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Apis mellifera vs Melipona beecheii Cuban polifloral honeys: A comparison based on their physicochemical parameters, chemical composition and biological properties

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ABSTRACT

Several polifloral honeys from the *Apis mellifera* and *Melipona beecheii* bee were analyzed to evaluate their pollen profile, physicochemical parameters, chemical composition and their antioxidant and antimicrobial activity. Flavonoids and other phenolic derivatives, 16 compounds in *Apis mellifera* honeys and 19 compounds in *Melipona beecheii* honeys, were identified using the HPLC-DAD-ESI-MS/MS analysis. The physicochemical parameters were within the ranges reported for these honeys, showing some of the distinctive characteristics of *M. beecheii* honeys compared to *A. mellifera* honeys, such as their high moisture and acidity. *M. beecheii* honey showed the highest values of total antioxidant capacity and total phenolic, flavonoid, carotenoids, ascorbic acid, free amino acid and protein contents compared to *A. mellifera* honey. *M. beecheii* honey also exhibited a higher antimicrobial activity. Our result shows that *M. beecheii* honeys is an important source of bioactive compounds with relevant biological properties compared to *A. mellifera* honey.

1. Introduction

Honey has been used by humans both as food and medical product from ancient times to the modern civilization (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010a), where its use in human nutrition and health is due to its chemical composition. Honey composition is varied and is linked to several factors that directly affect its composition and quality such as the bee species, floral origin, environmental and storage conditions (Gheldof, Wang, & Engeseth, 2002). Honey is a rich source of carbohydrates, making it widely used as a natural sweetener, as well as an important source of other minors constituents, which are more related to its biological properties such as polyphenols, carotenoids, minerals, proteins, free amino acid, enzymes and vitamins (Alvarez-Suarez, Giampieri, & Battino, 2013).

The world honey production and consumption is based on the product obtained from the species *Apis mellifera*, whose producers are principally located in Europe and Asia. However, there are other small productions that are based on products obtained from other species of bees, such as the stingless bee, better

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known and mainly used in Australia, Africa and South America. Stingless bee honey production is limited compared with *A. mellifera* honeys, since it does not reach industrial levels, has lower shelf life, lack of quality standards and little knowledge about the product (Vit, Medina, & Enríquez, 2004). In Cuba, the main production of honey is obtained from *A. mellifera* and, similar to the countries of South America, Africa and Australia, the production of honey from the stingless bees is scarce and without any type of industrialization, with *Melipona beecheii* as the principal stingless bees species distributed throughout the country (Fonte, 2007). The principal studies in Cuban honeys have focused mainly on the physicochemical parameters, chemical composition and biological properties of the main monofloral honeys from *A. mellifera* (Alvarez-Suarez, Giampieri, Damiani, Astolfi, Fattorini, Regoli et al., 2012; Alvarez-Suarez, González-Paramás, Santos-Buelga, & Battino, 2010b; Alvarez-Suarez, Tulipani, Díaz, Estevez, Romandini, Giampieri et al., 2010c), while studies on polyfloral honeys have not gone beyond simple quality controls, such as physicochemical parameters. On the other hand, studies on the physicochemical parameters, chemical composition and biological properties in stingless bee honeys are still very few (Fonte et al., 2013).

Therefore, the aim of the present study was to determine and compare the botanical origin, physicochemical parameters, chemical composition and biological properties in several polyfloral honeys from *A. mellifera* and *M. beecheii* from the central region of Cuba. This work constitutes the first report on the chemical composition and biological properties of stingless bees honey from Cuba.

2. Materials and methods

2.1. Honey samples, melissopalynologycal and physiochemical analysis

A total of 16 polifloral honey samples, 8 for each honey type, of two different bee species (*A. mellifera* and *M. beecheii*) were used in the study. Honey samples were provided by beekeepers and collected between April and August 2013 in the municipalities of Sancti Spíritus, Cabaiguán and Fomento, belonging to the province of Sancti Spíritus in the central region of Cuba. The municipalities were selected on the basis of their melliferous and polliniferous potential, as well as their geographic proximity, according to the floral maps designed by the National Center of Apiculture Research of Cuba, which ensures the similarity between plants serving as sources of pollen and nectar to the bees. On the other hand, the sampling period was selected according to the predominance of several blooms, which guarantees the polyfloral character of honeys. The hives sampled belonged to fixed apiaries, located in the same area, at 100 m distance between both bee species. Samples were stored in sterilized containers and maintained at 6–8 °C in the dark until analysis. The polyfloral character of honeys was confirmed according to the melissopalynologycal methods as previously reported (Louveaux, Maurizio, & Vorwohl, 1978), while physicochemical analysis for quality [ashes (%), electrical conductivity (mS/cm), color (mm Pfund), pH, free acidity (mequiv/kg), humidity (%)], diastases index (U Schade) and HMF test (mg/kg)] were verified by the official methods (AOAC, 1990; IHC, 2002).

A solution of artificial honey (1.5 g of sucrose, 7.5 g of maltose, 40.5 g of fructose and 33.5 g glucose in 17 mL of de-ionized water) (Cooper, Molan, & Harding, 2002) was included in the study to evaluate the contribution of the predominant sugars to the assayed activities.

2.2. Determination of total phenolic content (TPC), total flavonoid content (TFC), total carotenoids content (TCC), free amino acids and protein content

For TPC analysis honey samples (1 g) were diluted to 10 mL with distilled water, filtered through Minisart filter of 45 μm (PBI International) and analyzed using a Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). TPC results were expressed as mg of gallic acid equivalents (GAE) per 100 g of honey (mg GAE/100 g of honey). For TFC determination the samples were diluted in methanol (50% w/v), analyzed using the aluminum trichloride method (Chang, Yang, Wen, & Chern, 2002) and results were expressed as mg of (+)-catechin equivalents per 100 g of honey (mg CE/100 g of honey). TCC was determined spectrophotometrically as previously reported (Alvarez-Suarez et al., 2010c). The samples (1 g) were vigorously shaken (500 r m^{-1}) with 10 mL of *n*-hexane–acetone mixture (6:4) for 10 min at room temperature and filtered through Whatman No. 4 filter paper. The absorbance was measured spectrophotometrically at 450 nm and results were expressed as mg of β-carotene equivalents per kilogram of honey (mg βcarotE/kg of honey).

Total free amino acids content was determined spectrophotometrically using the Cd-ninhydrin method (Doi, Shibata, & Matoba, 1981) using L-Leucin as standard and results were expressed as mg of L-leucine equivalents (mg LE/100 g of honey), while the protein content in honey was determined as previously reported (Alvarez-Suarez et al., 2010c). Bovine serum albumin (BSA) was used for the calibration curve and results were expressed as mg of bovine serum albumin equivalents (mg BSAE/100 g of honey).

2.3. HPLC-DAD-ESI-MS/MS analysis of honey flavonols and other phenolic derivatives

Honey samples were fractionated into Sep-Pak C18 Plus Short SPE Cartridge (Waters S.p.A., Milan, Italy) as previously reported (Truchado, Ferreres, Bortolotti, Sabatini, & Tomás-Barberán, 2008) and phenolic eluent was analyzed using an HPLC-DAD-ESI-MS system. An Agilent Poroshell 120 EC-C18 column (2.7 μm, 150 mm × 4.6 mm) thermostated at 35 °C was used. The solvents were: (A) 0.1% formic acid, and (B) acetonitrile. The elution gradient established was isocratic 15% B for 5 min, 15–20% B over 5 min, 20–35% B over 10 min, 35–50% B over 10 min, 50–60% B over 5 min, isocratic 60% B for 5 min and re-equilibration of the column to initial solvent conditions. The flow rate was 0.5 mL/min. Double online detection was carried out in the DAD at 280, 330 and 370 nm as preferred wavelengths and in the MS operated in the negative ion mode. Spectra were recorded between m/z 100 and m/z 1500. Zero grade air served as the nebulizer gas (30 psi) and as turbo gas (400 °C) for solvent drying (40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). Both quadrupols were set at unit resolution and EMS and EPI analyses were also performed. The EMS parameters were: ion spray voltage 4500 V, DP -50 V, EP -6 V, CE -10 V and cell exit potential (CXP) −3 V, whereas EPI settings were: DP -50 V, EP -6 V, CE -25 V and CES 0 V.

2.4. HPLC analysis of vitamin C

Vitamin C content in honey samples was determined as previously described by our group (Alvarez-Suarez et al., 2010b) using a reversed-phase HPLC system. Honey samples (5 g) were diluted in 10 mL of a dithiothreitol solution (4.2 mM in 0.1 M K_2HPO_4, pH 7.0) mixing thoroughly and filtered through a Minisart filter of 45 μm. The filtrate (1 mL) was mixed with a 4.5% *m*-phosphoric acid solution (1 mL) and 20 μL of this solution was injected onto the HPLC system. The HPLC system (Shimadzu Corporation, Kyoto, Japan)