ELECTROCHEMICAL EVALUATION OF QUALITY CHARACTERISTICS IN HONEY FROM *Meliponini* AND *Apis mellifera* BEES

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Abstract
The objective of this work was to use electrochemical analytical methods to evaluate and compare the main quality parameters for samples of honey of Apis mellifera (Apis) and Meliponini honeys from five different regions of Mexico and one from Argentina.There is few information about the composition of stingless bee honey, and no official quality control standards have been developed in Mexico or any country. Existing honey standards in Mexico and the world cannot be applied to Meliponini honey.
The quality parameters quantified in the honey samples were: hydroxymethylfurfural (HMF), moisture, ash, reducing sugars, and pH.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.The principal difference between the Apis and Meliponini honeys was the relatively high moisture content in the later. The Meliponini samples had a lower mean ash content and pH than the Apis samples. As expected, reducing sugars content was lower in the Meliponini honey samples. Mean HMF content for the Apis honeys was below established limits while mean content for the Meliponini honeys was above them; however, both types varied widely.
This is a preliminary study which aims to help developing quality standards for honey Meliponini and expand their marketing potential.

Keywords: Honey quality, electrochemistry, stingless bees, *Apis*, physicochemical analysis

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1. INTRODUCTION

Apiculture is practiced worldwide wherever honey-producing bee species are found. In the Americas, it is attracting increasing numbers of producers both as an income-generating activity and as the source of numerous compounds with myriad health applications. Honey is the principal product of apiculture, but others include wax, propolis, pollen, and venom. The European honey bee *Apis mellifera* is the most widely cultivated due largely to its high honey yield. However, bees *Meliponini* tribe of the *Apidae* subfamily also produce honey, as well as wax and pollen. Known as stingless bees, these species are social, and inhabit warm subtropical regions throughout the world. They are called stingless because their stinger is atrophied, which facilitates their handling compared to *Apis*.
Research interest in stingless bee species is growing. Researchers in Brazil (Oliveiera Alves, 2005; Almeida Souza, 2006, 2009) and Venezuela (Vit, P. 2009; Vit et al, 2006) are currently most active in studying their characteristics and honey. Studies have also

begun in Guatemala (Dardon, 2008) and Peru (Rasmussen, 2003). Limited research on stingless bees in Mexico and a consequent lack of knowledge about them has limited effective marketing of their products in this country. Honey from *Meliponini* bee species is popularly believed to have medicinal properties, and is known for being produced in small quantities by local artisanal producers. Stingless bee honey is normally not used as a sweetener, and its low production level and putative medicinal properties can drive its cost up to five to fifteen times as much as *Apis* honey (Contreras, 2008). There is few information about the composition of stingless bee honey, and no official quality control standards have been developed in Mexico or any other country. Existing honey standards in Mexico (*NMX-F-036-NORMEX-2006*) and the world (*CODEX ALIMENTARIUS*; European Union Council Directive 2001/110/EC) cannot be applied to *Meliponini* honey because its moisture content exceeds the 20% maximum level stipulated in them. Most *Meliponini* honeys also have a reducing sugars content ≤ 60%, which is below the minimum established in these standards (reducing sugars content in *Apis* honeys is ≥63%).

In response, the objective of this work was to use electrochemical analytical methods to evaluate and compare the main quality parameters for *Apis* and *Meliponini* honeys:

- Quantification was done of hydroxymethylfurfural (HMF), reducing sugars, fructose, moisture and ash contents, as well as pH in *Meliponini* and *Apis* honey samples.
- A comparison was then done of the two types of honey, their main composition differences recorded and quality parameter recommendations for *Meliponini* honey made based on these results.

2. MATERIALS AND METHODS

A flow-chart for honey analysis is shown in Figure 1. The quality parameters quantified in honey samples were: HMF, moisture, ash, reducing sugars and pH.

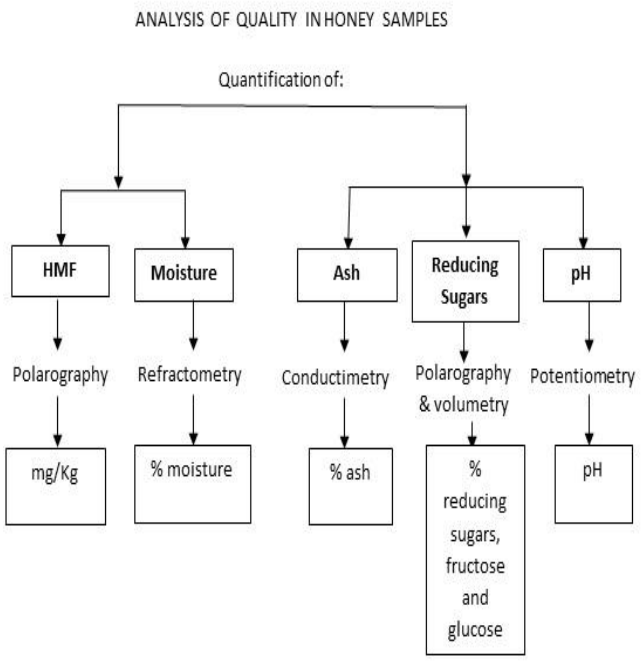

Fig. 1 Flow-chart for analytical measurement of main quality parameters of honey

2.1. Honey samples

The objective of this study was to use electrochemical methods to quantify main quality parameters of *Meliponini* honey samples from five regions in Mexico (Michoacán, Sierra de Puebla, Guerrero, Yucatan and the Huasteca Potosina) and one in Argentina, and compare them to *Apis* honeys to begin developing quality control parameters for *Meliponini* honeys.

2.2. Honey quality analyses

In 2009, the International Honey Commission (IHC) developed *Apis* honey quality identification and characterization standards based on the work of Bogdanov *et al.* (1997). These include parameters with potential applications for *Meliponini* honeys.

Temperature-corrected refractometry (Atago N-3E refractometer) was employed to calculate the moisture content. Ash content and pH were measured using standardized methods recommended by the IHC. Ash content was calculated from conductimetry measurements (Metrohm 664 conductimeter, cell constant = Kc = 1.0 cm^{-1}). pH values were registered using a Metrohm 715 potentiometer. Reducing

sugars content was measured following IHC recommendations using the classic Fehling method. However, solution equilibrium potential was simultaneously measured to avoid typical errors in equivalency point identification due to deficient sensing of indicator dye veer, which is frequently altered by the cuprous oxide precipitate and indicator reoxidation. Potentiometric monitoring (Tacussel S6N potentiometer) of the progression of the Fehling reaction confirmed the reaction stoichiometry and produced more accurate and reproducible results.

Voltammetry using a mercury drop electrode, known as polarography, was used to quantify HMF and fructose levels in the honey samples. Polarographic signals were recorded using a Metrohm 797 VA Computrace potenciostat. A method reported in the literature (Heyrovsky J., Zuman, P., 1968) and tested in our laboratory (Reyes-Salas *et al* 2006; Gazcón O. N., 2012) helped to detect both the HMF and fructose reduction signals, and then their levels in the honey were quantified with the calibration curve method. This allows for improved results accuracy and reproducibility, as well as shorter analysis time. Fructose was measured using a direct reduction signal in which glucose does not interfere under the conditions used here. Quantification of HMF was done with differential pulse polarography (DPP) under conditions of direct HMF reduction. This provides the benefits stated above, and, because the electrochemical method is direct, has advantages over commonly used quantification methods. It eliminates all the classic inaccuracies of previous chemical derivatization or transformation reactions, avoids kinetic problems in the reactions used to quantify HMF, and, because no other chemical reagents are used, lowers costs and health risks.

3. RESULTS AND DISCUSSION

3.1. HMF quantification

For each honey sample a solution was made of 0.4-0.5 g honey/mL deionized water. The reference was a HMF standard solution (1.168 $\times$ 10^{-2} mol/L in deionized water), and a borate buffer (pH 10) was used as supporting electrolyte. These were then analyzed with DPP, producing electrochemical signals like that in Figure 2.

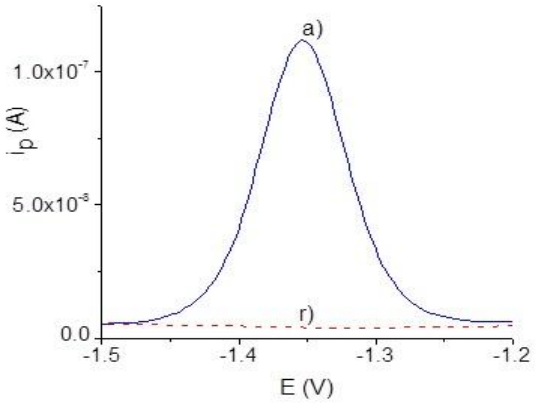

Fig. 2. Typical HMF reduction polarogram produced by DPP in a 0.5 mol/L boric acid/sodium borate medium, pH = 10, sweep rate = 2 mV/s, ΔE = 50 mV. a) 2.8x10^{-5} mol/L HMF; r) Residual current

When sample HMF was quantified using the electrochemical method, graphics like that in Figure 3 were produced.

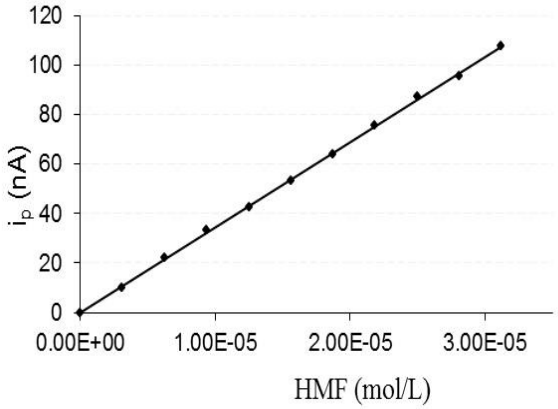

Fig. 3 DPP calibration curve of HMF in a boric acid/sodium borate solution (pH 10)

The points follow a linear trend expressed by the equation Y = 3.0x10^{6}X + 0.0777, where X represents HMF concentration, and Y peak current intensity. This trend has a very good linear correlation (R^2 = 0.9996). Sample HMF quantification was done using the straight line equation.

3.2. Fructose quantification

Fructose was quantified by first preparing solutions of approximately 0.1 g honey/mL deionized water. Supporting electrolyte was a $CaCl_2$ 1 mol/L solution, and the fructose standard solution was 1.2×10^{-2} mol/L in deionized water. These were then analyzed with DPP, producing electrochemical signals like that in Figure 4. Two reduction peaks were observed but the -1.562 V peak has better analytical qualities, which coincides with Gazcón (2012).

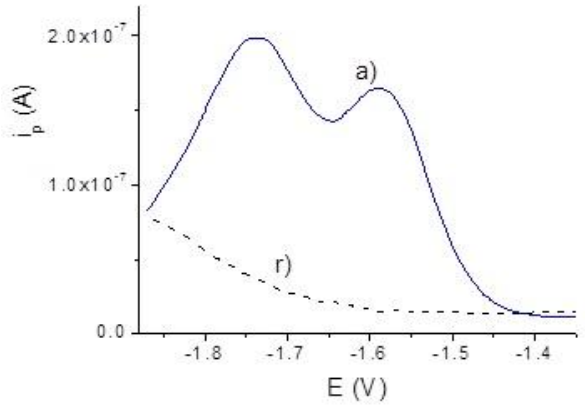

Fig. 4. Typical fructose reduction polarogram produced by DPP in a 1 mol/L $CaCl_2$ medium, sweep rate = 2 mV/s, $\Delta E_{impulse}$ = 50 mV; a) $6.1x10^{-4}$ mol/L Fructose. r) Residual, 1 mol/L $CaCl_2$

Table I shows the analytical quality characteristics of two electrochemical methods used to quantify fructose.

3.3. Sucrose quantification

Sucrose was quantified by difference between reducing sugars content before acid hydrolysis and sugars content after hydrolysis, also known as sucrose inversion. The acid medium hydrolyzes the disaccharide, resulting in glucose and fructose. These are then quantified using the reducing sugars quantification method described previously. Quantifications were done by preparing a solution of 1 g honey and deionized water added to complete a 200 mL volume. A 40 mL sample was taken from this solution, 2 mL 10% HCl added and the mixture left at room temperature (22- 25 °C) for 24 hours. Volume was then completed to 50 mL with deionized water and reducing sugars analysis run following the previously described method.

3.4. Discussion

All *Apis* honey samples (Ap1 to Ap8) were from the Spring 2010 harvest in Michoacán state, Mexico. *Meliponini* honey samples were from six different regions: Me1 from Misiones province, Argentina (*Tetragonisca angustula*); Me2 from Michoacán (*Scaptotrigona hellwegeri*); Me3 from Guerrero state, Mexico (*Geotrigona acapulconis*); Me4 and Me5 from San Luis Potosí state, Mexico (unspecified species); Me6 from Puebla state (*Scaptotrigona mexicana*); Me7 from Yucatan state (probably *Melipona beecheii*); and Me8 from Michoacán (*Friseomelita nigra*) (Table II).

The principal difference between the *Apis* and *Meliponini* honeys was the relatively high moisture content in the latter. The *Apis* samples had a moisture content ranging from 17 to 20%, within Mexican standards. In contrast, the *Meliponini* samples had moisture contents ranging from 25 to 34%, exceeding standard values. By no means does this mean that the *Meliponini* samples are lower quality or that the sugars in them would ferment more easily or rapidly than in the *Apis* samples. The *Meliponini* samples had a lower mean ash content and pH than the *Apis* samples.

As expected, reducing sugars content was lower in the *Meliponini* honey samples, largely due to their higher moisture content. This placed them below the reducing sugars levels stipulated in the Mexican standards, although this does not reflect on their quality. Fructose and glucose content was lower in the *Meliponini* samples than in the *Apis* samples. Sucrose content was slightly below that of the *Apis* samples, although one sample (Me3) did have content higher (6.65%) than the 5% allowed in *Apis* honeys. Worth noting is that the proportion fructose/reducing sugars was similar among the two sample types, accounting for 50.6% of reducing sugars. This applies only to the analyzed samples.

Table I. Detection (DL) and quantification limits (QL), linearity and equation produced with the reduction of fructose at -1.562 V using tast polarography and DPP in a 1 mol/L $CaCl_2$ medium.

Polarography mode	DL (mg/L)	QL (mg/L)	Linearity r	Linearity n-2	Equation i (nA); $C_{Fruct.}$ (mol/L)
DPP (E_{p1} = -1.562 V)	18.9	62.9	0.9972	19	$i_p = 1.85x10^5 C_{Fruct} - 29.1$
Tast	26.4	88.0	0.9959	5	$i = 3.02x10^6 C_{Fruct} - 463$

Table II. Compositions of *Apis* and *Meliponini* honey samples from six regions.

Sample	Moisture %	pH	Ash %	Reducing sugars %	Fructose %	Sucrose %	HMF mg/Kg
Ap1	19.6	3.83	0.0330	76.71	45.34	0.35	13.1
Ap2	19.0	4.59	0.1422	74.94	36.24	3.19	15.0
Ap3	18.8	6.20	0.2457	65.63	18.61	1.36	25.0
Ap4	17.8	4.91	0.1250	73.93	34.19	2.40	8.9
Ap5	18.0	6.33	0.2514	64.03	43.51	1.40	45.8
Ap6	19.6	4.73	0.1767	71.36	45.34	2.05	15.1
Ap7	18.8	3.56	0.1652	75.83	33.29	1.26	34.0
Ap8	19.6	4.31	0.1020	74.50	35.71	0.65	7.8
Me1	25.0	4.55	0.1250	61.70	32.79	0.87	18.1
Me2	33.0	3.23	0.0101	55.45	27.90	0.31	13.4
Me3	26.4	3.83	0.0848	50.57	45.34	6.65	35.4
Me4	26.0	3.40	0.0216	63.49	36.24	0.80	8.8
Me5	25.0	3.05	0.0330	63.17	17.10	1.22	**302
Me6	27.6	3.20	0.0100	52.39	28.10	1.26	58.8
Me7	21.6	3.40	0.0733	64.14	29.28	0.50	69.0
Me8	34.0	3.56	0.0101	45.70	16.86	0.64	27.6
Mean Ap	**18.9**	**4.8**	**0.1552**	**72.12**	**36.53**	**1.58**	**23.7**
*SD	0.71	1	0.0726	4.78	8.8	0.93	13.8
(%)	(3.8 %)	(20%)	(46.8%)	(6.6%)	(24%)	(59%)	(56%)
Mean Me	**27.3**	**3.5**	**0.0460**	**57.08**	**28.88**	**1.53**	**33.0**
SD	4.2	0.48	0.0433	7.03	9.3	2.09	23.0
(%)	(15.4%)	(13%)	(94.2%)	(12.3%)	(32%)	(136%)	(70%)

*SD: Standard deviation. **Sample collected in 2007 (aged)

Mean HMF content for the *Apis* honeys was below established limits while mean content for the *Meliponini* honeys was above them; however, both types varied widely. The updated standards for *Apis* honey from tropical regions allow for HMF levels as high as 80 mg/Kg, which would place all the tested *Meliponini* samples within allowed limits.

4. CONCLUSIONS

Moisture content is the parameter which most differs between the *Apis* and *Meliponini* honeys. Low moisture content is vital in *Apis* honeys to prevent sugar fermentation; however, their higher moisture content does not cause the *Meliponini* honeys to ferment.

Reducing sugars content was lower in the *Meliponini* honeys than in the *Apis* honeys; this is to be expected since water and sugars are the two principal components in honey and the *Meliponini* honeys have higher moisture content. Both honey types have similar sucrose contents.

Their high reducing sugars content demonstrates that the *Meliponini* honeys are excellent sweeteners.

Ash content was lower in the *Meliponini* honeys than in the *Apis* honeys. This parameter varies by geographic zone and flowers visited.
Electrochemical methods can be used for physicochemical analyses to measure honey quality, be it from *Apis* or *Meliponini* species.
Meliponini and *Apis* honeys have different physicochemical compositions. Therefore, Mexican *Apis* honey standards cannot be used to qualify *Meliponini* honeys.
Although the number of samples analyzed here was relatively small, it was clear that the *Apis* honeys exhibited minimal variation in moisture and reducing sugars contents while the *Meliponini* honeys exhibited wider variation. This greater variation may be associated with differences between *Meliponini* species, suggesting that species may need to be considered when analyzing *Meliponini* honeys.
The wide variation observed in HMF content among the honeys indicates the quality in the process of handling and storage of honey, i.e. of human work.
This is a preliminary study intended to help in developing quality standards for *Meliponini* honeys and broaden their marketing possibilities. Further study is still required to better understand the composition of honey from stingless bees.

5. ACKNOWLEDGEMENTS

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