Physical, chemical and organoleptic characterization of Melipona beecheii *honey collected in agroforestry systems*

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ABSTRACT

The research was conducted at the Pastures and Forages Research Station "Indio Hatuey" in order to determine the organoleptic, physical and chemical characteristics of the honey produced by *Melipona beecheii*. For the extraction and packaging of the honey; sterile syringes and dry sterilized flasks, properly labeled, were used. After that, in the samples, where the presence of pollen grains from *Gliricidia sepium* was detected, a sensorial, physical and chemical analysis was conducted based on the following indicators: aspect, color, smell, taste, texture, humidity (g/100 g), free acidity (meq/100 g), pH and soluble solids (g/100 g = %). Moreover, the content of different sugars in the honey was determined through a HPLC analysis. The pH values were around 3,6, the free acidity was 35,0 meq/100 g, and humidity, 24 %. On the other hand, the sensorial analysis was in agreement with the quality specifications recommended in the Cuban Quality Norm, because the honey did not show any unpleasant taste, smell or color. It is concluded that the *M. Beecheii* honey showed excellent quality, because it did not undergo any apparent degradation process; that is why it can be considered as fresh honey, which constitutes a key requisite for any feedstuff destined to human consumption.

Key words: agroforestry system, honey, *Melipona beecheii*

INTRODUCTION

According to Heard (1999), the potential of stingless bees (*Melipona beecheii*) in agroforestry systems, lies principally on their function as pollinators, although the honey obtained from the bees which visit shrubby legumes can be considered as an added value of such systems. Among the most visited legumes is *Gliricidia sepium*, from which, excellent-quality honey is obtained.

It is also known that this fodder plant stands out for some prominent attributes, such as: the production of high quality and digestibility foliage, as well as their ability to form balanced associations with grasses, with the subsequent increase of dry matter production in the associated pastures –thus achieving a higher stocking rate–. It also improves soil fertility, through the atmospheric nitrogen fixation. It can be used as green manure, for wood and firewood production and to provide shade for crops and animals (Pezo, Romero and Kass, 1993; Elevitch and Francis, 2006), for which it becomes an outstanding alternative for these purposes and for honey production.

Grajales *et al*. (2001) indicated that the Mexican aboriginal civilizations used the stingless bee honey with commercial, ritual and medicinal purposes; however, they indicated that just a few scientific studies have been conducted. These authors determined, in samples of *M. beecheii* honey that the pH values were approximately 4,5, the acidity values, 28 meq/kg; humidity, 24 %; and electric conductivity, 0,551 mS/cm; while the HMF was 64,79 mg/kg. As there is not a norm for the stingless bee honey, these authors took as reference the indicators established for the *Apis mellifera* honey.

The honey of stingless bees, in addition to its important feeding characteristics, is widely used in traditional medicine practices in Costa Rica, Mexico, Colombia, Argentina and Guatemala. In these countries it has been used to treat ocular diseases such as conjunctivitis, eye pterygium and cataract; and to treat the ones related to respiratory

(bronchial asthma), digestive (mouth ulcers) and gynecological problems (to relieve postpartum pains and to prevent vaginal hemorrhages and varicose veins); as well as to treat skin rash and urinary problems. This turns honey into a much appreciated product at local level, and of high demand in naturist stores and pharmacies (Cepeda, Nates Parra and Téllez, 2008; Meriggi, Lucía and Abrahamovich, 2008; González Acereto, 2008).

Although in Central American countries; like the ones mentioned above, rescue and conservation projects of this species are carried out, and in spite of the fact that their honey is highly demanded in the international market for its medicinal properties, the first steps are being taken in Cuba to commercialize it. For such reason, it is necessary to characterize this honey to establish the quality norms, as indispensable requisite for its market release. That is why this research was conducted in order to determine the physical, chemical and organoleptic characteristics of the honey produced by *M. beecheii*.

MATERIALS AND METHODS

Location. The experiment was conducted at the Experimental Station of Pastures and Forages (EEPF) "Indio Hatuey", located at 22º 48' and 7'' North latitude and 79º 32' and 2'' West longitude, at 19 m.a.s.l., in the Perico municipality, Matanzas province, Cuba (Academia de Ciencias de Cuba, 1989).

Edaphoclimatic conditions. The honey sampling was carried out during the dry season (November-April). The average monthly temperature was over 19 °C and did not exceed 28 °C, with an annual average of 23,7 °C. The relative humidity of the air was generally high and oscillated between 70-86 %, with an annual average of 79,3 %.

The soil is of plain topography, with slope of 0,5 to 1,0 %, and it is classified by Hernández *et al.* (2003) as lixiviated Ferralitic Red soil, hydrated humic ferruginous nodular, of fast desiccation, clayey and deep on limestone, with a slightly acid pH (6,2-6,4).

Procedure for the honey characterization. Six beehives were used, which were moved towards the area where the *G. sepium* trees were located, at the time of their flowering period and after a cleaning harvest was performed. From the hives a sealed amphora of pollen was extracted and 25 mL of honey were obtained from 50% of the total existing beehives

The samples were collected, with the highest possible hygiene, from the beehives that were in wooden boxes, 40 cm long by 10 cm wide; according to the Pablo Nogueira Neto –PNN– model (Nogueira Neto, 1997). Such beehives were at the center of a grove, which belongs to the small livestock area of the EEPF "Indio Hatuey". For that purpose, sterile syringes and dry sterilized flasks, properly labeled, were used. After that, the physical, chemical and organoleptic characterization of the honey from the sample that contained pollen grains of *G. sepium* was conducted. Also a sensory, physical and chemical analysis was conducted at the National Center of Food Hygiene, of Havana, according to the methodology described in the Cuban Norm 74-09 (Oficina Nacional de Normalización, 1986), based on the following indicators: aspect, color, smell, taste, texture, maximum humidity (g/100 g), free acidity (meq/100 g), pH and soluble solids (g/100 g = %).

Chromatographic determination of the sugars present in honey. For the determination of the sugars present in honey a Young Lin HPLC (Republic of Korea) was used. The glucose, fructose and sucrose were separated in an ICSep COREGEL-87 H355 column (7,8 x 300 mm) at 60 ± 1 ºC, with the utilization –as mobile phase– of deionized water at a flow of 0,4 mL/min. These sugars were detected with a differential refractometer (RID, YL 9170). The software for data acquisition Clarity (YL 9100 HPLC, USA) was used as interface for the analysis. The honey concentration that was prepared to be injected to the HPLC was 10,95 g/L; for that purpose 2,7394 g of honey were weighed and diluted in 250 mL of water.

RESULTS AND DISCUSSION

Honey is the product of the transformation of the flower nectar or the exudations of other live parts of the plants, which, after being capped and transported to the beehive in the melliferous stomach of the worker bee, is stored and matured in the honey comb to serve as feed for the bees and their brood (Codex Alimentarius Commission, 1990).

According to Umaña (2006), the water content is considered an indicator of purity, as well as of the degree of maturity and stability of honey during its storage, that is to say, whether it has –or not– risks of decomposing by fermentation. The free acidity can indicate if the honey has undergone any fermentative process, for which it is considered a criterion of freshness.

Table 1 shows that the physical and chemical tests made on the honey had a close relationship with the quality indicators, in correspondence with the criteria pointed out by Umaña (2006).

The Cuban Norm 371 (Oficina Nacional de Normalización, 2012) defines as "bee honey" just the one produced by *A. mellifera*; therefore, it would be wrong to establish rigid comparisons of the quality indicators values with those of the stingless bee honey. Nevertheless, nowadays there is a norm proposal (Vit, Medina and Enríquez, 2004) that serves as reference while there is not a corresponding norm. This allows, at least, to offer a valuation from a much studied and highly consumed product.

In this sense, if the results (table 1) are compared with the norm proposal suggested by Vit *et al*. (2004), the evaluated indicators (water content and free acidity) show an excellent quality of the sample from the beehive selected, by being even below the maximum values of the permissible indicators for the *Melipona* genus.

Moreover, the results of the sensory analysis are in correspondence with the Cuban Norm 371 (Oficina Nacional de Normalización, 2012). This norm states that bee honey should be presented as a dense, viscous and translucent, or well crystallized, liquid and it should not have any unpleasant taste, smell or color, which are acquired from strange matters during its processing, packaging or storage.

These results are similar to the ones obtained for the honey evaluated in the Matanzas province, Cuba, by Fonte (2007); as well as the ones reported by Grajales *et al*. (2001), in México, and by Díaz Mena *et al*. (2007), in Cuba. It follows that it was a honey harvested with the proper maturity degree, so that the fermentation during its storage (in closed recipients and in places of low environmental humidity) did not occur.

According to Umaña (2006), the sucrose content in mature honeys could be low, due to the invertase enzyme, which divides the disaccharide into two simple sugars (glucose and fructose). According to the norm of the Codex Alimentarius from Costa Rica, the sucrose percentage should not exceed 5 %. However, Vit *et al*. (2004) propose a maximum of 6 % for the *Melipona* honey; this value is close to the sucrose content of the sample obtained in this study (table 2) through high pressure liquid chromatography (HPLC: 6,54 %).

The content of simple sugars in both cases (table 2) was below 60 %, which is the maximum value allowed by the norm of the Codex Alimentarius from Costa Rica. These results

Table 1. Physical, chemical and organoleptic characteristics of *M. Beecheii* honey.

Indicator	Beehive	Reference indicator for *Melipona* (Vit *et al*., 2004)
Water content (g/100 g = %)	24,0	30,0
Free acidity (meq/100 g)	35,0	70,0
pH	3,6	
Soluble solids (g/100 g = %)	74,6	
Sucrose (g/100 g)	6,54	6,0
Aspect	Slightly turbid	
Smell	Characteristic	
Taste	Acid	
Texture	Moderate viscosity	
Color	Amber extra-clear (ELA)	

Table 2. Glucose, fructose and sucrose content present in *M. beecheii* honey.

Retention time (min.)	Area [mV.s]	Compound name	g/L	%
14,283	388,445	sucrose	0,76	6,54
17,492	1 067,297	glucose	3,21	29,30
20,367	60,024	unidentified	–	–
27,767	1 538,288	fructose	3,74	34,11

indicate that the sample of *M. beecheii* honey was harvested with optimal maturity and it was not adulterated with the addition of pure sugars or syrups, which can alter the normal values of carbohydrate composition in the honey (Fonte, 2012).

The sucrose, glucose and fructose peaks are observed clearly defined in the chromatogram (fig. 1). The prevalence of these sugars in the honey is justified –to a large extent– by the fact that, according to APIEXPA (2000), to carry out a charge in the recollection activity, the bee visits from few to numerous flowers and, if it is possible, it chooses nectaries of high sugar concentration, because it prefers those which contain, along with sucrose, the two monosaccharides: glucose and fructose.

CONCLUSIONS

Although the relatively high humidity content of *M. beecheii* honey with respect to that of *A. mellifera* honey makes it more vulnerable to fermentation processes –with the aggravating fact of the lack of standardized procedures for the harvest or quality specifications–, this honey showed an excellent quality, because it did not undergo any apparent degradation process. Therefore, it can be considered as fresh honey, which constitutes a key requisite for any feedstuff destined to human consumption.

In addition, this can be the starting point to elaborate the future quality norm for stingless bees, because such honey, according to its physical, chemical and organoleptic characterization, fulfills the required quality norms.

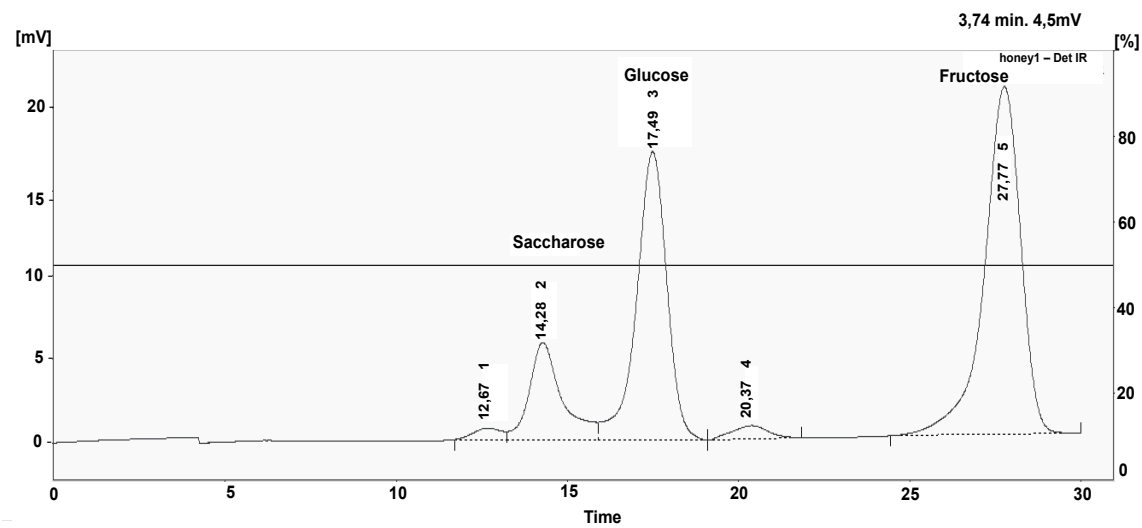

Figure 1. Chromatogram, by HPLC, of the sucrose, glucose and fructose of *M. beecheii* honey.

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