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Physico-Chemical, Enzymatic, Mineral and Colour Characterization of Three Different Varieties of Honeys from Kashmir Valley of India with a Multivariate Approach

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Key words: Kashmir, honey, acacia, enzymatic, pine honeydew, multifloral

The present study was undertaken to determine the physico-chemical properties (moisture content, reducing sugars, proline content, electrical conductivity, ash content, pH, titrable acidity, HMF, water activity, total soluble solids and total solids), enzymatic activity (diastase and invertase), colour characteristics (mmpfund, ABS_{450} and CIE $L^*a^*b^*$) and mineral content (Cu, Mn, Fe, Zn, Pb and Cd) of three different varieties of honeys from Kashmir valley of India (acacia honey, pine honeydew and multifloral honey). Of the honey samples analysed, only pine honeydew were grouped in dark category of honey ($L^*<50$) while acacia honey and multifloral honey were confirmed as light coloured honeys ($L^*>50$) and possessed both red and yellow components. The concentrations of mineral content were found highest in pine honeydew followed by multi floral and least in acacia honey. All the physico-chemical properties and enzymatic activity indicated that all the three analysed varieties of honey met the criteria set by the International Honey Commission and revised codex standards for honey. The source of honey had a significant effect ($p<0.05$) on physico-chemical characteristics, enzymatic activity, mineral content and colour properties. Strong and positive correlations exhibited among minerals, colour (mmpfund) and L^* value indicated that dark coloured honey contained high mineral content. Multivariate analysis proved to be an effective tool in classifying the three varieties of honey based on physico-chemical characteristics, enzymatic activity, mineral content and colour properties.

INTRODUCTION

Honey, the wonderfully rich golden liquid is the miraculous product of honey bees and a naturally delicious alternative to sugar. Chemically, honey is mostly dominated by sugars in the form of fructose and glucose (70–80%), water (10–20%) and other minor constituents such as organic acids (gluconic acid, acetic acid), mineral salts (potassium, calcium, sodium, phosphorus *etc.*), vitamins (ascorbic acid, niacin), proteins, enzymes (invertase, glucose oxidase, catalase, phosphatases), volatile chemicals, phenolic acids and flavonoids [Bouseta *et al.,* 1996; Terrab *et al.,* 2001; Gheldof *et al.,*2002; Blasa *et al.,* 2006; Ouchemoukh *et al.,* 2007; Nayik & Nanda, 2015]. The composition and physico-chemical properties of honey are wholly dependent on the plant species visited by the honeybees as well as the processing, storage, regional, and climatic conditions [Saxena *et al.,* 2010]. The colour, which serves as an indicator of floral origin ranges from light to dark often reddish or yellowish [Soria *et al.,* 2004; Terrab *et al.,* 2004]. It is well known fact that there is a strong correlation among colour, antioxidant activity, electrical conductivity and ash content [Marghitas *et al.,* 2009]. Generally dark coloured honey has higher phenolic content and consequently higher antioxidant activity as compared to honey with light colour [Berreta *et al.,* 2005].

Multivariate analysis viz. principal component analysis (PCA) and linear discriminate analysis (LDA) have been extensively used to classify honey based on physico-chemical data, mineral analysis and colour characteristics. Conti *et al.* [2007] classified three Italian honey samples (acacia, multifloral, honeydew) on the basis of their physico-chemical data and mineral content by applying multivariate statistical analysis. Chudzinska & Baralkiewicz, [2010] analysed fifty-five Polish honey samples to rationalize and interrupt the analytical data for the determination of thirteen metallic and nonmetallic elements by using PCA tool. Similar study was reported by Devillers *et al.* [2002] who examined French honeys.

Rearing of honey bees for production of honey (apiculture) is one of the prevalent agricultural activities done in India. As per the data published by Press Information Bureau [2012–2013], there are more than 1600 honey producing units in Jammu and Kashmir where honey production is near about 2000 metric tons. The physicochemical characterisation of different honeys from different parts of the world has been carried out extensively [Azeredo *et al.,* 2003; Finola *et al.,* 2007; Ouchemoukh *et al.,* 2007]. Honeys produced in India from various unifloral sources as well as from commercial honeys were already studied by various authors [Ahmed *et al.,* 2007; Nanda *et al.,* 2009; Saxena *et al.,* 2010]. After successful studies of characterisation of quality parameters of *Eucalyp-*

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tus, Brassica, Helianthus, L. chinensis, Z. mauritiana, C. sinensis and *P. persica* [Nanda *et al.,* 2003, 2006, 2009], the present study was undertaken, not only to study the physico-chemical parameters, mineral content, enzymatic analysis and colour characteristics from three different varieties from Kashmir valley of India viz. acacia honey, pine honeydew and multifloral honey but also to perform pattern recognition methods by using PCA and LDA to classify honey varieties which has never been performed till now.

MATERIALS AND METHODS

Honey sample

The present study was carried out using 30 samples (10 samples from each variety) of three different raw and fresh honey samples (acacia honey, pine honeydew and multifloral honey) collected from bee keepers during September 2012 to May 2014 from different regions (Pulwama, Srinagar, and Budgam) packed and sealed in glass bottles of Kashmir valley of India and stored at 4°C. The honey samples were kept at ambient temperature 25±1°C overnight before the analyses were performed. The origins of each honey sample were confirmed by microscopic pollen analysis (Melissopalynology). Honey samples were classified according to their botanical origin using the method described by Louveaux *et al.* [1978]. 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%).

Physico-chemical analysis

The samples of honey were analysed according to the methods established by AOAC [2012] and International Honey Commission [2009] for moisture content, ash content, electrical conductivity, diastase and invertase activity, reducing sugars, proline content, titrable acidity, pH, total solids and total soluble solids, hydroxymethylfurfural content and water activity. The colour characteristics of the honeys were assessed according to the CIE $L^*a^*b^*$ method described by Saxena *et al.* [2010]. Colour (mmpfund) was determined by the method described by White [1984] while colour intensity (ABS_{450}) was done by the method adopted by Beretta *et al.* [2005]. Mineral elements Cu, Mn, Fe, Zn, Pb and Cd were determined by using air acetylene flame atomic absorption spectrometer (AAS4141). The response from the equipment was periodically checked with known standards. Hollow cathode pump and air-acetylene flame were used for all the samples from three varieties. Calibration curves were constructed for each element by using appropriated standard solutions.

Statistical analysis

The results were expressed in triplicates, mean, standard deviation and correlation was obtained by using Microsoft Excel 2007. The significant differences were obtained by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) ($p<0.05$).

Principal component analysis and linear discriminate analysis were performed to classify honey samples from different botanical sources by using XLSTAT.

RESULTS AND DISCUSSION

The pollen spectra of honey samples studied have been briefly described and the percentages are related to pollen of nectar producing plants. Acacia honey contained 54–60% pollen of *R. 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,* other pollens found were those from *Prunus* sp., *Brassica* sp., *Thyme* sp. and *Ailanthus sp.* The mean (±S.D) results obtained from the physico-chemical, enzymatic and colour characteristics of honey samples are presented in Table 1 while mineral content is shown in Table 2. Moisture content was reported as an important parameter and honey with lower moisture content showed longer shelf life [Fredes & Montenegro, 2006]. In this study all the samples from three varieties showed the level of moisture content (18.2 to 19.11) which was lower than the limit prescribed by Codex standard for honey [Codex Almentarius, 2001] and International Honey Commission [2009] and the results were significantly different ($p<0.05$) for each honey obtained from different sources. Our results were in agreement with previous studies [Yilmaz & Kufrevioglu, 2000; Pan & Ji, 1998; Duman *et al.,* 2008]. The moisture content of honey was affected by various factors such as harvesting time, climatic factors and maturity period [Nanda *et al.,* 2003; Finola *et al.,* 2007]. The °Brix values ranged from 79.16 to 80.03 while the refractive index of the samples ranged from 1.4889 to 1.4908. With the increase in the solid content, there was increase in refractive index. The total solid content of the samples was in a range of 80.89% to 81.8% which were quite similar to the results (77.8–80.4%) reported by Saxena *et al.* [2010].

The pH values of all the honey varieties ranged from 3.52 to 3.78 which confirmed that all the honey varieties were acidic in nature. The pH values were consistent with the results reported by various authors [Azeredo *et al.,* 2003; Ouchemoukh *et al.,* 2007; Saxena *et al.,* 2010; Kamboj *et al.,* 2013]. Results indicated maximum acidity in the form of formic acid in pine honeydew variety (0.39%) followed by acacia honey and multifloral floral varieties in decreasing order with 0.17% and 0.14%, respectively. The titrable acidity and pH of all the samples analysed was found within the corresponding limits as described by Codex Almentarious Commission [2001]. A high strong positive correlation was found between pH and titrable acidity ($r=0.99$).The percentage ash content is an indicator of the mineral content present in a given sample. The maximum ash content was found in pine honeydew (0.39%) followed by acacia honey (0.06%) whereas ash content on multifloral floral honey was at 0.05%. Similar results were reported by Kamboj *et al*. [2013] (0.140–0.211%) and Nanda *et al.* [2009] (0.13 to 0.35%). As per previous studies, electrical conductivity (EC) was used to distinguish honeydew honeys from blossom honey [Mateo & Bosch-Reig, 1998]. The electrical conductivity of all analysed honey samples ranged from 0.25 to 0.79 mS/cm. As per

TABLE 1. Physico-chemical parameters of honey samples with Duncan's multiple range test results.

Parameter	Acacia honey (n=10)	Pine honeydew (n=10)	Multifloral honey (n=10)
Ash (%)	0.06 ± 0.03^{b}	0.35 ± 0.02^{a}	0.05 ± 0.01^{b}
Electrical Conductivity (mS/cm)	0.26 ± 0.05^{b}	0.79 ± 0.06^{a}	0.25 ± 0.04^{b}
Titrable acidity (%)	0.17 ± 0.02^{a}	0.39 ± 0.02^{b}	0.14 ± 0.02^{a}
pH	$3.55\pm0.0.0^{ab}$	3.78 ± 0.11^{a}	3.52 ± 0.02^{b}
Moisture (%)	18.6 ± 0.08^{b}	18.2 ± 0.12^{b}	19.11 ± 0.20^{a}
Total soluble solids (°B)	79.7 ± 0.10^{a}	80.03 ± 0.15^{a}	79.16 ± 0.25^{b}
Total solids (%)	81.40 ± 0.08^{a}	81.8 ± 0.12^{a}	80.89 ± 0.55^{a}
HMF(mg/kg)	5.49 ± 0.07^{b}	6.79 ± 0.10^{b}	22.64 ± 0.30^{a}
Water activity	0.507 ± 0.00^{c}	0.523 ± 0.00^{b}	0.566 ± 0.00^{a}
ABS_{450} (mAU)	188.05 ± 2.30^{b}	525.39 ± 2.98^{a}	148.31 ± 3.07^{c}
Color (mmpfund)	43.32 ± 1.36^{b}	143.04 ± 3.10^{a}	39.16 ± 1.45^{b}
L^*	61.43 ± 1.60^{a}	15.49 ± 0.3^{c}	54.64 ± 0.81^{b}
a^*	4.94 ± 0.08^{a}	2.25 ± 0.08^{c}	3.49 ± 0.29^{b}
b^*	12.32 ± 0.07^{a}	5.28 ± 0.10^{c}	9.39 ± 0.30^{b}
Diastase Number (DN)	15.51 ± 1.40^{b}	25.99 ± 2.79^{a}	14.93 ± 2.10^{b}
Invertase Number (IN)	9.40 ± 1.27^{b}	15.83 ± 2.82^{a}	12.58 ± 1.94^{b}
Proline (mg/kg)	292.02 ± 2.65^{b}	570.95 ± 2.15^{a}	168.05 ± 3.66^{b}
Reducing Sugars (%)	66.24 ± 3.05^{b}	60.6 ± 3.94^{a}	72.81 ± 2.97^{c}

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.

Codex Alimentarius [2001] electrical conductivity value for the nectar honey must be less than 0.80 mS/cm while according to the EU, minimum electrical conductivity for pine honey should be near 0.80 mS/cm. The results of electrical conductivity were consistent with reported results of Escuredo *et al.* [2012] in blossom and honeydew honeys. Positive and strong correlation ($r=0.90$) between ash content and electrical conductivity (Table 3) indicated that higher ash content resulted in higher electrical conductivity.

Tosi *et al.* [2002] reported that hydroxymethylfurfural (HMF) as a quality parameter to check the honey freshness and high temperature processing. All the three honey varieties showed an HMF level lower than the limit (40 mg/kg), recommended by the Codex Alimentarius [Codex Alimentarius, 2001] and statistical analysis revealed a significant difference ($p<0.05$) among the varieties. The maximum content of HMF was found in multifloral source (22.64 mg/kg) followed by pine honeydew (6.79 mg/kg) and minimum in acacia honey (5.49 mg/kg) as shown in Table 1. These results suggested that all the samples from three varieties of honey were raw and unprocessed. The HMF values of the analysed samples were consistent with the values reported by Yilmaz & Kufrevioglu [2000] and Duman *et al.* [2008]. The water activity is an important factor for stability of food by preventing microbial growth. The water activity of the honey samples was in the range of 0.507 to 0.566 and these values were statistically different from each other ($p<0.05$) (Table 1). Our results were quite similar to those of Greek honeys and Indian honeys [Lazaridou *et al.,* 2004; Saxena *et al.,* 2010].

The acceptability of honey by the consumer and its market price mainly depends on colour of honey. According to the classification of honey based on Pfund mm values set by USDA [1985] colour of honey ranges from water white (<8 mm) to dark amber (>114 mm). Based on this classification, the pine honeydew variety (143.04 mm) was classified as dark amber while multifloral honey (39.16 mm) and acacia honey (43.32 mm) as extra light amber. It was reported that colour of honey was affected by chemical composition, primarily due to the presence of pigments such as chlorophylls, carotenoids, flavonoids and polyphenols [Lazaridou *et al.,* 2004; Finola *et al.,* 2007; Juszczak *et al.,* 2009]. The honeys with light colour usually contained low ash content and low antioxidant activity while those with dark colour showed reverse of these values [Gheldof *et al.,* 2002; Marghitas *et al.,* 2009]. There was high positive correlation found between colour and ash content ($r=0.98$) and colour values of all samples from different sources were significantly different ($p<0.05$). Colour intensity (ABS_{450}) is an index for confirming the presence of pigments such as carotenoids and some flavonoids. ABS_{450} values of the tested samples ranged from 148.31 to 525.39 mAU and were statistically different ($P<0.05$) (Table 1). All obtained values were in the range as reported by Beretta *et al.* [2005] in different commercial honey varieties of Italy.

TABLE 2. Statistical analysis of mineral content (mg/kg) of honey from different sources.

Sources	Cu	Mn	Fe	Zn	Pb	Cd
Pine honeydew	0.12 ± 0.01^b	1.08 ± 0.01^a	2.11 ± 0.01^a	1.04 ± 0.02^a	0.21 ± 0.02^a	0.19 ± 0.02^a
Acacia honey	0.12 ± 0.01^b	0.95 ± 0.01^b	1.42 ± 0.03^b	0.06 ± 0.03^b	0.04 ± 0.04^b	0.07 ± 0.02^b
Multifloral honey	0.38 ± 0.02^a	1.01 ± 0.02^a	1.26 ± 0.03^b	0.07 ± 0.03^b	0.09 ± 0.02^b	0.08 ± 0.03^b

Results are expressed as mean values±standard deviations. Means in a column with same superscripts are not significantly different (P<0.05).

TABLE 3. Correlation among some physicochemical parameters and minerals (Pearson correlation coefficients, p<0.05).

	pH	Ash	Color	L^*	a^*	b^*	Cu	Mn	Fe	Zn	Pb	Cd	[a]EC
pH	1.00	–	–	–	–	–	–	–	–	–	–	–	–
Ash	0.99	1.00	–	–	–	–		–	–	–	–	–	–
Color	0.66	0.90	1.00	–	–	–	–	–	–	–	–	–	–
L^*	-0.65	-0.89	-0.98	1.00		–	–	–	–	–	–	–	–
a^*	-0.51	-0.76	-0.81	0.87	1.00	–	–	–	–	–	–	–	–
b^*	-0.54	-0.81	-0.87	0.92	0.95	1.00	–	–	–	–	–	–	–
Cu	0.66	0.78	0.51	-0.80	-0.04	0.08	1.00	–	–	–	–	–	–
Mn	0.59	0.77	0.77	-0.82	-0.89	-0.84	-0.08	1.00	–	–	–	–	–
Fe	0.64	0.90	0.99	-0.95	-0.72	-0.81	-0.62	0.69	1.00	–	–	–	–
Zn	0.64	0.91	1.00	-0.99	-0.83	-0.90	-0.47	0.80	0.98	1.00	–	–	–
Pb	0.64	0.74	0.89	-0.91	-0.85	-0.90	-0.25	0.70	0.84	0.89	1.00	–	–
Cd	0.66	0.70	0.87	-0.87	-0.75	-0.82	-0.32	0.58	0.85	0.86	0.92	1.00	–
[a]EC	0.66	0.90	0.98	-0.97	-0.81	-0.88	0.88	0.79	0.96	0.98	0.88	0.85	1.00

[a]EC= Electrical Conductivity

The L^*, a^*, b^* values obtained for three different varieties of honeys are shown in Table 1. According to L^* values, Gonzalez-Miret *et al.* [2005] classified honey into two groups: light honey with $L^*>50$ and the dark honey with $L^*<50$. On the basis of this classification, the pine honeydew was placed in group of dark category while acacia and multifloral honey in light category. It is evident from the Table 1 that acacia honey (61.43) and multifloral floral (54.64) honeys showed higher L^* values than pine honeydew (15.49). It established that acacia honey and multifloral floral were of light colour while pine honeydew was of dark colour. In all the honey samples from three varieties of honey, a^* values were ranged from 2.25 to 4.94 while b^* values from 5.28 to 12.37. These values were helpful in concluding that all the honey samples possessed both red and yellow components. Similar results were reported for Slovak honeys [Kasperova *et al.,* 2012].

The diastase activity, indicator of high temperature exposure of the analysed samples ranged from 14.93 DN (multifloral honey) to 25.99 DN (pine honeydew) (Table 1). All the honey samples showed the values within the Codex Standard *i.e.* more than 8 DN. Similar results were published by Meda *et al.* [2005] for Burkina Fasan honey, and by Saric *et al.* [2008] for Croatian honeys. The presence of high diastase number in the analysed samples might be due to moderate climate of Kashmir valley. According to the Codex standard, invertase number, indicator of honey freshness, should be more than 4. The invertase number ranged from 9.40 IN (acacia honey) to 15.83 IN (pine honeydew). Thus both IN were >4 and DN>8 which determined that all honey samples were fresh and unprocessed. Our results were consistent with reported results of Hasan *et al.* [2013] for Iraqi honeys and Dinkov *et al.* [2014] for acacia, sunflower and tilia honeys. All the honey samples had a high proline content ranging from 268.05 to 570.95 mg/kg (Table 1) thus could be considered as ripened and unadulterated ones. Similar results were published for Algerian honeys, Malaysian honeys and Indian honeys [Ouchemoukh *et al.,* 2007; Saxena *et al.,* 2010]. The total reducing sugars value in the analysed samples ranged from 60.6 to 72.81% (Table 1), which is in agreement with the standards proposed by EU Directive 2001/110, which should be more than 60% for blossom honeys and at least 45% for honeydew honey. Similar results were reported by Khalil *et al.* [2012] for Algerian blossom honeys.

Mineral analysis

The minerals found in the analysed honey samples are expressed in Table 2. Statistically significant differences (p<0.05) were observed among all analysed samples. Copper (Cu) content was found highest in multifloral honey (0.38 mg/kg) and lowest in both acacia honey and pine honeydew (0.12 mg/kg). The analysed honey varieties showed low concentration of Cu as compared to honey studied by Conti *et al.* [2007], Nanda *et al.* [2009] and Kamboj *et al.* [2013], but similar concentration of copper was found in Brazilian honey [Liberato *et al.,* [2013]. The manganese (Mn) concentration of all analysed samples ranged from 0.95 mg/kg to 1.08 mg/kg with highest concentration in pine honeydew (1.08 mg/kg). Similar findings were reported by Nanda *et al.* [2009]. Among the other minerals, iron (Fe) content was found in higher concentration in all samples with highest value in pine honeydew (2.11 mg/kg) and lowest in multifloral honey (1.26 mg/kg). The concentrations of zinc (Zn), lead (Pb) and cadmium (Cd) were found very low (<1mg/kg) except zinc in pine honeydew (1.04 mg/kg) (Table 2). The results for Mn, Fe, Zn, Pb and Cd were in agreement with those reported by Liberato *et al.* [2013] for Brazilian honey of *Apis mellifera* from different floral origins. The mineral content of the honey depends on geographical area, climatic conditions and soil type of the floral source. It was reported that soil in Kashmir valley of India did not contain high amount of minerals like Cu, Fe, Zn and Co [Bhat *et al.,* 2011; Yatoo *et al.,* 2011] which might be the reason of low level of mineral content in the studied samples of honey varieties.

Strong and positive correlations were found between all minerals and colour (pfund) while strong negative correlations with L^* value, which indicated that honey with dark colour contained high amount of ions (Table 3). All minerals also showed strong positive correlation with electrical conductivity and ash, which demonstrated that higher mineral content, resulted in higher ash content and electrical conductivity. A positive correlation was also found among all minerals, electrical conductivity and pH (Table 3), which showed that both pH and conductivity were dependent on the amount of ions in honey. Similar correlations were observed by Acquarone *et al.* [2006] between total mineral content and electrical conductivity.

Multivariate analysis

In our study, the PCA was applied to achieve a reduction of original data matrix while retaining the maximum amount of variability present in data. The factor loading obtained for the first three components (PCs) and the percentage of variance along with cumulative variance is shown in Table 4. The first three principal components accounted for 86.92% of the variance in the honey samples analysed. The first, second and third principal components (PC1, PC2, and PC3) explained 65.72%, 16.26% and 4.93% of the variance, respectively (Table 4). According to loading matrix (Table 5), it was observed that 65.72% variability explained by PC1 was positively correlated with variables viz. colour intensity, colour (mmpfund), iron, zinc, proline, electrical conductivity and negatively with colour L^*, a^* and b^* values (Figure 1) which could be specified as indicators of colour and minerals. Thus such variables could be used to distinguish pine honeydew from acacia honey. Figure 1 also illustrated that all acacia samples located on the left side of PC1 were linked to L^*, a^* and b^* values while all pine honeydew samples positioned on its upright were linked to minerals and electrical conductivity. The second component (PC2=16.26%) was positively correlated with water activity and HMF, which could be interpreted as an indicator of the maturity of honey. Such variables could be used to distinguish multifloral honey from acacia honey and pine honeydew. The third component with less contribution (PC3=4.93%) was positively correlated with TSS and pH but negatively with reducing sugar. Similar results were reported by Kadar *et al.* [2010] for colour (mmpfund), CIE L^* a^* b^* and conductivity, while Saric *et al.* [2008] published simi-

TABLE 4. Principal component analysis

Total variance explained			
PC	Initial Eigen values	% of variance	Cumulative %
1	15.77	65.72	65.72
2	3.90	16.26	81.98
3	1.18	4.93	86.92

TABLE 5. Principal component analysis. Loading of the first three components.

	Principal components		
Factor loading	1	2	3
pH	0.660	-0.014	0.424
Moisture	-0.657	0.481	-0.163
Total solids	0.657	-0.481	0.163
Color Intensity	0.994	-0.027	-0.048
Total soluble solids	0.333	-0.317	0.700
HMF	-0.479	0.840	0.111
Ash	0.903	0.042	-0.137
Titrable acidity	0.923	-0.082	0.092
Color (mmpfund)	0.993	0.034	-0.043
Water activity	-0.279	0.912	0.192
Color (L^*)	-0.978	-0.159	0.025
Color (a^*)	-0.746	-0.577	0.026
Color (b^*)	-0.872	-0.440	-0.008
Cu	-0.524	0.782	0.104
Mn	0.746	0.490	-0.245
Fe	0.985	-0.102	-0.063
Zn	0.990	0.078	-0.075
Pb	0.901	0.258	0.208
Cd	0.880	0.130	0.250
Diastase number	0.934	0.000	-0.080
Invertase number	0.674	0.435	-0.069
Proline	0.994	-0.002	-0.053
Reducing sugar	0.658	-0.307	-0.420
Electrical conductivity	0.978	0.072	-0.036

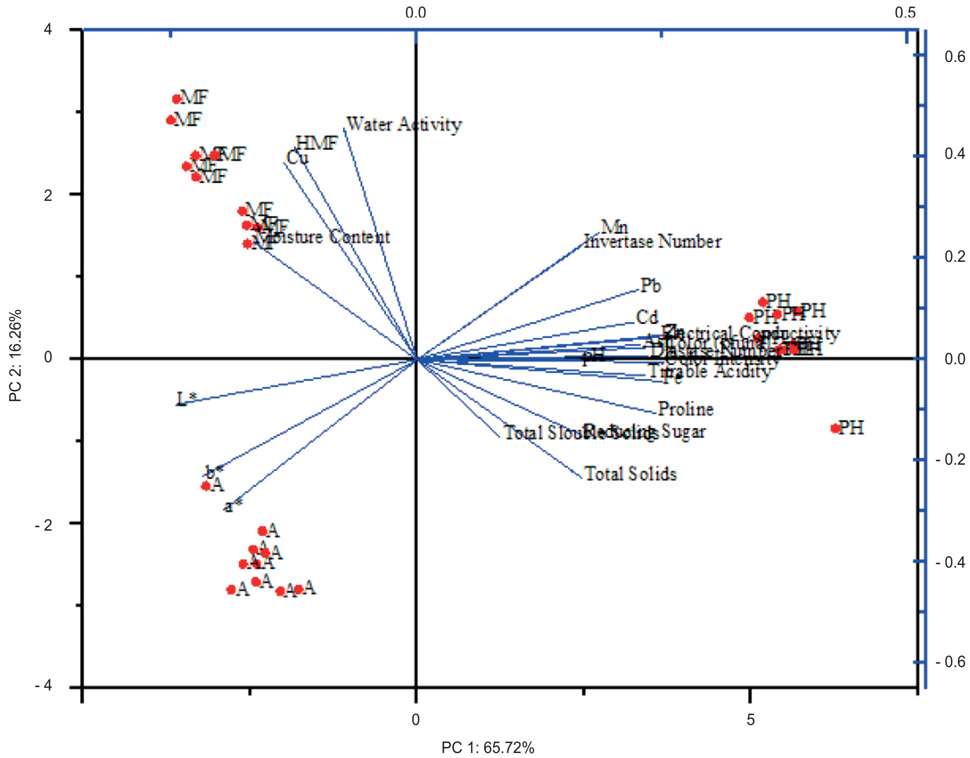

FIGURE 1. Projections of the variables on the factor plane for the three honeys (Botanical origins: A: Acacia, PH: Pine Honeydew, MF: Multifloral).

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	10	0	0	10	100.00
Pine honeydew	0	10	0	10	100.00
Multifloral honey	0	0	10	10	100.00
Total	10	10	10	30	100.00

lar results on Croatian honey varieties from three harvesting seasons in which he concluded that in all seasons PC1 was mainly dominated by conductivity, proline and diastase number. Similar results for HMF were obtained by Isopescu *et al.* [2014] for Romanian honeys. As shown in Figure 1, a natural separation between honeys of different botanical origin was obtained. All the three analysed honey samples from different botanical origins were correctly classified (100%) by using LDA (Table 6).

CONCLUSION

The analysed three honey varieties from Kashmir valley of India revealed that floral origin significantly affects all the physico-chemical parameters, enzymatic properties, mineral content and colour characteristics except total solids. The concentration of minerals found in all three honey varieties was low as compared to other varieties of honey of Indian origin. Application of multivariate techniques confirmed the validity of physico-chemical analysis as a tool for classification and characterization of honey obtained from different botanical sources. PCA revealed 86.92% of the variance with the first three principal components with minerals, colour and electrical conductivity dominating variables. LDA proved to be an effective tool which classified the all honey samples 100% correctly.

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REFERENCES

1. Acquarone C., Buera P., Elizalde B., Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food Chem., 2006, 101, 695–703.

2. Ahmed J., Prabhu S.T., Raghavan G.S.V., Ngadi M., Physicochemical, rheological, calometric and dielectric behavior of selected Indian honey. J. Food. Eng., 2007, 79, 1207–1213.
3. AOAC., Official Method of Analysis of the Association of the Analytical Chemists. 2012.
4. Azeredo L.C., Azeredo M.A.A., Souza S.R., Dutra V.M.L., Protein contents and physicochemical properties in honey samples of Apis mellifera of different floral origins. Food Chem., 2003, 80, 249–254.
5. Beretta G., Granata P., Ferrero M., Orioli M., Facino R.M., Standardization of antioxidant properties of honey by a combination of spectrophotometric/ fluorimetric assays and chemometrics. Anal. Chim. Acta., 2005, 533, 185–191.
6. Bhat M.S., Shaheen M., Zaman R., Muhee A., Mineral Inter-Relationship among soil, forage and dairy cattle in Kashmir India. Vet. World., 2011, 4, 550–553.
7. Blasa M., Candiracci M., Accorsi A., Piacentini M.P., Albertini M.C., Piatti E., Raw Millefiori honey is packed full of antioxidants. Food Chem., 2006, 97, 217–222.
8. Bouseta A., Scheirman V., Collin S., Flavor and free amino acid composition of lavender and eucalyptus honeys. J. Food Sci., 1996, 61, 683–694.
9. Chudzinska M., Baralkiewicz D., Estimation of honey authenticity by multielements characteristics using inductively coupled plasma-mass spectrometry (ICP-MS) combined with chemometrics. Food Chem. Toxicol., 2010, 48, 284–290.
10. Codex Alimentarius Commission., Revised standards for honey. Codex Standard 12–1981. Rev 1 (1987), Rev 2 (2001), Rome: FAO.
11. Conti M.E., Stripekis J., Campanella L., Cucina D., Tudino M.B., Characterization of Italian honeys (Marche Region) on the basis of their mineral content and some typical quality parameters. Chem. Cent. J., 2007, 1, 1–14.
12. Devillers J., Dore J.C., Marenco M., Poirier-Duchene F., Galand N., Viel C., Chemometrical analysis of 18 metallic and non metallic elements found in honeys sold in France. J. Sci. Food. Agric., 2002, 50, 5998–6007.
13. Dinkov D.H., Quality parameters of Bulgarian kinds of bee honey. Mac. Vet. Rev., 2014, 37, 35–41.
14. Duman A., Sezer C., Oral N.B., Kars'ta Satis a Sunulan Suzme Ballarin Kalite Niteliklerinin Aras tirilmasi. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2008, 14, 89–94.
15. Escuredo O., Fernandez-Gonzalez M., Carmen S.M., Differentiation of blossom honey and honeydew honey from Northwest Spain. Agriculture., 2012, 2, 25–37.
16. Finola M.S., Lasagno M.C., Marioli J.M., Microbiological and chemical characterization of honeys from central Argentina. Food Chem., 2007, 100, 1649–1653.
17. Fredes C., Montenegro G., Heavy metal and other trace elements contents in honey bee in Chile. Ciencia e investigacion agrarian., 2006, 33, 50–58.
18. Gheldof N., Wang X.H., Engeseth N.J., Identification and quantification of antioxidant components of honeys from various floral sources. J. Agric. Food Chem., 2002, 50, 5870–5877.
19. Gonzalez-Miret M.L., Terrab A., Hernanz D., Fernandez-Recamales M.A., Heredia F.J., Multivariate correlation between colour and mineral composition of honeys and by their botanical origin. J. Agric. Food Chem., 2005, 53, 2575–2580.
20. Hasan S.H., Effect of storage and processing temperature on honey quality. J. Babylon Univ. Pure Appl. Sci., 2013, 21, 2244––2253.
21. International Honey Commission., Harmonized methods of the International Honey Commission. 2009. URL: http://www.bee-hexagon.net/en/network.htm. Accessed 14–02–2013.
22. Isopescu R.D., Josceanu A.M., Minca I., Colta T., Postelnicescu P., Mateescu C., Characterization of Romanian honey based on physicochemical properties and multivariate analysis. Revista de Chimie –Bucharest., 2014, 65, 381–385.
23. Juszczak L., Socha R., Roznowski J., Fortuna T., Nalepka K., Physicochemical properties and quality parameters of herb-honeys. Food Chem., 2009, 113, 538–542.
24. Kadar M., Juan-Borras M., Hellebrandova M., Domenech E., Escriche I., Differentiation of Acacia, Sunflower and Tilia honeys from different countries based on sugar composition, physicochemical and colour parameters. Bulletin USAMV Agriculture., 2010, 67, 252–258.
25. Kamboj R., Bera B.M., Nanda V., Evaluation of physico-chemical properties, trace metal content and antioxidant activity of Indian honeys. Inter. J. Food Sci. Technol., 2013, 48, 578–587.
26. Karabagias I.K., Badeka A., Kontakos S., Karabournioti S., Kontominas M.G., Characterization and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. Food Chem., 2014, 146, 548–557.
27. Kasperova J., Nagy J., Popelka P., Dicakova Z., Nagyova, A., Mala P., Physico-chemical indicators and identification of selected Slovak honeys based on colour measurement. Acta. Vet. Scand., 2012, 81, 57–61.
28. Khalil M.I., Moniruzzaman M., Boukraa L., Benhanifia M., Islam M.A., Islam M.N., Sulaiman S.A., Gan S.H., Physicochemical and antioxidant properties of Algerian honey. Molecules, 2012, 17, 11199–11215.
29. Lazaridou A., Biliaderis C.G., Bacandritsos N., Sabatini A.G., Composition, thermal and rheological behaviour of selected Greek honeys. J. Food Eng., 2004, 64, 9–21.
30. Liberato M.T.C., Morais S.M., Magalhaes C.E.C., Magalhaes I.L., Cavalcanti D.B., Silva M.M.O., Physico-chemical properties, mineral, and protein content of honey samples from Ceara state, Northeastern Brazil. Food Sci. Technol. (Campas), 2013, 33, 38–46.
31. Louveaux J., Maurizio A., Vorwohl G., Methods of melissopalynology. Bee World, 1978, 59, 139–157.
32. Marghitas L.A., Dezmirean D., Adela M., Otilia B., Laura L., Stefan B., Physicochemical and bioactive properties of different floral origin honeys from Romania. Food Chem., 2009, 112, 863–867.
33. Mateo R., Bosch-Reig F., Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, colour, water content, sugars, and pH. J. Agric. Food Chem., 1998, 46, 393–400.
34. Meda A., Lamien C.E., Romito M., Millogo J., Nacoulma O.G., Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem., 2005, 91, 571–577.
35. Nanda V., Bera M.B., Bakhshi A.K., Optimization of process parameters to establish the quality attributes of hydroxymethylfurfural content and diastatic activity of sunflower (*Helianthus*

annus) honey using response surface methodology. Eur. Food Res. Technol., 2006, 222, 64–70.
36. Nanda V., Sarkar B., Sharma H., Bawa A., Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. J. Food Comp. Anal., 2003, 16, 613–619.
37. Nanda V., Singh B., Kukerja V.K., Bawa A.S., Characterization of honey produced from different fruit plants of northern India. Inter. J. Food Sci. Technol., 2009, 44, 2629–2636.
38. Nayik G.A., Nanda V., Characterization of the volatile profile of unifloral honey from Kashmir valley of India by using solid-phase microextraction and gas chromatography–mass spectrometry. Eur. Food Res. Technol., 2015, In Press, Accepted Manuscript, DOI: 10.1007/s00217–015–2413–2.
39. Ouchemoukh S., Louaileche H., Schweitzer P., Physico-chemical characteristics and pollen spectrum of some Algerian honeys. Food Contr., 2007, 18, 52–58.
40. Pan J.Z., Ji C.Y., General rheological model for natural honeys in China. J. Food Eng., 1998, 36, 165–168.
41. Press Information Bureau, Government of India. URL: http://pib.nic.in/newsite/erelease.aspx?relid=96239. Accessed 23–12–2013.
42. Saric G., Matkovic D., Hruskar M., Vahcic N., Characterization and classification of Croatian honey by physicochemical parameters. Food Technol. Biotechnol., 2008, 46, 355–367.
43. Saxena S., Gautam S., Sharma A., Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem., 2010, 118, 391–397.
44. Soria A.C., Gonzalez M., De Lorenzo C., Martinez-Castro I., Sanz J., Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. Food Chem., 2004, 85, 121–130.
45. Terrab A., Escudero M.L., Gonzalez-Miret M.L., Heredia F.J., Colour characteristics of honeys as induced by pollen grain content: A multivariate study. J. Sci. Food Agric., 2004, 84, 380–396
46. Terrab A., Valdes B., Diez D.M.J., Pollen analysis of honey from the Gharb region (NW Morocco). Grana., 2001, 40, 210–216.
47. Tosi E., Ciappini M., Re E., Lucero H., Honey thermal treatment effects on hydroxymethylfurfural content. Food Chem., 2002, 77, 71–74.
48. USDA: United States Standards for Grades of Extracted Honey., USDA. Washington DC Agricultural Marketing Service, 1985.
49. White J.W., Instrumental colour classification of honey: Collaborative study. J. AOAC, 1984, 67, 1129–1131.
50. Yatoo M.I., Devi S., Kumar P., Tiwari R., Sharma M.C., Soil plant- animal micro mineral status and their interrelation in Kashmir valley. Indian J. Anim. Sci., 2011, 81, 628–630.
51. Yilmaz H., Kufrevioglu I., Composition of honeys from eastern and southern Anatolia and effect of storage on hydroxymethylfurfural content and diastase activity. Turk. J. Agric. For., 2000, 25, 347–349.

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