A chemometric approach to evaluate the phenolic compounds, antioxidant activity and mineral content of different unifloral honey types from Kashmir, India

Gulzar Ahmad Nayik[*], Vikas Nanda

Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal 148106, Punjab, India

ABSTRACT

Phenolic and flavonoid compounds, color, mineral content and antioxidant properties were determined for 37 honey samples belonging to different flora (apple, cherry, saffron and wild bush) from Kashmir, India. Among the minerals, potassium was the most predominant mineral followed by calcium, sodium and phosphorus. The CIELAB value indicated that all honey types were dark colored (L*≤50) with red and yellow components of color. Six phenolic acids (gallic acid, caffeic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid and ferulic acid) and seven flavonoids (myriceten, naringenin, kaempferol, pinocembrin, chrysin, apigenin and quercetin) were identified in four unifloral honey types. The result for total phenolic content (37–117 mg GAE/100 g) and total flavonoid content (8–17 mg QE/100 g), revealed that honeys from Kashmir valley have high antioxidant activity than the other honeys available in different parts of world. The first three principal components explained more than 83% of the variance with minerals having highest discriminating power while hierarchical cluster analysis (HCA) successfully classified all the unifloral honey samples.

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1. Introduction

The composition of honey mostly depends on the plant species visited by the honeybees and the environmental factors, processing and storage conditions (Nayik et al., 2014; Saxena, Gautam, & Sharma, 2010). Honey, a natural supersaturated sugar solution, is mainly composed of fructose and glucose, which constitutes 65–80% of total honey sugars (Bogdanov, Jurendic, Sieber, & Gallmann, 2008; Gheldof, Xiao-Hong, & Engeseth, 2002) and also contains certain important minor constituents viz. enzymes (diastase and invertase), organic acids (gluconic acid, acetic acid, etc.), vitamins, volatile compounds, phenolic compounds and minerals (Alvarez-Suarez, Giampieri, & Battino, 2013; Nayik & Nanda, 2015a). The antioxidant activity is mainly due to presence of major polyphenols in the form of phenolics acids (chlorogenic, ferulic, caffeic, ellagic, vanillic, benzoic, cinnamic, coumaric acid etc.) and flavonoids (pinocembrin, apigenin, hesperitin, chrysin, quercetin, luteolin, myricetin, pinobanksin, galangin, kaempferol etc) (Aljadi

& Kamaruddin, 2004; Baltrusaityte, Venskutonis, & Ceksteryte, 2007; Gheldof & Engeseth, 2002; Gheldof et al., 2002; Nayik & Nanda, 2016a). Such polyphenols have been known to exhibit health-benefiting properties like antiatherogenic, anticarcinogenic, antithrombotic and anti-inflammatory. The variance in antioxidant properties among honeys from different sources is due to difference in composition of the polyphenols (Andrade, Ferreres, Gil, & Tomas-Barberan, 1997; Nayik, Dar, & Nanda, 2016b). The color of honey ranges from pale yellow to dark reddish to light black as degree of darkening depends on temperature and duration of storage (Holderna-Kedzia & Kedzia, 2006). The dark colored honeys possessed higher phenolic content and consequently higher antioxidant activity as compared to honey with light color (Bertoncelj, Dobersek, Jamnik, & Golob, 2007; Holderna-Kedzia & Kedzia, 2006). A significant correlation has been reported between antioxidant activity and color of honey.

The wide spread floral plants and fruit crops like apple (Production: 1.9 MT), cherry (Production: 10 kT), saffron (Production: 9.6 T) and *Plectranthus rugosus* (data not available) in Kashmir valley act as catalyst for the farmers to take beekeeping as a side business and has encouraged them to produce honey from such crops (Directorate of Horticulture Kashmir, 2015–16). There are about 1621 honey-producing units in Jammu & Kashmir and honey

production is about 2000 metric tons (Press Information Bureau, Government of India, 2013). The uniqueness and the authenticity of saffron (Crocus sativus), cherry (Prunus avium) and apple (Malus domestica) honey has already been established by identification and quantification of forty-two different volatile compounds using SPME-GCMS (Nayik & Nanda, 2015a). Therefore, the present research work was conducted with the aim to study the phenolic compounds, color, antioxidant properties and mineral content of four unifloral honey types (saffron, apple, cherry and wild bush) with a chemometric approach and to study the correlation among these physic-chemical characteristics.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals, solvents and reagents were of analytical grade. Ascorbic acid, ellagic acid, chlorogenic acid, caffeic acid, p-coumaric acid, gallic acid, naringenin, quercetin, kaempferol, pinocembrin, chrysin and HPLC-grade methanol 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 four different raw and fresh honey types (n = 37) apple (Malus domestica), cherry (Prunus avium), saffron (Crocus sativus) and wild bush (Plectranthus rugosus) collected between March 2013−October 2014 from different areas (Pulwama, Pampore, Budgam and Srinagar) of Kashmir valley. All the honey samples were packed and sealed in glass bottles collected from local beekeepers 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, Oddo, Piana, Morlot, and Martin (2004). The following terms were used for frequency classes: predominant pollen (>45%), secondary pollen (16−45%), important minor pollen (3−15%) and minor pollen (<3%).

2.3. Color

2.3.1. Color measurement (L*, a*, b*)

Homogenized honey samples were used without any dilution and the color parameters (L*, a*, b*) were determined using a color spectrophotometer (CM-3600d, Konica Minolta). L* is the lightness (L* = 100 for white and 0 for black), a* indicates red for a positive value and green for a negative value, b* indicates yellow for a positive value and blue for a negative value (Gonzalez-Miret, Terrab, Hernanz, Fernandez-Recamales, & Heredia, 2005).

2.3.2. Color (mm Pfund)

The honey samples were heated at 40 °C to dissolve sugar crystals, and the color was determined by spectrophotometric measurement of the absorbance of a 500 g/L honey solution at 635 nm. The honeys were classified according to the Pfund scale after conversion of the absorbance (Abs) values (White, 1984):

$$mm\ Pfund = -38.70 + 371.39 \times Abs$$

where mm Pfund is the intensity of honey color in the Pfund scale, Abs is the absorption of honey solution.

2.3.3. Color intensity: ABS_{450}

The color intensity of the honey samples was determined by the method of Beretta, Granata, Ferrero, Orioli, and Facino (2005). The honey samples were diluted to 500 g/L with warm (45−50 °C) distilled water and the solution was then filtered through a 0.45 μm filter. The absorbance was measured at 450 and 720 nm using a spectrophotometer (Hach Lange DR6000 UV-VIS, Dusseldorf Germany) and the difference in absorbance was expressed as mAU.

2.3.4. Electrical conductivity and ash content

Electrical conductivity measures the electrical resistance, which was measured by a conduct meter. 0.1 mol/L of 40 mL potassium chloride solution was prepared in a beaker. The conductivity cell was connected to the conductivity meter after rinsing the cell thoroughly with the potassium chloride solution. The cell was then immersed in the solution, together with a thermometer. The electrical conductance of honey solution (200 g/L) was measured after the temperature was equilibrated to 20 °C. Results were expressed in mS/cm. (International Honey Commission, 2009).

2.4. Mineral analysis

The ash content obtained was dissolved in 0.1 mol/L of HCl and then diluted to 100 mL distilled water (Kamboj, Bera, & Nanda, 2013). Mineral elements (K, Ca, P, Na, Cu, Mn, Fe, Zn, Pb and Cd) were determined by using air acetylene flame atomic absorption spectrometer (Perkin Elmer Analyst 700 Model, Singapore). The response from the equipment was periodically checked with known standards. Calibration curves were constructed for each element using by appropriate standard solutions by diluting stock solutions of 1000 mg/L of each element supplied by Fluka.

2.5. Analysis of phenolic compounds

2.5.1. Extraction of phenolic compounds

Honey sample (5 g) were dissolved in 50 mL volumetric flask containing 5 mL acidified distilled water (pH 2 by HCl). The flask was placed in water bath for 10 min at room temperature. The honey samples were homogenized and filtered through a 0.45 μm PTFE membrane filter. The phenolic compounds present in honey remained in the column while sugars and other polar compounds were eluted with the aqueous solvent. The column was first washed with acid water followed by with distilled water. The phenolic compounds were eluted with 250 mL of methanol. Then the methanol extracts were concentrated under vacuum at 40 °C in a rotary evaporator. The residue dissolved in 5 mL of distilled water was extracted three times by 5 mL of diethyl ether. The dried residue redissolved in 1 mL of methanol (HPLC grade) was then filtered through a membrane filter with a 0.45 μm pore size.

2.5.2. Identification and quantification of phenolic compounds

Analyses of the extracts were carried with Waters iso-cratic HPLC system (Chandigarh, India) equipped with diode array detector (DAD). The separation was performed by Waters X-bridge Amide HPLC Column, 5 μm (250 × 4.66 mm) using a mobile phase of water with 50 mL formic acid (solvent A) and methanol (solvent B) at a constant solvent flow rate of 1 mL/min. According to the method adopted by Martos, Ferreres, and Tomas-Barberan (2000), the following gradient modified programme was used: 30% solvent B was allowed to flow through the column isocratically for 15 min and then was increased to 40% methanol at 20 min followed by 45% methanol at 30 min and 60% methanol at 50 min. Finally, the iso-cratic elution was done until 55 min with 80% methanol (Yao, Jiang, Singanusong, Datta, & Raymont, 2004). The honey extracts were injected (5 μL) and phenolic compounds were detected using a photodiode-array detector to obtain the UV spectra of phenolic compounds. In addition, the chromatograms were monitored at