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Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil

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ABSTRACT

Honey contains compounds with antioxidant and antibacterial capacities, such as phenolic compounds and carotenoids. Current analysis evaluates the antioxidant and antibacterial activity and determines the phenolic compounds and carotenoids content in 24 honey samples from the state of Rio Grande do Sul, Brazil. Total content of phenolic compounds (from 11.37 to 54.01mgGAE·100 g^{-1}), flavonoids (from 2.97 to 10.46 mgQE·100 g^{-1}), phenolic acids (from 0 to 65.47mgCAE·100 g^{-1}) and carotenoids (from 0.56 to 6.19 mg β-carotene·Kg^{-1}) were found, and the antioxidant activities measured by ABTS (between 8.24 and 111.48 mgtrolox·100 g^{-1}) and by DPPH (between 2.48 and 17.21 mgQEA·100 g^{-1}) differed significantly. All samples showed antibacterial activity, where the H5 and H23 showed the best results (MIC: 10 mg mL^{-1}) against the four pathogenic bacteria tested. A significant and positive correlations between the carotenoids and phenolic compounds content was found; likewise, between antioxidant activity (ABTS) and carotenoids content and between color and flavonoids content.

1. Introduction

Honey is the product of beekeeping that has great market potential. Honey contains more than 200 compounds comprising approximately 38% fructose, 31% glucose, 10% other sugar types, 18% water and 3% of other compounds. However, precisely the great mixture of compounds in these 3% is the product's greatest feature, with special reference to phenolic and carotenoids compounds (Alvarez-Suarez et al., 2010).

Honey as one of the most complete food for humans, due to its therapeutic (Blasa, Candiracci, Accorsi, Piacentini, & Piatti, 2007), antioxidant (Lachman, Orsak, Hejtmankova, & Kovarova, 2010; Meda, Lamien, Romito, Millogo, & Nacoulma, 2005), antimicrobial (Escuredo, Silva, Valentão, Seijo, & Andrade, 2012; Vandamme, Heyneman, Hoeksema, Verbelen, & Monstrey, 2013), antitumoral (Jaganathan, Mazumdar, Mondhe, & Mandal, 2011), anti-inflammatory (Van Den Berg et al., 2008), antiviral (Watanabe, Rahmasari, Matsunaga, & Kobayashi, 2014) and antiulcer (Vandamme et al., 2013) activities.

Most studies on the effects of honey are concentrated on the activities of bioactive compounds, especially phenolic compounds, in the human organism. The most relevant are those widely distributed in nature, including the phenolic acids and flavonoids (Vermerris & Nicholson, 2006).

Carotenoids were found in small concentrations in the dark honey (10 mg β-carotene·Kg^{-1}) but they were not found in light colored honey. This fact reveals the effect that carotenoids (Alvarez-Suarez et al., 2010; Ferreira, Aires, Barreira, & Estevinho, 2009; Tomasik, 2004) and phenolic compounds have in the honey color (Estevinho, Prereira, Moreira, Dias, & Pereira, 2008).

Honeys therapeutic activities are mainly related to its antioxidant and antimicrobial properties (Martos, Ferreres, & Tomas-Barberan, 2000). The antimicrobial activity of honey has been showed in a recent study with 60 types of honey from different botanic origins against 16 clinically-derived pathogenic bacteria (Voidarou et al., 2011).

Several authors also studied the correlations between color and antioxidant and antibacterial activities with content of the bioactive compounds of honey (Beretta, Granata, Ferrero, Orioli, & Facino, 2005; Bertoncelj, Doberšek, Jamnik, & Golob, 2007;

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Canadanovic-Brunet et al., 2014).

Data on these properties in Brazilian honeys are limited, therefore, it is currently very important to determine parameters in honey samples from the state of Rio Grande do Sul, Brazil, especially due to the productive and economic relevance on the Brazilian honey market. Current analysis assessed the antibacterial and antioxidant activities and the content of bioactive compounds in 24 honey samples.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Honeys from entirely randomized different botanic origins were analyzed (Table 1). Honeys were provided by beekeepers and collected from several regions in the state of RS, Brazil. The samples were from the following regions: west (H1, H2, H3, H4, H5, H10, H11, H14), metropolitan (H6, H16, H19, H20, H21, H22, H23), southwest (H7, H12, H13), southeast (H8, H9, H17, H18), northeast (H15) and northwest (H24).

Samples were acquired between January and November 2013 and kept in sterilized dark polyethylene flasks under refrigeration (5 °C).

2.1.2. Standards and reagents

All chemical products were of the highest analytic degree. Quercetin, caffeic acid, gallic acid, DPPH and ABTS were supplied by Sigma–Aldrich (St. Louis, USA), methanol, ciprofloxacin and Folin Ciocalteu 2 N solution were obtained from Merck (Darmstadt, Germany) and tryptone soya agar, tryptone soya broth and bacteriologic peptone were obtained from Oxoid, Basingstone, Hampshire, UK. Water was purified by an Ultra Purification System (*Mega Purity*).

2.2. Methods

Prior to all measurements, the samples were previously homogenized and were sonicated for 10 min (45 °C) until the complete dissolution of the sugar crystals.

2.2.1. Color

Samples were diluted in water (1:1; w/v) and the absorbance was measured at 635 nm. Results were calculated (PFund = 38.70 + 371.39*ABS) (Ferreira et al., 2009) and *expressed* in millimeters on a Pfund *scale* (Fell, 1978).

2.2.2. Bioactive compounds and antioxidant activity

2.2.2.1. Total content of phenolic compounds (TCPC). Honey solution (100 mg mL^{-1}) was previously homogenized and filtered through quantitative filter, 500 μL of honey solution was added to 2.5 mL of Folin Ciocalteu (0.2 N). After 5 min, 2 mL of sodium carbonate solution (Na_2CO_3-75 g L^{-1}) was added and incubated for 2 h in the dark. The absorbance was measured at 760 nm in a spectrophotometer (Singleton, Orthofer, & Lamuela-Raventos, 1999). Standard curve was defined by known concentrations of gallic acid, ranging between 0 and 200 mg L^{-1} ($R^2$0.9923) and results expressed in milligrams of gallic acid equivalents (mgGAE·100 g^{-1}).

2.2.2.2. Total content of flavonoids (TCF). Honey solution (100 mg mL^{-1}) was prepared with methanol 50% and previously homogenized and filtered through quantitative filter, 5 mL of honey solution was mixed with 5 mL of $AlCl_3$ (2%) in methanol. The mixture was homogenized and allowed to stand for 30 min. The absorbance was measured at 415 nm (Arvouet-Grand, Vennat, Pourrat, & Legret, 1994). Standard curve was defined by known concentrations of quercetin, between 0 and 40 mg L^{-1} ($R^2$0.9989) and expressed in milligrams of quercetin equivalents (mgQE·100 g^{-1}).

2.2.2.3. Total content of phenolic acids (TCPA). Phenolic acids were determined according to the method of Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999) with some modifications. Appropriate aliquots of solutions prepared with honey (250 μL; 100 mg mL^{-1} in 50% ethanol solution), 250 μL of acidified ethanol solution (0.1% HCl in 95% ethanol) and 4.55 mL of solution 2%HCl

Table 1
Honey samples with respective collection data: municipality, botanic origin and color.

Samples	Town	Botanic origin	Pfund scale[a]	Color
H1	Nova Esperança	Wild	89	Amber
H2	Unistalda	Wild	74	Light amber
H3	Mata	Wild	150	Dark amber
H4	Bom Respiro	Eucalyptus	116	Dark amber
H5	Cacequi	Wild	65	Light amber
H6	Ivoti	Wild	123	Dark amber
H7	Bagé	Eucalyptus	75	Light amber
H8	Pelotas	Eucalyptus	74	Light amber
H9	Pedras Altas	Wild	150	Dark amber
H10	São Pedro do Sul	Wild	98	Amber
H11	Santiago	Wild	89	Amber
H12	Livramento	Wild	79	Light amber
H13	São Francisco de Assis	Wild	90	Amber
H14	Jaguari	Wild	122	Dark amber
H15	Farroupilha	Wild	84	Light amber
H16	Nova Petropolis	Wild	149	Dark amber
H17	Rio Grande	Wild	90	Amber
H18	Cerrito Alegre	Wild	122	Dark amber
H19	Camaquã	Wild	84	Light amber
H20	Santo Antônio da Patrulha	Wild	149	Dark amber
H21	Taquara	Eucalyptus	70	Light amber
H22	Gramado	Wild	74	Light amber
H23	Três Coroas	Wild	67	Light amber
H24	Teutônia	Eucalyptus	103	Amber

[a] In millimeters.