Food Research International 84 (2016) 61–68

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Polyphenolic profile and antioxidant and antibacterial activities of monofloral honeys produced by *Meliponini* in the Brazilian semiarid region

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ARTICLE INFO

Article history:
Received 23 September 2015
Received in revised form 25 February 2016
Accepted 6 March 2016
Available online 9 March 2016

Keywords:
Meliponini
Honey
Radical scavenger
Antimicrobial effect

ABSTRACT

This study assessed the polyphenolic profile and the antioxidant and antibacterial activities of monofloral honeys produced by *Meliponini* in the Brazilian semiarid region. Honeys from *Ziziphus joazeiro* Mart. (juazeiro) and *Croton heliotropiifolius* Kunth (velame branco) showed the highest total phenolic contents (TPCs) and the greatest antioxidant activity in assays with DPPH and ABTS$^{\bullet+}$ radicals. Honeys from *Mimosa quadrivalvis* L. (malícia) presented the strongest anti-peroxyl activity in ORAC assay. Juazeiro honeys showed the highest quantities of *trans*-cinnamic, *p*-coumaric, ellagic and ferulic acids, as well as of catechin, rutin, hesperetin and chrysin when compared to the other honeys produced by the same bee species. Malícia honeys showed the greatest quantities of myricetin, quercetin and kaempferol among the studied honeys. Honeys with the highest TPCs presented the highest antimicrobial activity. The results showed the impact of the floral source on the polyphenolic profile as well as on the antioxidant and antimicrobial properties of *Meliponini* honeys.

1. Introduction

Honeys are members of a class of natural substances that have recently attracted attention for having high therapeutic interest. In the long human tradition, honey has been used not only as a nutrient but also as a medicine. Despite the relevant importance of polyphenolic compounds, which are recognized as the major constituents and responsible for the health-promoting properties of honey (Meda Lamien, Romito, Millogo & Nacoulma, 2005; Habib, Meqbali, Kamal, Souka & Ibrahim, 2014), their identification and quantification are of great interest for understanding their contributions to the overall bioactivity of honey (Manzanares, García, Galdón, Rodríguez & Romero, 2014). In honeys, polyphenolic compounds, comprising phenolic acids and flavonoids, are also considered potential markers of botanical origin (Alvarez-Suarez et al., 2012).

Antioxidant activity, or simply the antioxidant capacity of honeys, is the ability and potential of these products to reduce oxidative reactions within food systems and human health (Frankel, Robinson, & Berenbaum, 1998). The antioxidants that naturally occur in honey contribute to its antioxidant capacity (Silva et al., 2013b). Furthermore, the phenolic profiles of honeys and consequently their antioxidant capacity depend on the floral sources visited by the bee (Meda et al., 2005; Habib et al., 2014).

Apart from their antioxidant activity, honeys also possess broad antimicrobial activity that is capable of inhibiting the growth of many foodborne pathogens and bacteria of clinical importance (Vorlova, Karpiskova, Chabiniokova, Kalabova, & Brazdova, 2005). The antimicrobial properties of honeys were first attributed to their high sugar concentration and the presence of peroxide compounds (primarily hydrogen peroxide) (Weston, Brocklebank & Lu, 2000). However, polyphenols have been strongly related to the antimicrobial properties of monofloral honeys (Rodriguez, Mendoza, Iturriga & Castaño-Tostado, 2012). Moreover, studies have found that honeys from specific floral sources present stronger antibacterial activity than other types of honey (Silici, Sagdic, & Ekici, 2010; Hussain et al., 2015). The predominance of particular botanical species in honey is influenced by geographical, seasonal and environmental factors (Andrade, Ferreres & Amaral, 1997). The phenolic profile of honeys is variable depending primarily on their floral source but also on their entomological origin (Kirnpal-Kaur, Tan, Boukraa & Gan, 2011; Belitz, Grosch & Schieberle, 2009). Thus, honeys that are produced by distinct bee species or that are collected from different locations possess different active

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http://dx.doi.org/10.1016/j.foodres.2016.03.012
0963-9969/

compounds, and they consequently exhibit differences in their biological properties (Kirnpaul-Kaur et al., 2011).

Monofloral honeys are of interest because they have specific phenolic profiles related to the honey-producing plant (Habib et al., 2014; Cavazza, Corradini, Musci, & Salvadeo, 2013; Silva et al., 2013b). This characteristic is particularly relevant in the Brazilian semi-arid region, where a unique biodiversity of stingless bees (*Meliponini*) and native flora is found. The occurrence of only two seasons during the year (dry and rainy) with availability of specific major botanical sources favors the production of different monofloral honeys with particular characteristics in this region (Silva et al., 2013b; Sousa et al., 2015).

Only a few studies on the bioactivity of monofloral honeys produced by the *Meliponini* are available, although it is well-known that these products possess differences, including their phenolic profile, when compared with honeys produced by bees belonging to the *Apis* genera (Silva et al., 2013b; Sousa et al., 2013). Among the *Meliponini* species, *Melipona subnitida* Ducke (jandaíra) and *Melipona scutellaris* Latrelle (uruçu) typically visit botanical species that are available only during the rainy or dry season in the semiarid region of Brazil, thus producing different types of honeys during the year (Sousa et al., 2013). Considering these aspects, the aims of the present study were to determine for the first time a) the antioxidant activity of rare monofloral honeys produced by distinct stingless bee species in the Brazilian semi-arid region by using distinct methods; b) the total phenolics and flavonoids as well as the individual phenolic profile of the studied monofloral honeys; and c) the antibacterial activity of the whole honeys against different pathogenic bacterial strains.

2. Materials and methods

2.1. Honey samples

The experimental design included four different monofloral honeys produced by two different stingless bee species in the semiarid region of Brazilian northeastern (Fig. 1) collected in three different occasions ($4 \times 2 \times 3$). Each of the 24 samples analyzed was composed by a mixture of honeys collected in four different beekeeper's meliponaries specific for each stingless bee species. The honey samples from *Ziziphus joazeiro* Mart. (juazeiro) and *Mimosa quadrivalvis* L. (malícia) produced by the stingless bees *M. subnitida* Ducke (jandaíra) and *M. scutellaris* Latrelle (uruçu) were collected during the 2012 dry season, while the honey samples from *Mimosa arenosa* Willd Poir (jurema branca) and *Croton heliotropiifolius* Kunth (velame branco) produced by the both bee species (jandaíra and uruçu) were collected during the 2013 rainy season. The samples were stored in sterilized amber glass containers, shipped to the laboratory and maintained at 6–8 °C in the dark until analysis (Sousa et al., 2015). When the analysis was delayed for more than a month after sampling, the honeys were frozen at −18 °C. To ensure the botanical source, the monofloral honeys were submitted to melissopalynological analysis (Louveaux, Maurizio & Vorwohl, 1978). Briefly, a total of 10 g of each honey sample was diluted in 20 mL of distilled water and centrifuged at 4000 rpm for 20 min. The sediment was dried at 40 °C and mounted in microscope slides with Entellan Rapid (Merck, 1.07961.0500). The honeydew elements and pollen grains ($n = 500$) were counted and identified in 20 distinct optical areas (Nikon Optiphot II microscope; 400× and 1000×). The pollen grains were compared to reference images of Pollen and Apicultural Plants of Laboratory of Entomology, ESALQ, São Paulo, Brazil (ESALQ, 2013). All honey samples included in the study contained more than 85% pollen grains of the same botanical origin, being characterized as monofloral honey samples (Table 1).

2.2. Test strains

The strains *Listeria monocytogenes* 3375, *Staphylococcus aureus* 18N, *Escherichia coli* CINF1, *Salmonella* spp. CINF2, and *Pseudomonas aeruginosa* CINF3 that were isolated from food samples were obtained from the Collection of Innovation Center and Business Support of Porto (CINATE, Porto, Portugal). The strains were stored in cryovials with glycerol at 15% (v/v) and maintained at −80 °C before use. The inoculum of each bacteria strain used in the antimicrobial testing was obtained after preparing the suspensions in sterile saline solution (0.85% NaCl w/v) from overnight cultures grown in Mueller–Hinton (MH) agar (Biokar Diagnostics, Beauvais, France) at 37 °C. Each strain was grown in MH broth (Biokar Diagnostics, Beauvais, France) at 37 °C for 18–20 h (late exponential growth phase), harvested by centrifugation (4500 *g*, 15 min, 4 °C), washed twice in sterile saline solution and resuspended in MH broth to obtain standard cell suspensions at which the OD reading at 660 nm (OD_{660}) was 0.1, which provided viable cell counts of approximately 8 log CFU/mL when they were pour-plated onto MH agar (McMahon et al., 2008).

2.3. Total phenolic contents (TPCs)

The concentration of total phenolics was measured by using Folin–Ciocalteu phenol reagent (Habib et al., 2014). Briefly, each honey (1 g) was diluted in ultrapure water (H_2O) (10 mL) and filtered through grade 1 Whatman® qualitative filter paper. An aliquot of 0.5 mL (filtrate) was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu reagent (Sigma Aldrich, Germany) for 5 min, and then 2 mL of 7.5% sodium

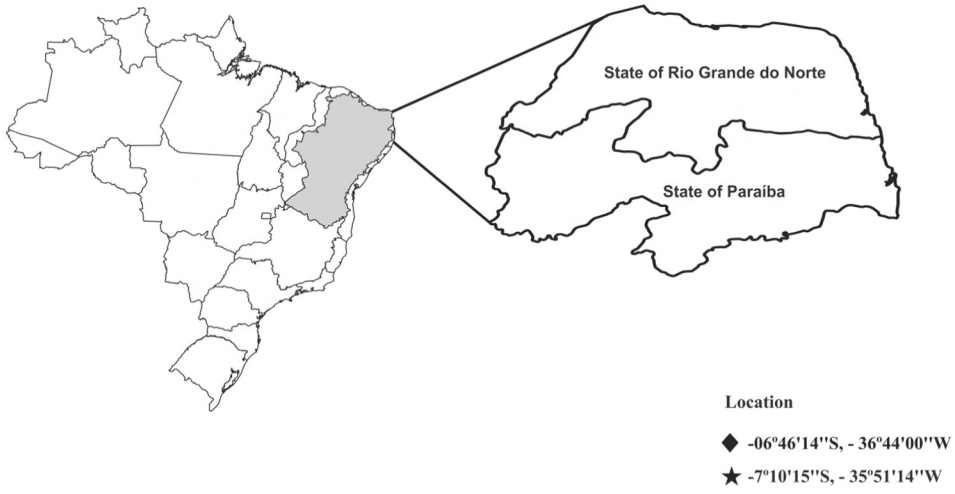

Fig. 1. Map of the semiarid region of northeastern Brazil showing the distribution of study honey samples and their respective geographic coordinates. ⬢ Seridó region, state of Rio Grande do Norte; ★ Agreste region, state of Paraíba.