sucrose (max. 5%), (4) Free acidity (max 40 meq/100 g), (5) Ash (max 0.5%), (6) Hydroxymethylfurfural HMF (negative), (7) Diastase activity (positive). These last two parameters are qualitative and refer

Table 25.2 Composition of *Melipona favosa* pot-honey from Venezuela highlighted values are different from *Apis mellifera* honey standards

Physicochemical parameter	*N*	Mean ± SD	Min	Max
Moisture (g/100 g honey)	40	28.0 ± 2.7	22.1	32.0
Ash (g/100 g honey)	40	0.14 ± 0.13	0.01	0.61
Diastase (DN)[a,b]	6	2.86 ± 0.36	2.64	3.50
Free acidity (milliequivalents/kg honey)	40	51.7 ± 25.2	12.7	97.1
Invertase (IU)[c]	6	90.08 ± 48.03	31.80	150.70
Nitrogen (mg/100 g honey)	39	45.7 ± 18.3	10.5	102.0
HMF (mg/kg honey)	21	17.7 ± 8.5	5.04	24.69
Sugars (g/100 g honey)				
Reducing sugars	40	67.3 ± 4.1	60.9	78.6
Apparent sucrose	40	2.1 ± 1.3	0.5	5.1

[a]The Diastase Number (DN) indicates g starch hydrolyzed/100 g honey/h, at pH 5.2 and 40°C
[b]Semiquantitative data not included
[c]An Invertase Unit (IU) indicates μmoles p-nitrophenyl glucopyranoside hydrolyzed/kg honey/min, at pH 6.0 and 40°C

to the heating and aging of the honey. Findings in previous works indicated the low diastase activity of *M. favosa* honey, as well as an HMF content similar to that of *A. mellifera* honey (Vit et al. 1994, 1998b). The natural low diastase activity values found in previous qualitative (Vit 1992) and quantitative (Vit et al. 1994, 1998b) measurements suggest this is not a quality indicator for *M. favosa* honey. For this reason, diastase activity was measured in half of the samples. The average composition and variations of 40 samples of *M. favosa* honey studied from samples taken over 20 years are indicated in Table 25.2.

Free acidity, ash, reducing sugars, sucrose, and water content of honey are useful quality indicators for *M. favosa,* as they are for *A. mellifera*, although standards may differ. Flavonoid and polyphenol contents, antioxidant and antibacterial activities, and sensory analysis are biochemical, biological, and consumer analyses which also contributed to *M. favosa* honey characterization.

25.6 Suggested Standards for *Melipona favosa* Honey Compared to *Apis mellifera*

Compared to Venezuelan honey standards for *A. mellifera* (COVENIN 1984b), the following changes in reference values may be adopted for *M. favosa* honey (see Table 25.3): (1) No variation for HMF values, (2) Increased maximum values for water content (up to a maximum of 35%), apparent sucrose (up to a maximum of 6%), free acidity (up to a maximum of 100 meq/100 g), and ash (up to a maximum of 1.0%), (3) Decreased minimum for reducing sugars (down to a minimum of

Table 25.3 Suggested standards for *Melipona favosa* honey, compared to *A. mellifera*

Quality factor	*Melipona favosa* suggested standard	Relation	*Apis mellifera* standard
Moisture (g/100 g)	Max 35.0	>	Max 20.0
Ash (g/100 g)	Max 0.5	=	Max 0.5
Free acidity (meq/100 g)	Max 100.0	>	Max 40.0
Nitrogen (mg/100 g)	10.0–105.0	New	–
Reducing sugars (g/100 g)	Min 60.0	<	Min 65.0
Apparent Sucrose (g/100 g)	Max 6.0	>	Max 5.0
HMF (mg/kg)	Max 40.0	=	Max 40.0

60%), (4) The nitrogen content is not included in the standards for *A. mellifera* honey, but a range 10–100 mg N/100 g honey would be useful for protection against adulteration and falsification, (5) Diastase activity is not included because the activity of this enzyme is very low in *M. favosa* honey; therefore, it is not a practical quality factor to measure freshness or heating.

25.7 The Inclusion of Biological Activity Descriptors

In addition to compositional quality factors, the biological activity of honey could also become a useful descriptor for medicinal use. However, there are no simple descriptors for that purpose. For instance, the variable contents of flavonoids and polyphenols in *A. mellifera* unifloral honeys (Frankel et al. 1998) did not correlate with antioxidant capacity. The flavonoid content is lower than the polyphenols, as generally observed in the honey produced by other species of stingless bees, such as *T. carbonaria* from Australia (Persano Oddo et al. 2008), *M. beecheii* and *M. solani* from Guatemala (Gutiérrez et al. 2008), *M. crinita*, *M. eburnea*, *M. grandis*, *M. illota*, *Nannotrigona melanocera*, *Partamona epiphytophyla*, *Ptilotrigona lurida*, *Scaptotrigona polysticta*, *Scaura latitarsis*, and *Tetragonisca angustula* from Peru (Rodríguez-Malaver et al. 2009), *Tetragonisca fiebrigi* from Argentina and Paraguay (Vit et al. 2009a), and also in *M. favosa* from Venezuela (Vit et al. 2012). This means that other polyphenol types in pot-honey may explain their antioxidant activities. Seminal findings on greater contents of flavonoid glycosides than aglycones in *M. favosa* honey strongly differentiate them from *A. mellifera* honey. Pot-honey of *M. favosa* has more aglycones, from hydrolyzed *O*-glycosides in the nectar and propolis (Truchado et al. 2011). Values of 45.9–227.92 μmole Trolox equivalents/100 g honey, positioned *M. favosa* honeys in the categories low (0–100) and high (200–300) reported for unifloral *A. mellifera* Czech honeys (Vit et al. 2008a).

Considering antibacterial activity, a successful marker of antibacterial activity is the unique manuka factor (UMF) described by Prof. Peter Molan from Waikato University in New Zealand (Molan 2005). However, this is a useful marker for honey of a botanical origin including only Myrtaceae, genus *Leptospermum*. More conservative are the tests to measure inhibition of bacterial growth under controlled condition. The Gram positive *S. aureus* is more resistant to these honeys than the Gram negative *E. coli*, because lower MICs of honey were needed to kill *E. coli* than *S. aureus*. This was also observed in Venezuelan honeys of *A. mellifera* (Vit et al. 2008b) and *M. favosa* (Vit et al. 2012), other stingless bee species from Argentina (Vit et al. 2009a), and *Geotrigona acapulconis* from Guatemala (Dardón and Enríquez 2008). Although *E. coli* and *S. aureus* MICs were similar to those found in other *Melipona* species, *E. coli* was more resistant than *S. aureus* to *Tetragonisca angustula* honey from Guatemala.

The anticancer activity of two *M. favosa* honeys (IC_{50} 3.39–16.50 mg/mL) was measured in vitro using a model based on ovarian cancer (see Vit et al. chapter 35, this book). Considering that both samples were collected in the same meliponary but in different months, the effect of the botanical origin (see Obregón et al. chapter 23 in this book) becomes relevant to the bioactive properties of pot-honey.

Melissoplaynology will be useful in the future, for understanding the contribution of botanical origin to the composition, sensory and biological properties of *M. favosa* honey. Denomination of unifloral honeys of each stingless bee species is not envisaged, but some exceptions may be valid, as well as for the geographical origin.

25.8 Contemporary Interactions to Value *Melipona favosa* Honey

Expert scientists, technicians, and keepers of traditional meliponiculture can benefit consumers in search of information. Emotion, cognition, and communication are relevant components to spread the tradition and to foster technological progress. Observing a living stingless bee hive is the ultimate learning experience concerning pot-honey and the meliponines. *M. favosa* is a gentle bee that could be easily kept in schools, where young people can observe them. However, this bee thrives in the plains and coastal regions, and other species will be needed in different locations of Venezuela. The *M. favosa* bee can be kept by women, children, and the elderly.

The entomological origin of honey should be on the label (common and scientific name of the bee). Consumers and stingless bee-keepers should be protected from producers of false meliponine honeys without stingless bee apiaries (meliponaries) to back up their honey production. Labels of organic certified honey may help to safeguard the reputation of pot-honey and be useful to promote this industry, but they demand great organization to be reliable.

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