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Effect of botanical and physicochemical composition of Argentinean honeys on the inhibitory action against food pathogens

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ABSTRACT

Honey is a natural food with functional properties such as antioxidant and antimicrobial activities. These properties vary greatly depending on floral source, climate, and environmental and processing conditions. In this work, we characterized honeys on the basis of their botanical composition and clustered them according to their physicochemical parameters in order to find similarities, and assess their antibacterial action against microorganisms isolated from contaminated food. All honeys studied complied with international quality standards. The data showed differences between multifloral and unifloral honeys in their physicochemical parameters, as well as a direct correlation between colour, phenolic compounds, and antioxidant activity. -Antimicrobial activity resulted from hydrogen peroxide effect. Multifloral honeys with similar phenolic compounds and a botanical composition of eucalyptus and blueweed had greater inhibitory power against *E. coli, P. aeruginosa, Salmonella* spp., *S. aureus* and *B. cereus*.

1. Introduction

Honey has been used for both medical and nutritional purposes. In terms of the first, it has been used for its therapeutic action, which includes antimicrobial and anti-inflammatory properties (Libonatti, Varela, & Basualdo, 2014; Pascoal, Feás, Dias, Dias, & Estevinho, 2014, pp. 221–234). In terms of the second, it is a nutritive food widely used in the food industry, with antimicrobial and antioxidant properties that make honey a natural food preservative.

Honey is a supersaturated solution of sugars, mainly composed of fructose and glucose, and a wide range of minor components such as minerals, proteins, free amino acids, vitamins, enzymes -glucose-oxidase, and catalase-, phenolic acids, and flavonoids (Alvarez-Suarez, Tulipani, Romandini, Bertoli, & Battino, 2010; Saxena, Gautam, & Sharma, 2010). Its composition is variable and its properties vary greatly depending on floral source, climate, and environmental and processing conditions (Libonatti et al., 2014; Liu, Ye, Lin, Wang, & Peng, 2013).

The literature has widely reported on the antibacterial properties of honey (Allen, Molan, & Reid, 1991; Molan, 1992; Fangio, Iurlina, & Fritz, 2010; AL-Waili et al., 2013) which may stem from variations in plant source (Liu et al., 2013; Mundo, Padilla-Zakour, & Worobo, 2004). On the other hand, antimicrobial activity of honey is given by peroxide and non-peroxide factors. As to the first ones, some researchers have concluded that the major one is hydrogen peroxide, formed out of the oxidation of glucose by glucose oxidase during the ripening of honey (White et al., 1963). As to the non-peroxide antimicrobial factors, physicochemical characteristics -high osmolarity, acidity, peptides, lysozyme, phenolic acids, and flavonoids-are included (Feás, Iglesias, Rodrigues, & Estevinho, 2013; Kwakman et al., 2010; Molan, 1992). Some authors have shown a relationship between colour -given by carotenoids and flavonoids-phenolic compounds, and antioxidant and antibacterial activity of honey and their relation to floral source (Bueno-Costa et al., 2016; Isla et al., 2011; Liu et al., 2013).

Antibacterial activity of unifloral and mutlifloral honey against

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foodborne pathogens has been reported by various studies (Isla et al., 2011; Mundo et al., 2004). Multiflora honeys do not have any type of predominant pollen and could be mainly composed by nectar of two or more species in certain proportions. This mixture of botanical species could give honey particular physicochemical and antibacterial characteristics. However, researchers have not focused on this aspect. Thus, we hypothesized that there could be a relationship between honey inhibitory power and botanical composition in both qualitative and quantitative terms.

As a natural complex food, many variables are needed to characterize honey. The chemo metrics techniques, as principal component analysis, are the most commonly used ones to identify the natural clustering pattern and groups of variables on the basis of similarities between samples (Silvano, Varela, Palacio, Ruffinengo, & Yamul, 2014). In this work, we characterized Argentinean honey samples out of their botanical composition, and clustered them according to physicochemical parameters in order to find similarities, and assess their antibacterial action against microorganisms isolated from contaminated food. We focused on the relation between inhibition power of honey and the proportion of botanical species present in honey.

2. Materials and methods

2.1. Honey samples

Twenty four honey samples were harvested in 2014 from apiaries from three regions of Argentina (Northwestern region, Cuyo region and Pampean region) and classified according to their botanical origin. The samples were provided by beekeepers who obtained the honey by cold extraction, kept in plastic containers, and stored in a fresh, dry place until their analysis. Honey samples were clustered according to their botanical origin and classified into 10 different types of honey: 7 multifloral and 3 unifloral. The results are expressed in relation to these samples.

2.2. Botanical origin

To determine botanical composition, ten grams of honey were diluted in 20 mL of distilled water and centrifuged at 3000 rpm for 5 min. The supernatant was poured off and the sediment was acetolyzed according to Louveaux, Maurizio, and Vorwhol (1978). The sediment was removed with a stylet, embedded in glycerin jelly, deposited on a microscopic slide, and then sealed with paraffin wax. The slides were examined using an optical microscope (40X). At least 200 pollen grains in each honey sample were counted. Pollen grains were identified using the reference collection of the Apicultural Laboratory, Veterinary Sciences Faculty, National University of Buenos Aires Province Centre. Morphological pollen types were determined with the greatest possible taxonomic approximation, achieving genus or species level when possible. Other than that, botanical families or group were determined. For multifloral honeys (MH), occurrence frequencies of pollen types were determined according to Basualdo, Pereda, and Bedascarrasbure (2006), who considered as dominant pollen (D: frequency $\geq$45% of total counted pollen grains), secondary (S: 16 < frequency< 45%) and minor importance (M: 3 < frequency< 15%). Unifloral honeys (UH) were classified following Argentinean standards (SAGPYA, 1994) according to which honeys are considered as unifloral *Eucalyptus* if the relative frequency of occurrence (RF) of *Eucalyptus* pollen reaches a minimum value of 70%, while for unifloral *Lotus* sp. the RF should be 20%, and for unifloral clovers the RF should be 45% mixture of *Trifolium* sp., *Medicago* sp. *Melilotus* sp., and *Lotus* sp. pollens (SAGPYA, 1994). Thus, a total of 10 honey types were classified according to botanical associations considering the RF.

2.3. Physicochemical analysis

Moisture was determined with an Abbé refractometer (American optical corporation), reading at 20 °C and the corresponding moisture value was obtained from the Chataway Table (1932) as cited by Wedmore (1955).

The acidity of honey was determined according to A.O.A.C. (1990). For pH determination, strips indicators (DF®) were used.

Colour measurements were performed using HI 96785 HANNA colorimeter (IRAM 15941-2, 1997). Crystallized honey was melted at 56 °C in thermostatic bath until complete dissolution of the crystals and elimination of dissolved air. The liquid honeys without air bubbles thus obtained were placed in plastic buckets and the colour was read, results being expressed in mm Pfund scale (Fell, 1978). Honey colour grades on Pfund readings are: average scale reading $\leq$8 mm: water-white; 8 < reading $\leq$16: extra white; 16 < reading $\leq$34: white; 34 < reading $\leq$50: extra light-amber; 50 < reading $\leq$85: light-amber; 85 < reading $\leq$114: amber; reading >114: dark.

2.4. Sugar profile analysis

Glucose, fructose, and sucrose were determined with high performance liquid chromatograph (Waters 1525), equipped with a differential refractive index detector (Waters 2414), (Bogdanov & Baumann, 1988). Five grams of honey were prepared with 500 μL of solution of Carrez I ($K_4Fe(CN)_6 \cdot 3H_2O$) and II ($Zn(AcO)_2.2H_2O$), in a volumetric flask of 25 mL. distilled water was added to complete the volume. The dissolution was then filtered through 0.45 μm cellulose filter prior to HPLC analysis. The injection volumes of the samples were 20 μL with a flow rate of 1.3 mL/min. The separation was performed by using a Polyamine II (4.6 × 250 mm, YMC HPLC Column) column. A mobile phase of acetonitrile/water HPLC grade; 8:2 (v/v) was used. The system was maintained at 35 °C. Identification of individual compounds was made by comparing the retention times of the honey compounds identified with commercial standards (Fluka, Switzerland). For quantification, calibration curves were developed for each compound.

2.5. Antioxidant activity and total phenolic content

The antioxidant activity of honey was evaluated by the method of inhibiting the radical $ABTS^+$ (2,2′-azinobis- [3-ethylbenzothiazol-6-sulfonic acid]). The ABTS test was performed according to Re et al. (1999). The cation radical ABTS+ was synthesised by the reaction of a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The mixture was kept at 23 °C in the dark for 16 h. Afterwards, the ABTS + solution was diluted with ethanol until an absorbance (A) of 0.7 at 734 nm was achieved in a UV–Vis spectrophotometer. First the sample was conveniently diluted, then Aliquots of 2.7 mL from the ABTS + solution were immediately added to the sample. After 6 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration relative to the blank absorbance (ethanol).

The scavenging capability of the ABTS + radical (%AS) was calculated using the following equation:

$$\% AS = 100(Acontrol - Asample)/Acontrol$$

Where “A control” is the absorbance control obtained from the ABTS + radical alcoholic solution, and “A sample” is the absorbance radical in presence of the sample or the trolox standard. The results were expressed as SC50 ± sd, where SC50 represents the sample