Scientific Note

A SCIENTIFIC EVENT TO PROMOTE KNOWLEDGE REGARDING HONEY FROM STINGLESS BEES: 1. PHYSICAL-CHEMICAL COMPOSITION

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ABSTRACT: When compared to honey produced by *Apis mellifera*, honey from stingless bees is little known by the general public. Also, analysts and supervision agencies are faced with difficulties due to the lack of standards for honey produced in “pots” instead of “honeycomb”, although it has been widely appreciated before Colombus. A scientific event about honey was promoted in the city of Merida, Venezuela, 2005 in order to obtain honey samples from worldwide stingless bees, whereas six entries were received for the analysis. The identification of the meliponines that produced the honey samples was carried out in order to confirm their entomological origin. This scientific event was suggested to broaden the data bank regarding honey produced by Meliponines, for the International Honey Commission, featuring its standardization. Honey samples produced by six meliponine species from four countries were analyzed as follows: *Melipona favosa* (Venezuela), *Tetragonisca fiebrigi* (Argentina), *M. compressipes manaosensis* and *M. seminigra pernigra* (Brazil), *Trigona carbonaria* and *Austroplebeia symei* (Austrália). The physico-chemical properties of their honey varied as follows: antioxidant activity (0.0-299.7) M Trolox equivalents, ash (0.01-0.90) g.100 g^{-1} honey; diastase activity (0.0-34.7) DN, free acidity (14.0-257.8) meq.kg^{-1} honey, HMF (15.0-33.2) mg.kg^{-1} honey, moisture content (23.0-29.8) g.100 g^{-1} honey, nitrogen (27.7-131.5) mg.100 g^{-1} honey, reducing sugars (48.0-73.7) g.100 g^{-1} honey, sucrose (0.7-2.8) g.100 g^{-1} honey, vitamin C (17.7-26.9) mg.100 g^{-1} honey. Besides promoting the knowledge regarding honey from stingless bees, this work is a contribution for the data bank in order to adjust the quality standards of honey from stingless bees.

Key words: antioxidant activity, Meliponini, physico-chemical analysis, quality control, scientific honey event, stingless bees

EVENTO CIENTÍFICO PARA PROMOVER O CONHECIMENTO SOBRE O MEL DE ABELHAS SEM FERRÃO: 1. COMPOSIÇÃO FÍSICO-QUÍMICA

RESUMO: Quando comparado ao mel produzido por *Apis mellifera*, o mel de abelhas sem ferrão é pouco conhecido pelo público em geral. Também os analistas e órgãos fiscalizadores enfrentam dificuldades devido à falta de padrões para o mel produzido em “potes” ao invés de “favos”, embora este tenha sido amplamente apreciado desde antes de Colombo. Uma mostra científica sobre mel foi promovida na cidade de Mérida, Venezuela, 2005, para obter amostras de méis de abelhas sem ferrão do mundo, sendo recebidas seis amostras para análises. A identificação dos meliponíneos que produziram as amostras de méis foi feita para confirmar sua origem entomológica. A realização desta mostra científica foi sugerida para ampliar o banco de dados sobre o mel produzido por Meliponini, para a Comissão Internacional de Mel, enfocando a sua padronização. Amostras de méis produzidas por seis espécies de meliponíneos de quatro países foram analisadas: *Melipona favosa* (Venezuela), *Tetragonisca fiebrigi* (Argentina), *M. compressipes manaosensis* e *M. seminigra pernigra* (Brasil), *Trigona carbonaria* e *Austroplebeia* *symei* (Austrália). As propriedades físico-químicas de seus méis variaram como segue: atividade antioxidante (0,0-299,7) µM equivalentes de Trolox, cinzas (0,01-0,90) g.100 g^{-1}; atividade diastásica (0,0-34,7) DN, acidez livre (14,0-257,8) meq.kg^{-1}, HMF (15,0-33,2) mg.kg^{-1}, umidade (23,0-29,8) g.100 g^{-1}, nitrogênio (27,7-131,5) mg.100 g^{-1}, açúcares redutores (48,0-73,7) g.100 g^{-1}, sacarose (0,7-2,8) g.100 g^{-1}, vitamina C (17,7-26,9) mg.100 g^{-1}. Além de promover o conhecimento sobre o mel das abelhas sem ferrão, este trabalho é uma contribuição para o banco de dados para ajuste de padrões de qualidade do mel de abelhas sem ferrão.

Palavras-chave: atividade antioxidante, Meliponini, análise físico-química, controle de qualidade, mostra científica sobre mel, abelhas sem ferrão

INTRODUCTION

Quality standards of *Apis mellifera* honey have an international Codex Alimentarius Commission (1969, 1987, 2001) reference. However, honey is also produced by stingless bees, Tribe Meliponini (Michener, 2000), in the tropical and subtropical regions (Crane, 1992). This honey was widely relished in the Americas before Columbus. Paradoxically, there are no standards for meliponine honey production. Meliponiculture probably needs more biological information in order to become profitable (Cortopassi-Laurino et al., 2006) and also to reduce the cost of marketing stingless bee honey. The large quantity of stingless bee species producing honey and the species of bee flora that need pollen identifications to assign a botanical origin, increase the complexity of an attempt to set quality standards and bioactive properties of stingless bee honey. The International Honey Commission (IHC), chaired by Dr. Werner von der Ohe is also interested in creating standards for stingless bee honey. For this purpose, besides a review of already published data, a new honey database also needs to be created with agreeing methods to confirm the composition of honey produced by different species of stingless bees.

Working with a database of 27 samples from Venezuela, honey quality factors became useful to discriminate stingless bee honey from different entomological groups (Vit et al., 1998), but this preliminary observation needs follow up studies with a larger collection, including stingless bee honey produced in other tropical and subtropical locations, prior to further conclusions.

The objective of the present work was to promote the knowledge of stingless bee honey by organizing a scientific honey event, with the cooperation of an international multidisciplinary team for sample collection and analysis, towards a long term goal to propose stingless bee honey quality standards. The identification of bees that produce meliponine honey was needed to confirm the entomological origin of the honey samples received from Argentina, Australia, Brazil and Venezuela. Besides the physicochemical parameters accepted for honey quality control (ash, diastase activity, free acidity, HMF, moisture content, nitrogen, reducing sugars and sucrose), the antioxidant activity and vitamin C content were also measured.

MATERIAL AND METHODS

Scientific honey event

The scientific honey event presented in this work was initially programmed as a honey competition in order to gather samples from different locations where stingless bees produce honey. A honey competition only needs HMF and moisture content for the physicochemical analysis. However, due to the limited number of honey samples received, more analyses were done and discussed with the public in order to contribute to the knowledge of this bee product.

Stingless bee samples

The collection of stingless bees was necessary for their entomological identification. The bees (15-20) were sent either in ethanol 95° or point-mounted. Table 1 shows the information kindly requested by Camargo JMF and Pedro SRM, for the bee collection.

Honey samples

Six stingless bee honey samples were received from R. Álvarez (Venezuela), A.E. Tricio (Argentina), S. Meneses and S. Foçassa (Brazil), and T.A. Heard and T. Carter (Australia), codified as follows: 3 *Melipona* 3 VEN 2, 4 *Tetragonisca* 1 ARG 1, 5 *Melipona* 4 BRA 1, 6 *Melipona* 5 BRA 2, 7 *Trigona* 1 AUS 1, 8 *Tetragonisca* AUS 2. Their entomological origin as well as their common names and the geographical origin are given in Table 2.

Physicochemical analyses

The antioxidant activity was measured in triplicates and the follow,ing analyses were conducted in duplicates:

Table 1 - Information of stingless bee samples[1].

Collection Number	Country	
NEST		
Location		
Geographic coordinates (if possible use GPS)		
Hive type (feral or rational)		
Meliponary type (modern or traditional)		
Origin of the nest (location of collection of the nest, if possible with GPS)		
Substrate description (tree cavity, underground, termite, ant or bird nest, exposed on the branches, exposed on the wall, etc.)		
Entrance (tubular shape, etc.) and material (resin, cerumen, vegetal particles, clay, etc.)		
BEE		
Common name		
Identification		
COLLECTOR		
Name		
Date		
Address		
e-mail		

[1] Kindly suggested by JMF Camargo and SRM Pedro, FFCLRP/USP, Brazil.

Table 2 - Geographical and entomological origin of honey samples.

Honey samples	Country of origin	Stingless bee species	Common name
1	Venezuela	*Melipona favosa* Fabricius, 1789	erica
2	Argentina	*Tetragonisca fiebrigi* Schwarz, 1938	yatei
3	Brazil	*Melipona compressipes manaosensis* Schwarz, 1932	jupará
4	Brazil	*Melipona seminigra pernigra* Moure & Kerr, 1950	uruçu
5	Australia	*Trigona carbonaria* Smith, 1854	no local name
6	Australia	*Austroplebeia symei* (Rayment, 1932)	no local name

Antioxidant activity - decolorization of the ABTS$^{+\bullet}$radical cation is known as total antioxidant activity (TAA). Following the method of Re et al. (1999) 7 mM ABTS and 4.9 mM ammonium persulfate was mixed 1:1, covered with foil for 16 h, and diluted up to an absorbance of 0.7 ± 0.2 at 734 nm, which was approximately 40 L of reagent + 760 L ethanol, 10 L of the diluted honey (0.1 g of honey plus 1 mL of 20% v/v ethanol) were added, shaken vigorously and variations of absorbance were recorded at 734 nm during 6 min. A calibration curve with 0.625-1.25-2.5 M Trolox was used to measure the percentage of decolorization, to estimate M Trolox equivalents (n=3).

Ash - A honey sample of 2.5 ± 0.1 g was incinerated in a crucible, in order to obtain g ash.100 g^{-1} honey (COVENIN, 1984a).

Diastase activity - Diastase activity was measured using a buffered solution of soluble starch and honey, which was incubated in glass tubes in a thermostatic bath. The time required to reach a specified reaction endpoint (absorbance less than 0.235 nm) was determined spectrophotometrically (Metrolab 1700 UV/VIS Spectrophotometer). The diastase activity, as Gothe's degrees, is expressed as mL of 1% starch hydrolyzed by the enzyme in one gram of honey per one hour, also known as the diastase number (DN) (CAC, 1990).

Free Acidity - A honey sample of 2.5 ± 0.1 g was diluted with distilled water and titrated with a 0.1 N NaOH solution (COVENIN, 1984a).

Hydroxymethylfurfural (HMF) - The HMF was determined after clarifying honey samples with Carrez reagents (I and II) and the addition of sodium bisulphate in reference solution (AOAC, 1990). UV absorbance in samples was determined at 284 and 336 nm in a 1 cm^3 quartz cuvette in a spectrophotometer (Metrolab 1700 UV/VIS Spectrophotometer). Results are expressed in mg $HMF.kg^{-1}$ honey.

Moisture content - The refractometric index of honey was converted into moisture content using the Chataway Table (COVENIN, 1984a).

Nitrogen - A standard microKjeldahl digestion of 200 ± 10 mg honey followed by distillation and titration, to express mg nitrogen.100 g^{-1} honey (AOAC, 1990).

Reducing sugars and apparent sucrose - The Lane-Eynon hot titration method was used to measure the content of reducing sugars and apparent sucrose (COVENIN, 1984a).

Vitamin C - The determination of vitamin C was carried out by the 2,4-dinitrophenylhidrazine method. Briefly, in the protein-free supernatant obtained by the treatment with metaphosphoric acid, ascorbic acid was oxidized by copper ions to dihydroascorbic acid and diketogulonic acid. These acids reacted with 2,4-dinitrophenylhidrazine in the presence of sulphuric acid given phenylhydrazones that absorbed at 520 nm (Pesce and Kaplan, 1990).

Statistical analyses

SPSS 12.0 software was used to calculate mean ± SD for all variables and for bivariate correlation with Pearson coefficient.

RESULTS AND DISCUSSION

Honey event

During the scientific stingless bee honey event held at Consejo de Desarrollo Científico, Humanístico y Tecnológico from Universidad de Los Andes, on December 8th, 2005, participants received information on stingless bee honey physicochemical composition, compared to *A. mellifera*. Participants also tasted the honey displayed in goblets for a comparative sensorial evaluation. Dr. Alberto Ortiz Valbuena, the international guest from Centro Apícola Regional in Guadalajara - Spain conducted the professional degustation of honey. This type of event should continue in several cities, in order to promote the scientific knowledge of stingless bee honey.

Stingless beekeepers received special recognition for their work. Instead of a final prize for the competition, all participants received the compositional data of their honey, and were acknowledged with a plate for their important contribution to promote the quality standards of stingless bee honey. The multidisciplinary team facilitated the participation of stingless bee honeys from Argentina, Australia, Brazil and Venezuela. Stingless bee entomologists from Brazil and Australia kindly identified the bees. Analysts from Brazil and Venezuela determined the physicochemical data presented in this work. Different botanical origins were illustrated with pollen plates of the honey samples. However, melissopalynological analysis will be done by Dr. OM Barth from the Instituto Oswaldo Cruz in Brazil, and Dr. DW Roubik from the Smithsonian Tropical Institute in Panama.

Table 3 shows the data on honey components of the six stingless bee honey samples, which varied as follows: antioxidant activity (0.0-299.7) M Trolox equivalents, ash (0.01-0.90) g.100 g^{-1} honey, diastase activity (0.0-34.7) DN, free acidity (14.0-257.8) $meq.kg^{-1}$ honey, HMF (15.0-33.2) $mg.kg^{-1}$ honey, moisture content (23.0-29.8) g.100 g^{-1} honey, nitrogen (27.7-131.5) mg.100 g^{-1} honey, reducing sugars (48.0-73.7) g.100 g^{-1} honey, sucrose (0.7-2.8) g.100 g^{-1} honey and vitamin C (17.7-26.9) mg.100 g^{-1} honey. Values out of standards compared with *A. mellifera* honey are indicated.

Comparing the data of stingless bee honey analyzed here with the international standards for *A. mellifera* honey (CAC, 2001), it is noted that: 1. Moisture content of the six stingless bee honey samples were higher than the standard for *A. mellifera* honey (max 20% of moisture content). 2. The standards for sucrose content of not more than 5 g.100 g^{-1} honey and for the reducing sugars content (sum of fructose and glucose) of not less than 60 g.100 g^{-1} blossom honey and 45 g.100 g^{-1} honeydew honey or blends with honeydew honey, were met by all stingless bee honey samples participating in the scientific honey event. However, the method of sugar analysis used here is not the HPLC recommended in the new regulation (CAC, 2001). Although the maximum of 5 g sucrose.100 g^{-1} honey remained unchanged from that suggested in the Codex Alimentarius Commission (CAC, 1969), the standard for the reducing sugars considered as a minimum of 65 $g.100g^{-1}$ honey with the Lane-Eynon method is now lower, therefore, our data should be compared with local regulations (COVENIN, 1984a) or international standards with traditional methods for sugar analysis (CAC, 1969). Only the Brazilian *Melipona* spp. honey contained more than the suggested minimum of 65 g reducing sugars.100 g^{-1} honey.

Table 3 - Components of stingless bee honey samples. Values are average ± (SD), n = 2.

Honey components	Stingless bee[1] honey samples					
	1	2	3	4	5	6
Antioxidant activity[2]	45.8	62.5	35.3	0.0	299.7	193.4
(M Trolox equivalents)	(18.89)	(24.41)	(4.42)	(0.00)	(101.91)	(136.08)
Ash	0.18	0.31	0.01	0.03	0.45	0.90
($g.100\ g^{-1}$ honey)	(0.01)	(0.01)	(0.00)	(0.00)	(0.01)	(0.01)
Diastase activity	0.0*	34.7	0.0*	0.0*	0.0*	1.3*
(DN)	-	(1.29)	-	-	-	(0.27)
Free acidity	63.6*	62.3*	14.0	28.0	257.8*	45.0
($meq.kg^{-1}$ honey)	(0.67)	(0.07)	(0.00)	(0.00)	(3.18)	(0.21)
Hydroxymethylfurfural	21.5	15.0	19.8	23.4	33.2	21.6
($mg.kg^{-1}$ honey)	(3.61)	(1.02)	(0.07)	(4.08)	(0.69)	(6.71)
Moisture content	29.8*	23.0*	23.4*	23.0*	25.6*	23.6*
($g.100\ g^{-1}$ honey)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Nitrogen	52.8	76.2	27.7	42.7	131.5	123.1
($mg.100\ g^{-1}$ honey)	(3.59)	(0.47)	(0.07)	(0.35)	(0.00)	(0.30)
Reducing sugars[3]	63.8*	61.0*	73.7	71.5	48.0*	61.2*
($g.100\ g^{-1}$ honey)	(0.23)	(0.21)	(0.00)	(0.00)	(0.16)	(0.26)
Sucrose[3]	0.8	2.8	0.8	0.7	1.3	2.6
($g.100\ g^{-1}$ honey)	(0.21)	(0.18)	(0.00)	(0.00)	(0.01)	(0.02)
Vitamin C	20.6	21.4	23.1	26.9	17.7	18.1
($mg.100\ g^{-1}$ honey)	(8.08)	(3.95)	(14.38)	(19.90)	(8.35)	(6.90)

[1] See stingless bee species in Table 2.
[2] n=3.
[3] Reducing sugars and sucrose are compared to local standards (COVENIN, 1984b).
* Values do not fulfill *A. mellifera* international honey standards (CAC, 2001).

From the additional composition and quality factors included in the annex of the Codex Alimentarius Commission (CAC, 2001), diastase activity, free acidity and hydroxymethylfurfural content were analyzed for the honey event. These values are suggested for voluntary application but not for official use, although they are still present in the official regulations of Venezuela (COVENIN, 1984b). Compared to the *A. mellifera* honey values: 1. Only the *T. fiebrigi* honey from Argentina had a diastase activity not lower than the standard. 2. The suggested free acidity of not more than 50 $meq.kg^{-1}$ honey, was easily fulfilled by the two Brazilian honey samples of *M. compressipes manaosensis* and *M. seminigra pernigra*, with values of 14.0 and 28.0 $meq.kg^{-1}$ honey, respectively, but was close to the limit in the *A. symei* Australian honey sample, with 45.0 $meq.kg^{-1}$ honey. However, for the Venezuelan *M. favosa* and the Argentinian *T. fiebrigi* honey samples, the free acidity was slightly higher than the suggested limit, with values of 63.6 and 62.3 $meq.kg^{-1}$ honey, respectively. These minor discrepancies on free acidity, become conspicuous for the *T. carbonaria* honey, with 257.8 $meq.kg^{-1}$ honey. 3. Although all the samples studied for the honey event, came from regions with tropical environmental temperatures, none of them exceeded the suggested lowest limit (40-80 mg $HMF.kg^{-1}$ honey).

In a seminal report where stingless bee honey composition is compared with that of *A. mellifera*, values on higher water content and lower diastase activity were the main differences, as well as higher total acidity for some stingless bee species such as *Scaptotrigona postica* and *M. quadrifasciata anthidioides* (Gonnet et al., 1964). These observations were confirmed in more recent papers (Vit et al., 1994; Vit et al., 2004; Villas-Bôas and Malaspina, 2005), whereas the water content of stingless bee honey tended to be higher than in *A. mellifera* honey, the diastase activity is lower, as well as the reducing sugars. Acidity varies according to the samples; some fulfill the maximum of 50 $meq.kg^{-1}$

honey, wheareas others are excessively high.

The use of honey in the diet and in traditional medicine could provide protection to reduce the damage caused by reactive oxygen species (Schramm et al., 2003). In this work, the antioxidant activity of four honey samples increased with the acidity ($r = 0.97$, $p < 0.01$), therefore more investigation is needed to explain this correlation because high acidity is characteristic for some stingless bee honey. Vitamin C ranged from 18.1 to 26.9 mg.100 g^{-1} honey, and total antioxidant capacity ranged from 0.0 to 299.7 µM Trolox equivalents. Although there are no reports regarding the concentration of vitamin C in stingless bee honey, it is known that *A. mellifera* honey has a low concentration of this vitamin; less than 5 mg.100 g^{-1} (White, 1975).

The lack of correlation between vitamin C and total antioxidant activity confirms the idea expressed by other authors that the total antioxidant capacity of honey is likely the result of the combined activity and interactions of a wide range of compounds, including phenolics, peptides, organic acids, enzymes, Maillard reaction products, and possibly other minor components (Gheldof et al., 2002). It is worth noting that some authors have suggested that organic acids, such as gluconic, citric, and malic acids, might contribute to the antioxidant capacity of honey. Organic acids chelate metals and therefore can synergistically enhance the action of other antioxidants, such as phenolics (Rajalakshmi and Narasimhan, 1996).

Descriptive data for the 10 physicochemical parameters analyzed in honey produced by six species of stingless bees collected in this work, show compositional variations of honey according to the stingless bee species (Table 3). Therefore, the collection of more honey samples per species, such as that provided by 11 honey samples of *M. asilvai* (Souza et al., 2004), is needed for forthcoming work aiming at the proposal of stingless bee honey standards, as suggested by S Bogdanov (Personal Communication). The contribution of this work for the IHC database was the increase of six honey samples produced by six different species of stingless bees from Argentina, Australia, Brazil and Venezuela.

The contribution of this work for the IHC database was its increase on six honey samples produced by six different species of stingless bees from Argentina, Australia, Brazil and Venezuela.

CONCLUSIONS

1. The stingless bee honey IHC database increased in six samples, and variations were observed in the physicochemical composition of honey from six stingless bee species (*Austroplebeia symei*, *Melipona compressipes manaosensis*, *M. favosa*, *M. seminigra pernigra*, *Tetragonisca fiebrigi* and *Trigona carbonaria*). Consequently, further honey sampling is needed to reach at least 10 honey samples of these important stingless bee species from Argentina, Australia, Brazil and Venezuela, to substantiate a proposal of their quality standards.

2. Diastase activity, free acidity, and reducing sugars of some stingless bee honeys were different from the suggested standards for *A. mellifera*, whereas moisture content was higher than the maximum for *A. mellifera* in all the samples (23.0 - 29.8 g.100 g^{-1} honey).

3. In addition to traditional standards for physicochemical honey quality control, such as ash, diastase activity, free acidity, hydroxymethylfurfural, moisture content, nitrogen, reducing sugars and sucrose, expanded indicators of antioxidant activity and vitamin C were also suggested to support the medicinal uses of meliponine honey.

4. The physicochemical composition of stingless bee honey was discussed in the scientific honey event, in order to promote the knowledge of this bee product by comparing it with *A. mellifera* honey.

5. A multidisciplinary team of experts from Argentina, Australia, Brazil, Panama and Venezuela cooperated with the stingless bee entomological identification, honey collection and quality control analyses.

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