

## Multi-Element Composition of Honey as a Suitable Tool for Its Authenticity Analysis

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The aim of this study was to evaluate the composition of 36 honey samples of 4 different botanical origins (acacia, sun flower, tilia and honeydew) from the North East region of Romania. An inductively coupled plasma-mass spectrometry (ICP-MS) method was used to determine 27 elements in honey (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn). We would like to achieve the following goal: to demonstrate that the qualitative and quantitative multi-element composition determination of honey can be used as a suitable tool to classify honey according to its botanical origin. The principal component analysis allowed the reduction of the 27 variables to 2 principal components which explained 74% of the total variance. The dominant elements which were strongly associated with the principal component were K, Mg and Ca. Discriminant models obtained for each kind of botanical honey confirmed that the differentiation of honeys according to their botanical origin was mainly based on multi-element composition. A correct classification of all samples was achieved with the exception of 11.1% of honeydew honeys.

### INTRODUCTION

Honey, according to the Council Directive 2001/110/EC, is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature.

Romania has an ancient tradition of beekeeping. The honey production of Romania, according to the National Institute of Statistics, is about 18,000 tons/year, 85% of the production being exported. The most common unifloral honeys produced in Romania are acacia (*Robiniapedudoacacia*), tilia (*Tiliaeuropea*), sunflower (*Helianthus annuus*) and honeydew. The North-East region of Romania produces around 2,700 tons honey/year.

Honey authenticity is an important issue for honey consumers; therefore it should comply with its declared botanical and geographical origin. Unifloral honeys have always higher commercial value than the polyfloral ones; therefore, finding reliable chemical markers to ascertain the floral origin of honey is a priority research objective in the apiculture industry. Melissopalynological analysis, based on the identification and quantification of the percentage of pollen by microscopic examination, has traditionally been accepted to authenticate the botanical origin of honey and therefore, it is considered to be a reference method [Ohe, 2005]. Physicochemical parameters have also been suggested as complementary informa-

tion to characterise honey [Anklam, 1998]. Additionally, such parameters as sugars, amino acids, proteins and flavours are among markers which are able to characterise various types of honey in conjunction with a number of techniques [Arvanitoyannis *et al.*, 2005].

Gas chromatography coupled to mass spectrometry (GC-MS) combines high sensitivity and efficacy required by the analysis of the very complex mixtures of volatiles present in honey at low concentrations and provides structural information (mass spectrum) for their qualitative analysis [Soria *et al.*, 2008]. The aroma profile can be considered to be a “chemical marker” of monofloral honey due to the fact that it is directly related to the plant nectar extracted by bees [Amtmann, 2010; Overton & Manura, 1994]. For this reason, volatile fraction assessment could be a useful tool to characterise geographical or botanical origins [Castro-Vazquez *et al.*, 2010; Cuevas-Glory *et al.*, 2007].

Honey authenticity was studied by analysing trace elements presented in honeys. There are many studies that use the multi-elements to classify honeys. Chudzinska & Baralkiewicz [2011] have used Al, B, Ba, Ca, Cd, Cu, K, Mg, Mn, Na, Ni, Pb and Zn to classify honeydew, buckwheat and rape honeys from Poland. They observed that K, Al, Ni and Cd were the parameters that best predicted the authenticity of honey. Also Pisani *et al.* [2008] studied the elemental composition (23 elements) of 51 Italian honey samples using ANOVA and PCA. The results confirmed the highly significant influence of the botanical origin of honey on their chemical composition. The element composition of honey is influenced by: the environment and soil type where the nectar plants grow, and by anthropogenic factors (*e.g.* pollu-

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tion). In other study, the characterisation of Hatay honeys was made according to their multi-element composition (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, Sr and Zn) by Yucel & Sultanoglu [2013]. The study revealed that cluster analysis and principal component analysis were useful tools to differentiate the authenticity of honey samples using the profile of mineral content, highlighting the relationship between the elements' distribution and honey type.

Fernandez-Torres *et al.* [2005] applied the multi-element analysis to classify honey according to its botanical origin. They analysed eleven elements (Zn, P, B, Mn, Mg, Cu, Ca, Ba, Sr, Na and K) and made a classification into four different botanical origins: eucalyptus, heather, orange and rosemary. They observed a good prediction of the botanical origins of honey using the multi-element analysis (greater than 97%).

The Northwest Morocco honeys (multifloral honey, *Apiaceae*, eucalyptus, citrus, *Lythrum* and honeydew) have been classified using the K, Mg, Mn, Cu, Fe and Zn according to their botanical origin by Terrabet *et al.* [2003]. The classification of eucalyptus and honeydew honeys using the multi-element content has been higher than 97%.

All the multi-element classifications of honeys could not be made without the chemometrics approach. These authors have used different approaches in honey classification: Principal Component Analysis, Cluster Analysis, Linear Discriminant Analysis and Multilayer Perceptrons [Terrabet *et al.*, 2003; Fernandez-Torres *et al.*, 2005; Yucel & Sultanoglu, 2013].

To the authors' knowledge no other study related to the multi-element composition of Romanian honeys has been reported so far.

The aim of this study was to evaluate, from a qualitative and quantitative point of view, the multi-element composition of four honey types from the North-East region of Romania using an ICP-MS technique to determine simultaneously elements and get the possibility to classify honey samples according to their multi-element composition using chemometric analysis.

## MATERIAL AND METHODS

### Honey samples

To carry out this study, 36 honey samples of different origins: acacia (9 samples), tilia (9 samples), sunflower (9 samples) and honeydew (9 samples) were purchased from local beekeepers of North East region of Romania. All the samples were placed and stored in glass bottles and kept at 4–5°C in dark prior to analysis.

### Melissopalynological analysis

The pollen analysis was made according to the method of Louveaux *et al.* [1970], using a non-acetolytic method. Ten grams of honey were mixed with about 40 mL of distilled water; then centrifuged at 4500 rpm (3383×g) for 15 min, the supernatant being carefully removed. The residue was re-dissolved again and centrifuged for other 15 min. The full sediment was used to prepare the slide. The pollen spectrum of each honey sample was determined by a light microscopy (Motic ×40) by counting at least 800 pollen grains. For all pollen types the individual occurrence was expressed as percentage.

### Electrical conductivity

Electrical conductivity was determined in accordance with the harmonised methods of the International Honey Commission [Bogdanov, 2002].

### Sample preparation

Approximately 1 g of each honey sample was weighed into PTFE vessels and dissolved in 9 mL 65% HNO<sub>3</sub> and 1 mL 30% H<sub>2</sub>O<sub>2</sub>. The digestion procedures were carried out in a micro-wave oven (Speed wave MWS-2, Berghof Products + Instrument GmbH, Germany) according to instrumental parameters and settings reported previously (in a part Apparatus). Blank solutions were prepared in the same way.

### Reagents and solutions

All reagents were of analytical grade. Double deionised water (18 MΩ cm resistivity) produced by a water purification system (ThermoFisher, Germany) was used in all solutions. The element standard solutions were prepared by diluting a stock solution of 1000 mg/L of Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn. Honey samples were digested with concentrated nitric acid (65% HNO<sub>3</sub>, Sigma Aldrich, Germany) and hydrogen peroxide (30% H<sub>2</sub>O<sub>2</sub> pure p.a, Sigma Aldrich, Germany).

### Apparatus

The mineral elements analysis was performed using an Agilent Technologies 7500 Series (Agilent, USA) system coupled plasma-mass spectrometer. The ICP-MS parameters were: nebulizer 0.9 mL/min, RF power 1500 W, carrier gas 0.92 L/min, makeup gas 0.17 L/min, mass range 7–205 uma, integration time 0.1 s, acquisition 22.76 s. Detector parameters were: discriminator 8 mV, analogue HV 1770 V and pulse HV 1070 V.

### Statistical analysis

Statistical analysis was performed using the version 5.1 of the Statgraphics Plus software system. The data corresponding to each variable were analysed by one-way analysis of variance (ANOVA). Multiple comparisons were performed using the least significant difference test (LSD) and Fisher ratio (F), and statistical significance was set at  $\alpha=0.05$ .

The Principal Component Analysis (PCA) was performed using Unscrambler X 10.1 (CAMO Process AS, Oslo, Norway), all the multi-elements were weighed and normalised to perform the cluster analysis. The Principal Components Analysis (PCA) was applied to describe the relations among the multi-element composition. The discriminant analysis was made using SPSS trial version (USA).

## RESULTS AND DISCUSSION

### Melissopalynological analysis

The pollen content of the three types of honey ranged between 620 and 6598 pollen grains. According to the classification made by Maurizio [1939], the honey samples analysed can be classified in the 1<sup>st</sup> (less than 2000 pollen grains per gram) and 2<sup>nd</sup> class (between 2000–10,000 pollen grains per

gram). According to the number of pollen grains, it seems that the acacia honey had the smallest number (the number of pollen grains per gram ranged between 620 and 5389). In the case of tilia honey, the number of pollen grains per gram ranged between 825 and 5231, while in the case of sunflower ranged between 784 and 6598 pollen grains per gram. The honey samples have been classified, according to the mellissopalynological analysis, into four main classes as follows: acacia (*Robinia pseudoacacia*), sun flower (*Helianthus annuus*), tilia (*Tilia europea*) and honeydew.

The pollen grains found in the acacia honeys were: *Robinia pseudoacacia*, *Brassica napus*, *Plantago*, *Prunus*, *Trifolium* and *Rubus*. The *Brassica napus* pollen was the main pollen. The pollen grains of *Robinia pseudoacacia* were placed in the 2<sup>nd</sup> place as frequency; the percentage of this type of pollen ranged between 7% and 37%.

The following types of pollen grains were found in sunflower honeys: *Helianthus annuus*, *Taraxacum officinale*, *Trifolium*, *Fragaria*, *Tilia*, *Brassica napus* and *Robinia pseudoacacia*. The major type of pollen was *Helianthus annuus*, ranging between 52.5% and 67.2%.

In the case of tilia honey, the following were observed: *Tiliaeuropea*, *Brassica napus*, *Helianthus annuus*, *Galium* and *Trifolium pollen grains*. The major pollen was *Tilia europea* (31.2–87.4%).

Honeydew honey is a poor pollen honey type, having an average concentration of pollen grains of 2241 grains. The major pollen grains present in honeydew honeys were: *Castanea sativa* and *Quercus*, followed by *Brassica napus*, *Helianthus annuus* and *Trifolium repens*.

The electrical conductivity of acacia, sunflower and tilia honeys ranged between 0.122–0.198, 0.420–0.520 and respectively 0.608–0.730 mS/cm. Honeydew electrical conductivity ranged between 0.92 and 1.26 mS/cm. A higher value than 0.80 mS/cm is not an acceptable one for floral honeys, being specific to honeydew honeys, therefore this parameter can be used as a quality parameter to distinguish honeydew and floral honeys [Bogdanov et al., 2004]. The electrical conductivity values for each honey type are in agreement with those presented in the literature [Kadar et al., 2010; Oroian, 2012; Escriche et al., 2009].

## METHOD OF VALIDATION

The 27 elements were simultaneously determined using ICP-MS after acid mineralization. The capability of the method as a routine analysis method was estimated through the determination of the detection limits of each element studied. The limits of detection (LOD) and limits of quantification (LOQ), were calculated with three and ten times the standard deviation of the blank divided by the slope of the analytical curve, respectively [Thompson et al., 2002; Khan et al., 2014]. The values of LOD were in the range of 0.251–18.321 µg/kg as it is presented in Table 1. The LOQs ranged between 0.761 and 385.513 µg/kg. Precision is described as the degree of variability given by the expression of results, not taking into account the influence of the sample (sample variability). The precision was evaluated as the relative standard deviation of 10 repeated determinations for one

TABLE. 1. Limit of detection (LOD), limit of quantification (LOQ), precision, recovery for the 27 elements analysed using ICP-MS.

Analyte	LOD (µg/L)	LOQ (µg/L)	Precision (CV %)	Recovery (%)
Ag	19.512	59.121	1.29	99
Al	3.812	11.55	3.21	97
As	0.751	2.276	2.75	98
Ba	0.915	2.772	1.21	94
Be	0.351	1.064	2.54	96
Ca	3.156	9.563	4.87	96
Cd	62.624	189.751	1.95	104
Co	86.254	261.35	4.09	103
Cr	0.592	1.794	2.93	97
Cs	0.51	1.545	1.29	105
Cu	0.346	1.048	4.21	98
Fe	0.829	2.512	4.87	99
Ga	0.325	0.985	2.41	99
In	36.214	109.728	2.21	105
K	118.321	358.513	4.89	101
Li	0.271	0.821	4.21	98
Mg	1.212	3.672	4.05	99
Mn	0.456	1.382	4.21	99
Na	115.125	348.829	3.89	98
Ni	0.261	0.791	3.26	95
Pb	1.598	4.842	1.35	103
Rb	0.251	0.761	2.65	97
Se	1.61	4.878	1.92	104
Sr	87.916	266.385	2.98	101
Tl	5.104	15.465	1.89	102
U	0.924	2.8	1.98	102
V	0.271	0.821	1.51	99
Zn	22.659	68.657	2.98	98

sample [Chudzinska & Baralkiewicz, 2011]. Table 1 shows the coefficient of variation for each element. The coefficient of variation for the 27 elements analysed ranged between 1.21 and 4.89%, complying with the required criteria of 5%.

Analytical quality control was also verified by the recovery experiments for the 27 selected elements, spiking at two selected concentration levels, 10 and 100 mg/kg. The recoveries, depicted in Table 1, were in the range of 94–105%.

## Multi-element content in honey samples

Table 2 shows the elemental composition of the honey samples analysed. The values of elements were not homogeneous. The highest total element content was observed

TABLE. 2. Elemental composition of acacia, honeydew, sun flower and tilia honeys.

Element (mg/kg)	Honey type				F-value
	Acacia	Honeydew	Sun flower	Tilia	
Ag	0.037 <sup>a</sup>	0.017 <sup>b</sup>	0.015 <sup>b</sup>	0.019 <sup>ab</sup>	2.10 <sup>ns</sup>
Al	11.045 <sup>b</sup>	27.038 <sup>a</sup>	13.561 <sup>b</sup>	11.155 <sup>b</sup>	9.82 <sup>***</sup>
As	0.009 <sup>a</sup>	0.007 <sup>ab</sup>	0.005 <sup>bc</sup>	0.003 <sup>c</sup>	6.26 <sup>**</sup>
Ba	0.228 <sup>b</sup>	0.506 <sup>a</sup>	0.349 <sup>ab</sup>	0.174 <sup>b</sup>	4.73 <sup>*</sup>
Be	0.001 <sup>b</sup>	0.002 <sup>a</sup>	0.001 <sup>b</sup>	0.001 <sup>b</sup>	16.42 <sup>***</sup>
Ca	52.914 <sup>c</sup>	101.518 <sup>b</sup>	163.878 <sup>a</sup>	137.854 <sup>ab</sup>	13.49 <sup>***</sup>
Cd	0.001 <sup>b</sup>	0.004 <sup>a</sup>	0.003 <sup>ab</sup>	0.001 <sup>b</sup>	3.62 <sup>*</sup>
Co	0.008 <sup>b</sup>	0.017 <sup>a</sup>	0.010 <sup>b</sup>	0.009 <sup>b</sup>	3.63 <sup>*</sup>
Cr	0.051 <sup>a</sup>	0.049 <sup>a</sup>	0.037 <sup>b</sup>	0.029 <sup>b</sup>	7.38 <sup>***</sup>
Cs	0.003 <sup>b</sup>	0.013 <sup>a</sup>	0.007 <sup>b</sup>	0.004 <sup>b</sup>	12.21 <sup>***</sup>
Cu	1.822 <sup>b</sup>	3.354 <sup>a</sup>	2.390 <sup>b</sup>	1.563 <sup>b</sup>	7.36 <sup>***</sup>
Fe	19.387 <sup>b</sup>	28.285 <sup>a</sup>	24.009 <sup>ab</sup>	19.156 <sup>b</sup>	2.76 <sup>ns</sup>
Ga	0.015 <sup>bc</sup>	0.030 <sup>a</sup>	0.021 <sup>b</sup>	0.012 <sup>c</sup>	7.03 <sup>***</sup>
K	553.867 <sup>b</sup>	1648.16 <sup>a</sup>	849.36 <sup>b</sup>	955.289 <sup>a</sup>	23.85 <sup>***</sup>
Li	11.157 <sup>b</sup>	19.693 <sup>a</sup>	13.677 <sup>b</sup>	12.055 <sup>b</sup>	4.07 <sup>*</sup>
Mg	51.212 <sup>b</sup>	75.415 <sup>a</sup>	63.772 <sup>ab</sup>	50.549 <sup>b</sup>	3.64 <sup>*</sup>
Mn	1.715 <sup>ab</sup>	2.529 <sup>a</sup>	1.001 <sup>ab</sup>	0.868 <sup>b</sup>	2.04 <sup>ns</sup>
Na	171.149 <sup>ab</sup>	229.333 <sup>a</sup>	154.068 <sup>b</sup>	123.754 <sup>b</sup>	4.64 <sup>**</sup>
Ni	0.191 <sup>b</sup>	0.325 <sup>a</sup>	0.183 <sup>b</sup>	0.122 <sup>b</sup>	9.48 <sup>***</sup>
Pb	0.062 <sup>ab</sup>	0.078 <sup>a</sup>	0.040 <sup>bc</sup>	0.026 <sup>c</sup>	8.23 <sup>***</sup>
Rb	0.442 <sup>c</sup>	2.246 <sup>a</sup>	1.097 <sup>b</sup>	0.895 <sup>bc</sup>	11.78 <sup>***</sup>
Se	0.009 <sup>c</sup>	0.013 <sup>a</sup>	0.014 <sup>a</sup>	0.011 <sup>b</sup>	14.47 <sup>***</sup>
Sr	0.264 <sup>b</sup>	0.414 <sup>a</sup>	0.351 <sup>ab</sup>	0.304 <sup>ab</sup>	2.58 <sup>ns</sup>
Tl	0.001 <sup>b</sup>	0.003 <sup>a</sup>	0.002 <sup>b</sup>	0.002 <sup>ab</sup>	4.19 <sup>*</sup>
U	0.002 <sup>b</sup>	0.002 <sup>a</sup>	0.001 <sup>b</sup>	0.001 <sup>b</sup>	3.77 <sup>*</sup>
V	0.006 <sup>b</sup>	0.023 <sup>b</sup>	0.798 <sup>a</sup>	0.004 <sup>b</sup>	3.32 <sup>*</sup>
Zn	2.421 <sup>a</sup>	3.871 <sup>a</sup>	3.241 <sup>a</sup>	2.655 <sup>a</sup>	1.20 <sup>ns</sup>

ns – not significant ( $P > 0.05$ ), \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ; a, b, c, d – statistical groups.

in the case of the honeydew sample (2805.08 mg/kg) and the lowest one was observed in the case of the sunflower sample (663.65 mg/kg). The high content of total element in the case of honeydew is mainly due to the presence of potassium in high concentration (2108.21 mg/kg); the same observation was made by others scientists [Lachman *et al.*, 2007; Chua *et al.*, 2012]. Golob *et al.* [2005] and Vanhanen *et al.* [2011] observed higher total element contents in the case of honeydew honeys from New Zealand (4060 mg/kg) and Slovenia (3680 mg/kg), respectively.

The first group of elements had higher concentrations than 30 mg/kg, such as: K, Na, Ca and Mg. The major concentration was observed in the case of potassium, which ranged between 380.91 and 2108.21 mg/kg. The potassium content covered the elemental composition between 56.16 and 80.56% and was in agreement with the previous studies [Pisani *et al.*, 2008; Terrab *et al.*, 2003; Chua *et al.*, 2012; Vanhanen *et al.*, 2011]. The potassium content ranged between 57.39 and 68.64% in the case of sunflower with a medium concentration of 64.82%, between 70.84 and 75.61% in the case of tilia honey with a medium concentration of 72.59%, between 56.16 and 67.71% in the case of acacia honey with a medium concentration of 63.07%, and between 72.45 and 80.56% in the case of honeydew honey with a medium concentration of 77.06%, respectively. The potassium content decreased as follows: honeydew honey (77.06%) > tilia honey (72.59%) > sunflower honey (64.82%) > acacia honey (63.06%).

Sodium and calcium were the second and the third predominant minerals in honey samples with a total content ranging between 7.23 to 25.66% and 2.98 to 15.32%, respectively. The next element was Mg with a total content ranging between 2.88% and 9.40%, followed by iron which ranged between 0.95% and 4.57%. The content of Ca was in agreement with the data reported by Lachman *et al.* [2007].

The second group of elements included Li, Al, Mn, Fe, Cu and Zn, all of them having higher concentrations than 1 mg/kg and lower than 30 mg/kg. Honeydew samples had the highest concentration of elements from the second group. Lithium effects include leukocytosis, polyuria, dry mouth, confusion, nausea, vomiting, muscle twitch, however it is recommended in bipolar disorder treatment. Aluminium is an unwanted element for humans, due to its neurological, lungs, fertility and cancer effects.

The copper content was three times higher in honeydew than in the other three honey types, as it was observed by Chua *et al.* [2012] and Chudzinska & Baralkiewicz [2011], ranging between 0.644 and 5.491 mg/kg. Still trace amount of copper is essential for the formation of haemoglobin, namely oxygen carrying blood component. Furthermore, it helps in the production of melanin which is responsible for pigmentation of eyes, hair and skin.

Out of a total of 27 elements, 16 elements were trace elements: Be, V, Cr, Co, Ni, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Tl, Pb and U, having lower concentrations than 1 mg/kg in honeys; they belonged to the third group of elements. Selenium was found in all the four honey types, it is a micronutrient which is very important in proper functioning of the immune system, especially thyroid function in humans.

Honeydew samples were richer than the other samples not only in the case of elements from the 1<sup>st</sup> and 2<sup>nd</sup> groups. It can be observed that Al content was much higher than 2, Mn was much higher than 1.5, Fe much higher than 1.2, Ni much higher than 1.6, Cu much higher than 1.4, Zn much higher than 1.2, Rb much higher than 2, Cs much higher than 1.6, Ba much higher than 1.4 and Pb much higher than 1.2 times in the case of honeydew samples than in the case of acacia, sunflower and tilia honeys, respectively.

Heavy metals (Cr, Zn, As, Cd and Pb) in the composition of the honeys under study were registered as well. Cr con-



tent ranged between 0.013 and 0.074 pm, Zn content ranged between 0.741 and 8.011 mg/kg, As content from 0.002 to 0.015 mg/kg, Cd content from 0.001 to 0.011 mg/kg and Pb content from 0.020 to 0.142 mg/kg, respectively. Contents of heavy metals were in the same range with those reported by Chua *et al.* [2012] in the case of honey samples from Malaysia. Lead and arsenic are the most severe environment contaminants. Mostly, these contaminant elements come from industrial activities or automobile exhaust gas emission. Contact with stainless steel surfaces during harvesting, processing and/or preparation of honey for the market, can generate high Cr content, due to corrosive effect of honey acidity [Przybylowski & Wilczynska, 2001].

The analysis of variance was applied to all the elements found in the honey samples (Table 2). In the case of five elements (Mn, Fe, Zn, Sr and Ag), no statistically significant difference was found among honey samples ( $P > 0.05$ ). For twelve elements (Be, Al, K, Ca, Cr, Ni, Cu, Ga, Se, Rb, Cs and Pb), there has been noticed a highly statistically significant difference between honey samples ( $P < 0.001$ ). Considering the Fisher ratio, K content is the most influential element depending on honey type ( $F = 23.85$ ).

**Chemometric analysis**

The chemometric analysis is commonly used in science today, so variance analysis (ANOVA), principal component

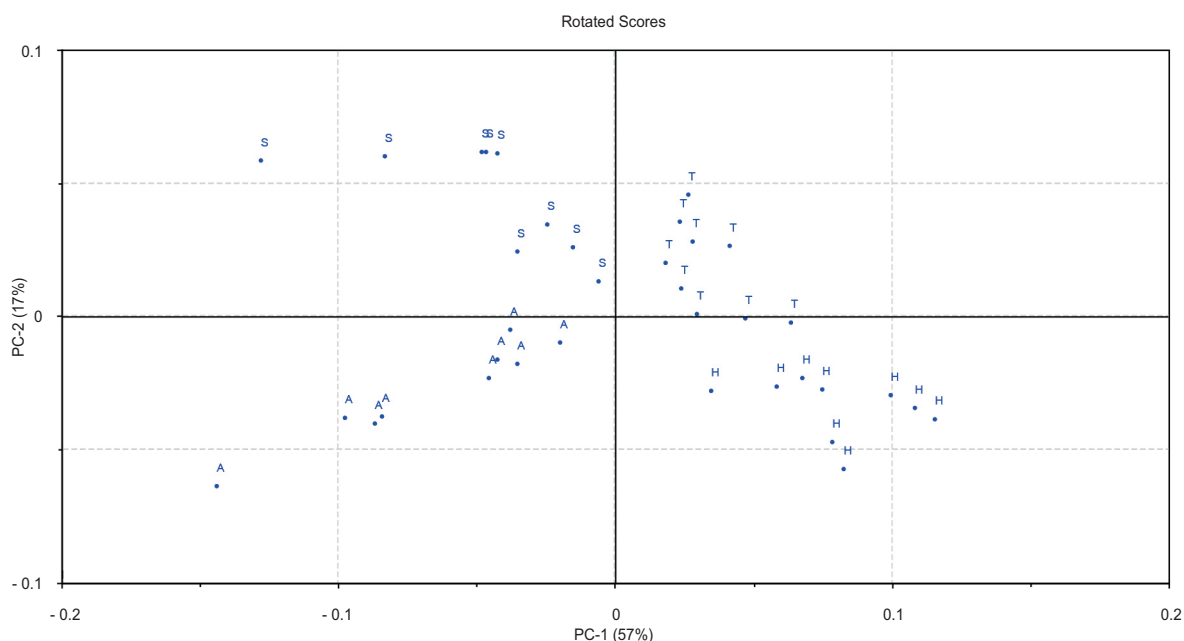


FIGURE 1. Principal component analysis of the multi-elements scores of acacia, sunflower, tilia and honeydew honeys.

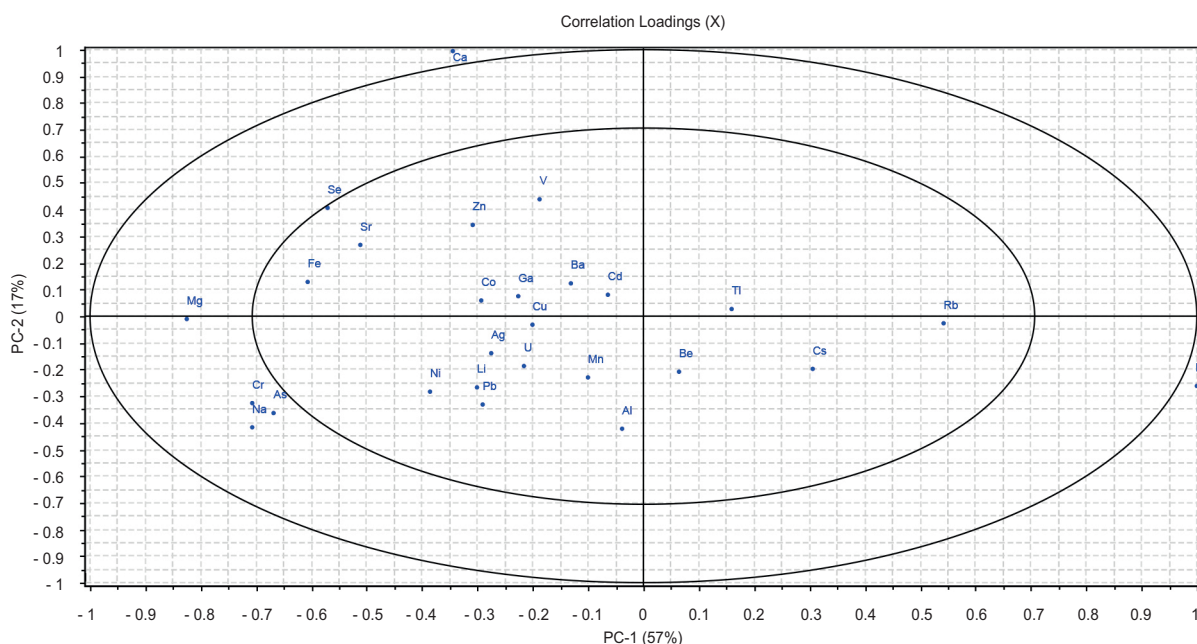


FIGURE 2. Principal component analysis of the multi-element composition of acacia, sunflower, tilia and honeydew honeys.

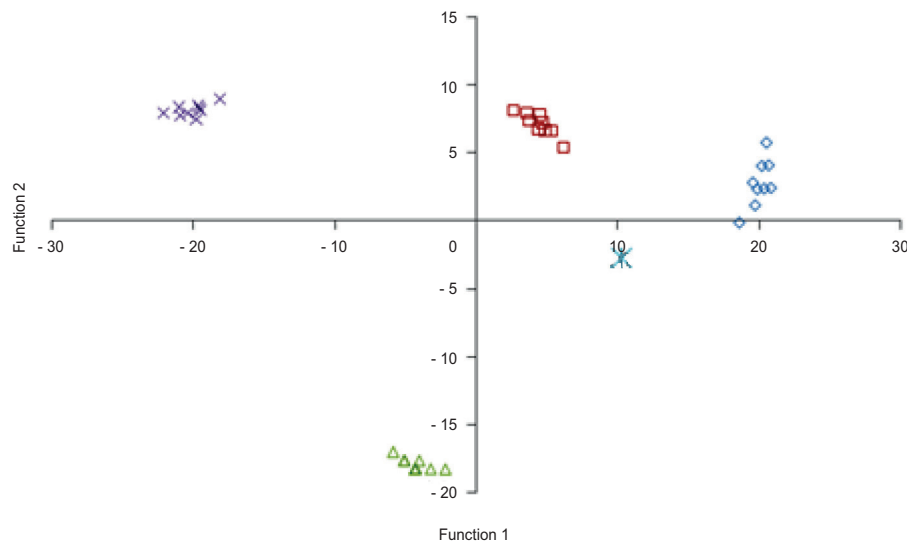


FIGURE 3. Linear discriminant score plot: rhombus– acacia honeys, triangle – tilia honeys, square – sunflower honeys, cross – honeydew samples.

TABLE 3. Results obtained from the discriminant analysis applied to elemental composition in order to differentiate acacia, tilia, sunflower and honeydew honeys.

	Count	Type	Predicted group membership				Total (%)
			Acacia	Sunflower	Tilia	Honeydew	
Original	%	Acacia	100	–	–	–	100
		Sunflower	–	100	–	–	100
		Tilia	–	–	100	–	100
		Honeydew	–	–	–	100	100
Cross validated	%	Acacia	100	–	–	–	100
		Sunflower	–	100	–	–	100
		Tilia	–	–	100	–	100
		Honeydew	–	–	11.1	88.9	100

analysis (PCA) and stepwise discriminant analysis (SDA) were used to check the similarities between samples according to botanical origin.

### Principal component analysis

The principal component analysis was conducted to evaluate the global effect of elemental composition on honey type, from a descriptive point of view. Figures 1 and 2 present the scores and compound loadings of PCA analysis performed. It was found that the two principal components (PCs) explained 74% of the variations in the data set. The PC1 explained 57% of the variability and the PC2 explained 17%. It can be observed that the honey samples are divided into 4 groups by the two principal components. Magnesium influences the projection of acacia honeys; potassium influences the projection of honeydew honeys, while calcium influences the projection of sunflower honeys. The elements placed in the outer ellipse of the correlation loadings have a higher influence on the projection than those placed in the inner ellipse.

### Stepwise discriminant analysis

A stepwise discriminant analysis was applied, out of which six classification models were constructed. All the elemental components analysed were used for this purpose, and the discriminant functions were constructed using all the variables (Table 3). In order to evaluate the model classification capacity, the percentage of samples classified correctly was considered: original grouped (using all samples to estimate the classification model) and cross-validated grouped (leaving one out) to estimate its robustness. This procedure calculates the model with all samples minus one, after which the prediction is performed. This data processing was repeated as many times as the number of samples was. In this way, it was possible to evaluate the capacity of predicting correctly the group that unknown samples belong to. In all the cases, the same classification of groups was observed. Irrespective of the parameters chosen the percentage of cases correctly classified were 100% in the case of the original classification while in the case of the cross-validation classification the samples were 97.2% correctly classified (Table 3). Acacia, sunflower

and honeydew samples were correctly classified, while a honeydew sample was classified as tilia. This fact can be due to the low content of potassium found in that honeydew sample. The linear discriminant analysis applied to all the physico-chemical parameters resulted into two canonical functions with the Eigen values of 237.126 and 125.682 and the Wilks's lambda values of 0.001 and 0.003, respectively. The linear discriminant analysis is shown in Figure 3. Function 1 explains 65.1%, while function 2 explains 34.44% of the total variance. The bi-dimensional plot (Figure 3) of the first two functions shows four groups for the four honey types. The SDA allows visualisation of data in botanical origin representations, simplifying the observation and interpretation of information. The highest absolute value which dominated the first discriminant function is represented by Be content (F1=16.91, F2=7.57), followed closely by the Ca content (F1=14.74, F2=4.73). These two parameters dominated and the second discriminant function did, too. The V content (F1=-0.36, F2=-2.24) had the lowest influence on the first discriminant function, while Fe content (F1=4.41, F2=0.88) had the lowest influence on the second discriminant function.

## CONCLUSIONS

The multi-element composition of honey provided us with useful information on the differentiation of acacia, honeydew, sunflower and tilia. Therefore, the honey type has a great influence on the multi-element composition. Potassium is the element with the highest concentration in all the honeys irrespective of their botanical origin. The multivariate analysis allowed the discrimination of honey types according to their botanical origin using the multi-element composition. The cross validation of honey samples was correct for the 97.2% of the honey samples (11.1% of the honeydew samples were classified as tilia honeys). Having in view the chemometric approach, we can consider that the multi-element composition of honeys is a suitable tool in predicting their botanical origin.

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