

Determination of Floral Origin Common Honey in Khorasan Razavi Province Based on Color Characteristics, Salinity, Electrical Resistance and TDS using Chemometrics Methods

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Abstract

Nowadays, food adulteration is important to marketing and production. The purpose of this study was to classify different types of honey (Thyme, Chaste tree, Jujube, Coriander, Barberry, Acacia and Alfalfa) with different herbal origin based on color characteristics, salinity, electrical resistance and total dissolved solids (TDS). For classification of honey, principal component analysis (PCA), linear discriminant analysis (LDA) and cluster analysis (CA) of chemometrics method, were used. The results showed the two principal components (PC1 and PC2) comprise 77.69% of the total variance, which indicates the distinction between different honeys based on physicochemical characteristics. It was also shown in the LDA method was able to classify different honeys with an accuracy of 75%. On the other hand, the method of CA in the distance of 85.58, honey was placed in 7 groups that contained thyme, coriander, acacia, barberry and jujube honey in separate groups and alfalfa honey and chaste tree honey were scattered among the groups.

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Introduction

Honey cannot be a major meal for humans, but it can be considered as a nutritional supplement. Honey has also been used in traditional medicine from ancient times and has been reported to treat burn, asthma, infection, gastrointestinal disorders, skin lesions, various inflammatory processes, as well as cataracts and other eye diseases (Khalafi *et al.*, 2016). Honey is a natural product that contains a large amount of sugars and minor amounts of minerals, proteins, vitamins, organic acids,

flavonoids, phenolic acids, enzymes and volatile compounds. The amount of these different compounds varies depending on the source of the flower and the geographical origin of the honey. In addition, honey combinations are also affected by processed and storage time (Gheldof *et al.*, 2002).

Color is a variable feature of honey and is mainly influenced by the flower's source, but also depends on the ash content, temperature and storage time. Color is the first sensory assessment that consumers take, which can

determine whether or not to buy a product (Bogdanov *et al.*, 2004). Honey lighting plays a very important role in consumer acceptance. Honey specialists are aware that honey color varies from bright to dark violet, but yellow amber, red and green are the most colorful colors of honey. In many countries, the price of honey is associated with its color. Honey with a light color has a higher price than dark honey, and in some areas dark honey is more popular (Tuberoso *et al.*, 2014).

The electrical conductivity of honey has a high correlation with the amount of minerals, ash and acidity, indicating ion, protein and organic acids. Therefore, the presence of these compounds increases the electrical conductivity, which can be used as a quality indicator in the detection of counterfeit honey from natural honey (Karabagias *et al.*, 2014).

Misleading labeling and cheating on honey has unfortunately become a problem all over the world. Cheating is usually the addition of water solution with sugar and syrup (such as corn syrup and high fructose corn syrup), as well as cheating another feeding bee with sugar and syrup, which produces artificial honey. Because of the difference in the price of honey on the basis of herbaceous origin, there is another type of fraud that is deliberately referred to as a flower or geographical origin. For this reason, regulatory agencies, manufacturers, retailers and consumers are interested in knowing the origin and quality of honey (Tewari & Irudayaraj, 2004).

Chemometrics is a method that extracts useful information from a chemical and biochemical data set. Its application in applied chemistry has been well known since the 1960's. But its application in the field of food is almost new (Granato *et al.*, 2018). More recently, chemometrics methods such as principal component analysis

(PCA), linear discriminant analysis (LDA) and cluster analysis (CA) have been used to classify honey types based on physicochemical properties.

Conti *et al.* (2007) classified 3 Italian honey samples (acacia, several flowers and honeydew) based on chemometrics method and physicochemical properties (Conti *et al.*, 2007). Nayik & Nanda (2015) classify honey from the Kashmir region of India by measuring the properties of color, enzyme, minerals, and physicochemical properties (Nayik & Nanda, 2015). In a similar study, Fechner *et al.* (2016) classification of honey in the city of Argentina Corrientes, and Manzanares *et al.* (2017) measured the physicochemical properties of Spain Tenerife honey and classification by using PCA and LDA methods.

Bee breeding does not require much found and facilities, special tools and equipment, and can be easily grown in different areas. Therefore, production of honey and its other side products can be a good opportunity for the country, and determining the quality control of honey can provide market security. Therefore, the main goal of this research is to study the methods of chemometrics to evaluate and predicting the quality of honey to determine its herbaceous origin and reducing the supply of counterfeit honey and protecting consumers.

Material and methods

Honey samples

44 samples from 7 different types of honey (Thyme, Chaste tree, Jujube, Coriander, Barberry, Acacia and Alfalfa honey) were obtained from the Agricultural Jahad of Mashhad, with emphasis on single flowering. The samples were stored in plastic containers within a refrigerator.

Determination salinity, electrical resistance and TDS

Electrical resistance, salinity, and total dissolved solids (TDS) were measured at 20 °C using a conductivity meter (Seven Easy, Mettler Toledo, Switzerland) and a solution prepared by dissolving 20 g of honey (dry matter basis) in 100 mL of distilled water twice (Saxena *et al.*, 2010).

Colorimetric

To measure the color characteristics L (brightness), a (red-green), b (blue-yellow) and H (hue angle) was used colorimetric (Colorimeter-WF-30, Iwave, China) with D65 light source and specular component included (SCI) optical mode for 9 °C.

Data preprocessing

Due to the lack of uniformity of the units and the variation in the range of variations of variables, the pre-processing problem was solved to solve this problem, and the data scale was changed and made identical. Eq. (1) was used for this.

$$Y_i = \frac{x_i^2}{\sum x_i^2} \quad (1)$$

Chemometric methods

Principal component analysis is based on the concentration of the data variance into a small number of principal components (PCs) by means of mathematical transformation. As a result, the first PC describes the maximum information from the data; the second PC describes the maximum amount of the residual variance. Each successive principal component is an orthogonal combination of the original variables which cover the maximum of the variance not accounted for by the previous component (Yucel & Sultanoglu, 2013). The aim of the method is to reduce the dimensionality of multivariate data whilst preserving as much of the relevant information as possible (Tsankova & Lekova, 2015). The plot of scores of PC1 versus PC2 is a common method for classifying samples

from their properties measured (Yucel & Sultanoglu, 2013).

Cluster analysis was performed to classify samples on the basis of the similarities of their chemical properties. The method allowed displaying different classes from botanical origins according to the mineral profiles. In cluster analysis, the groups showed the relation of botanical origin of the honey samples. The similarity between samples was calculated from the Eq. (2) distance as follows:

$$D_{ik} = \sum_{j=1}^n \sqrt{(X_{ij} - X_{kj})^2} \quad (2)$$

Where x_{ij} and x_{kj} are the values of variables j for the samples i , k and n is the number of variables (Lachman *et al.*, 2007).

In cluster analysis methods, samples are grouped in high dimensional space and form dendrogram. Gradually samples are joined into clusters up to the final cluster with all the samples. In the first step each sample forms a cluster, and then two objects closest together are joined. In the next step, either a third sample joins the first two or two other samples join together in a different cluster. Each step results in one lesser cluster than the step before until at the end all samples are in one cluster (Yucel & Sultanoglu, 2013).

LDA is used to determine which variables discriminate between two or more naturally occurring groups. This mathematical procedure maximizes the variance between groups and minimizes the variance within each group (Corbella & Cozzolino, 2006). For validation of the model, cross-validation was used.

Result and discussion

Honey color based on the color standards USDA (United States Department of Agriculture) is one of the early features of honey classification. Honey color, which has not been processed, depends primarily on the source of the origin

flower (Moniruzzaman *et al.*, 2014). Table (1) shows color indicators (L, a*, b* and H) of different honey. The L indicator represents the brightness. Among the evaluated honey, Acacia honey had the highest L-value, which had a significant difference ($P<0.05$) with other honey (except Alfalfa honey). Alfalfa honey is also considered as light honey, and the darkest honey is Chaste tree honey. In addition, in terms of a redness index a*, yellowing index b* and Hyo H index respectively, Jujube, Chaste tree and Acacia honey had the highest amount.

And the range of changes in L*, a*, b* and H indexes was 22.20-28.52, 2.08-4.34, 5.81-11.08 and 61.76-78.29 respectively. Gonzalez-Miret *et al.* (2005) generally divided honey into two categories they were classified honey with a L index of more than 50, as bright honey, and honey with a L index of less than 50 as dark honey. Based on this categorization, honey is considered to be dark in this study. On the other hand, the color of honey is also associated with its taste, bright honey has a mild taste, while dark honey has a bitter taste. Honey contains potassium, calcium, sodium and phosphorus salts that are responsible for salinity production (Gheldof *et al.*, 2002). The

amount of salinity is correlated with electrical resistance and electrical conductivity, and the amount of these compounds is dependent on the source of honey (Corbella & Cozzolino, 2006). In the evaluation of honey in this study, honey with the highest salinity has the least amount of electrical resistance. Acacia honey had the lowest salinity (0.09 ± 0.009) and the highest electrical resistance (5.03 ± 0.87) and had a significant difference ($P<0.05$) with other honey. On the other hand Barberrry and Jujube honey, unlike Acacia, had the highest amount of salinity and the least amount of electrical resistance.

Total dissolved solids (TDS) content in honey include all organic and inorganic compounds that are molecular, ionized, and micro granular (colloidal solution) (Khalil *et al.*, 2012). The range of total solids changes was measured in honey samples between 305.12 and 96.85 ppm, witch Barberrry and Alfalfa honey having the highest and lowest total dissolved solids respectively. Islam *et al.* (2017) reported the amount of TDS in Bangladesh's honey ranging from 150-100 ppm (Islam *et al.*, 2017). In a study by Nweze *et al.* (2017), the TDS was between 209-399 ppm was variable (Nweze *et al.*, 2017).

Table 1. Characteristics of color, salinity, electrical resistance and TDS of different honey

Honey	L	a*	b*	H	TDS (mg/L)	Salinity (ppm)	Electrical resistance
Thyme	25.94±1.33 ^{bc}	3.1±0.51 ^{bc}	7.15±1.31 ^{cd}	68.54±4.36 ^{bc}	162.52±4.78 ^d	0.156±0.011 ^d	3.02±0.09 ^c
Chaste tree	25.45±2.4 ^c	3.68±0.8 ^{ab}	11.08±2.63 ^a	70.99±5.77 ^b	163.72±7.42 ^d	0.15±0.076 ^d	3.61±1.55 ^b
Jujube	25.9±0.67 ^{bc}	4.34±1.39 ^a	8.27±1.58 ^{cd}	61.76±3.51 ^d	258.89±8.75 ^b	0.25±0.008 ^a	1.19±0.07 ^b
Coriander	25.52±1.42 ^b	3.37±0.21 ^{bc}	9.69±2.91 ^{bc}	71.73±7.12 ^b	204.83±8.42 ^c	0.19±0.008 ^c	2.44±0.08 ^{cd}
Barberrry	22.2±1.38 ^d	2.89±0.44 ^{cd}	5.81±0.6 ^d	63.61±2.82 ^{cd}	305.12±10.17 ^a	0.29±0.011 ^a	1.64±0.05 ^d
Acacia	28.52±0.71 ^a	2.08±0.35 ^d	10.05±0.65 ^{ab}	78.29±2.13 ^a	96.85±14.44 ^e	0.09±0.009 ^e	5.03±0.87 ^a
Alfalfa	27.63±1.2 ^{ab}	2.47±0.22 ^{cd}	9.56±3.21 ^{bc}	71.15±6.25 ^b	131.4±27.99 ^d	0.12±0.025 ^e	3.77±0.96 ^b

Data are means ± standard deviation. In each column, values with different letters indicate significant differences ($P<0.05$)

Chemometrics

Principal component analysis (PCA)

Principal component analysis (PCA) is one of the methods of chemometrics and is commonly used to reduce high-dimensional data and display it in two or three-dimensional environments (Jandric *et al.*, 2015). According to Table (2) and Figure (1), we consider components with a specific value greater than 1 as the main components.

The first main component comprise the 61.91% of the variance and the second main component comprise the 15.78% of the variance of the data. In total, the two main components comprise 77.69% of the total data variance. Jandric *et al.* (2015) evaluated the chemical properties of different honey and determined the main components that showed the two main components comprised 87.5% of the variance.

Table 2. Variance and initial eigenvalues of the main components evaluated

Component	Initial eigenvalues			Extraction sums of squared loadings		
	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %
1	4.334	61.911	61.911	4.334	61.911	61.911
2	1.105	15.787	77.698	1.105	15.787	77.698
3	0.954	13.634	91.332			
4	0.394	5.628	96.960			
5	0.119	1.695	98.655			
6	0.091	1.307	99.962			
7	0.003	0.038	100.000			

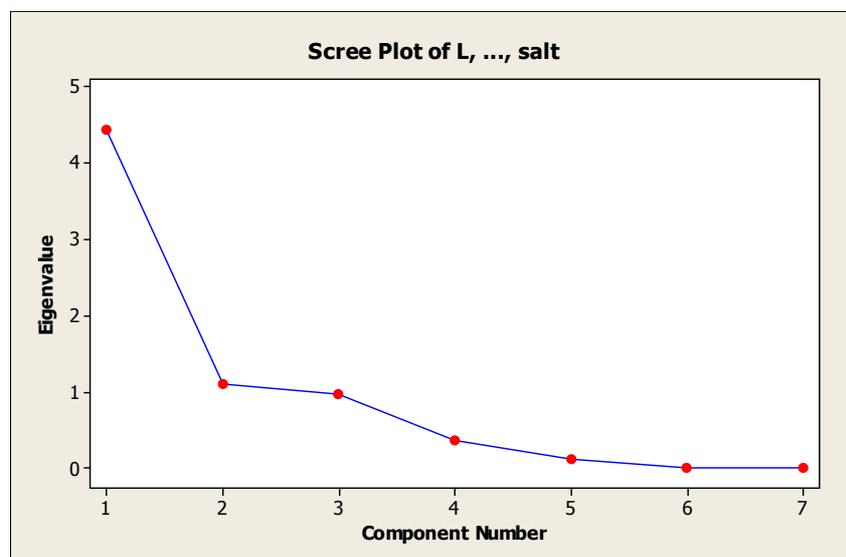


Figure 1. Initial eigenvalues changes in the main components

Figure (2) shows the dispersal of 7 different honey types in the two-dimensional space PC1 and PC2. Based on the main components, Barberry honey, Acacia, Alfalfa, Jujube, Thyme and Coriander were placed in separate groups and only Chaste tree honey was shown sporadically. The reason for the overlap of some honey

can be due to the beekeeping method. In the case of migrating bees, the possibility of mixing honey is present and, given that the honey is prepared from different beekeepers, there is a potential for mixing. However, the main components were able to distinguish between different honeys.

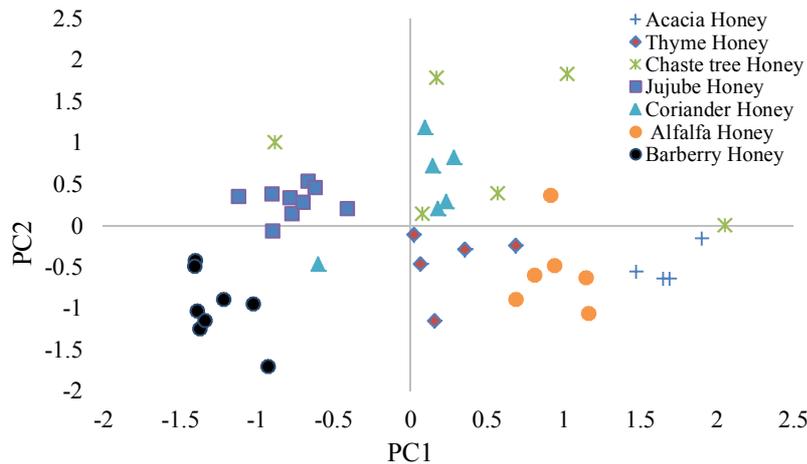


Figure 2. Principal component analysis. Distribution of honey samples on scores plot

Table (3) shows the effect of each of the characteristics evaluated on the two main components. According to this Table (3), the characteristics of L, H, salinity and electrical resistance are affected by the first main component and the characteristics a* and b* affect the second main component.

Table 3. Effect of the evaluated variables on the two main components

	Component	
	1	2
L	0.711	-0.003
a*	-0.418	0.812
b*	0.588	0.658
H	0.782	-0.055
TDS	-0.962	0.051
Salinity (ppm)	-0.962	0.055
Electrical resistance	0.910	-0.151

Linear discriminant analysis (LDA)

Before the analysis with the LDA method, a step-by-step method was used to determine the effective variables, and in each step, the variable with the highest F value was selected (Ciappini *et al.*,

2016). According to Table (4), the variables TDS, electrical resistance and a* were selected as effective variables in the honey grouping. Based on LDA analysis, 75% of honey was correctly grouped. As shown in Table (5), Thyme, Jujube, Coriander and Barberry honey were correctly (100%) in there. And only Chaste tree honey was scattered among different groups, as can be seen in Figuer (2).

Manzanares *et al.* (2017), by studying the physicochemical properties of various Spanish honey, were able to group honey with 95% accuracy by using the LDA method (Manzanares *et al.*, 2017). Rios *et al.* (2014) grouped Argentina honey with 84% accuracy (Rios *et al.*, 2014).

Cross-validation method was used to validate the model. In this method, the model was first created with all samples. Then all samples were placed in order to validate in the model. In this study, out of, 31 samples from 44 honey samples in the validation phase were correctly predicted, indicating that the provider is 70.45% of the model's validity.

Table 4. F value of each variable in each step

Step	Tolerance	F to Remove	Wilks' Lambda
1	TDS	1.000	34.796
	TDS	0.242	18.353
2	Electrical resistance	0.242	6.874
	TDS	0.242	17.743
3	Electrical resistance	0.241	6.051
	a*	0.948	4.200

Table 5. Grouping of different honey based on LDA

	Honey	Predicted group membership						Total	
		Thyme	Chaste tree	Jujube	Coriander	Barberry	Acacia		Alfalfa
Original content	Thyme	5	0	0	0	0	0	0	5
	Chaste tree	0	1	0	2	1	1	1	6
	Jujube	0	0	9	0	0	0	0	9
	Coriander	0	0	0	6	0	0	0	6
	Barberry,	0	0	0	0	8	0	0	8
	Acacia	0	0	0	0	0	2	2	4
	Alfalfa	2	0	0	0	0	2	2	6
% Original	Thyme	100.0	0	0	0	0	0	0	100.0
	Chaste tree	0	16.7	0	33.3	16.7	16.7	16.7	100.0
	Jujube	0	0	100.0	0	0	0	0	100.0
	Coriander	0	0	0	100.0	0	0	0	100.0
	Barberry,	0	0	0	0	100.0	0	0	100.0
	Acacia	0	0	0	0	0	50.0	50.0	100.0
	Alfalfa	33.3	0	0	0	0	33.3	33.3	100.0

75.0% of original grouped cases correctly classified.

Cluster analysis (CA)

The cluster analysis is a multivariate analysis method used to find real groups. In this way, members who are more similar are grouped together (Santos *et al.*, 2008). Figure (3) shows the dendrogram diagram of the grouping of honey. The ward method was used for cluster analysis and Euclidean distance was used to determine the distance between groups. In the distance of Euclidean 322.6, the honey was divided into 5 groups: Alfalfa honey and Thyme in one group, Chaste tree and Coriander honey in the other group, and Jujube, Acacia and Barberry honey in separate groups.

While at 85.58 Euclidean, the honey was placed in 7 different groups: Thyme, Barberry, Acacia, Jujube and Coriander honey were almost in separate groups, and Chaste tree honey and Alfalfa honey were scattered among the groups. Kivrak *et al.* (2017) measured the physicochemical properties of Turkish honey by means of the average linkage and rescaled spacing, they were able to divide the honey into 3 different groups. Group A included: Cedar, Lentil and Pine; group B: Multicolored, Lavender, Crank and Chestnut; group C: Peony, Five Fingers, Sunflower and Heather (Kivrak *et al.*, 2017).

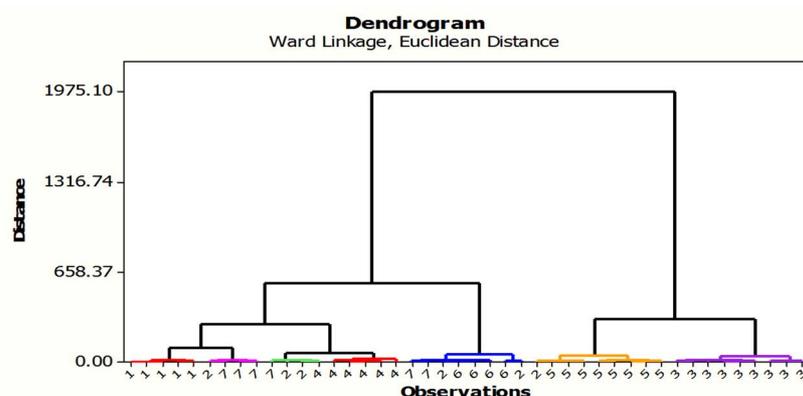


Figure 3. Cluster analysis dendrogram diagram

(1: Thyme, 2: Chaste tree, 3: Jujube, 4: Coriander, 5: Barberry, 6: Acacia, 7: Alfalfa)

Conclusion

The results of color characteristics, electrical resistance, salinity and TDS of single-flowered honey with the aid of multivariate analysis showed that, honey can be grouped according to its origin flower but using a feature, cannot distinguish between different honeys.

Given the varying price of honey based on its origin flower, this method can be used to determine adulteration and ensure consumer food security. It is also possible to find out that PCA, LDA and CA chemometric methods have the ability to separate various honey.

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تعیین منشأ گیاهی عسل‌های رایج استان خراسان رضوی براساس خصوصیات رنگ، شوری، مقاومت الکتریکی و مواد جامد تام با استفاده از روش‌های کمومتریکس

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چکیده

در حال حاضر اصالت‌سنجی در مواد غذایی یک چالش مهم در بازاریابی و تولید آن می‌باشد. هدف از این مطالعه تقسیم‌بندی انواع عسل (آویشن، افاقیا، گشنیز، زرشک، عناب، یونجه و هندبید) با منشأ گیاهی متفاوت براساس خصوصیات رنگ، شوری، مقاومت الکتریکی و مواد جامد محلول تام (TDS) می‌باشد. برای تقسیم‌بندی عسل‌های تک‌گل از روش‌های کمومتریکس تحلیل مؤلفه اصلی (PCA)، آنالیز تشخیصی خطی (LDA) و آنالیز خوشه‌ای (CA) استفاده گردید. نتایج نشان داد دو مؤلفه اصلی PC1 و PC2، ۷۷/۶۹ درصد از کل واریانس را شامل می‌شوند. همچنین در بررسی صورت‌گرفته با متد LDA نشان داد که این روش توانایی تقسیم‌بندی عسل‌های مختلف با صحت ۷۵ درصد را دارد. از طرف دیگر متد CA در فاصله اقلیدسی ۸۵/۵۸ عسل‌ها در ۷ گروه قرار گرفتند که عسل‌های آویشن، زرشک، افاقیا، عناب و گشنیز تقریباً در گروه مجزا بودند و عسل‌های هندبید و یونجه در بین گروه‌ها پراکنده بودند.

واژه‌های کلیدی: آنالیز چندمتغیره، اصالت‌سنجی، تقلبات، عسل، کمومتریکس