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Research Article

Phenolic compounds, antioxidant capacity and bioaccessibility of minerals of stingless bee honey (*Meliponinae*)

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ABSTRACT

The phenolic composition, antioxidant capacity and bioaccessibility of the minerals of thirteen honey samples from nine different species of stingless bees (*Meliponinae*) were determined in this study. Twenty-six phenolic compounds were found. The major phenolic compounds were salicylic acid (8.02–94.8 μg 100 g^{1}), *p*-coumaric acid (4.54–64.3 μg 100 g^{1}), naringin (4.00–32.0 μg 100 g^{1}) and taxifolin (12.0–1920 μg 100 g^{1}). Moreover, the presence of mandelic acid, caffeic acid, chlorogenic acid, rosmarinic acid, aromadendrin, isoquercetrin, eriodictyol, vanillin, umbelliferone, syringaldehyde, sinapaldehyde and carnosol in stingless bee honeys was reported for the first time. The study also states that the abundant minerals in the samples were potassium (263–4980 μg g^{1}), followed by calcium (88.7–138 μg g^{1}), sodium (12.7–261 μg g^{1}) and magnesium (25.9–231 μg g^{1}). The estimation of the minerals bioaccessibility demonstrated high fractions (73.62–107.6% of the total concentrations). Stingless bee honey has considerable concentrations of phenolic compounds and macro minerals (K, Ca, Na and Mg) as well as a related antioxidant capacity, suggesting a source of natural antioxidants.

1. Introduction

Bees collect nectar, which is transformed through combination with their own substances, as a salivary secretion. It is then deposited, dehydrated, stored and left in the honeycomb to ripen and mature into honey. As a natural, unprocessed and easily digested food, honey can be regarded as an important part of the human diet (Arvanitoyannis and Krystallis, 2006).

Presently, the *Apis mellifera* L. honey bee dominates the world trade market. However, honey is also produced by other bee species, such as the *Meliponin*ae subfamily, known as “stingless bees” (Chuttong et al., 2016a). These bees are inhabitants of the tropics, with about 600 species distributed across 56 genera (Vit et al., 2004). This species produces a rare honey that has gained attention in recent years, due to its particular characteristics and exotic flavor (Chuttong et al., 2016b; Da Silva et al., 2013; Ramón-Sierra et al., 2015; Sousa et al., 2016). Furthermore, it receives special attention because it shows resistance in the formation of 5-(hydroxymethyl)furfural (5-HMF) when subjected to elevated temperatures (Biluca et al., 2014). This feature increases the interest of pharmaceutical and food companies, since honey can be added as a complement to other products with no health risks due to excess 5-HMF.

Stingless bee honey is also seen as a health-promoting product, although its composition has never been studied in detail (Vit et al., 2013). The properties of honey are usually related to its minor components, such as enzymes, ascorbic acid, Maillard reaction products, carotenoid-like substances, organic acids and amino acids, proteins, minerals and polyphenols, especially flavonoids and phenolic acids (Da Silva et al., 2013; Ramón-Sierra et la., 2015; Toydemir et al., 2015; Chuttong et al., 2016b). The phenolic compound concentration reflects

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the inherent quality of honey and is responsible for its color, sensory features and antioxidant activity. Likewise it can serve as a naturally occurring marker for the botanical origin of some types of honeys (Campone et al., 2014; Sergiel et al., 2014). In this context, data on the phenolic profile appear to be a valuable in enhancing the characterization of stingless bee honey.

Other chemical compounds particularly important in terms of the characterization and classification of honey are minerals, since they are stable and dependent on plant absorption from the soil and environment (De Alda-Garcilope et al., 2012). In the human body, these nutrients play a critical role in biological systems, since they maintain normal physiological reactions, induce general metabolism and circulatory systems and influence reproduction as catalysts of various biochemical reactions (Staniškienė et al., 2006). However, some heavy metals, such as lead, cadmium and aluminum, can be toxic if maximum residue levels are exceeded (ATSDR, 2016).

It is known that the amount of nutrients present in the human diet differs from the quantity thereof utilized by in the body (Khouzam et al., 2011; Pohl et al., 2012). In the fields of nutrition and food science, bioaccessibility studies are performed *in vivo* and *in vitro*, to indicate the proportion of a dietary nutrient that is absorbed and used by an organism, relative to the total content consumed (Khouzam et al., 2011; Sulibursk a and Krejpcio, 2014).

Considering the lack of studies reporting phenolic compounds and the bioaccessibility of the minerals found in stingless bee honey, the objective of this work was to evaluate the phenolic contents using LC–ESI–MS/MS and antioxidant capacity (ORAC) in 13 Brazilian stingless bee honeys of nine different species and investigated the inorganic content, including major minerals, trace elements and heavy metals using ICP-MS and AAS, followed by an evaluation of the *in vitro* bioaccessibility of the minerals.

2. Materials and methods

2.1. Samples

Thirteen multifloral stingless bee (*Meliponinae*) honey samples were obtained directly from producers in three municipalities of Santa Catarina, Brazil: Florianópolis, Santo Amaro da Imperatriz and São Miguel do Oeste (Table 1) during the harvest of 20122013. The samples of the stingless bee honey were collected and transported at room temperature to the laboratory and stored at 18 ± 2 °C in the dark until experiments were conducted.

2.2. Reagents and solutions

All reagents were of analytical grade. Digestive enzymes and bile salts (pepsin, pancreatin, glycodeoxycholate, taurodeoxycholate and sodium taurocholate) were obtained from Sigma-Aldrich Chemical S.A. (Madrid, Spain). Methanol, ethyl acetate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and monosodium phosphate monohydrate, sodium fluorescein and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were obtained from Sigma-Aldrich (St Louis, MO). Sodium bicarbonate was obtained from Vetec (Rio de Janeiro, Brazil). Nitric acid (65% m/m) was purchased from Merck (Darmstadt, Germany) and purified by sub-boiling distillation in a quartz still (Kürnern Analysentechnik, Rosenheim, Germany). A standard multi-element ICP III solution was purchased from Perkin Elmer (Waltham, MA). Ultra-pure phenolic standards and Rh, Ca and K stock solutions were purchased from Sigma-Aldrich Co. (St. Louis, MO). Argon gas with a purity of 99.996% was purchased from White Martins (Sao Paulo, Brazil). Based on the fact that some isotopes suffer from interference and considering the relative abundance of the isotopes (low concentrations of elements being present in honey samples), the isotopes monitored were ^{27}Al, ^{138}Ba, ^{112}Cd, ^{59}Co, ^{63}Cu, ^{56}Fe, ^{7}Li, ^{24}Mg, ^{55}Mn, ^{23}Na, ^{61}Ni, ^{208}Pb, ^{39}K, ^{40}Ca, ^{103}Rh and ^{66}Zn. Deionized water with a resistivity of 18.2 MΩ/cm was obtained from a Milli-Q Plus system (Millipore, Bedford, MA).

2.3. Phenolic compounds by LC–ESI–MS/MS

2.3.1. Extraction procedure

The extraction of phenolic compounds was performed according to Trautvetter et al. (2009), with adjustments. Samples of honey (1 g) were homogenized in 1 mL of 2% sodium chloride solution and mixed for 1 minute under constant stirring (Vortex tube stirrer, Fisatom 774). The diluted honey was extracted five times with 2 mL of ethyl acetate (HPLC grade). The organic phases were combined and dried with sodium sulfate for 15 minutes and then filtered, followed by concentration on a rotary evaporator (40 °C). The dried extract was reconstituted in 1 mL of methanol and microfiltered through 0.45-µm membranes (Millipore, Bedford, MA). For injection in LC-ESI–MS/MS the extract was diluted in methanol:water (70:30), and the result expressed in µg 100 g^{-1}.

2.3.2. Identification and quantification of phenolic compounds by LC-ESI–MS/MS

Identification and quantification of phenolic compounds in stingless bee honey were performed according to Schulz et al. (2015). Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI–MS/MS) was used and chromatographic separation was achieved using a mobile phase gradient consisting of (**A**) 95% methanol in water and (**B**) 0.1% formic acid in water. Phenolic compounds were separated on a Synergi column (4.6 µm particle size, 150 mm, 2.0 mm), with the temperature maintained at 30 °C, using segmented gradient elution as follows: 0–5 min, 10% **A**; 5–7 min, 90% **A**; 7–10 min, 90% **A**;

Table 1
Identification of species of stingless bees (*Meliponinae*) and geographical description of the municipalities of origin of the samples.

Sample	Popular name	Scientific name	Origin	La.	Lo.	Al.
A	Mandaçaia	*Melipona quadrifasciata*	FLN	27°35′49″	48°32′56″	0
B	Tujuba	*Melipona mondury*	FLN	27°35′49″	48°32′56”	0
C	Guaraipo	*Melipona bicolor*	FLN	27°35′49″	48°32′56″	0
D	Manduri	*Melipona marginata*	FLN	27°35′49”	48°32′56”	0
E	Mandaçaia	*Melipona quadrifasciata*	SAI	27°41′18”	48°46′45”	19
F	Manduri	*Melipona marginata*	SMO	26°43′08”	53°31'18”	676
G	Vorá ou Borá	*Tetragona clavipes*	SMO	26°43′08”	53°31'18”	676
H	Jataí	*Tetragonisca angustula angustula*	SMO	26°42′51”	53°30'53”	680
I	Guaraipo	*Melipona bicolor*	SMO	26°43′08”	53°31'18”	676
J	Uruçu Nordestina	*Melipona scutellaris*	SMO	26°43′08”	53°31'18”	676
K	Tubuna	*Scaptotrigona bipunctata*	SMO	26°43′08”	53°31'18”	676
L	Uruçu Amarela	*Melipona rufiventris mondory*	SMO	26°43′08”	53°31'18”	676
M	Mandaçaia	*Melipona quadrifasciata*	SMO	26°43′08”	53°31'18”	676

Municipalities: FLN = Florianópolis, SAI = Santo Amaro da Imperatriz, SMO = São Miguel do Oeste. ***La** = latitude, ***Lo** = longitude, ***Al** = Altitude (m).