

Characterization of honeys by their botanical and geographical origins based on physico-chemical properties and chemo-metrics analysis

Nuru Adgaba¹ · Ahmed A. Al-Ghamdi¹ · Awraris Getachew¹ · Yilma Tadesse¹ · Abera Belay³ · Mohammed J. Ansari¹ · Sarah E. Radloff² · Deepak Sharma¹

Received: 31 October 2016 / Accepted: 6 February 2017 / Published online: 7 March 2017
© Springer Science+Business Media New York 2017

Abstract Honey, because of its nutritional and medicinal values, is in high demand and has become one of the important commodities. However, the issue of its quality and authenticity remain as important factors in consumption and marketing of honey. To assess the possibility of discriminating honeys by their geographical and botanical origins; 30 fresh honey samples of different botanical and geographical origins were collected and their major physico-chemical properties such as: total dissolved sugar (TDS), total ash, sugar profile, acidity, metallic ions and

electric conductivity (EC) were investigated. The data was subjected to different chemo-metric (Hierarchical Cluster, Principal components and stepwise discriminant) analysis. Among the 23 characters used in the analysis; only 11 (TDS, EC, acidity, total ash, colour, and some specific metallic ions) characters have showed significant variations among different origin honeys. According to the stepwise discriminant analysis; 11 variables confirmed the grouping of the honey samples into four cluster groups based on their botanical and geographical origins. The clustering of the honeys associated with dominant plant source & climatic conditions of their origins. The study generally revealed the successful discrimination of honeys into their botanical and geographical provenances using fewer physico-chemical characters supported with melissopalynological data through applying suitable chemo-metric analysis.

✉ Nuru Adgaba
nuruadgaba@gmail.com

Ahmed A. Al-Ghamdi
aalkhazim1@gmail.com

Awraris Getachew
awrariskiy@gmail.com

Yilma Tadesse
yiltadesse@gmail.com

Abera Belay
ab.berabelay@gmail.com

Mohammed J. Ansari
mjavedansari@gmail.com

Sarah E. Radloff
s.radloff@ru.ac.za

Deepak Sharma
deepak.sharmatinoo@gmail.com

Keywords Honey characterization · Authenticity · Chemo-metric · Physico-chemical properties · Botanical origins · Ethiopia

Introduction

The nutritional and therapeutic values of honey are very high [1]. As a result honey becomes a highly demanded product for both domestic consumptions and export markets. Now a day honey is one of the important world commodities and large volume of honey is traded annually. However, the issue of its quality and authenticity of its geographical and botanical origins remain as important factors in the marketing and consumption of honey [2]. The composition of honey largely depends on the types of source vegetation, environmental factors, processing and storage conditions [2]. Depending on the types and amount

¹ Bagshan Chair for Bee Research, Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

² Department of Statistics, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa

³ Center for Food Science and Nutrition Program, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia

of substances found in it, every honey is unique by its character.

Honey because of its relatively high prices, it has been targeted to be adulterated with other low prices products [3]. Moreover, due to the preferences of consumers towards some mono-floral honeys or honey's of specific geographical region, such honeys may fetch high prices and their demands are high [3, 4]. As a result, mislabeling of botanical and geographical sources of honey is very common [5]. Authentication of honey by its natural physico-chemical properties, botanical source and geographical origin is important to protect genuine honeys from fraud products and to create consumers trusts [6].

Different approaches have been used to characterize honeys of specific floral and geographical origins, however, so far there is no one universal approach that unequivocally discriminate one honey from the others. So multiple approaches, that complement each other would be more reliable to characterize honeys of different botanical and geographical origins.

Physico-chemical properties of honey such as sugar profile, water content and others have been widely used to characterize honeys of different botanical and geographical regions [6, 7]. However, these major constituents (sugars and water) are shared by all honeys and their discriminatory role is relatively low [2, 4]. The minor constituents such as minerals can be varied depending on the botanical and geographical origins of honeys [2]. Moreover, metallic ions are reported to be consistent with environmental conditions [8]. In this regard, many studies indicated the presence of strong correlation between physical properties and mineral compositions of honeys [8, 9]. Hence analysis of some minor constituents such as metallic ions have been successfully used to characterize honeys of different origins [2].

Generally, different physico-chemical properties of honey such as sugar profiles, water content, acidity, EC, HMF, enzymes, color, amino acids and total ash and mineral profiles have been widely used to authenticate the botanical and geographical origins of honey in different parts of the world [2, 6, 10–13]. Recently more than 600 volatile compounds from different chemical families that originated from various biosynthetic pathways have been identified in different honeys [14]. The volatile compounds in general and phenolics in particular have been widely used as potential finger prints to authenticate the botanical and geographical origins of honeys of different regions [15–19].

Using phenolic profiles as possible markers, TOMÁS-BARBERÁN, et al. [20] tried to authenticate the botanical origins 52 unifloral honeys from different regions of Europe. Moreover, different volatile compounds: flavonoids and phenolic and abscisic acids have been used to authenticate the botanical and geographical origins of

Australian and New Zealand's honeys [21–23]. Moreover, recently the presence of variations in rheological properties among different unifloral honeys and its potential in discriminating the floral origins of honey well demonstrated [24].

Despite the various novel and advanced instrumental methods (GC-MS, EC-MS, NMR and NIR) of detection of adulteration, classification and authentication of honeys; applying of different multivariate analysis/chemometric techniques also proved to be extremely useful in authenticating the botanical and geographical origins of honeys [25–28]. Moreover, many researchers have used melissopalynology techniques, to authenticate the botanical and geographical origins of honeys [29, 30]. Pollen analysis along with other techniques, have been suggested as important means for determination of the botanical origin of honey [7].

Characterization of honeys following a combination of approaches, have been largely used to discriminate honeys of different botanical and geographical origins [10, 30]. Combining the palynological data with physico-chemical properties [31, 32] mineral profile and physical properties with multivariate analysis [33] and multi-elemental analysis with the support of multivariate analysis [29] have been used to successfully authenticate the botanical and geographical origins of honeys. Moreover, different analytical results of honey, have been subjected to chemometric analysis techniques using different statistical analysis and these have been used to identify honey characters with the most discriminatory power that allow the accurate classification of honey samples according to their botanical and geographical origins [2, 10].

Generally, BOGDANOV, et al. [34] suggested that currently a reliable determination of the botanical and geographical origins can be achieved only by a global interpretation of sensory, pollen and physico-chemical analyses carried out by experts.

In Ethiopia beekeeping is widely practiced throughout the country by about one million farmer households and large volume of honey is produced annually [39]. The type of honey production system is dominantly traditional. Due to the presence very diverse ecologies and over 7000 flowering plant species, several and distinctive monofloral and multi-floral honeys are produced in different seasons and regions. However, so far the properties of the honeys are not yet characterised and documented. With this general background the aim of the current study was to assess, the possibilities of characterization of some major Ethiopian honeys by their botanical and geographical origins based on their physico-chemical properties and metallic ion contents using chemo-metric analysis.

Materials and methods

Sample collection and analysis

Thirty fresh honey samples of different botanical sources and geographical regions of the country were collected from farm gates following their harvested seasons. The samples were kept in air tight glass containers and in refrigerator at 4–5 °C until required for analysis.

Physico-chemical analysis

The major physico-chemical properties such as: total dissolved substances (TDS), total ash (mineral) content, sugar profile, acidity and electric conductivity were investigated following the standard protocols [35].

Sugar profile analysis

The major honey sugars (fructose, glucose and sucrose) amounts were determined using high pressure liquid chromatography (HPLC) with RI-detector (Perkin Elmer 200 Series). Before the analysis, suitability of the methods, validation parameters (limit of determination, limit of quantification, linearity and recovery) were conducted as regular procedures for conformity of the test. Accordingly, the HPLC with RI detection proved to be very responsive as reflected from the values of average recovery (96–98%), limits of detection (0.05–0.18/100 g) and limits of quantification (0.07–0.24/100 g) with linearity response index of between 0.97 and 1.03. The testing conditions were: flow rate: 1.3 ml/min, mobile phase: Acetonitrile: water (80:20, v/v), column and detector temperature: 30 °C, sample volume: 10 µl. For each test 5 g of honey sample was dissolved in 40 ml water and 25 ml of methanol and added into a 100 ml volumetric flask and the honey solution was transferred to the flask and made to the mark with water. Quantitation was performed according to the external standard method on peak areas or heights following harmonized methods of the International Honey Commission protocols [36]. The honey sugars were identified and quantified through comparison of the retention times and the peak area of the honey sugars with those of the standard sugars. Pure standard sugars (fructose, glucose, sucrose) were purchased from Sigma chemicals (Germany). The mass percentage of the sugars, (W), to be determined in g/100 g was calculated following the above mentioned protocol.

Other properties

The ash (total mineral) content, electric conductivity (EC) and acidity of the honey samples were determined following standard protocols [35].

Total dissolved substance (TDS)

The TDS was obtained through measuring the total dissolving sugar content of the honey samples using a refractometer (Atago, No. 3840, Japan). For this a few drops of honey were placed on the refractometer prism and a reading was taken directly from the scale. The readings were further corrected for a standard temperature of 20 °C by adjusting the correction factor of 0.00023/°C.

Color

The colour of the honey samples was measured by digital honey color analysis from 0 to 150 mm P-fund using Hanna HI96785 Honey Color Portable Photometer. The determination was made while the samples were liquid and fresh.

Metallic ion type and amount determination

The mineral types and amount found in the honey samples was determined using Perkin Elmer analyst 700 atomic absorption spectrometer (FAAS) equipped with HGA graphite furnace and a deuterium lamp background corrector. For graphite furnace measurement argon was used as inert gas. For digestion of the samples, microwave digestion system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. The microwave digestion of the honey samples and analytical procedures were done following TUZEN & SOYLK [37] protocols.

Melissopalynological analysis

Besides the collection of the honey samples following the harvesting seasons of particular honey samples at farm gates; their botanical origins were confirmed through the analysis of major pollen grains following [38] protocols. The identification of the pollen grains was made based on long period collection of pollen reference slides of the study regions which is available as published material [39].

Statistical analysis

In addition to the univariate statistical analysis of each parameter; the pool analytical results of the honey samples have been subjected to multivariate chemometric analysis. Different multivariate statistical analysis (principal component analysis, cluster analysis, discriminant analysis and logistic regression and others) have been used to identify honey characters with the most discriminatory power that allows the accurate classification of honey samples according to their origins [2, 6].

In this study, the analytical results of 23 major honey characters (sugars profiles, total ash contents, metallic ions,

color, acidity, electric conductivity, and total dissolved substance) data have been subjected to chemometric processing techniques using different statistical analyses.

To determine the mean, range and standard deviations of each variable and also to detect extreme values; first a descriptive statistical analysis was carried out. Along with this, the normality of the data was also verified using Kolmogorov–Smirnov test. Then analysis of variance (one way ANOVA) was made to detect the presence of significant variations in the means of the variables depending on the origins of the honeys. To characterize the honeys by their specific botanical and geographical regions; the data was subjected to multivariate analysis. The multivariate analysis used includes principal component analysis, hierarchical cluster analysis and linear discriminant analysis.

Hierarchical cluster analysis using the Ward’s minimum variance linkage with squared Euclidian distance was used to detect the clustering of the sample data. Principal Components Analysis was also used to detect if any clustering exists within the sample data. Then stepwise discriminant analysis was employed to confirm the separation of these clusters, to determine the most discriminatory variables and to calculate the percentages of correctly classified honeys. This procedure may provide an overly optimistic estimate of the probability of correct classification. A jackknife procedure was therefore carried out that classified each sample into a group with the highest *a posteriori* probability according to the discrimination functions computed from all the data except the honey samples being classified. Finally, Wilk’s lambda test was used to compare multivariate cluster group means among the different botanical and geographical cluster groups. All statistical analyses were performed using the SPSS software program version 23.

Results

Univariate analysis

All the variables passed tests of normality Kolmogorov–Smirnov test: $p > 0.20$; for Mg, Cu and Rb (Kolmogorov–Smirnov test: $p > 0.06$). The one-way ANOVA considering the geographical factor effect showed significant differences for parameters such as: TDS, EC, Acidity, Ash, Color, Na, Mg, K, Ca, Mn and Zn ($p < 0.05$; Table 1).

Honey sugar profile

Based on HPLC sugar profile analysis of the honey samples; the glucose amount was ranging from 22.28 to 34.21/100 g with a mean of $27.68 \pm 3.32/100$ g while the fructose amount was ranging from 28.06 to 48.30/100 g with a mean of $36.60 \pm 4.94/100$ g. The average glucose plus fructose amount of the tested honey samples was 64.28% by weight and the values were within the Codex Alimentarius Commission, [40] limits. In most of the tested honey samples; the fructose amount was higher than the glucose as in (Fig. 1a); however in some honey samples such as from *Becium grandiflorum* the glucose amount was higher than the fructose (Fig. 1b). The sucrose amount of honey samples was ranging from 0.12 to 7.69/100 g with mean of $3.76 \pm 1.77/100$ g.

Total dissolved substance

The total dissolved substances of the honey samples were ranging from 75.60 to 85.00% with mean of $80.94 \pm 2.63\%$.

Table 1 Descriptive statistics values of some major properties of honey samples from four geographical origin

Variables	Northern (n=7) Mean & SD	Central (n=11) Mean & SD	Southern highland (n=4) Mean & SD	South Western (n=8) Mean & SD
TDS%	84.00 ± 0.81 ^a	82.00 ± 0.35 ^b	79.50 ± 0.79 ^c	77.51 ± 1.4 ^d
EC mS/Cm	0.31 ± 0.18 ^a	0.56 ± 0.17 ^{ab}	0.33 ± 0.15 ^{ab}	0.65 ± 0.28 ^b
Acidity*	12.4 ± 3.5 ^a	30.1 ± 3.2 ^b	19.9 ± 1.9 ^c	38.7 ± 4.1 ^d
Ash%	0.38 ± 0.02 ^a	0.48 ± 0.03 ^b	0.16 ± 0.02 ^c	0.29 ± 0.02 ^d
Color mm	6.14 ± 1.35 ^a	49.81 ± 3.22 ^b	16.50 ± 2.38 ^c	88.75 ± 7.44 ^d
Na	493.3 ± 21.4 ^a	590.6 ± 19.9 ^b	297.9 ± 46.78 ^c	405.0 ± 33.12 ^d
Mg	1.51 ± 0.46 ^a	3.15 ± 0.35 ^b	2.93 ± 0.15 ^b	3.37 ± 0.38 ^b
K	513.7 ± 43.5 ^a	661.75 ± 39.47 ^b	267.8 ± 47.23 ^c	379.5 ± 44.62 ^d
Ca	8.14 ± 1.55 ^a	16.35 ± 6.31 ^b	13.32 ± 2.14 ^{ab}	18.52 ± 4.84 ^b
Mn	0.06 ± 0.06 ^a	0.14 ± 0.03 ^b	0.10 ± 0.06 ^{ab}	0.14 ± 0.04 ^b
Zn	0.157 ± 0.132 ^a	0.289 ± 0.076 ^b	0.234 ± 0.112 ^{ab}	0.281 ± 0.079 ^{ab}

The values for metallic ion are in mg/kg of honey

Different letter in a row indicates a significant difference ($p < 0.05$)

± is standard deviation

*acidity is as meq.acid/kg of honey

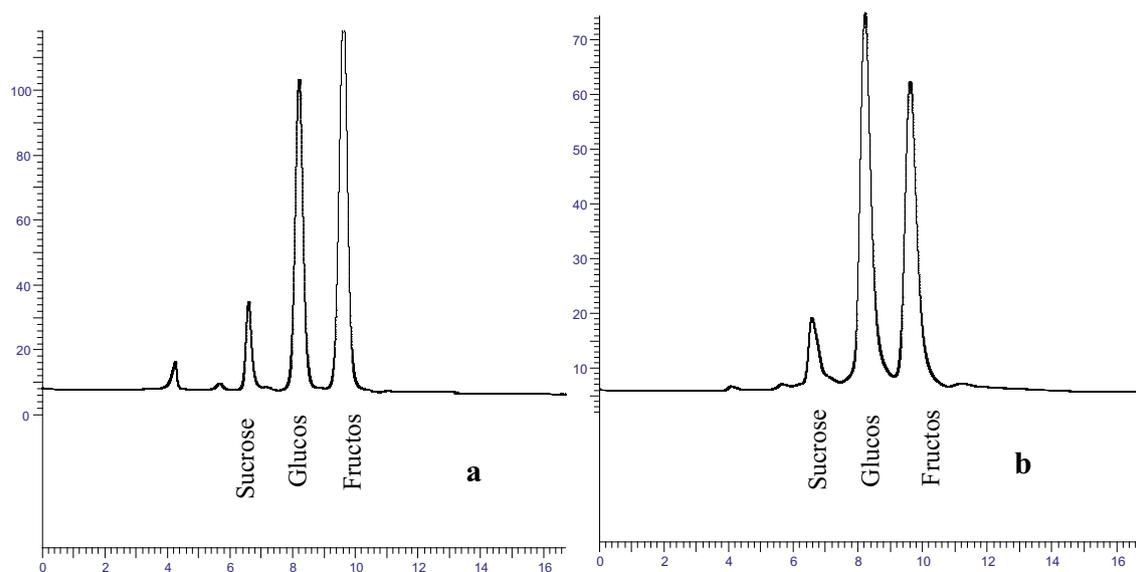


Fig. 1 Sugar profiles of some honey samples: **a** *Croton macrostachyus* and **b** *Becium grandiflorum*

Electric conductivity

The EC values were varied from 0.173 to 1.134 mS/cm with mean of 0.493 ± 0.24 mS/cm. Relatively strong correlation ($r=0.60$, $p=001$) was observed between EC and total ash contents of honeys, in which honey with more mineral content showed higher conductivity. Moreover, 82.85% of the honey samples EC values were between the range of ≥ 0.2 mS/cm and ≤ 0.8 mS/cm indicating the large majority of the samples were within the acceptable standard ranges.

Acidity

The acidity of tested honey samples varied from 7.50 to 43.50 mEq. acid/kg with mean of 26.93 ± 10.52 mEq. acid/kg of honey.

Color

Based on the honey color measurements, the colors of the honey samples were varying from 4.0 to 100 mm with mean of 45.57 ± 32.11 mm.

Total mineral (ash) content

The total ash (mineral) percentage of honey samples varied from 0.14 to 0.55% with mean of $0.36\% \pm 0.11$. The

obtained values of all the tested samples were within mineral content standards limits [40].

Metallic ion types and amount

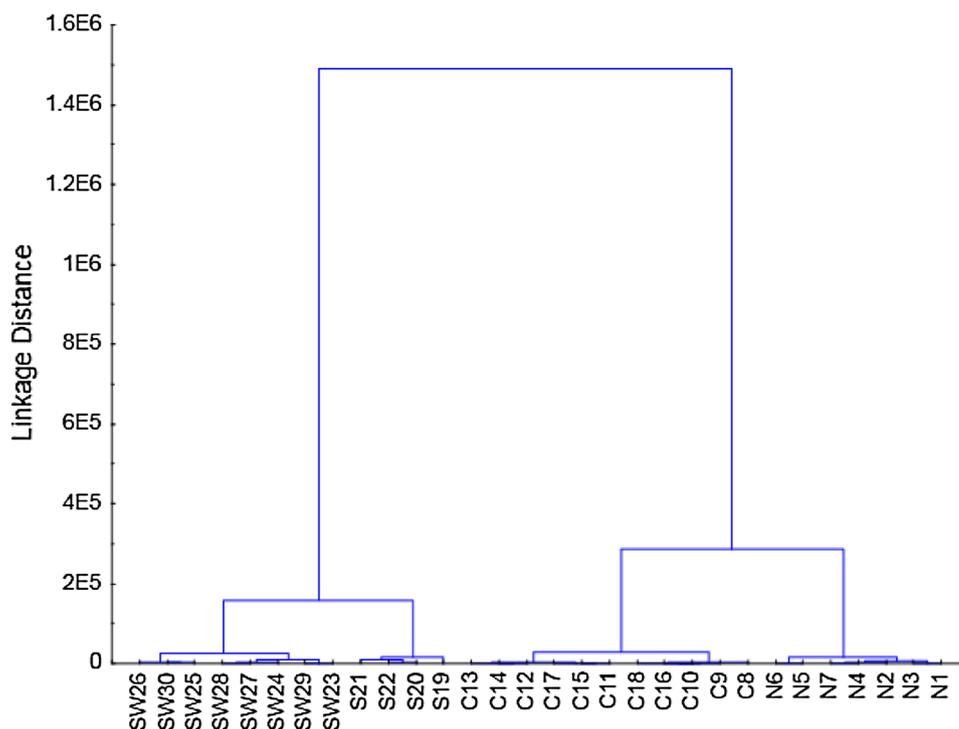
In all 30 honey samples tested; 15 different types of minerals (metallic ions) (Na, Mg, Al, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Ba, Pb) were identified and quantified. However, the quantities recorded varied from mineral to mineral and also sample to sample. A minimum value of 0.001 mg/kg and a maximum of 710.00 mg/kg were recorded for Pb and K respectively. Generally, relatively high amount of minerals were recorded for K, Na, Ca and Al with average amount of 499.44, 479.38, 15.01 and 3.81 mg/kg of honey respectively. The lowest average amount of minerals obtained were Co and Pb with mean of 0.004 and 0.005 mg/kg of honey respectively.

Multivariate analysis

Hierarchical cluster analysis

According to the hierarchical clustering analysis, four phenetically separate cluster groups of honeys were recognized. The first hierarchical clustering linked honey samples from the northern region (cluster-1), the central region (cluster-2), the southwestern highland region (cluster-3) and the south western midland region (cluster-4). The second hierarchical clustering linked clusters (1) and (2) (northern and central regions), and clusters (3) and (4) (southwestern highland and south western

Fig. 2 Hierarchical clustering dendrogram for sampled honeys, derived from Ward's method and squared Euclidean distance linkage based on honey characters SW southwestern; S southern highland; C central plus northeastern regions and N northern parts of the country



midland regions); finally the northern and central regions clusters linked with southern highland and south western midland regions clusters (Fig. 2). These results showed the clustering of the Ethiopian honey samples were according to their geographical and botanical origins.

Principal components analysis

Based on the principal component (PC) analysis; three components that account for 68.5% of the variance in the data were obtained. PC 1, includes: TDS, EC, Acidity, Color, Mg, Al, Ca, Cr, Mn, Co, Ni, Cu, Zn, Sr, Ba and Pb, accounted for 41.7% of the total variance; PC 2, TDS, Ash, Na and K, accounted 15.9% and PC 3, Mg and Rb, accounted 10.9%. Out of the 23 variables used in PC analysis the loading values of the three sugars (sucrose, fructose and glucose) were relatively low. The graph of the two component scores from PC 1 and 2 (Fig. 3) showed the formation of two clusters: honey samples from northern and central regions forming a cluster (1 and 2) in the top half of the plot and honey samples from southern highland and south western midland regions forming a cluster (3 and 4) in the bottom half of the plot. These PC analysis results confirmed the clustering of the honey samples obtained using cluster analysis.

Stepwise discriminant analysis

Out of the 23 characters used in the analysis; only 11 of them have showed significant variations among the different geographical and botanical origin honeys. The characters include: TDS, EC, acidity, total ash, color, and some specific metallic ions (Na, Mg, K, Ca, Mn & Zn). The mean, standard deviation, minimum and maximum values of characters in cluster groups are shown in (Table 1). Moreover, the correlations among the 11 variables were also significant at $p < 0.05$ ($N = 30$).

According to the stepwise discriminant analysis; these 11 variables also confirmed the grouping of the honey samples in to four distinct cluster groups based on their botanical and geographical origins (Fig. 4). The canonical root scores plot for roots 1 and 2, together with the 95% confidence ellipses showed that the groups are distinct. The linear discriminant functions obtained correctly classified 100% of the samples in each group. The jackknife procedure gave the same classification results. A significant difference was also found between the group means (Wilks $\Lambda = 0.001$, approximate $F = 49.1$ with 33, 47 df , $p < 0.001$).

According to the stepwise discriminant analysis; seven honey samples from northern Ethiopia (Tigray and Waghumra) form one cluster (group 1). According to the honey pollen analysis; the honey samples in the cluster

Fig. 3 Principal component scores plot for PC 1 and PC 2
Legend: 1 northern; 2 central;
3 southern highland; 4 south
western midland

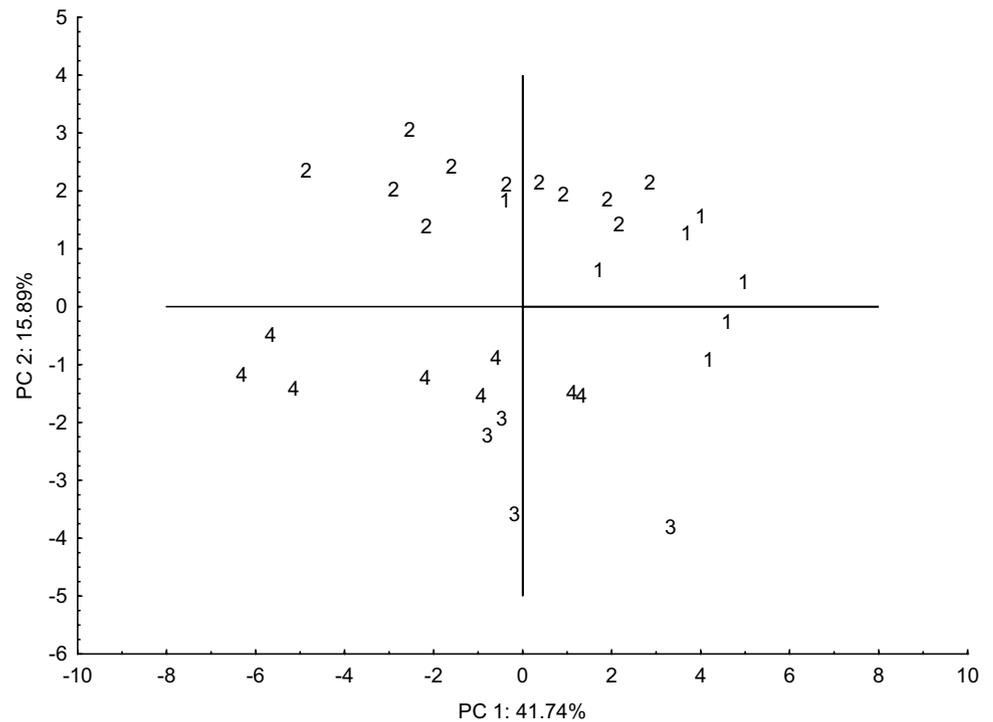
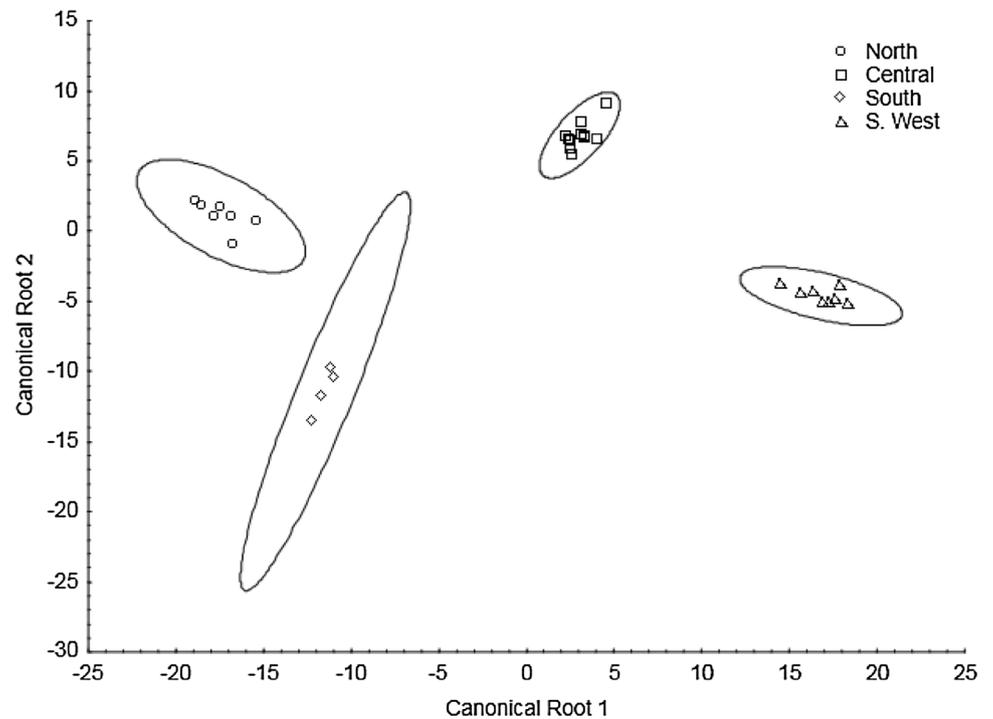


Fig. 4 Canonical root scores plot for root 1 and root 2, with 95% confidence ellipses

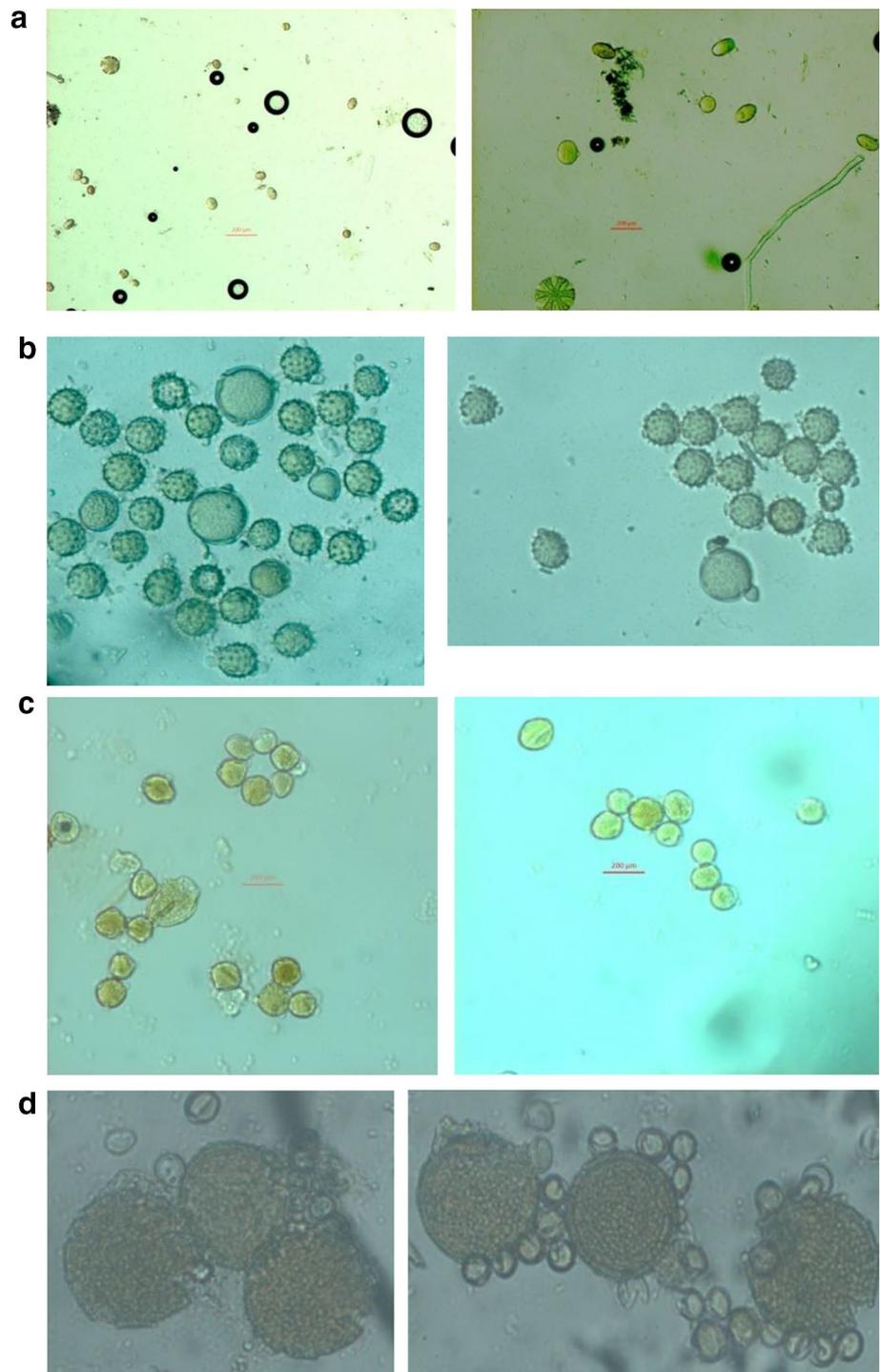


group 1 are dominantly from *Hypoestes forskoolii* (Vahl) R. Br. (Plate 1a) and *Becium grandiflorum* (Lam.) Pic. Serm. *H. forskoolii* averagely accounts for more 85.2% of the pollen grains analyzed in the samples. The honeys in this cluster group are extra white with average colour value of 6.14 ± 1.35 mm and with lowest average values

of acidity (12.42 ± 3.48 mEq. acid/kg) and with the highest total dissolved substances ($84 \pm 0.81\%$). The extra white color of the honey could be due to its botanical origin.

Eleven honey samples from central part of the country (Shoa) and northwestern Ethiopia (Gojam) form another cluster (group 2). According to the honey pollen analysis

Plate 1 Pollen grains of honey samples of different botanical and geographical origins **a** Pollen grains of honey samples from group 1 with dominant pollen grains of *Hypoestes forskalli* (pollen grains with oval shapes). **b** Pollen grains of honey samples from group 2 which is dominated by *Guizotia scabra* pollen grains with spines. **c** Pollen grains of honey samples from group 3 dominated with *Schefflera abyssinica* pollen grains. **d** Pollen grains of honey samples from cluster group 4 with *Croton macrostachys* (bigger size) & *Aningeria* sp. pollen grains (smaller size)



of the honey samples under this cluster (group 2) are multi-floral sources but dominantly from *Guizotia scabra* (Vis.) Chiov. (Plate 1b) and some cultivated pulses. The pollen from *Guizotia scabra* accounts for about 65.9% pollen grains of analyzed samples. *G. scabra* is widely growing in most highlands of Ethiopia both in cultivated lands and fallow fields as weeds. The dominant color of this honey is dark amber with average color value of 49.82 ± 3.22 mm,

with relatively high acidity of 30.13 ± 3.24 mEq. acid/kg, with mean mineral content of $0.48 \pm 0.03\%$ and average electric conductivity value of 0.56 ± 0.17 mS/cm.

Four honey samples from southwest parts of the country (Mash and Kaffa) and southeast area (Bure) humid highland forests formed the third cluster (group 3). The honey samples in cluster 3 are mono-floral type dominantly from *Schefflera abyssinica* (A. Rich) Harms (Plate

1c). *S. abyssinica* pollen grains averagely accounts for 75.6% the pollen grains of the honey samples. The honeys are lighter in color with 16.5 mm; with moderate acidity of 19.9 mEq. acid/kg and relatively low TDS (79.5%) and the lowest ash content. The remaining eight honey samples from southwest forest midlands form the fourth cluster (group 4). Honeys in this cluster group 4, are also multi-floral sources dominantly from tree species such as *Croton machrostachys* and *Aningeria spp.* (Plate 1d). The honeys are characterized with the lowest TDS (77.5%), high acidity (38.7 mEq. acid/kg), high EC (0.645mS/cm) and darkest in color (88.75 mm).

Discussion

The glucose and fructose values recorded in this study are more or less closer to values of [41] who reported 35.99–42.57 and 24.63–35.06% by weight, for fructose and glucose respectively in Algerian honeys. It is also closer to fructose and glucose values of 35.99–42.57 and 24.63–35.06%, respectively reported for Lithuania honeys [42]. Generally the mean glucose and total invert sugars values of honey samples recorded in this study were slightly lower than other study reports; however it was closer to study result [10] who reported a glucose value of 21.5–34.7% by weight with mean of 29.4 ± 3.7 for northern region of Argentina. Similarly TADESSE and GEBREGI-ZIABHER [43] recorded low level of invert sugars (<60%) for some honey samples collected from Adigrat area, northern Ethiopia. However, the current study results for glucose and invert sugars values were within the acceptable standard ranges of 23–32% (by weight) for glucose and >60/100 g for that of invert sugars [18]. In some monofloral honey samples such as *Bacium glandifolia* the glucose values were greater than the fructose values. More glucose values than fructose were reported in many honey samples from Lithuania [42]. Honeys with high glucose values were extra white which may be related to the physical properties of glucose which is white in color upon its crystallization and tends to granulate faster than fructose. The association of white color with the crystallization of the glucose in honey was well documented [44].

The total TDS of honey samples were ranging from 75.6 to 85.0% with mean of 80.94%. The majority of the TDS values obtained are closer to values recorded for Argentina [10], Portugal [45] and Cameroon honeys [46]. Generally, 74.28% of the honey samples had more than 80% TDS and 88.57% of the honey samples were within acceptable standard limits [18]. The TDS of honey is an important indicator of the degree of maturation (ripening) of a honey which is important for its long shelf life span. Generally, honey samples from low humid areas

(central, northern, and northwestern regions of the country) consisted relatively higher TDS than honeys from high humid areas. High moisture content of honey from humid parts of the country may relate to the difficulties of evaporation of moisture from a honey by the bees against the high relative humidity of the surrounding air. The presence of equilibrium between moisture contents of honeys and relative humidity of the surrounding air and its difficulties to evaporate honey moisture against the high relative humidity of air well demonstrated [32].

The acidity values obtained in this study are nearer or less than the values reported for Argentina [10] and Portugal [45] honeys. More than 86% of the honey samples acidities were less than 40 mEq. acid/kg of honey which falls within the acceptable range of honey standard limit [18]. Generally the mean ash % obtained in this analysis is relatively higher than the values recorded for Argentina [10], Portugal [45] and Malaysian [33] honeys but average amount of minerals found in this analysis were within the acceptable standard range ($\leq 0.6\%$).

The average EC value of 0.493 ± 0.24 mS/cm recorded in this study are relatively higher than the mean EC values of 1.35 mS/cm obtained for Malaysian honeys [33] but lower than the average EC values 0.66 mS/cm recorded for Portugal honeys [45]. The EC value of the tested honey samples were significantly correlated with the total ash contents of the honey samples tested ($N=64$, $r=0.59$, $F_{1,63}=33.34$, $p<0.001$) indicating the interdependence of the ash content of honey with its electrical conductivity. Besides the free acids found in the honey, the major minerals (K, Na, Ca & Al) recorded in the honey would play major roles in EC of the tested honeys. The presence of strong correlation between the total element and EC was also reported [33].

The minerals that occur in averagely large amount were K=(499.44 mg/kg, Na=(479.39 mg/kg), Ca=15.01 mg/kg and Al=3.81). Similarly high amount of these elements have been reported in other studies [2, 33] indicating the relative abundance of these elements in most honeys. Moreover, RODRIGUEZ, et al. [47] reported high Potassium content (1500 mg/kg) in Spanish honeys.

Moreover the average amount of heavy metal like (Pb) identified was =0.005 mg/kg and the amount was far below the acceptable maximum residue limit (MRL) of 0.1 mg/kg [40]. Since the samples were collected far from industrial areas; the occurrences of the metal seems natural. The possibility of presence of heavy metals even in non-polluted areas has been reported [4]. Generally the majority of honey samples test results (Total dissolved substance, mineral (ash) content, electric conductivity and acidity) were within the acceptable range of world honey standards, which indicate the high opportunity for exporting of honey from the country.

The clustering of the honey samples into four discrete groups generally seems related with their major properties which are associated with their botanical and geographical origins. The strong association of EC, acidity, color, total ash and some metallic ions with botanical and geographical origins is well demonstrated [2, 10]. Moreover, the great association of honey color with botanical origin is also well documented [48]. The highest total dissolved substances of honey in the cluster group (1) could be due to its geographical origin, in which the area from where the honey samples collected are known for having relatively low annual precipitation and low RH compared to the geographical areas of the other cluster groups. The low acidity of the honey in this cluster group could be also related to the high concentration of the honeys and the absence of fermentation that favors the production of alcohol and its further breaking down in to acetic acid which leads to increase the acidity of a honey. The association of high acidity with high moisture content and subsequent fermentation of honey is well presented [48].

The relatively high mineral content of honeys in cluster group 2 could be due to their high K content which may be related to the application of K fertilizer in cultivated crop fields. In this regard the major sources of honeys in the cluster group 2 were mainly from cultivated and fallow land annual weeds (*Guizotia scabra*). The high electric conductivity of the honeys in this cluster group could be due to their high mineral content and acidity. The strong correlation of electric conductivity with mineral content and acidity is well established [45]. Moreover, CHUA, et al. [33] reported the high abundance of potassium and sodium in honey and he mentioned their importance as mineral markers to distinguish the geographical origins of honey. Moreover, HERNÁNDEZ, et al. [2] reported the presence of correlation between mineral content and geographical origins and they reported its potential in differentiating the origin of honeys.

The lighter color of the honeys in the cluster group 3 could be due to their botanical origin in which the dominant source of the samples in the area was *Schefflera abyssinica*. The association of color with botanical origins and its important role in characterization of honey is well reported [48]. The relatively low TDS values of honeys in this cluster group could be due to high precipitation and relative humidity of the area and these may have contributed for high moisture content of the honeys. The lowest ash content of the honeys in this cluster group could be due to the origin of the honeys which is mainly from forest trees where farming and application of artificial fertilizer are limited.

The study indicated that metallic ions in general were accounting for major variations among the different geographical and botanical origins honeys. Analysis of metallic profiles and trace elements of honeys has been used to

effectively authenticate their geographical and botanical origins [25, 28, 49].

The low TDS values of honey in the cluster group 4 could be also associated with the high precipitation and humidity of the area. However the honeys in this cluster group were darker, acidic with high EC values. The high EC value of the honeys could be due to its high acidity. The positive correlation of EC with acidity is well documented [45]. The role of EC in discriminating the botanical origins of honey is well reported [6].

Conclusion

Generally the study indicated the possibilities of characterization and classification of honeys into geographical and botanical origins based on their physico-chemical properties (color, acidity, TDS and AC) and contents of some metallic ions applying chemo-metric analysis supported with melissopalynological data. Moreover the application of stepwise discriminant analysis showed successful classification of honeys into their geographical provenance using fewer characters. Combination of different analytical approaches which are complements each other would be more efficient to discriminate and authenticate the origins of honeys. Further studies based on analysis of volatile compounds in honey as finger prints would be more important to authenticate major monofloral honeys of the country.

Acknowledgements The authors are grateful to the Deanship of Scientific Research and College of Food and Agricultural Science Research Center, King Saud University Riyadh, for providing financial and material supports this research.

References

1. P.C. Molan, A. Mizrahi, Y. Lensky, Honey as an antimicrobial agent. In A. Mizrahi & Y. Lensky (eds.), *Bee products: properties, applications and apitherapy*. (Plenum Press, New York, 1997), pp. 27–37 <http://www.springer.com/gp/book/9780306455025>
2. O.M. Hernández, J.M.G. Fraga, A.I. Jiménez, F. Jiménez, J.J. Arias, Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. *Food Chem.* **93**, 449–458 (2004). doi:10.1016/j.foodchem.2004.10.036
3. J.F. Cotte, H. Casabianca, J. Lhéritier, C. Perrucchiotti, C. Sanglar, H. Waton, Study and validity of ^{13}C stable carbon isotopic ratio analysis by mass spectrometry and ^2H site-specific natural isotopic fractionation by nuclear magnetic resonance isotopic measurements to characterize and control the authenticity of honey. *Anal. Chim. Acta.* **582**, 125–136, <http://www.sciencedirect.com/science/journal/00032670/582/1> (2007)
4. J. Wang, Q.X. Li, Chemical composition, characterization, and differentiation of honey botanical and geographical

- origins. *Adv. Food Nutr. Res.* **62**, 89–137 (2011). doi:[10.1016/B978-0-12-385989-1.00003-X](https://doi.org/10.1016/B978-0-12-385989-1.00003-X)
5. E. Alissandrakis, P.A. Tarantilis, P.C. Harizanis, M. Polissiou, Comparison of the volatile composition in thyme honeys from several origins in Greece. *J. Agric. Food Chem.* **55**, 8152–8157 (2007). doi:[10.1021/jf071442y](https://doi.org/10.1021/jf071442y)
 6. S. Serrano, M. Villarejo, R. Espejo, M. Jodral, Chemical and physical parameters of Andalusian honey: classification of citrus and eucalyptus honeys by discriminant analysis. *Food Chem.* **87**, 619–625 (2004). doi:[10.1016/j.foodchem.2004.01.031](https://doi.org/10.1016/j.foodchem.2004.01.031)
 7. O. L. Persano, M.G. Piazza, A.G. Sabatini, M. Accorti, Characterization of unifloral honeys. *Apidologie* **26**, 453–465 (1995), <https://hal.archives-ouvertes.fr/hal-00891311>
 8. P. Przybylowski, A. Wilczynska, Honey as an environmental marker. *Food Chem.* **74**, 289–291 (2001). doi:[10.1016/S0308-8146\(01\)00153-4](https://doi.org/10.1016/S0308-8146(01)00153-4)
 9. European Union, Council Directive 2001/EC relating to honey. *Off. J. Eur. Communities L* **10**, 47–52 (2001), <http://faolex.fao.org/docs/pdf/eur37441.pdf>
 10. M.V. Baroni, C. Arrua, M.L. Nores, P. Fayé, M.P. Díaz, G.A. Chiabrando, D.A. Wunderlin, Composition of honey from Córdoba (Argentina): assessment of North/South provenance by chemometrics. *Food Chem* **114**, 727–733 (2009). doi:[10.1016/j.foodchem.2008.10.018](https://doi.org/10.1016/j.foodchem.2008.10.018)
 11. E. Corbella, D. Cozzolino, Classification of the floral origin of Uruguayan honeys by chemical and physical characteristics combined with chemometrics. *LWT* **39**, 534–539 (2006). doi:[10.1016/j.lwt.2005.03.011](https://doi.org/10.1016/j.lwt.2005.03.011)
 12. O.A. Naab, M.A. Tamame, M.A. Caccavari, Palynological and physicochemical characteristics of three unifloral honey types from central Argentina. *Span. J. Agric. Res.* **6**, 566–576 (2008)
 13. C. Cimpoi, A. Hosu, V. Miclaus, A. Puscas, Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties. *Spectrochim. Acta. A.* **100**, 149–154 (2013). doi:[10.1016/j.saa.2012.04.008](https://doi.org/10.1016/j.saa.2012.04.008)
 14. G. Montenegro, M. Gómez, G. Casaubon, A. Belancic, A.M. Mujica, R.C. Peña, Analysis of volatile compounds in three unifloral native Chilean honeys. *Int. J. Exp. Bot.* **78**, 61–65 (2009)
 15. C.E. Manyi-Loh, R.N. Ndip, A.M. Clarke, Volatile compounds in honey: a review on their involvement in aroma, botanical origin determination and potential biomedical activities. *Int. J. Mol. Sci.* **12**, 9514–9532 (2011). doi:[10.3390/ijms12129514](https://doi.org/10.3390/ijms12129514)
 16. M. Juan-Borrás, E. Domenech, M. Hellebrandova, I. Escriche, Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Res. Int.* **60**, 86–94, <http://hdl.handle.net/10251/59309> (2014)
 17. I. Escriche, M. Kadar, M. Juan-Borrás, E. Domenech, Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. *Food Chem.* **142**, 135–143 (2014). doi:[10.1016/j.foodchem.2013.07.033](https://doi.org/10.1016/j.foodchem.2013.07.033)
 18. I. Sergiel, P. Pohl, M. Biesaga, A. Mironczyk, Suitability of three-dimensional synchronous fluorescence spectroscopy for fingerprint analysis of honey samples with reference to their phenolic profiles. *Food Chem.* **145**, 319–326 (2014)
 19. G.A. Nayik, V. Nanda, 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.* **240**, 1091–1100 (2015). doi:[10.1007/s00217-015-2413-2](https://doi.org/10.1007/s00217-015-2413-2)
 20. F.A. Tomás-Barberán, I. Martos, F. Ferreres, B.S. Radovic, E. Anklam, HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agric.* **81**, 485–496 (2001). doi:[10.1002/jsfa.836](https://doi.org/10.1002/jsfa.836)
 21. L. Yao, N. Dattaa, F.A. Toma's-Barbera'nb, F. Ferreresb, I. Martosb, R. Singanusongc, v Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand Leptospermum honeys. *Food Chem.* **81**, 159–168 (2003)
 22. L. Yao, Y. Jiang, R. Singanusong, N. Datta, K. Raymont, Phenolic acids and abscisic acid in Australian *Eucalyptus* honeys and their potential for floral authentication. *Food Chem.* **86**, 169–177 (2004). doi:[10.1016/j.foodchem.2003.08.013](https://doi.org/10.1016/j.foodchem.2003.08.013)
 23. L. Yao, Y. Jiang, R. Singanusong, N. Datta, K. Raymont, Phenolic acids in Australian Melaleuca, Guioa, Lophostemon, Banksia and Helianthus honeys and their potential for floral authentication. *Food Res. Int.* **38**, 651–658 (2005). doi:[10.1016/j.foodres.2005.01.002](https://doi.org/10.1016/j.foodres.2005.01.002)
 24. G.A. Nayik, B.N. Dar, V. Nanda, Physico-chemical, rheological and sugar profile of different unifloral honeys from Kashmir valley of India. *Arab. J. Chem.* (2016). doi:[10.1016/j.arabjc.2015.08.017](https://doi.org/10.1016/j.arabjc.2015.08.017)
 25. C. Chalhou, P. Gotsiou, N. Lydakis-Simantiris, P. Kefalas, Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authentication. *Crit. Rev. Food Sci. Nutr.* **45**, 193–203 (2005). doi:[10.1080/10408690590956369](https://doi.org/10.1080/10408690590956369)
 26. C.H. Latorre, R.M.P. Crecente, S.G. Martín, J.B. García, A fast chemometric procedure based on NIR data for authentication of honey with protected geographical indication. *Food Chem.* **141**, 3559–3565 (2013). doi:[10.1016/j.foodchem.2013.06.022](https://doi.org/10.1016/j.foodchem.2013.06.022)
 27. G.A. Nayik, V. Nanda, 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.* **65**, 101–108 (2015). doi:[10.1515/pjfn-2015-0022](https://doi.org/10.1515/pjfn-2015-0022)
 28. G.A. Nayik, V. Nanda, A chemometric approach to evaluate the phenolic compounds, antioxidant activity and mineral content of different uni-floral honey types from Kashmir, India. *LWT—Food Sci. Technol.* **74**, 504–513 (2016). doi:[10.1016/j.lwt.2016.08.016](https://doi.org/10.1016/j.lwt.2016.08.016)
 29. E. Anklam, A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* **63**, 549–562 (1998). doi:[10.1016/S0308-8146\(98\)00057-0](https://doi.org/10.1016/S0308-8146(98)00057-0)
 30. S. Ouchemoukh, H. Louaileche, P. Schweitzer, Physico-chemical characteristics and pollen spectrum of some Algerian honeys. *Food Control* **18**, 52–58 (2007). doi:[10.1016/j.foodcont.2005.08.007](https://doi.org/10.1016/j.foodcont.2005.08.007)
 31. G. Downey, K. Hussey, J.D. Kelly, T.F. Walshe, P.G. Martin, Preliminary contribution to the characterization of artisanal honey produced on the island of Ireland by palynological and physico-chemical data. *Food Chem.* **91**, 347–354 (2005). doi:[10.1016/j.foodchem.2004.06.020](https://doi.org/10.1016/j.foodchem.2004.06.020)
 32. E. C. Martin, Some aspects of hygroscopic properties and fermentation of honey. *Bee World* **39**, 165–178 (1958). doi:[10.1080/0005772X.1958.11095058](https://doi.org/10.1080/0005772X.1958.11095058)
 33. L.S. Chua, N.L. Abdul-Rahaman, M.R. Sarmidi, R. Aziz, Multi-elemental composition and physical properties of honey samples from Malaysia. *Food Chem.* **135**, 880–887 (2012). doi:[10.1016/j.foodchem.2012.05.106](https://doi.org/10.1016/j.foodchem.2012.05.106)
 34. S. Bogdanov, K. Ruoff, O.L. Persano, Physico-chemical methods for the characterization of unifloral honeys: a review. *Apidologie* **35**, S4–S17 (2004). doi:[10.1051/apido:2004047](https://doi.org/10.1051/apido:2004047)
 35. AOAC, Official methods of analysis. In K. Helrich (ed.) 15th edn. (Association of official Analytical Chemists, Inc., Arlington, 1990), <https://archive.org/stream/gov.law.aoc.methods.1.1990/aoc.methods>
 36. International Honey Commission, Harmonized methods of the International honey commission, 2009, pp. 63, <http://www.bee-hexagon.net/en/network.htm>
 37. M. Tuzen, M. Soylak, Trace heavy metal levels in microwave digested honey samples from middle Anatolia, Turkey. *J. Food Drug Anal.* **13**, 343–347, <http://www.fda.gov/tw>

- [tc/includes/GetFile.ashx?mID=148&id=8299&chk=39c1a44e-f824-4a1f-a458-](#) (2005)
38. J. Louveaux, A. Maurizio, G. Vorwohl, Methods of melissopalynology. *Bee World* **59**, 139–157 (1978). doi:[10.1080/0005772X.1978.11097714](https://doi.org/10.1080/0005772X.1978.11097714)
 39. A. Nuru, Atlas of pollen grains of major honeybee flora of Ethiopia. (Ethiopian Society of Animal production, Addis Ababa, Ethiopia, 2007) pp. 152
 40. Codex Alimentarius Commission: “Revised Codex Standard for Honey Codex Stan 12-1981, Rev. 1 (1987), Rev. 2 (2001),” Codex Standard, 12, 1–7 (2001), <http://teca.fao.org/sites/default/files/resources/Annex%20A%20Codex%20Alimentarius%20Honey%20Standard.pdf>
 41. S. Ouchemoukh, P. Schweitzer, M.B. Bey, H. Djoudad-Kadji, H. Louaileche, HPLC sugar profiles of Algerian honeys. *Food Chem* **121**, 561–568 (2010). doi:[10.1016/j.foodchem.2009.12.047](https://doi.org/10.1016/j.foodchem.2009.12.047)
 42. V. Kaškonienė, P.R. Venskutonis, V. Čeksterytė, Carbohydrate composition and electrical conductivity of different origin honeys from Lithuania. *LWT—Food Sci. Tech.* **43**, 801–807 (2010). doi:[10.1016/j.lwt.2010.01.007](https://doi.org/10.1016/j.lwt.2010.01.007)
 43. G. Tadesse, B. Gebregziabher, Determination of quality and adulteration effects of honey from Adigrat and its surrounding areas. *Int. J. Techno. Enhanc. Emerging Eng. Res.* **2**, 71–76, <http://www.ijteee.org/final-print/oct2014/Determination-Of-Quality-And-Adulteration-Effects-Of-Honey> (2014)
 44. T. Eteraf-Oskouei, M. Najafi, Traditional and modern uses of natural honey in human diseases: a review. *Iran J. Basic. Med. Sci.* **16**, 731–742, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3758027/> (2013)
 45. X. Feás, J. Pires, A. Iglesias, M.L. Estevinho, Characterization of artisanal honey produced on the Northwest of Portugal by melissopalynological and physico-chemical data. *Food Chem. Toxicol.* **48**, 3462–3470 (2010). doi:[10.1016/j.fct.2010.09.024](https://doi.org/10.1016/j.fct.2010.09.024)
 46. T. Joseph, A. Julius, F. Florence, D.N. Delphine, P. Jonnas, M.Z. Antoine, Physico-chemical and microbiological characteristics of honey from the sudano-guinean zone of West Cameroon. *Afr. J. Biotech.* **6**, 908–913, <http://www.academicjournals.org/AJB> (2007)
 47. J.L. Rodriguez-Otero, P. Paseiro, J. Simal, A. Cepeda, Mineral content of the honeys produced in Galicia (north-west Spain). *Food Chem* **49**, 169–171 (1994). doi:[10.1016/0308-8146\(94\)90154-6](https://doi.org/10.1016/0308-8146(94)90154-6)
 48. N. Prica, M. Živkov-Baloš, S. Jakšić, Ž. Mihaljev, B. Kartalović, J. Babić, S. Savić, Moisture and acidity as indicators of the quality of honey originating from Vojvodina region. *Arh. Vet. Med.* **7**, 99–109, <http://niv.ns.ac.rs/wp-content/uploads/2014/12/Nadezda-Prica.pdf> (2014)
 49. M.J. Latorre, R. Peña, S. García, C. Herrero, Authentication of Galician (N.W. Spain) honeys by multivariate techniques based on metal content data. *Analyst* **125**, 307–312 (2000). doi:[10.1039/A905978D](https://doi.org/10.1039/A905978D)