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FULL LENGTH ARTICLE

Discrimination of high altitude Indian honey by chemometric approach according to their antioxidant properties and macro minerals

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Abstract The study was intended to characterize three honeys (acacia, pine honeydew and multi-floral) from high altitude Kashmir valley of India according to their macro minerals (K, Ca, Na and P), antioxidant properties and sugar parameters. The result for total phenolic content (22.68–59.84 mg GAE/100 g) and total flavonoid content (6.10–8.12 mg QE/100 g), revealed that honeys from Kashmir valley have high antioxidant activity. Principal component analysis (PCA), explained more than 81% of the variance. Four sugars were identified and quantified by HPLC, which include monosaccharides and disaccharides. Chemometric methods such as principal component analysis and linear discriminate techniques were applied on the data in order to differentiate the honeys. PCA explained more than 81% of the variance with the first two PC variables with minerals and antioxidant properties having highest discriminating power while LDA successfully classified all the unifloral honey samples.

1. Introduction

Honey is a sweet, flavorful and complex natural viscous product produced by honeybees (*Apis mellifera*) either from nectar of flowers (blossom honey) or from secretion of living part of plants (honeydew honey). The honey is composed of 65–70% carbohydrates, mainly monosaccharides (glucose, fructose) followed by disaccharides (sucrose) and a low concentration of trisaccharides (Nayik et al., 2015a,b; De La Fuente et al., 2011). The honey is considered as a rich source of minerals (1/3rd potassium), amino acids (mainly proline), proteins, vitamins, enzymes, etc. (Gheldof et al., 2002; Bentabol Manzanares et al., 2011). Being a complex food product, the composition of honey depends on not only floral source, but also many factors viz. geographical origin, climatic conditions, storage period, temperature as well as environmental factors (Nayik et al., 2015a,b). The honey has been reported as a rich source of natural antioxidants (Nayik and Nanda, 2016a). The antioxidant activity of honey is mainly due to phenolic and flavonoid compounds; thus, a considerable variation of antioxidant activity is found among different honey varieties around

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the world (Beretta et al., 2005; Aljadi and Kamaruddin, 2004; Nayik and Nanda, 2016b). This variation is mainly due to different floral as well as different geographical origins while there is a little effect of storage on antioxidant activity of honey. The color of honey is reliable index of antioxidant activity; more dark the honey is more is the antioxidant activity of that honey (Nayik and Nanda, 2015; Beretta et al., 2005; Holderna-Kedzia and Kedzia, 2006).

Honey is considered a part of apitherapy since early humans, and in more recent times, it has been used in treatment of burns, gastrointestinal disorders, chronic wounds, asthma, skin ulcers, cataracts, etc. due to its antimicrobial, antioxidant, antiviral, antiparasitic, anti-inflammatory, anticancer and immunosuppressive activities (Subrahmanyam et al., 2001; Kucuk et al., 2007; Gomez-Caravaca et al., 2006). The health benefit of honey is mentioned in various holy books of different religions and is widely embraced by all cultural and religious beliefs (Nayik et al., 2014).

Chemometric techniques such as principal component analysis (PCA) and linear discriminate analysis (LDA) have been used for classification of wines, olive oils and juices and different types of milk (Latorre et al., 1994; Giansante et al., 2003; Rinaldi et al., 2009). Such techniques have also been employed in classifying honey according to its type and origin based on physico-chemical data. Silvano et al. (2014) have classified twenty-four honey samples from Buenos Aires province (Argentina) by physico-chemical and sensory characteristics using chemometric technique. Yucel and Sultanoglu (2013) classified and characterized forty-five honey samples from Hatay region of Turkey by applying chemometric technique. The comparative physicochemical, mineral, color, antioxidant and enzymatic characterization of different honeys from other regions of the world has been carried out extensively (Azeredo et al., 2003; Finola et al., 2007; Guler et al., 2007).

Kashmir valley located in the Indian state of Jammu and Kashmir at a latitude of 32°44'N and longitude of 74°54'E is phyto-geographically the most complex and diverse zone. The intermediate climate and varied geographical conditions provide a great potential in the production of various fruits and spices in this zone. According to data reported by Press Information Bureau, Government of India (2013), there are about 1621 honey-producing units in Jammu and Kashmir with honey production capacity of 2000 metric tons. Although in our previous study we determined the physicochemical and trace mineral analysis of three varieties (acacia, pine and multifloral) from Kashmir valley, the antioxidant and macro minerals of such varieties were yet to be studied. Thus, the main aim of the present study was to classify the three honey varieties from Kashmir valley based on antioxidant and macro minerals using multivariate techniques.

2. Materials and methods

2.1. Chemicals and reagents

Ascorbic acid, gallic acid, quercetin and HPLC-grade methanol of the analytical grade were purchased from Acros Organics, New Jersey, USA. Folin–Ciocalteu reagent, DPPH (1,1-diphenyl-2-picrylhydrazyl), $AlCl_3$ and sodium carbonate were purchased from Fluka Goldie, Mumbai, India.

2.2. Honey sample collection and pollen analysis

The present study was carried out using three different raw and fresh honey varieties ($n = 24$): acacia honey, pine honeydew and multifloral honey collected from bee keepers during September 2012 to May 2014 from different areas (Pulwama, Budgam and Srinagar) of Kashmir valley. All the honey samples were packed and stored at 4 °C. The origins of each honey sample were confirmed by microscopic pollen analysis. Honey samples were classified according to their botanical origin using the method described by Von der Ohe et al. (2004). The following terms were used for frequency classes: predominant pollen (>45% of pollen grains counted), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen (<3%).

2.3. Moisture content

The moisture content was determined based on the refractometric method using an Atago hand refractometer and the readings were further corrected for a standard temperature of 20 °C by adding the correction factor of 0.00023/°C. Moisture content was determined in triplicate and the % moisture content values corresponding to the corrected refractive index values were calculated using Wedmore table.

2.4. Determination of different sugars by HPLC–Refractive Index

The sugar composition of all the three varieties was determined by using HPLC. The determination of sugar was performed with Waters isocratic HPLC system (USA) equipped with refractive index (RI) detector. The separation was performed using Waters X-bridge Amide HPLC Column, 5 µm (250 × 4.66 mm). The injection volumes of the sample were 20 µl, with a flow rate of 0.6 ml/min, using as mobile phase prepared by dissolving 80% of acetonitrile in ultra pure water. The separated sugar peaks were identified by comparing the retention times obtained from standards.

2.5. Antioxidant properties

2.5.1. Determination of total phenolic and flavonoid content

Folin–Ciocalteu method was used to determine the total phenolic content in honey (Noor et al., 2014). 1 mL honey solution (10% w/v in methanol) was mixed with 5 mL of 0.2 N Folin Ciocalteu reagent followed by addition of 4 mL (75 g/L) of sodium carbonate. The mixture was incubated for 2 h and the absorbance of reaction mixture was measured at 760 nm against methanol blank by using Hach Lange DR6000 UV–VIS Spectrophotometer (Dusseldorf Germany). The total phenolic content was determined by comparing with the standard curve using gallic acid (0–100 µg/mL). The results were expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey. A method modified by Arvouet-Grand et al. (1994) was used for total flavonoid determination. Briefly, 0.1 mg of honey dissolved in 5 mL of methanol was mixed with 5 mL of 2% aluminum chloride ($AlCl_3$) and incubated for 10 min. The absorbance was measured at 415 nm (Hach Lange DR6000 UV–VIS Spectrophotometer) against a blank sample (5 mL honey solution + 5 mL methanol without $AlCl_3$). The total flavonoid content was expressed as mg quercetin/

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