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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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KEYWORDS

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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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100 g honey (mg QE/100 g) using standard curve of quercetin (0–100 μg/mL).

2.5.2. DPPH radical scavenging activity

Antioxidant activity of all honey samples was described by modified method adopted by Ordonez et al. (2006) based on DPPH radical scavenging activity. To make the honey solution, 0.6 g of sample was dissolved in 4 mL of methanol. After this, 1.5 mL of DPPH reagent solution (0.02 mg/mL) was added to 0.75 mL of honey solution, then the samples were kept in the dark for 15 min at room temperature. The absorbance of the mixture was measured at 517 nm against methanol blank by using Hach Lange DR6000 UV–VIS Spectrophotometer (Dusseldorf Germany). The radical scavenging activity of DPPH radical expressed as % inhibition was calculated from the following equation (Meda et al., 2005):

$$\%\ \text{Inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

where Abs control is the absorbance of control (1.50 mL DPPH and 0.75 mL methanol) at 517 nm and Abs sample is the absorbance of sample at 517 nm.

2.5.3. Antioxidant content

The antioxidant content in terms of antioxidant equivalent ascorbic acid content (AEAC) was determined by the method described by Meda et al., 2005. The antioxidant content was expressed as mg of ascorbic acid equivalent antioxidant content per 100 g of honey (mg AEAC/100 g) using standard curve of ascorbic acid (0–100 μg/mL).

2.6. Mineral analysis

Macro mineral elements (K, Ca, P and Na) were determined by using air acetylene flame atomic absorption spectrometer (AAS4141). Calibration curves were constructed for each element using appropriated standard solutions.

3. Statistical analysis

Analyses were made in triplicate and the data are presented as mean ± SD. The statistical differences were obtained through one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at 95% of confidence level ($P < 0.05$). Multivariate analysis [principal component analysis (PCA), linear discriminate analysis (LDA)] was performed by using Statistica.v.12. (StatSoft India Pvt. Ltd., New Delhi, India). The antioxidant, sugar and minerals parameters were also subjected to LDA in order to evaluate the potential of these parameters for classification of the samples. Prior to multivariate analysis, the entire data matrix was auto-scaled for column, subtracting the median of each column to every sample and dividing it for their standard deviation to ensure that all the elements had equal weightage in the results.

4. Results and discussion

4.1. Pollen analysis

Melisssopalynological determination of botanical origin of honey is based on the relative frequency of the pollen from the nectar-secreting plants. Acacia honey contained 54–60% pollen of *Robinia pseudoacacia* sp. The honeydew element/pollen grain (HDE/P) ratio was in a range of 2.79–3.01 in pine honeydew (*Pinus wallichiana*), which was in good agreement with Louveaux et al. (1978). The microscopic analysis revealed some fungal spores in pine honeydew which is in good agreement with those found in Greek pine honeys (Karabagias et al., 2014). Multifloral honey contained 2–5% pollens of *plectranthus rugosus*, and other pollens found were those from *Prunus* sp., *Brassica* sp., *Thyme* sp. and *Ailanthus* sp.

4.2. Moisture content

The results obtained for moisture content and sugar analysis of three honey varieties are presented in Table 1. Moisture content is considered as valid criterion of honey quality; honey with lower moisture content showed longer shelf life (Fredes and Montenegro, 2006). The moisture content of honey depends on various factors such as harvesting time, climatic factors geographical area, processing conditions, storage temperature and maturity period (Nanda et al., 2003 and Finola et al., 2007). In this study all the honey samples from three varieties showed the low level of moisture content (18.2–19.11%) which was in agreement with the values (<20%) set by Codex standard for honey (Codex Almentarius, 2001). Yılmaz and Kufrevioglu (2000) and Duman et al. (2008), reported similar results.

4.3. Sugar analysis

The sweet nature of honey is due to the presence 65–70% that mostly depends on flower visited by the bee as well as geographical origins and less on seasonal, processing and storage conditions (Dobre et al., 2012; Ouchemoukh et al., 2010). In this study, only four sugars were identified and quantified as shown in Table 1, and the other sugars could not be identified due to the lack of standards. Statistical differences were observed in identified sugars and moisture content among the three honey varieties ($P < 0.05$). The concentration of fructose ranged between 31.96% and 39.27% (Table 1) with highest percentage

Table 1 Sugar composition of honey from different sources assessed.

Parameter	Acacia honey ($n = 7$)	Pine honeydew ($n = 8$)	Multifloral honey ($n = 9$)
Moisture (%)	18.6 ± 0.53^{b}	18.2 ± 0.62^{b}	19.11 ± 0.33^{a}
Fructose (%)	35.64 ± 1.80^{b}	31.96 ± 1.27^{c}	39.27 ± 1.13^{a}
Glucose (%)	31.66 ± 1.42^{b}	32.84 ± 1.21^{b}	33.97 ± 1.27^{a}
Maltose (%)	1.57 ± 0.08^{b}	1.02 ± 0.06^{c}	1.97 ± 0.05^{a}
Sucrose (%)	1.33 ± 0.09^{b}	1.10 ± 0.02^{c}	1.92 ± 0.05^{a}
F/G ratio	1.12 ± 0.05^{b}	1.13 ± 0.09^{b}	1.16 ± 0.05^{a}
G/M ratio	1.70 ± 0.1^{b}	1.80 ± 0.08^{a}	1.78 ± 0.08^{a}

Results are expressed as mean values ± standard deviations. Means in a row with same superscripts are not significantly different ($P < 0.05$). F/G = fructose/glucose; G/M = glucose/moisture.

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in multifloral and lowest in pine honeydew. Although the similar range for fructose was reported by De La Fuente et al. (2011) in Spanish unifloral honey and Nayik et al., 2015a,b in unifloral Kashmiri honey but higher ranges for fructose 41.3–43.30% (acacia honey) and 39.70–49.10% (black locust honey) were reported by Escuredo et al. (2014) and Primorac et al. (2011), respectively. All the analyzed honey varieties showed the glucose content more than 30% (31.66–33.97%). Escuredo et al. (2014) reported the similar glucose values in lime honey from Northwest of Spain and different regions of Romania. Both floral honeys (acacia and multifloral) showed the value of glucose plus fructose higher than 60 g/100 g, which is the value, required for all the kinds of honey in the European and Codex standards. Soria et al. (2004) reported that the sum of glucose plus fructose could be used as discriminatory variable for distinguishing blossom and honeydew honeys. The sum of glucose plus fructose in pine honeydew honey was less than 60 g/100 g, thus proving its non-floral nature. The sucrose concentration of all the honey samples ranged from 1.10% in pine honeydew honey to 1.92% in multifloral honey which was in agreement with the limit (5 g/100 g), prescribed by European Community Directive (European Economic Community, 2002) which confirmed that these honeys are at an advanced stage of ripening. The concentration of maltose ranged between 1.02% and 1.97%, which agrees with results reported by Juszczak et al. (2009) and Nayik et al. (2015a,b). Crystallization or granulation of honey is a natural and spontaneous phenomenon by which honey turns its liquid state to a semi-solid state. Sometimes crystallized honey is referred as the set honey. Crystallization of honey is little understood by the consuming public. Many assume that crystallized honey is adulterated or unnatural product but some honey users like it in semi-solid state since it is easy to spread on bread or toast without dripping off. Crystallization affects only color and texture of honey but preserve the flavor and quality characteristics of the liquid honey. Sugar ratios were calculated and evaluated to check their contribution to the crystallization tendencies. The tendency of honey to granulate is explained by fructose/glucose (F/G) ratio because glucose is less water soluble than fructose (Laos et al., 2011). In the present study, F/G ratio was 1.16 (multifloral honey), 1.12 (acacia honey) and 1.13 (pine honeydew honey) as shown in Table 1 which is common F/G ratio found in most of the honey varieties around the world (Bentabol Manzanares et al., 2011; Dobre et al., 2012). The values for F/G ratio were significantly lower than those found for chestnut and honeydew honeys (>1.4). According to Venir et al. (2010), F/G ratio of $\leqslant 1.14$ indicates a faster crystallization; thus, acacia honey crystallizes faster than other varieties while F/G ratio of $\geqslant 1.58$ results no crystallization. Apart from F/G ratio, glucose/moisture (G/M) ratio is another important parameter that affects the crystallization of honey (National Honey Board, 2010). Lower the moisture content and higher the glucose content of honey, faster the crystallization will take place. According to the Dobre et al. (2012), crystallization of honey is very slow or null when the G/M ratio is <1.7, and it is rapid when the ratio is >2. The G/M ratio ranged between 1.7 and 1.8 (Table 1).

4.4. Mineral analysis

The mean content of all mineral (mg/kg) found in three analyzed honey varieties is shown in Table 2 and statistical significant differences ($P < 0.05$) were also observed among the three analyzed varieties. The most abundant macro minerals found in analyzed honey varieties were potassium (K) followed by calcium (Ca), sodium (Na) and phosphorus (P), which ranged from 353.56 to 752.22 mg/kg, 88.40 to 122.94 mg/kg, 35.28 to 93.72 mg/kg and 24.36 to 69.28 mg/kg, respectively. The results for K and Ca in the analyzed varieties were consistent with the results reported in Italian honey, in which 75% of the total mineral content was occupied by potassium (Silva et al., 2009). The concentration of calcium was found highest in pine honeydew honey (752.22 mg/kg) and lowest in acacia (353.56 mg/kg). These were in agreement with Hungarian and Croatian honeys (Czipa et al., 2015; Ursulin-Trstenjak et al., 2015). Our results for sodium (35.28–93.72 mg/kg) and phosphorus (24.36–69.28 mg/kg) were low as compared to results reported by Sulbaran de Ferrer et al. (2004) in Venezuelan honey. The results displayed in Table 2 showed that pine honeydew being dark colored honey was richest in macro minerals followed by multifloral honey and then acacia honey.

4.5. Antioxidant activity

Honey is considered as rich source of antioxidant activity mainly due to presence of phenolic acids and flavonoids. The total phenolic content (TPC) ranged from 22.68 mg GAE/100 g in acacia honey to 59.84 mg GAE/100 g in pine honeydew honey (Table 2). Dark colored honey possessed higher phenolic content and consequently higher antioxidant activity as compared to honey with light color (Frankel et al., 1998). The phenolic content of pine honeydew and acacia honey was similar to that reported by Can et al. (2015) in Turkish pine and acacia honey. Flavonoid compounds are also present in honey, which are too responsible for antioxidant activity of honey. The total flavonoid content (TFC) of three

Table 2 Antioxidant properties and mineral content of different honey varieties.

Parameter	Pine honeydew ($n = 7$)	Acacia honey ($n = 8$)	Multifloral honey ($n = 9$)
Total phenolic content mg GAE/100 g	59.84 ± 0.33[a]	22.68 ± 1.98[c]	32.29 ± 0.43[b]
Total flavonoid content mg quercetin /100 g	8.12 ± 1.05[a]	6.10 ± 1.03[c]	8.08 ± 2.23[b]
AEAC mgAA/100 g	23.74 ± 2.06[a]	14.13 ± 1.37[c]	20.99 ± 1.57[b]
DPPH RSA (%)	55.37 ± 0.68[a]	52.27 ± 1.42[c]	56.91 ± 3.92[b]
Potassium (mg/kg)	752.22 ± 3.99[a]	353.56 ± 7.20[c]	439.93 ± 7.92[b]
Calcium (mg/kg)	122.94 ± 3.06[a]	88.40 ± 5.21[c]	109.34 ± 4.16[b]
Sodium (mg/kg)	93.72 ± 3.93[a]	35.28 ± 3.57[c]	67.79 ± 3.00[b]
Phosphorus (mg/kg)	69.28 ± 2.61[a]	24.36 ± 4.18[c]	44.30 ± 2.58[b]

Results are expressed as mean values ± standard deviations. Means in a row with same superscripts are not significantly different ($P < 0.05$). n = Number of samples; GAE: Gallic Acid Equivalents; AEAC: Antioxidant Equivalent Ascorbic acid Content; DPPH RSA: 2,2,diphenyl-1-picrylhydrazyl Radical Scavenging Activity.

analyzed honey varieties ranged from 6.10 to 8.12 mg QE/100 g (Table 2). The total flavonoid content of all the analyzed varieties was high as compared to Malaysian honey (1.1–3.4 mg CEQ/100 g) (Khalil et al., 2011). The TFC of pine honeydew was high as compared to Turkish pine honey (1.58 mg QE/100 g) (Can et al., 2015) which could be due to variance in geographical origin as well as environmental factors. The DPPH radical scavenging activity (DPPH-RSA) of analyzed three honey varieties ranged from 52.27% to 55.37%) which is in agreement with the results published for Romanian honeys (35–64%) (Azeredo et al., 2003). The antioxidant content determined in terms of antioxidant equivalent ascorbic acid content (AEAC) values, ranged from 14.13 to 23.74 mg AEAC/100 g of honey (Table 2) which is in agreement with the AEAC values published for commercial Indian honeys (15.1–29.5 mg AEAC/100 g) (Saxena et al., 2010), Malaysian honeys (24.2–32 mg AEAC/100 g) (Khalil et al., 2011) and Pakistani honey (8.3–22.10 mg AEAC/100 g) (Noor et al., 2014). The pine honeydew honey being the dark colored honey among the three analyzed honey varieties was found high in TPC, TFC, DPPH-RSA and AEAC as well.

The Pearson's correlation observed between TPC and DPPH-RSA ($r = 0.86$) indicated that phenolic content was the strongest contributing factor to the radical scavenging activity of the three analyzed honey varieties (Table 3). The significant correlation between antioxidant properties and total mineral content (Table 3) indicated that minerals can also contribute in the antioxidant activity of honey. Escuredo et al. (2014) observed similar correlation in honeydew and chestnut honeys that possessed highest antioxidant activities because of high macro mineral content.

5. Multivariate analysis

To confirm whether the macro minerals and antioxidant and sugar parameters could differentiate the honeys according to their botanical origin, principal component analysis (PCA) and linear discriminate analysis (LDA) were applied to data. The first two principal components (PCs), with eigen values greater than one (7.49 & 3.06) were extracted (Table 4) by using Kaiser criterion of PCA. The first two principal components accounted for more than 81% of the variation in the analyzed honey samples (Table 4). The first (PC1) and second (PC2) principal component explained 57.68%, and 23.56% of the variance, respectively (Table 4). The first component explained 57.68% of the variance was mostly dominated by macro minerals viz. potassium, phosphorus and TPC as shown in bold in Table 4. Similar results for potassium, phosphorus and calcium were reported by Fernandez-Torres et al. (2005) for PC1 in four different Spanish honeys. The second component with variance of 23.56% was dominated by sucrose, DPPH-RSA and AEAC; therefore, in the present study most of the minerals and antioxidant properties were important variables which were capable of discriminating as compared to sugar parameters. Figs. 1 and 2 showed that all pine honeydew honey samples on upleft of PC1 were linked to antioxidant properties and macro minerals while all multifloral honey samples positioned on its downright were linked to sugar parameters and moisture content. Thus, antioxidant properties and minerals could be used to distinguish pine honeydew from multifloral honey. To compare the relative importance of the independent variables, the standardized discriminate coefficients were used by applying least discriminate analysis. The higher the absolute value of a standardized coefficient, the more significant was the related selected variable. The first discriminant function accounts for 94.5% of total variance (Table 5). Potassium contributed the most to the first canonical variable (standardized coefficient = −1.730), accounting for most of the discrimination between honey classes followed by sodium. The second canonical variable was related to calcium (standardized coefficient = 1.6214) variable in explaining the separation among the honey samples according to botanical origin (Table 5). To predict the group

Table 4 Principal component analysis. Loading of first two components.

Factor number	1	2
Initial Eigen values	7.49	3.06
% of variance	57.68	23.56
Cumulative %	57.68	81.24
Factor loadings		
Moisture content	0.4908	−0.5557
TPC	−0.9884	−0.0511
TFC	−0.3331	−0.5162
DPPH-RSA	−0.3897	−0.6234
AEAC	−0.7519	−0.5799
Fructose	0.7911	−0.4677
Glucose	0.7334	−0.5164
Maltose	0.7350	−0.5316
Sucrose	0.5306	−0.7898
Potassium	−0.9926	−0.0039
Calcium	−0.8723	−0.4202
Sodium	−0.8998	−0.3660
Phosphorus	−0.9547	−0.2410

Table 3 Correlation among antioxidant properties and macro minerals (Pearson correlation, $P < 0.05$).

	TPC	TFC	DPPH-RSA	AEAC	K	Ca	Na	P
TPC	1.00							
TFC	0.82	1.00						
DPPH-RSA	0.86	0.88	1.00					
AEAC	0.87	0.84	0.67	1.00				
K	0.99	0.74	0.35	0.74	1.00			
Ca	0.88	0.82	0.85	0.88	0.86	1.00		
Na	0.93	0.86	0.80	0.88	0.91	0.95	1.00	
P	0.96	0.81	0.80	0.86	0.95	0.92	0.96	1.00

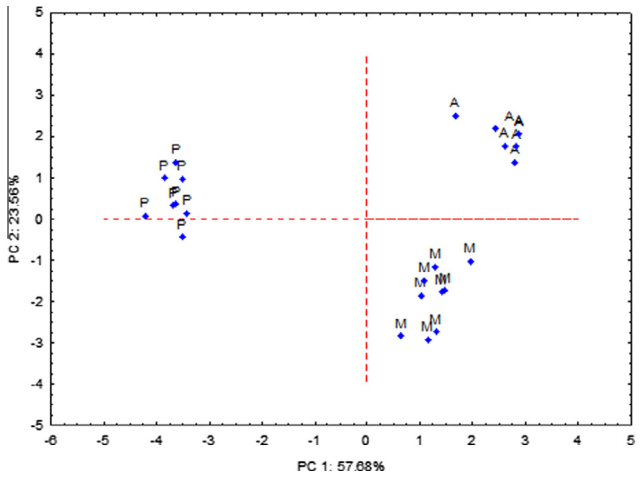

Figure 1 Principal component analysis. Distribution of honey samples on scores plot. (Botanical origins: A: Acacia, M: Multifloral P: Pine honeydew.)

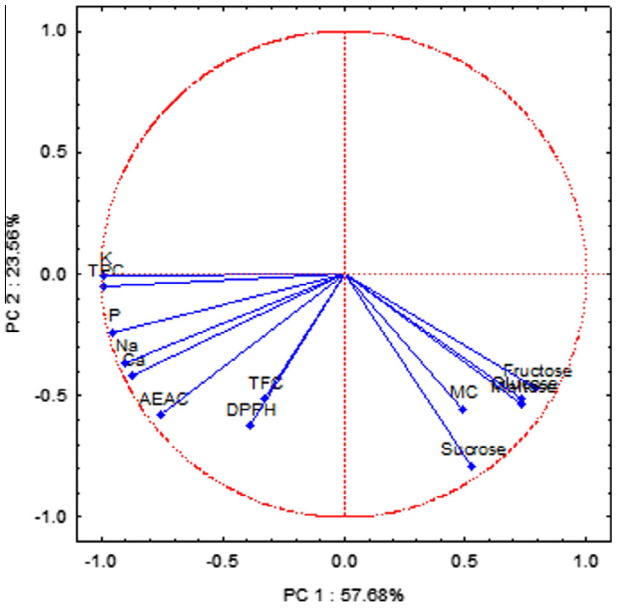

Figure 2 Projections of the variables on the factor plane for the three honeys.

membership of honeys, LDA, showed that 100% of samples were correctly classified into each honey type (on the diagonal matrix) (Table 6). By observing the graphical distribution of the honey samples on the reported scatterplot (Fig. 3), a natural and complete separation between three honeys of different botanical origins was obtained by the discriminant functions DF1 and DF2. All the samples corresponding to multifloral honey were located as a compact group at the top right side of the scatterplot. The pine honeydew samples were positioned in middle upright side forming a defined and separate group while at the lower right side of the scatterplot, all the acacia honey samples appeared to be more dense and separate group (Fig. 3).

Table 5 Standardized coefficient for canonical variables obtained by discriminate analysis.

Variable	Root 1	Root 2
Moisture content	0.124	0.3645
TPC	0.232	0.7098
TFC	−0.066	−0.1732
DPPH-RSA	−0.228	−0.7491
AEAC	−0.012	−0.1076
Fructose	1.266	1.4896
Glucose	1.074	0.3674
Maltose	0.591	0.8435
Sucrose	0.569	0.8980
Potassium	−1.730	−0.4531
Calcium	0.946	1.6214
Sodium	−1.422	0.6043
Phosphorus	0.649	1.1976
Eigen value	2296.792	132.4150
Cumulative (%)	94.5	100.0

Table 6 Classification result of LDA of variables in three types of honeys.

From/to	Acacia honey	Pine honeydew	Multifloral honey	Total	% Correct
Acacia honey	07	0	0	07	100.00
Pine honeydew	0	08	0	08	100.00
Multifloral honey	0	0	09	09	100.00
Total	07	08	09	24	100.00

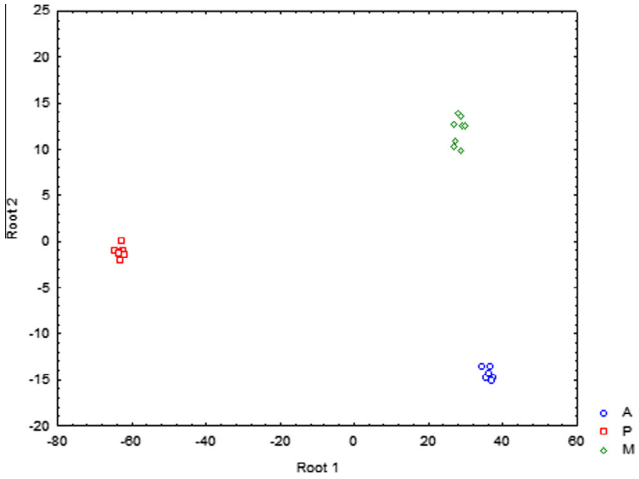

Figure 3 Scatterplot of canonical discriminant scores of three different honey varieties using LDA.

6. Conclusion

The results obtained in this study showed that all honey varieties were rich in reducing sugars (fructose and glucose). It was concluded that among the three honey varieties from high altitude

Kashmir valley, pine honeydew honey was rich in macro minerals and possesses high antioxidant properties. By applying multivariate techniques (PCA and LDA), the study confirmed that macro minerals and antioxidant properties possessed the high discriminating power than sugar parameters. These results suggested that the application of multivariate analyses (PCA and LDA) on the macro minerals, antioxidant and sugar parameters is a useful tool to characterize different types of honey.

References

Aljadi, A.M., Kamaruddin, M.Y., 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem. 85, 513–518.

Arvouet-Grand, A., Vennat, B., Pourrat, A., Legret, P., 1994. Standardization of propolis extract and identification of principal constituents. J. Pharam. Belg. 49, 462–468.

Azeredo, L.C., Azeredo, M.A.A., de Souza, S.R., Dutra, V.M.L., 2003. Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins. Food Chem. 80, 249–254.

Bentabol Manzanares, A., Hernandez Garcia, Z., Rodriguez Galdon, B., Rodriguez Rodriguez, E., Díaz Romero, C., 2011. Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chem. 126, 664–672.

Beretta, G., Granata, P., Ferrero, M., Orioli, M., Facino, R.M., 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. Anal. Chim. Acta 533, 185–191.

Can, Z., Yildiz, O., Sahin, H., Turumtay, E.A., Silici, S., Kolayli, S., 2015. An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chem. 180, 133–141.

Codex Alimentarius Commission, 2001. Revised standards for honey. Codex Standard 12–1981. Rev 1 (1987), Rev 2 (2001), Rome, FAO.

Czipa, N., Andrasi, D., Kovacs, B., 2015. Determination of essential and toxic elements in Hungarian honeys. Food Chem. 175, 536–542.

De la Fuente, E., Ruiz-Matute, A.I., Valencia-Barrera, R.M., Sanz, J., Martinez-Castro, I., 2011. Carbohydrate composition of Spanish unifloral honeys. Food Chem. 129, 1483–1489.

Dobre, I., Georgescu, L.A., Alexe, P., Escuredo, O., Seijo, M.C., 2012. Rheological behavior of different honey types from Romania. Food Res. Int. 49, 126–132.

Duman, A., Sezer, C., Oral, N.B., 2008. Kars'ta Satis_a Sunulan Suzme Ballarin Kalite Niteliklerinin Aras tirilmasi. Kafkas Universitesi Veteriner Fakultesi Dergisi 14, 89–94.

Escuredo, O., Dobre, I., Fernandez-Gonzalez, M., Seijo, M.C., 2014. Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. Food Chem. 149, 84–90.

European Economic Community, 2002. EEC council directive of 20 December 2001 relating to honey. Off. J. Eur. Commun. 110, 47–50.

Fernandez-Torres, R., Perez-Bernal, J.L., Bello-Lopez, M.A., Callejon-Mochon, M., Jimenez-Sanchez, J.C., Guiraum-Perez, A., 2005. Mineral content and botanical origin of Spanish honeys. Talanta 65, 686–691.

Finola, M.S., Lasagno, M.C., Marioli, J.M., 2007. Microbiological and chemical characterization of honeys from central Argentina. Food Chem. 100, 1649–1653.

Frankel, S., Robinson, G.E., Berenbaum, M.R., 1998. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J. Apic. Res. 37 (1), 27–31.

Fredes, C., Montenegro, G., 2006. Heavy metals and other trace elements contents in Chilean honey. Cienc. Investig. Agrar. 33, 50–58.

Gheldof, N., Xiao-Hong, W., Engeseth, N.J., 2002. Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food Chem. 5, 5870–5877.

Giansante, L., Vincenzo, D.D., Bianchi, G., 2003. Classification of monovarietal Italian olive oils by unsupervised (PCA) and supervised (LDA) chemometrics. J. Sci. Food Agric. 83, 905–911.

Gomez-Caravaca, M., Gomez-Romero, M., Arraez-Roman, D., Segua-Carretero, A., Fernandez-Gutierrez, A., 2006. Advances in the analysis of phenolic compounds in products derived from bees. J. Pharm. BioMed. 41, 1220–1234.

Guler, A., Bakan, A., Nisbet, C., Yavuz, O., 2007. Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup. Food Chem. 105, 1119–1125.

Holderna-Kedzia, E., Kedzia, B., 2006. Research on an antioxidant capacity of honeys. Acta Agrobot. 59, 265–269.

Juszczak, L., Socha, R., Roznowski, J., Fortuna, T., Nalepka, K., 2009. Physicochemical properties and quality parameters of herb-honeys. Food Chem. 113, 538–542.

Karabagias, I.K., Badeka, A., Kontakos, S., Karabournioti, S., Kontominas, M.G., 2014. Characterization and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem. 146, 548–557.

Khalil, M.I., Alam, N., Moniruzzaman, M., Sulaiman, S.A., Gan, S. H., 2011. Phenolic acid composition and antioxidant properties of Malaysian honeys. J. Food Sci. 76 (6), 921–928.

Kucuk, M., Kolayli, S., Karaoglu, S., Ulusoy, E., Baltaci, C., Candan, F., 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem. 100, 526–534.

Laos, K., Kirs, E., Pall, R., Martverk, K., 2011. The crystallization behaviour of Estonian honeys [Special Issue II]. Agron. Res. 9, 427–432.

Latorre, M.J., Garcia-Jares, C., Medina, B., Herrero, C., 1994. Pattern recognition analysis applied to classification wines from Galacia (NW Spain) with certified brand origin. J. Agric. Food Chem. 42, 1451–1455.

Louveaux, J., Maurizio, A., Vorwohl, G., 1978. Methods of melissopalynology. Bee World. 59, 139–157.

Meda, A., Lamien, C.E., Romito, M., Millogo, J., Nacoulma, O.G., 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91, 571–577.

Nanda, V., Sarkar, B.C., Sharma, H.K., Bawa, A.S., 2003. Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. J. Food Compos. Anal. 16, 613–619.

National Honey Board, Honey Varietals, 2010. Available at: <http://www.honey.com>.

Nayik, G.A., Dar, B.N., Nanda, V., 2015a. Physico-chemical rheological and sugar profile of different unifloral honeys from Kashmir valley of India. Arab J. Chem., <http://dxdoi.org/10.1016/j.arabjc.2015.08.017>..

Nayik, G.A., Dar, B.N., Nanda, V., 2015b. Optimization of the process parameters to establish the quality attributes of DPPH radical scavenging activity, total phenolic content and total flavonoid content of apple (*Malus domestica*) honey using response surface methodology. Int. J. Food Prop., Doi:101080/10942912.2015.1107733.

Nayik, G.A., Nanda, V., 2015. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from Kashmir valley of India with a multivariate approach. Pol. J. Food Nutr. Sci. 2 (65), 101–108.

Nayik, G.A., Nanda, V., 2016a. Effect of thermal treatment and pH on antioxidant activity of saffron honey by using response surface methodology. J. Food Meas. Charact. 10 (1), 64–70.

Nayik, G.A., Nanda, V., 2016b. Application of response surface methodology to study the combined effect of temperature time and

pH on antioxidant activity of cherry (*Prunus avium*) honey. Pol. J. Food Nutr. Sci. 66 (4), Doi: 10.1515/pjfns-2015-0055.

Nayik, G.A., Shah, T.A., Muzaffar, K., Wani, S.A., Gull, A., Majid, I., Bhat, F.A., 2014. Honey: its history and religious significance: a review. Uni. J. Pharam. 3 (1), 5–8.

Noor, N., Sarfraz, R.A., Ali, S., Shahid, M., 2014. Antitumour and antioxidant potential of some selected Pakistani honeys. Food Chem. 143, 362–366.

Ordonez, A.A.L., Gomez, J.D., Vattuone, M.A., Isla, M.I., 2006. Antioxidant activities of *Sechium edule* (Jacq). Food Chem. 97, 452–458.

Ouchemoukh, S., Schweitzer, P., Bachir Bey, M., Djoudad-Kadji, H., 2010. HPLC sugar profiles of Algerian honeys. Food Chem. 121, 561–568.

Press Information Bureau, Government of India, 2013. URL: <http://pib.nic.in> (accessed 23.12.13).

Primorac, L., Flanjak, I., Kenjeric, D., Bubalo, D., Topolnjak, Z., 2011. Specific rotation and carbohydrate profile of Croatian unifloral honeys. Czech J. Food Sci. 29, 515–519.

Rinaldi, M., Gindro, R., Barbeni, M., Allegrone, G., 2009. Pattern recognition and genetic algorithms for discrimination of orange juices and reduction of significant components from headspace solid-phase microextraction. Phytochem. Anal. 20, 402–407.

Saxena, S., Gautam, S., Sharma, A., 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118, 391–397.

Silva, L.R., Videira, R., Monteiro, A.P., Valentao, P., Andrade, P.B., 2009. Honey from Luso region (Portugal): physicochemical characteristics and mineral contents. Microchem. J. 93 (1), 73–77.

Silvano, M.F., Varela, M.S., Palacio, M.A., Ruffinengo, S., Yamul, D. K., 2014. Physico-chemical parameters and sensory properties of honeys from Buenos Aires region. Food Chem. 152, 500–507.

Soria, A.C., Gonzalez, M., De Lorenzo, C., Martinez-Castro, I., Sanz, J., 2004. Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melisssopalynological, physicochemical and volatile composition data. Food Chem. 85, 121–130.

Subrahmanyam, M., Sahapure, A.G., Nagane, N.S., Bhagwat, V.R., Ganu, J.V., 2001. Effects of topicalapplication of honey on burn wound healing. Ann. Burns Fire Dis. 14, 143–145.

Sulbaran de Ferrer, B., Ojeda de Rodríguez, G., Pena, J., Martinez, J., Moran, M., 2004. Mineral content of the honey produced in Zulia state. Venezuela. Arch. Latinoam Nutr. 54 (3), 346–348.

Ursulin-trstenjak, N., Levanic, D., Primorac, L., Bosnir, J., Vahcic, N., Saric, G., 2015. Mineral profile of Croatian honey and differences due to its geographical origin. Czech J. Food Sci. 33, 156–164.

Venir, E., Spaziani, M., Maltini, E., 2010. Crystallization in "Tarassaco" Italian honey studied by DSC. Food Chem. 122, 410–415.

Von Der Ohe, W., Oddo, L.P., Piana, M.L., Morlot, M., Martin, P., 2004. Harmonized methods of melissopalynology. Apidologie 35, 18–25.

Yılmaz, H., Kufrevioglu, I., 2000. Composition of honeys collected from Eastern and South-Eastern Anatolia and effect of storage on hydroxyl methyl furfural content and diastase activity. Turk. J. Agric. For. 25, 347–349.

Yucel, Y., Sultanoglu, P., 2013. Characterization of honeys from Hatay Region by their physicochemical properties combined with chemometrics. Food Biosci. 1, 16–2.

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