

# Physicochemical Parameters as a Tool for the Assessment of Origin of Honey

KRISTINA B. LAZAREVIĆ and MILICA S. JOVETIĆ

Centre for Food Analysis, Zmaja od Noćaja 11, 11000 Belgrade, Serbia

ŽIVOSLAV L.J. TEŠIĆ

University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, P.O. Box 51, 11158 Belgrade, Serbia

**Honey is a complex mixture of various substances, and its composition depends on both botanical and geographical origin, as well as anthropogenic factors. The accurate identification of honey origin guarantees the satisfaction of consumers' needs and has an impact on the honey market value. Physicochemical parameters, some of which are used in routine analysis of honey quality, could be useful for the assessment of its origin. In this review, special attention is paid to those studies that assessed the sugar and mineral composition of honey, whether they were investigated in terms of botanical or geographical origin, or for the characterization of honey type. The oligosaccharides present in honey and the electrical conductivity of honey correlate strongly with its botanical origin. Mineral content could be indicative for distinguishing honeys according to their botanical and geographical origins because it depends on both the soil composition and the floral type of melliferous plants. This review provides insight into the results obtained by various studies from approximately the last 10 years concerning the sugar profile and the mineral and trace element content of different types of honey. An attempt was made to statistically analyze the results regarding mineral and trace element content in order to identify indicators that could distinguish honey by origin.**

Honey is a sweet natural substance produced by honeybees (*Apis mellifera*) from various secretions of plants. According to European Union Council Directive No. 2001/110/EC, honey may be designated according to the botanical source “if it comes wholly or mainly from that particular source and has the organoleptic, physicochemical and microscopic properties corresponding with that origin.” The accurate identification of botanical and geographical origin is important in order to guarantee a level of predictability in organoleptic properties for the consumer. It also has a significant impact on the honey market value, with monofloral honey, as well as honey from a specific geographical region that

has gained a reputation among consumers, generally having a greater value (1–3).

A great number of factors influence the composition and properties of honey. Absolutely pure unifloral honey does not exist, and there are numerous sources of nectar that can be incorporated in honey in variable relationships. The composition of honey is strongly influenced by both the geographical and the botanical origin of honey, which vary based on natural and anthropogenic factors. The properties of honey with the same floral source but a different geographical origin can vary to some extent as a consequence of different climate conditions and type of vegetation in the different geographical region (4).

Routine determination of physicochemical parameters [water content, electrical conductivity, sugar content, fructose/glucose ratio, enzyme activity, color, optical rotation, pH value, acidity, and hydroxymethylfurfural (HMF) content] is commonly used for both QC and processing control of honey. A number of factors influence the final values of these parameters in honey. For water content, the most important factors are air humidity, the abundance of nectar flow, colony strength, and the ventilation of the beehive. The variability in the amount of added enzymes depends on factors including nectar collection, the abundance of nectar flow and its sugar content, the age and diet of the bees, the strength of the colony, temperature, etc. Several factors influence the formation of HMF in honey: temperature and heating time, storage conditions, the use of metallic containers, and the chemical properties of honey, which are related to the floral source from which the honey has been extracted. Some physicochemical parameters, particularly electrical conductivity and sugar composition, also allow conclusions to be made regarding the botanical origin of honey (5).

The primary goal of our work is to give a review of the studies carried out in recent years (approximately the last 10 years) that have considered the sugar profile (with a special emphasis on oligosaccharides) and the mineral content of honey, whether they were dedicated to investigating botanical or geographical origin or to characterizing a certain honey type.

## Sugar Composition

Honey sugar composition is correlated with the initial profile of its precursor. The monosaccharide fraction of honey is basically composed of the simple sugars fructose and glucose, ranging from 65 to 80% of the total soluble solids. Fructose and glucose are present, to a greater or lesser degree, in nectar or in the carbohydrate excretion of insects that suck the fluid from the phloem. Saccharides present at high concentrations in nectar or in the excretion of insects will also be present in large quantities

Guest edited as a special report on “Assessment of the Authenticity of Honey” by Dušanka M. Milojković-Opsenica and Živoslav Lj. Tešić.

Corresponding author's e-mail: kristina.lazarevic@cin.co.rs

This work was supported by the Ministry of Education, Science, and Technological Development of Serbia, grant No. 172017.

DOI: 10.5740/jaoacint.17-0143

in the produced honey (6). Monosaccharides can be additionally produced by the inversion of sucrose by the enzyme invertase in honey.

In addition to monosaccharides, many di-, tri-, and tetrasaccharides have been identified in honey. Honey disaccharides, ranging from 10 to 15%, are mainly constituted by regioisomers of  $\alpha$ -glucosyl glucose and  $\alpha$ -glucosyl fructose. Disaccharides with  $\beta$ -glycosidic linkages are present in minor amounts, whereas fructosyl-fructoses are very scarce. The more abundant trisaccharides are derivatives of sucrose (7).

Although the carbohydrate composition of nectar is not fully examined, the origin of many oligosaccharides, whose presence is found in honey, cannot be associated with plant nectar. The large variety of honey oligosaccharides has been attributed to *trans*-D-glycosylation reactions. In this process, D-glucopyranosyl or D-fructofuranosyl groups are transferred to a receptor molecule, which may be a mono-, oligo-, or polysaccharide. These reactions are catalyzed by the same enzymes involved in the hydrolysis reactions of disaccharides in honey, mainly  $\alpha$ - and  $\beta$ -glucosidases (8). Along with enzymatic reactions, the nonenzymatic reaction called "acid reversion" (condensation of monosaccharides by acid catalysis) and the formation of compounds such as difructose anhydride, with concomitant loss of fructose, take place during storage. The high concentration of carbohydrates and the low pH value of the honey favor these reactions (7).

The main disaccharides in honey are maltose and sucrose, but another 12 compounds have been reported in the literature: trehalose, cellobiose, laminaribiose, maltulose, nigerose, turanose, kojibiose, trehalulose, palatinose, gentiobiose, isomaltose, and melibiose. The presence of the disaccharides gentiobiose, isomaltose, laminaribiose, kojibiose, maltose, maltulose, trehalose, nigerose, sucrose, and turanose, referred in some earlier works (6, 9), was confirmed by Cotte et al. (10), Nozal et al. (11), de la Fuente et al. (12), Ruiz-Matute et al. (13), Kaškoniënė et al. (14), Ruiz-Matute et al. (15), Primorac et al. (16), de la Fuente et al. (7), Bentabol Manzanares et al. (17), and Escuredo et al. (18). Other disaccharides also were detected in some of those works, such as cellobiose (12–14, 16), melibiose (6, 14), palatinose (7, 12–14), trehalose (7, 10–14, 17), and trehalulose (12, 13).

Although several works have been focused on the study of mono- and disaccharides in honey, knowledge about the tri- and tetrasaccharide fractions is still scarce. Siddiqui and Furgala (19) isolated and characterized up to 11 trisaccharides from honey, and a critical review by Doner (20) evidenced the presence of eight trisaccharides (isopanose, theandrose, erlose, melezitose, maltotriose, panose, 3- $\alpha$ -isomaltosyl-glucose, and isomaltotriose). All of them, with the exception of 3- $\alpha$ -isomaltosyl-glucose, were detected by chromatographic methods in further works (10, 13, 21–23). Other trisaccharides, such as raffinose (6, 23–26) and kestoses (10, 13, 22, 24), have also been identified by GC and HPLC.

Available data on higher oligosaccharide composition in honey are very scarce. Three tetrasaccharides (maltotetraose,  $\alpha$ -panosyl-D-fructofuranoside, and  $\alpha$ -maltosyl-D-fructofuranoside), two pentasaccharides, and one hexasaccharide have been detected in New Zealand honeydew honeys (27). The number of identified oligosaccharides in honey is constantly increasing. Ruiz-Matute et al. (15) detected 25 trisaccharides and tetrasaccharides in Spanish and New Zealand honey samples, wherein the

trisaccharide planteose and  $\alpha$ -3-glucosyl-isomaltulose were detected for the first time.

The amount of the monosaccharides fructose and glucose, as well as the fructose/glucose and glucose/water ratios, are identified as useful parameters for the classification of unifloral honeys by many authors (28, 29), but during recent years, controversy has existed about the relationship between the pattern of oligosaccharides in honey and its botanical origin. Some earlier works showed that minor sugars have a relatively low diagnostic value for the determination of botanical origin of honey (6, 30–33). The small differences between the minor sugar profiles of unifloral honey samples are explained by the fact that oligosaccharides are mainly a product of honey invertase action. The work of Mateo and Bosch-Reig (6) revealed a correlation between the botanical origin of nectar and honey oligosaccharide profile. The levels of various sugars (fructose, glucose, sucrose, maltose, maltulose, kojibiose, isomaltose, raffinose, erlose, and melezitose) were determined in different Spanish unifloral honey types (rosemary, orange blossom, lavender, sunflower, eucalyptus, heather, and honeydew) by GC of the trimethylsilyl oxime derivatives. There were significant differences among the honey types in relation to sugar composition, but the monosaccharides fructose and glucose, the disaccharides sucrose and maltose, and the glucose/water ratio were selected by discriminant analysis as the parameters significant for the correct classification of the honey samples. Erlose was detected in nearly all honey types, but not in sunflower honey. Sunflower honey was very poor in trisaccharides; in fact, erlose and melezitose were not detected in any sunflower honey sample. The origin of erlose is mainly associated with the reaction of *trans*-D-glycosylation in honey, and is formed from sucrose by transglucosylation of the  $\alpha$ -D-glucosyl group of a molecule of sucrose to the fourth position of the glucose moiety of another molecule of sucrose. Its level increases at first, and then decreases by the action of honey  $\alpha$ -glucosidase at the time new oligosaccharides are synthesized. The reported low concentration of sucrose in the nectar of Asteraceae may be the reason for the undetectable levels of erlose in sunflower honey. The chemometrics of sugar spectra, determined by ion-exchange chromatography, has shown better results in honey botanical source determination. Swallow & Low (9) introduced charcoal-Celite chromatography followed by anion-exchange chromatography in conjunction with a pulsed amperometric detection (PAD) system for qualitative and quantitative analyses of 20 minor structurally similar oligosaccharides present in four honeys of known botanical origin (alfalfa, alsike, canola, and trefoil). The largest variation in honey oligosaccharide profiles among the tested honeys was found in canola (rape) honey. The significantly different carbohydrate profile and the almost total absence of erlose in canola honey were explained by the low levels of sucrose in canola nectar. GC was used to analyze 18 sugars (fructose, sucrose, maltose, maltulose, turanose, trehalose, palatinose, laminaribiose, melibiose, isomaltose, gentiobiose, raffinose, neo-kestose, 1-kestose, erlose, melezitose, maltotriose, and panose) in 141 French honey samples belonging to three monofloral types: 50 acacia, 38 chestnut, and 53 lavender honeys (34). Chestnut honey was characterized by a low trisaccharide content, in contrast to acacia and lavender honeys that contained, e.g., considerable quantities of erlose (1.88 and 1.40%, respectively). In addition, after statistical processing by

principal component analysis (PCA) was applied to the acacia, chestnut, and lavender honey samples, the detection of 5 to 10% sugar syrup addition was enabled.

The same authors next investigated the correlation of sugar profile (19 sugars) with honey origin, through the chromatographic analyses of six monofloral honeys (acacia, chestnut, rape, lavender, fir, linden, and sunflower) and multivariate statistical processing of the sugar content (10). Fir honey samples were characterized by a high content of trisaccharides, particularly raffinose (2.1%), melezitose (5.7%), and erlose (2.1%). Erlose was practically absent in rape and sunflower honey samples, enabling them to be distinguished from other botanical varieties. Acacia honey was characterized by a high trisaccharide concentration (1.9%). Differences in the profiles of nectar and honeydew honeys were identified, undoubtedly arising from existing differences between the composition of the nectars and honeydews themselves, resulting from the work of aphids. The presence of melezitose in honeydew honeys, a sugar absent from the sap of the trees, is explained by the presence of an aphid enzyme that converts sucrose to melezitose. The complete distinction of each floral origin was not obtained, but PCA enabled zones of the different varieties to be differentiated (i.e., for samples that were completely separated).

In the work of Nozal et al. (11), 14 carbohydrates (fructose, glucose, turanose, erlose, nigerose, isomaltose, maltose, panose, trehalose, melezitose, maltotriose, gentiobiose, sucrose, and isomaltotriose) were quantified using HPLC–PAD in 77 honeys belonging to different botanical origins (ling, spike lavender, French lavender, thyme, forest, and multifloral). From the results of the descriptive statistics, it was observed that the most abundant disaccharides were turanose, nigerose, maltose, and isomaltose. Among the trisaccharides, erlose was the most abundant. Discriminant analysis achieved in a single step does not offer good results in the simultaneous discrimination of all types of honeys, possibly attributed to the monofloral honeys studied in this work having been collected from a small area in July (thyme), August (French lavender), September (spike lavender and forest), and November (ling), which sometimes confers similar characteristics for honeys of different botanical origins, hence making their characterization more difficult. PCA, used as a first approach to characterize the analyzed honey samples, showed similarities between spike lavender and multifloral honeys, in which melezitose, turanose, isomaltose, and nigerose were identified as sugars that may play an important role in distinguishing among samples of different botanical origins. The best discrimination among groups was obtained when four canonical discriminant analyses were carried out sequentially, origin by origin. By means of the use of only the content of six carbohydrates (erlose, nigerose, trehalose, melezitose, isomaltose, and panose) a discrimination of ling, spike lavender, French lavender, and forest honeys was achieved, with a global percentage of success of more than 90% after cross-validation.

In the work of Martins et al. (24), the correlations among production year, locality, and sugar composition of Serra da Lousã honey, a high-quality heather honey from central Portugal, were investigated. Serra da Lousã honey is characterized by the absence of both sucrose and melibiose, and the presence of these sugars in this honey is considered an adulteration. The sugar profiles of Serra da Lousã heather honey and Terra Quente

de Trás-os-Montes lavender honey were compared, allowing the following discrimination: Serra da Lousã honey samples do not contain sucrose and generally exhibit lower amounts of turanose, trehalose, and maltose and higher amounts of fructose and glucose.

Seventy authentic honey samples of nine different floral types (rhododendron, chestnut, honeydew, Anzer (*Thymus* spp.), eucalyptus, cotton (*Gossypium* spp.), citrus, sunflower, and multifloral) from 15 different geographical regions of Turkey were analyzed for their chemical composition (including sugar profile) and for indicators of botanical and geographical origin (25). Along with the oligosaccharide profile, the profiles of free amino acids and volatile components together with water activity were determined in order to characterize chemical composition, and those variables that most significantly affected the PLS and linear discriminant analysis (LDA) calculation were selected. Maltose and raffinose were identified as two oligosaccharide parameters to be used in the classification of Turkish honey, with the presence of maltose being characteristic of both rhododendron and honeydew honeys. Raffinose and maltose were ranked as second and fourth, respectively, in variable importance in the PLS-LDA of all the 51 variables. In another work, the sugar profiles were determined in multifloral and unifloral honey samples from different regions of Algeria (26). The sugar profiles of 50 honey samples (25 multifloral and 25 unifloral honeys) from different regions of Algeria were analyzed by HPLC–PAD. Eleven sugars (two monosaccharides and nine oligosaccharides) were quantified. Sucrose, maltose, isomaltose, turanose, and erlose were present in nearly all the samples, whereas raffinose and melezitose were detected in few samples. Trehalose was present only in two samples and none of the samples contained melibiose. PCA showed that the cumulative variance was approximately 40% and that the honey types were not well distinguished by their sugar profile, but glucose, isomaltose, turanose, and trehalose content showed some correlations. Samples of Apiaceae honey were correctly classified using factorial discriminant analysis.

The specific rotation and carbohydrate profile of Croatian black locust, sage, and chestnut honey samples were determined (16). Fructose, glucose, sucrose, maltose with cellobiose and trehalose, melezitose with erlose, raffinose, and xylose were evaluated and quantified by HPLC, whereas specific rotation was determined using a polarimeter. Significant differences in disaccharide and trisaccharide content were noticed between the analyzed honey types. The differences in the carbohydrate profiles, especially in disaccharide and trisaccharide content, reflected different specific rotation values of the selected honey types. Weak positive correlations between specific rotation and sucrose, melezitose with erlose, and raffinose content were found. Sage honey had the lowest content of sucrose, maltose with cellobiose, and trehalose, whereas chestnut honey had the lowest content of melezitose with erlose, and no raffinose. In this study, Chestnut honey was characterized by a low trisaccharide content, as was stated in the previously mentioned works by Cotte et al. (10, 34), in contrast to black locust honey, which is characterized by high di- and trisaccharide content. Black locust and chestnut honeys had similar fructose and glucose content, but the higher di- and trisaccharide content in black locust honey was expressed in its higher specific rotation values. The correlations between specific rotation and each determined carbohydrate content were calculated, and very weak positive

correlations were found only between specific rotation and content of sucrose, melezitose with erlose, and raffinose.

As part of a research project aiming to characterize the most important Spanish unifloral honeys (citrus, heather, eucalyptus, rosemary, echium, Rosaceae, and multifloral), the carbohydrate analysis of 109 honey samples was carried out (7). A GC method based on the use of two different stationary phases was used to analyze two monosaccharides, 14 disaccharides, and 21 trisaccharides, which were quantitatively determined. The number of identified and quantified di- and trisaccharides in this work was higher than those reported in previous chromatographic studies. Also, trehalulose, theanderose, and planteose were reported for the first time for a high number of samples. Trehalose was reported with the configuration of the glycosidic link specified:  $\alpha,\alpha$ -trehalose appeared in only five samples in low concentration, whereas  $\alpha,\beta$ -trehalose was present in all examined samples. Data regarding trisaccharides were also improved in comparison with previous reports. Multivariate statistical techniques were applied to the carbohydrate concentration data in order to study possible relationships among the floral sources of honey and sugar composition. Several carbohydrates were found to be characteristic for the most important honey types. Despite this, the broad concentration range of carbohydrates within a given source and the common presence of mixed-type honeys led to a high degree of intrasource variability that encumbered an unambiguous classification of the main unifloral types.

In the work of Rybak-Chmielewska et al. (35), coniferous (mainly *Abies alba*) honeydew honey was characterized by physicochemical parameters and sugar content (fructose, glucose, sucrose, maltose, turanose, trehalose, isomaltose, and melezitose). The following properties proved to be characteristic for this variety: high electrical conductivity, a lower percentage (by a few points) of monosaccharides in relation to other honey types, and a higher content of disaccharides and the trisaccharide melezitose. It was noted that the total content of disaccharides was about two times higher than in floral honeys.

The results of research on 63 samples of four types of Slovenian honey (acacia, spruce, multifloral, and forest) suggested that the carbohydrate profile (the presence of individual carbohydrates in honey) and the content of carbohydrates in honey may have a potentially valuable role in the assessment of botanical origin and as an indicator of putative adulteration with sugar mixes or syrups, respectively (23). The content of fructose, glucose, and 10 oligosaccharides was detected and quantified with high-performance anion-exchange chromatography coupled with PAD. Statistical comparison of the results showed statistically significant differences among some parameters. Disaccharide content did not differ much among the four types of honey, but there were significant differences in the content of trisaccharides between nectar (acacia and multifloral) and honeydew (forest and spruce) honeys.

Sixty-two honey samples from Turkey were examined on the basis of pollen analyses, including 11 unifloral honey types (chestnut, heather, chaste tree, rhododendron, common eryngo, lavender, Jerusalem tea, astragalus, clovers, and acacia), two different honeydew honeys (lime and oak), and seven different multifloral honey samples (29). Physicochemical parameters of the honey samples were assessed and seven saccharides were analyzed by HPLC with refractive index detection. In addition to monosaccharides, sucrose was detected, but only

in very few honeys. Maltose was detected at levels of 1% or lower in all honey samples, with the exception of chaste tree honey and clover honeys. Melezitose was detected at levels of approximately 0.5% in all honeys, apart from chaste tree honey and acacia honey, but at higher levels in oak and pine honey samples.

Sugar profiles of 45 Korean honey samples (15 acacia, 15 multifloral, 10 chestnut, and 5 artificial honey samples) were analyzed in the work of Jang et al. (36) using GC-MS through trimethylsilyl oxime and trimethylsilyl methoxime derivatization. The average content of total disaccharides was highest in chestnut honey and lowest in acacia honey. Seven trisaccharides were detected from the samples, and the average content of trisaccharides was highest in artificial honey samples, which had a high erlose content. The total content of di- and trisaccharides was highest in chestnut honey and lowest in acacia honey. These findings are in contrast to those of some earlier studies in which acacia honey was characterized by a high content of oligosaccharides and chestnut honey was characterized by a low trisaccharide content (10, 16, 34).

It may be concluded that oligosaccharide composition is a reliable indicator for honey classification and authentication only in the case of unifloral honeys, which are produced from very high amounts of the dominating plant, but even then, it is difficult to specify one or more carbohydrates that could serve as floral markers for honey botanical source. Instead, most authors have suggested using ratios of particular carbohydrates, as well as other criteria (such as electrical conductivity, polyphenol profile, etc.), together with the amount of carbohydrates for the differentiation of honey types.

The oligosaccharide content in honey samples from different countries and different botanical origins reported in various studies are shown in Table 1.

### Electrical Conductivity

The electrical conductivity of honey is a result of the minerals or total ash, organic acids, and proteins present in honey. The higher their content, the higher the resulting conductivity. This parameter is often included in routine analysis of honey quality (37–41). Electrical conductivity is frequently used for the characterization of the botanical origin of honey because it correlates significantly to mineral (ash) content (5, 42).

A linear regression model for the relationship between the ash content and electrical conductivity was demonstrated for 290 Slovenian honey samples (37). Honeydew is a rich source of minerals; therefore, these types of honey have the highest levels of electrical conductivity (41).

### Mineral Content

The content of minerals in honey is relatively low, ranging from 0.1 to 0.2% in nectar honey to over 1% in honeydew honey. The most abundant metal in honey is potassium (45–85% of the total mineral content). Other major metals are sodium, calcium, and magnesium. Copper, iron, zinc, and manganese are present in intermediate quantities. Honey also contains trace metals at much lower levels (<1  $\mu\text{g/g}$ ). The main concentrations of metals in honey are derived from the soil; accordingly, the composition and content of metals in honey are affected by soil composition (i.e., geographical origin of honey). The floral

**Table 1. Oligosaccharides in honey samples from different countries and different botanical origins reported in various studies**

Botanical origin	No. of samples	Country	Reported oligosaccharides	Method	Ref.
Alfalfa	1				
Alsike	1	Canada	Isomaltose, kojibiose, laminaribiose, maltose, melibiose, nigerose, palatinose, sucrose, erlose, isomaltotriose, isopanose, maltotriose, melezitose,	IC-PAD <sup>a</sup>	9
Canola	1				
Trefoil	1				
Rosemary	13				
Orange	16				
Lavender	15				
Sunflower	14	Spain	Isomaltose, kojibiose, maltose, maltulose, sucrose, erlose, melezitose, raffinose	GC-FID <sup>b</sup>	6
Eucalyptus	14				
Heather	13				
Honeydew	16				
Acacia	50	France	Isomaltose, laminaribiose, maltose, maltulose, melibiose, palatinose, sucrose, trehalose, turanose, erlose kestose, maltotriose, melezitose, panose, raffinose	GC-FID	34
Chestnut	38				
Lavender	53				
Acacia	50				
Chestnut	38				
Rape	28	France	Gentiobiose, isomaltose maltose, maltulose, melibiose palatinose, sucrose, trehalose, turanose, erlose kestoses, maltotriose, melezitose, panose, raffinose	GC-FID	10
Lavender	53				
Fir	37				
Linden	38				
Sunflower	36				
Ling	15				
Spike lavender	17	Spain	Gentiobiose, isomaltose, maltose, nigerose, sucrose, trehalose, turanose, erlose, isomaltotriose, maltotriose, melezitose, panose	HPLC-PAD	11
French lavender	8				
Thyme	10				
Multifloral	12				
Forest ( <i>Quercus</i> )	15	Portugal	Isomaltose, maltose, melibiose, sucrose, trehalose turanose, melezitose, raffinose	HPLC	24
Heather ( <i>Erica</i> spp.)	20				
Lavender	20				
Citrus	1	Spain	Cellobiose, gentiobiose, isomaltose, kojibiose, laminaribiose, maltose, maltulose, melibiose, nigerose, palatinose, sucrose, trehalose, turanose, erlose, kestose, maltotriose, melezitose, panose, raffinose, theanderose	GC-FID	13
Acacia	17	Slovenia	Isomaltose, maltose, palatinose, sucrose, turanose, erlose maltotriose, melezitose, panose, raffinose	HPAEC-PAD <sup>c</sup>	23
Multifloral	18				
Forest	15				
Spruce	13				
Anzer ( <i>Thymus</i> spp.)	8				
Rhododendron	20				
Eucalyptus	2	Turkey	Maltose, sucrose, raffinose	HPLC-RID <sup>d</sup>	25
Chestnut	6				
Honeydew	6				
Gossypium	2				
Multifloral	24				
Citrus	1				
Sunflower	1				
Multifloral	23				
Eucalyptus	10	Algeria	Isomaltose, maltose, melibiose, sucrose, trehalose, turanose, erlose melezitose, raffinose	HPLC-PAD	26
Erica	4				
Citrus	1				

Table 1. (continued)

Botanical origin	No. of samples	Country	Reported oligosaccharides	Method	Ref.
Sage	41				
Chestnut	17	Croatia	Maltose, sucrose, erlose + melezitose	HPLC–RID	16
Acacia	17				
Eucalyptus	6				
Echium	8				
Rosemary	14	Spain	Cellobiose, isomaltose, kojibiose, laminaribiose, maltose, maltulose, nigerose, sucrose, trehalose, turanose, erlose, melezitose, panose	GC-MS	7
Heather	13				
Citrus	12				
Rosacea	6				
Honeydew	24	Spain	Isomaltose, maltose, sucrose, trehalose, turanose, melezitose	HPLC–RID	17
Honeydew (coniferous)	27	Poland	Isomaltose, maltose, sucrose, trehalose, turanose, melezitose	IHC <sup>e</sup>	35
Chestnut	21				
Eucalyptus	19				
Heather	9	Spain	Maltose, sucrose, melezitose	IC–PAD	18
Acacia	11				
Lime	14				
Rape	11				
Sunflower	4				
Honeydew	14				
Chestnut	NI <sup>f</sup>				
Heather	NI				
Clover	NI				
Lavender	NI				
Lime	NI	Turkey	Maltose, melibiose, sucrose, trehalose, melezitose	HPLC–RID	29
Rhododendron	NI				
Oak	NI				
Acacia	NI				
Multifloral	NI				

<sup>a</sup> IC = Ion chromatography.

<sup>b</sup> FID = Flame ionization detector.

<sup>c</sup> HPAEC = High-performance anion-exchange chromatography.

<sup>d</sup> RID = Refractive index detector.

<sup>e</sup> IHC = International Honey Commission Method.

<sup>f</sup> NI = Not indicated.

type of melliferous plants, floral density, and composition of the nectar and pollen are also responsible for variations in the metal content of honey. Thus, the mineral content could be indicative for distinguishing honeys according to their botanical origin (42). Metals in honey can also result from different anthropogenic activities, such as nearby agricultural practices, industries, and waste dumps. For that reason, honey can be a useful indicator for assessing environmental pollution (43–47). Golob et al. (48) analyzed trace and minor elements in Slovenian honey by total reflection X-ray fluorescence spectroscopy. Statistically significant differences were established between different types of honey. Honeys were also separated according to their botanical origin as nectar honey or honeydew honey (48). NeĀemer et al. (49) performed a more extensive study on a total of 264 samples of Slovenian honey, also employing total reflection X-ray fluorescence spectroscopy. Chemometric methods were applied

for interpreting the results. It was shown that the characteristic key elements (chlorine, potassium, manganese, and rubidium) could be used to discriminate the botanical origin of various types of honey (49). The characterization of three types of Italian honey (acacia, multifloral, and honeydew) was carried out on the basis of their physicochemical parameters (pH value, sugar content, and humidity) and mineral content (Na, K, Ca, Mg, Cu, Fe, and Mn). PCA and LDA were used to classify honey samples. The most discriminant parameters were magnesium content and pH value. In addition, all samples of acacia and honeydew honey were correctly classified by using LDA (50).

Bogdanov et al. (43) investigated Swiss honey (a total of 95 samples of known botanical and geographical origin) and pointed out that variation in trace element content is primarily due to botanical factors.

Polish rape and honeydew honeys from different regions were analyzed for the content of 12 elements (Al, B, Ca, Cr, Cu, Fe,

K, Mg, Mn, Na, Ni, and Zn). The results showed that element content may allow discrimination between the botanical origin of honeys by using cluster analysis (51).

Fermo et al. (52) used ion chromatography for the determination of sodium, calcium, and magnesium in honeydew and nectar honey samples originating from Italy and the West Balkans. The results were processed by PCA and hierarchical cluster analysis, which indicated a difference between nectar and honeydew honey (52).

In a study of Croatian honey, Bilandžić et al. (53) confirmed, on the basis of trace element content, differences due to botanical origin.

Czipa et al. (54) analyzed 16 elements in a total of 34 various Hungarian honey samples, employing inductively coupled plasma (ICP) MS. Acacia, rape, and sunflower honeys were well distinguished based on mineral content and LDA (54).

Oroian et al. (55) evaluated the mineral composition of 36 honey samples (acacia, sunflower, linden, and honeydew honeys) from the North-East Region of Romania. Twenty-seven elements were determined by ICP-MS. The multivariate chemometric analysis allowed the discrimination of honey types according to their botanical origin. The dominant elements that were strongly associated with the principal components were potassium, magnesium, and calcium (55).

The geographical origin of three Slovenian unifloral honey types (black locust, lime, and chestnut) was investigated by analysis of physicochemical parameters, element content, and stable carbon and nitrogen isotope ratios, and the results were interpreted using chemometric methods. Lime honey samples were 100% correctly classified according to their regional origin, whereas the success rates for black locust and chestnut honey samples were slightly lower at 98.2 and 94.6%, respectively. Chestnut honey samples from different Slovenian macroregions differed primarily in ash, sulfur, and potassium content (56).

Uršulin-Trstenjak et al. (57) analyzed the mineral content of 200 samples of Croatian black locust honey from different areas of the country during two seasons. PCA showed that aluminum (Bjelovar-Bilogora County), iron (Bjelovar-Bilogora County and Istria Region), copper (Eastern Croatia), and potassium (Istria Region) were the mineral substances typical for the black locust honey of each area (57).

Di Bella et al. (58) concluded that mineral content could be used as a tool to assess the traceability of honeys after investigating 39 honey samples of different botanical origin from two Italian regions. The discrimination between Sicilian and Calabrian honey samples was achieved by PCA. Also, the results of canonical discriminant analysis indicated that 100% of the investigated samples were correctly classified (58).

Some studies pointed out that the mineral composition of honey could be attributed to both botanical and geographical origin (59–62).

Some of the studies were carried out in order to characterize certain types of honey by determining their mineral content. Pătruică et al. (63, 64) presented results for acacia honey from different regions of Romania, as well as for acacia, linden, sunflower, and polyfloral honey samples from southern Romania. Nowak et al. (65) analyzed different types of nectar honeys from Lower Silesia, Poland, and compared their metal and trace element content. Czipa et al. (66) investigated 44 samples of Hungarian acacia honey. Stihl et al. (67) characterized 36 samples of honey from different regions

of Romania, which were collected in 2 consecutive years. Physicochemical parameters, together with mineral content, were used for this purpose (67).

Bulgarian honeydew honey samples were characterized by determining the content of 19 elements (K, Ca, Mg, P, S, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Sr, V, and Zn), physicochemical parameters, and melissopalynological analysis (68).

Spanish chestnut honey samples were characterized on the basis of palynological analysis, physicochemical parameters, mineral composition, total phenolic content, flavonoid content, and radical scavenging activity (69).

The studies mentioned above, conducted in the past 10 years, present (among other data) the results of the mineral and trace element content of European honey samples. A total of 950 honey samples of six different botanical types (474 acacia, 206 chestnut, 125 linden, 87 honeydew, 31 rape, and 27 sunflower samples), from 13 countries were investigated in these studies. In Table 2 these 950 samples are represented by 74 sets of results, which involve the average amounts of minerals or trace elements characteristic for certain botanical species and certain geographical regions (i.e., countries). We considered average amounts of potassium, calcium, magnesium, sodium, copper, zinc, iron, manganese, nickel and chromium, although the studies often included other elements as well. It should also be emphasized that samples were analyzed by different methods.

We made an attempt to statistically analyze all of these results in order to get indicators that could distinguish honey samples by origin. The summarized parameters of descriptive statistics obtained from the mineral content analysis of acacia, chestnut, linden, honeydew, rape, and sunflower honey samples are presented by box-and-whisker plots in order to compare different data sets (Figure 1).

Concerning the major minerals, it can be seen that the highest variability in potassium concentration is found in chestnut honey, followed by honeydew, linden, and sunflower honeys, respectively. Acacia and rape honeys contain the lowest concentration of this element. Regarding magnesium, the highest concentration is found in chestnut honey, followed by honeydew and sunflower honeys. Linden, rape, and acacia honey contain relatively small amounts of magnesium. Higher amounts of calcium are found in chestnut honey as compared to the other honeys. Medians for sodium content are similar for all honey types. Acacia, chestnut, linden, rape, and sunflower honeys contain a similar amount of copper, whereas honeydew honey has a slightly higher amount. Medians for zinc concentrations are also similar for various honey types, but linden honey has the highest variability of results. The concentration of iron is higher in honeydew than in other honey types. Chestnut honey shows the highest content of manganese, and also the greatest variability of results. The results for nickel concentration in acacia, honeydew, linden, and rape honey samples are quite dispersed. Honeydew honey contains a higher amount of nickel in comparison to other honey types. Chromium is, generally, present in small amounts.

To detect whether any of the investigated parameters might be used for differentiation between the studied botanical types of honey, a Kruskal-Wallis test was applied to the results of the mineral content of honey samples with various botanical origins.

**Table 2. Mineral and trace metal content of honey reported in various studies<sup>a</sup>**

Botanical origin	Country	No. of samples	K	Ca	Mg	Na	Cu	Zn	Fe	Mn	Ni	Cr	Method	Ref.
	Slovenia	9	390	9.4	— <sup>b</sup>	—	—	—	—	1.5	—	—	TXRF <sup>c</sup>	48
	Slovenia	54	284 <sup>d</sup>	—	—	—	—	—	—	4.25 <sup>d</sup>	—	—	TXRF	49
	Slovenia	55	287 <sup>d</sup>	17.7 <sup>d</sup>	—	—	—	—	—	6.7 <sup>d</sup>	—	—	TXRF	56
	Croatia	19	304.7	349.3	8.02	33.9	18.6	0.55	2.77	—	—	—	FAAS, GFAAS <sup>e</sup>	53
	Croatia	200	298.8 <sup>d</sup>	96.72 <sup>d</sup>	19.93 <sup>d</sup>	84.34 <sup>d</sup>	0.28 <sup>d</sup>	5.6 <sup>d</sup>	1.11 <sup>d</sup>	0.17 <sup>d</sup>	0.36 <sup>d</sup>	—	ICP-MS	57
	Hungary	44	—	—	—	—	0.325	1.75	2.18	—	0.098	0.027	ICP-OES <sup>f</sup>	66
	Hungary	8	181	23.6	12.8	—	0.13	1.58	0.429	0.837	—	0.014	ICP-OES, ICP-MS	54
	Romania	3	136.35 <sup>d</sup>	192.65 <sup>d</sup>	20.872 <sup>d</sup>	27.102 <sup>d</sup>	0.1425 <sup>d</sup>	0.3253 <sup>d</sup>	3.7935 <sup>d</sup>	0.4002 <sup>d</sup>	—	—	AAS <sup>g</sup>	63
	Romania	3	454 <sup>d</sup>	7.06 <sup>d</sup>	13.40 <sup>d</sup>	17.25 <sup>d</sup>	0.26 <sup>d</sup>	2.09 <sup>d</sup>	2.01 <sup>d</sup>	0.10 <sup>d</sup>	0.005 <sup>d</sup>	—	AAS	64
	Romania	9	553.867	52.914	51.212	171.149	1.822	2.421	19.387	1.715	0.191	0.051	ICP-MS	55
	Romania	14	262 <sup>d</sup>	68 <sup>d</sup>	—	—	0.39 <sup>d</sup>	2.7 <sup>d</sup>	5.5 <sup>d</sup>	—	—	—	XRF, FAAS, GFAAS <sup>h</sup>	67
Acacia	Bulgaria	6	126	32	6	8.11	<0.01–0.15	0.22	0.83	0.11	<0.01–0.08	<0.01–0.01	ICP-AES <sup>i</sup>	60
	Serbia	3	503.8 <sup>d</sup>	61.5 <sup>d</sup>	12.5 <sup>d</sup>	12.2 <sup>d</sup>	—	—	—	—	—	—	IC <sup>j</sup>	52
	Kosovo	2	267.6 <sup>d</sup>	13.4 <sup>d</sup>	3.8 <sup>d</sup>	10.1 <sup>d</sup>	—	—	—	—	—	—	IC	52
	Albania	2	18.0 <sup>d</sup>	16.8 <sup>d</sup>	8.7 <sup>d</sup>	2.8 <sup>d</sup>	—	—	—	—	—	—	IC	52
	Turkey	1	—	—	—	—	0.29	0.84	2.81	0.35	0.53	0.024	AAS	70
	Italy	23	307	32.71	7.27	12.86	0.67	—	4.51	0.33	—	—	FAAS, GFAAS	50
	Italy	2	158.5 <sup>d</sup>	9.9 <sup>d</sup>	3.2 <sup>d</sup>	7.1 <sup>d</sup>	—	—	—	—	—	—	IC	52
	Italy	3	719	115	70	91	0.17	2.03	2.05	1.01	0.06	0.68	ICP-OES, ICP-MS	58
	Switzerland	7	—	—	—	—	0.180	0.217	0.278	0.453	0.056	0.003	ICP-MS	43
	Germany	1	324.6	60.75	88.4	15.69	—	3.44	67.18	4.15	0.15	—	AAS	71
	Poland	1	—	—	13.8	—	0.44	1.96	1.08	0.27	0.85	—	ICP-AES	65
	Poland	5	166	48.8	10.1	13	0.1	4.1	1.2	0.5	0.3	ND <sup>k</sup>	AAS	62
	Slovenia	25	3500	150	—	—	—	—	—	28	—	—	TXRF	48
	Slovenia	38	3483 <sup>d</sup>	—	—	—	—	—	—	23.61 <sup>d</sup>	—	—	TXRF	49
	Slovenia	37	3673 <sup>d</sup>	146 <sup>d</sup>	—	—	—	—	—	22.9 <sup>d</sup>	—	—	TXRF	56
	Slovenia	1	1575.6	108.6	24.3	8.7	—	—	—	—	—	—	IC	52
	Croatia	1	1812.1	129.8	30.6	7.8	—	—	—	—	—	—	IC	52
Chestnut	Croatia	9	2824.4	486.7	59.1	35.8	6.19	0.89	3.57	—	—	—	FAAS, GFAAS	53
	Bulgaria	1	1628	66	16	9.55	0.09	0.2	0.59	3.73	<0.01	<0.01	ICP-AES	60
	Kosovo	1	1099	67.6	20.7	8.7	—	—	—	—	—	—	IC	52
	Albania	1	1394.8	87.3	116.2	9.0	—	—	—	—	—	—	IC	52
	Turkey	15	5007	481	—	28.00	0.43	2.20	3.20	17.20	—	—	AAS	59
	Turkey	1	—	—	—	—	0.16	0.34	3.28	0.18	0.06	0.01	AAS	70
	Italy	16	—	—	—	—	—	17.869	27.294	—	—	0.830	ICP-OES	61
	Italy	3	2242.8 <sup>d</sup>	146.6 <sup>d</sup>	40.7 <sup>d</sup>	12.3 <sup>d</sup>	—	—	—	—	—	—	IC	52
	Italy	8	3100	179	122	84	0.27	3.5	3.97	2.06	0.14	0.18	ICP-OES, ICP-MS	58
	Spain	41	2602	158	171	34	2.0	2.0	3.0	—	—	—	AAS	69
	Switzerland	7	—	—	—	—	0.399	0.662	0.602	6.167	0.041	0.003	ICP-MS	43
	Poland	1	709	55	49.3	7.8	0.6	0.7	1.4	0.8	ND	0.5	AAS	62
	Slovenia	7	780	43	—	—	—	—	—	2.8	—	—	TXRF	48
	Slovenia	28	1805 <sup>d</sup>	—	—	—	—	—	—	3.71 <sup>d</sup>	—	—	TXRF	49
	Slovenia	30	1740 <sup>d</sup>	65.3 <sup>d</sup>	—	—	—	—	—	3.65 <sup>d</sup>	—	—	TXRF	56
	Croatia	11	1574.8	387.8	25.5	31.9	20.6	6.78	4.02	—	—	—	FAAS, GFAAS	53
	Hungary	1	955	45.7	28.6	—	0.320	2.15	0.612	1.36	—	0.037	ICP-OES, ICP-MS	54
	Romania	3	591 <sup>d</sup>	55.63 <sup>d</sup>	17.79 <sup>d</sup>	36.96 <sup>d</sup>	0.44 <sup>d</sup>	1.60 <sup>d</sup>	2.99 <sup>d</sup>	0.10 <sup>d</sup>	0.025 <sup>d</sup>	—	AAS	64
Linden	Romania	9	955.289	137.854	50.549	123.754	1.563	2.655	19.156	0.868	0.122	0.029	ICP-MS	55

Table 2. (continued)

Botanical origin	Country	No. of samples	K	Ca	Mg	Na	Cu	Zn	Fe	Mn	Ni	Cr	Method	Ref.
	Romania	12	291 <sup>d</sup>	43 <sup>d</sup>			0.62 <sup>d</sup>	2.9 <sup>d</sup>	6.9 <sup>d</sup>				XRF, FAAS, GFAAS	67
	Bulgaria	5	792	77	21	7.50	0.12	1.04	1.62	2.45	<0.01–0.92	<0.01–0.01	ICP-AES	60
	Turkey	1					0.14	0.48	2.97	0.14	0.22	0.004	AAS	70
	Italy	2	1653.5 <sup>d</sup>	86.2 <sup>d</sup>	31.3 <sup>d</sup>	16.8 <sup>d</sup>							IC	52
	Switzerland	8					0.382	0.999	0.654	1.292	0.040	0.003	ICP-MS	43
	Poland	1			13.0		0.52	13.41	2.11	1.39	0.89		ICP-AES	65
	Poland	7	393	39.7	16	18.9	0.3	8.4	2.5	1.1	0.4	0.2	AAS	62
	Romania	9	1648.16	101.518	75.415	229.333	3.354	3.871	28.285	2.529	0.325	0.049	ICP-MS	55
	Bulgaria	30	1331	103	83	17	0.55	1.2	3.0	12	<0.01–0.92	<0.01–0.04	ICP-AES	68
	Albania	1	340.6	41.2	16.3	21.0							IC	52
Honeydew	Italy	2	2569	397.9	64.3	62.45	1.94		8.65	0.45			FAAS, GFAAS	50
	Italy	1	1819.7	68.1	55.8	25.3							IC	52
	Switzerland	19					1.450	1.528	2.769	5.72	0.665	0.007	ICP-MS	43
	Poland	19	2387.6 <sup>d</sup>	25.7 <sup>d</sup>	3.44 <sup>d</sup>	28.99 <sup>d</sup>	1.02 <sup>d</sup>	3.18 <sup>d</sup>	7.2 <sup>d</sup>	4.09 <sup>d</sup>	0.965 <sup>d</sup>	0.0270 <sup>d</sup>	FAAS, ICP-MS	51
	Poland	6	621.0	53.4	45.2	20.0	0.90	5.2	2.70	3.5	0.4	ND	AAS	62
Rape	Hungary	6	332	51.2	17.7		0.164	3.66	1.35	0.614		0.01	ICP-OES, ICP-MS	54
	Romania	2	214 <sup>d</sup>	49 <sup>d</sup>			0.39 <sup>d</sup>	4.7 <sup>d</sup>	10.7 <sup>d</sup>				XRF, FAAS, GFAAS	67
	Bulgaria	6	105	46	11	8.49	<0.01–0.02	0.25	1.01	0.17	<0.01–0.04	<0.01–0.01	ICP-AES	60
	Poland	1			17.4		0.85	1.54	1.43	0.45	0.54	<0.005	ICP-AES	65
	Poland	11	200.3 <sup>d</sup>	52.4 <sup>d</sup>	6.17 <sup>d</sup>	29.30 <sup>d</sup>	1.69 <sup>d</sup>	0.94 <sup>d</sup>	2.9 <sup>d</sup>	0.3700 <sup>d</sup>	0.087 <sup>d</sup>	0.0298 <sup>d</sup>	FAAS, ICP-MS	51
	Poland	5	190.0	51.3	18.9	11.2	0.1	2.1	3.00	0.6	0.3	ND	AAS	62
	Hungary	5	439	111	22.4		0.27	3.35	0.648	1.1		0.010	ICP-OES, ICP-MS	54
	Romania	2	563 <sup>d</sup>	48.33 <sup>d</sup>	21.72 <sup>d</sup>	30.04 <sup>d</sup>	0.46 <sup>d</sup>	2.665 <sup>d</sup>	3.21 <sup>d</sup>	0.10 <sup>d</sup>	0.003 <sup>d</sup>	ND	AAS	64
	Romania	9	849.36	163.878	63.772	154.068	2.390	3.241	24.009	1.001	0.183	0.037	ICP-MS	55
Sunflower	Romania	4	179 <sup>d</sup>	50 <sup>d</sup>			0.22 <sup>d</sup>	3.6 <sup>d</sup>	10.5 <sup>d</sup>				XRF, FAAS, GFAAS	67
	Bulgaria	6	247	71	14	7.58	<0.01–0.07	0.61	1.93	0.36	<0.01–0.98	<0.01–0.01	ICP-AES	60
	Turkey	1					0.02	0.39	1.71	0.02	0.04		AAS	70

<sup>a</sup> Mineral and trace metal content reported in milligrams per kilogram.

<sup>b</sup> — = Not reported in original paper.

<sup>c</sup> TXRF = Total reflection X-ray fluorescence spectroscopy.

<sup>d</sup> Mean values were subsequently calculated, based on the data reported in the original paper.

<sup>e</sup> FAAS = Flame atomic absorption spectrometry; GFAAS = graphite furnace atomic absorption spectrometry.

<sup>f</sup> OES = Optical emission spectrometry.

<sup>g</sup> AAS = Atomic absorption spectrometry.

<sup>h</sup> XRF = X-ray fluorescence spectroscopy.

<sup>i</sup> AES = Atomic emission spectrometry.

<sup>j</sup> IC = Ion chromatography.

<sup>k</sup> ND = Not detected.

The results presented in Table 3 show statistically significant differences between honeys of various botanical origins. According to these results, the most influential factor with which to discriminate acacia honey from chestnut, honeydew, and linden honeys is the content of potassium. This element is also the most influential factor for distinguishing rape honey from acacia, chestnut, honeydew, and linden honeys,

as well as for distinguishing chestnut honey from sunflower honey.

Calcium, magnesium, and manganese are significant factors for the differentiation of chestnut honey. Magnesium could be used to discriminate acacia honey from chestnut and honeydew honeys, and manganese is the most influential factor for distinguishing honeydew honey from rape honey.

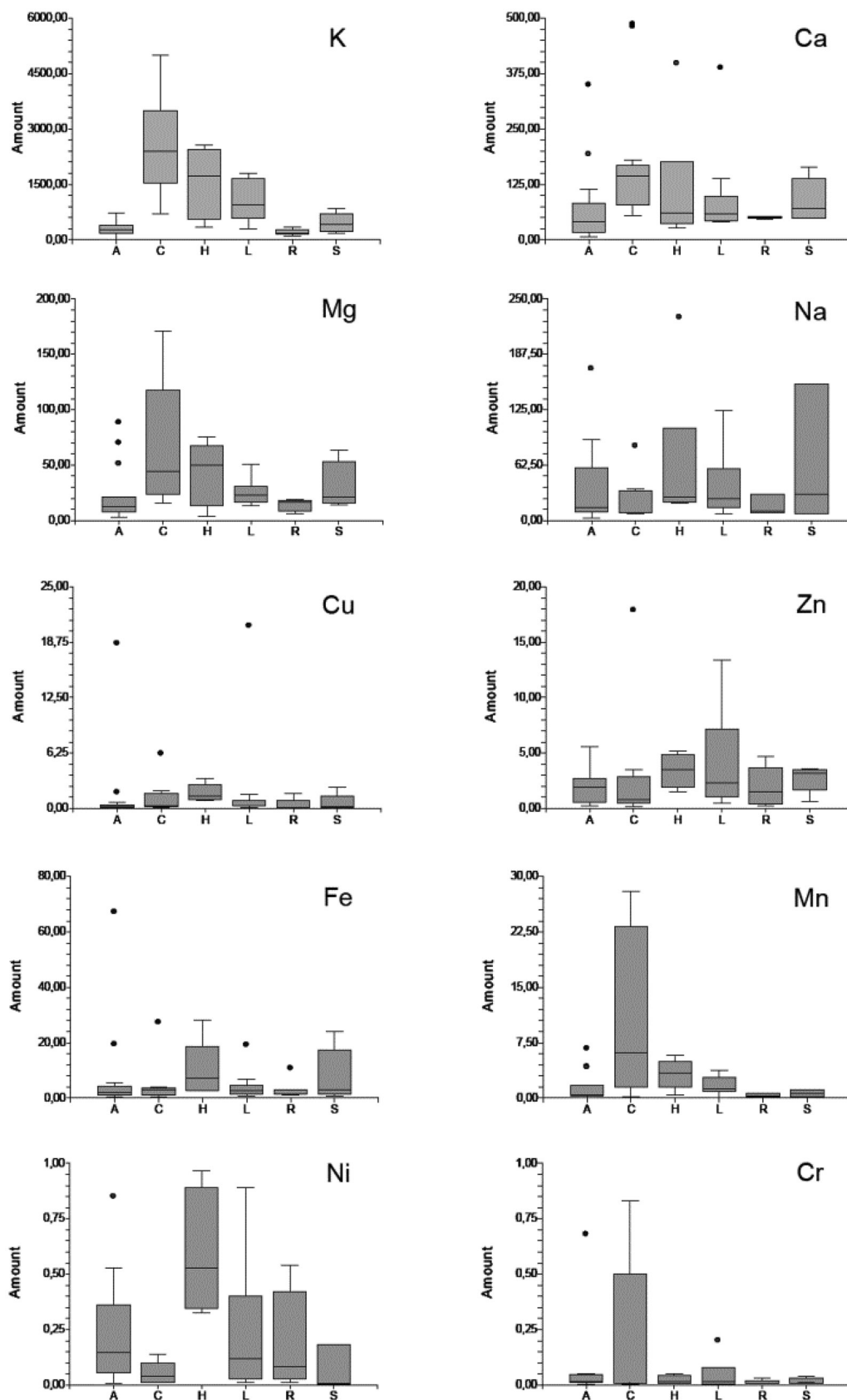


Figure 1. Mineral content of honey samples from Table 2.

**Table 3. Kruskal-Wallis test applied to the mineral content of honey with various botanical origins<sup>a</sup>**

Mineral	P	z-Value <sup>b</sup>
		A(C,H,L)
K	<0.0001	R(A,C,H,L) C(S)
Ca	0.0061	C(A,L,R)
Mg	0.0154	A(C,H) C(A,R)
Na	0.5104	— <sup>c</sup>
Cu	0.1010	H(A,R,S)
Zn	0.3869	—
Fe	0.5497	—
Mn	0.0069	C(A,R,S) H(R)
Ni	0.0667	H(C,S)
Cr	0.9340	—

<sup>a</sup> Botanical origin designations: A, acacia; C, chestnut; L, linden; H, honeydew; R, rape; and S, sunflower.

<sup>b</sup> Regular test: medians significantly different if z-value is >1.9600.

<sup>c</sup> — = Not calculated (samples belong to the same population according to Kruskal-Wallis test).

## References

- Consonni, R., Cagliani, L.R., & Cogliati, C. (2013) *Food Control* **32**, 543–548. doi:10.1016/j.foodcont.2013.01.038
- Juan-Borrás, M., Domenech, E., Hellebrandova, M., & Escriche, I. (2014) *Food Res. Int.* **60**, 86–94. doi:10.1016/j.foodres.2013.11.045
- Siddiqui, A.J., Musharraf, S.G., Choudhary, M.I., & Rahman, A.U. (2017) *Food Chem.* **217**, 687–698. doi:10.1016/j.foodchem.2016.09.001
- Karabagias, I.K., Louppis, A.P., Karabournioti, S., Kontakos, S., Papastephanou, C., & Kontominas, M.G. (2017) *Food Chem.* **217**, 445–455. doi:10.1016/j.foodchem.2016.08.124
- Bogdanov, S., Ruoff, K., & Oddo, L.P. (2004) *Apidologie (Celle)* **35**, S4–S17. doi:10.1051/apido:2004047
- Mateo, R., & Bosch-Reig, F. (1997) *Food Chem.* **60**, 33–41. doi:10.1016/S0308-8146(96)00297-X
- de la Fuente, E., Ruiz-Matute, A.I., Valencia-Barrera, R.M., Sanz, J., & Martinez-Castro, I. (2011) *Food Chem.* **129**, 1483–1489. doi:10.1016/j.foodchem.2011.05.121
- Da Costa Leite, J.M., Trugo, L.C., Costa, L.S.M., Quinteiro, L.M.C., Barth, O.M., Dutra, V.M.L., & De Maria, C.A.B. (2000) *Food Chem.* **70**, 93–98. doi:10.1016/S0956-7135(99)00115-2
- Swallow, K.W., & Low, N.H. (1990) *J. Agric. Food Chem.* **38**, 1828–1832. doi:10.1021/jf00099a009
- Cotte, J.F., Casabianca, H., Chardon, S., Lheritier, J., & Grenier-Loustalot, M.F. (2004) *Anal. Bioanal. Chem.* **380**, 698–705. doi:10.1007/s00216-004-2764-1
- Nozal, M.J., Bernal, J.L., Toribio, L., Alamo, M., Diego, J.C., & Tapia, J. (2005) *J. Agric. Food Chem.* **53**, 3095–3100. doi:10.1021/jf0489724
- de la Fuente, E., Sanz, M.L., Martinez-Castro, I., Sanz, J., & Ruiz-Matute, A.I. (2007) *Food Chem.* **105**, 84–93. doi:10.1016/j.foodchem.2007.03.039
- Ruiz-Matute, A.I., Ramos, L., Matinez-Castro, I., & Sanz, M.L. (2008) *J. Agric. Food Chem.* **56**, 8309–8313. doi:10.1021/jf8014552
- Kaškonienė, V., Venskutonis, R., & Čeksteryte, V. (2010) *Food Sci. Technol. (Campinas)* **43**, 801–807. doi:10.1016/j.lwt.2010.01.007
- Ruiz-Matute, A.I., Brokl, M., Soria, A.C., Sanz, M.S., & Martinez-Castro, I. (2010) *Food Chem.* **120**, 637–642. doi:10.1016/j.foodchem.2009.10.050
- Primorac, L.J., Flanjak, I., Kenjerić, D., Bubalo, D., & Topolnjak, Z. (2011) *Czech J. Food Sci.* **29**, 515–519
- Bentabol Manzanares, A., Herdández García, Z., Rodríguez Galdón, B., Rodríguez Rodríguez, E., & Díaz Romero, C. (2011) *Food Chem.* **126**, 664–672. doi:10.1016/j.foodchem.2010.11.003
- Escuredo, M., Dobre, I., Fernández-González, M., & Seijo, M.S. (2014) *Food Chem.* **149**, 84–90. doi:10.1016/j.foodchem.2013.10.097
- Siddiqui, I.R., & Furgala, B. (1970) *Adv. Carbohydr. Chem. Biochem.* **25**, 285–309. doi:10.1016/S0065-2318(08)60430-8
- Doner, L.W. (1977) *J. Sci. Food Agric.* **28**, 443–456. doi:10.1002/jsfa.2740280508
- Weston, R.J., & Brocklebank, L.K. (1999) *Food Chem.* **64**, 33–37. doi:10.1016/S0308-8146(98)00099-5
- Sanz, M.L., Gonzalez, M., de Lorenzo, C., Sanz, J., & Martinez-Castro, I. (2004) *J. Sci. Food Agric.* **84**, 1577–1584. doi:10.1002/jsfa.1823
- Korošec, M., Bertonec, J., Gonzales, P.A., Kropf, U., Golob, U., & Golo, T. (2009) *Acta Aliment.* **38**, 459–469. doi:10.1556/AAlim.38.2009.4.6
- Martins, R.C., Lopes, V.V., Valentao, P., Carvalho, J.C.M.F., Isabel, P., Amaral, M.T., Batista, M.T., Andrade, P.B., Branca, M., & Silva, B.M. (2008) *Nat. Prod. Res.* **22**, 1560–1582. doi:10.1080/14786410701825004
- Senyuva, H.Z., Gilbert, J., Silici, S., Charlton, A., Dal, C., Rel, N.G., & Cimen, D. (2009) *J. Agric. Food Chem.* **57**, 3911–3919. doi:10.1021/jf900039s
- Ouchemoukh, S., Schweitzer, P., Bey, M.B., Djoudad-Kadji, H., & Louaileche, H. (2010) *Food Chem.* **121**, 561–568. doi:10.1016/j.foodchem.2009.12.047
- Astwood, K., Lee, B., & Manley-Harris, M. (1998) *J. Agric. Food Chem.* **46**, 4958–4962. doi:10.1021/jf980720d
- Persano Oddo, L., & Piro, R. (2004) *Apidologie (Celle)* **35**, S38–S81. doi:10.1051/apido:2004049
- Can, Z., Yildiz, O., Sahin, H., Turumtay, E.A., Silici, S., & Kolay, S. (2015) *Food Chem.* **180**, 133–141. doi:10.1016/j.foodchem.2015.02.024
- Low, N.H., & Sporns, P. (1988) *J. Food Sci.* **53**, 558–561. doi:10.1111/j.1365-2621.1988.tb07755.x
- Sabatini, A.G., Persano Oddo, L., Piazza, M.G., Accorti, M., & Marazzan, G. (1990) *Apicoltura* **6**, 63–70
- Goodall, I., Dennis, M.J., Parker, I., & Sharman, M. (1995) *J. Chromatogr. A* **706**, 353–359. doi:10.1016/0021-9673(94)01074-0
- Radovic, B., Goodacre, R., & Anklam, E. (2001) *J. Anal. Appl. Pyrolysis* **60**, 79–87. doi:10.1016/S0165-2370(00)00163-7
- Cotte, J.F., Casabianca, H., Chardon, S., Lheritier, J., & Grenier-Loustalot, M.F. (2003) *J. Chromatogr. A* **1021**, 145–155. doi:10.1016/j.chroma.2003.09.005
- Rybak-Chmielewska, H., Szezęsna, T., Was, E., Jańkiewicz, K., & Teper, D. (2013) *J. Apic. Sci.* **57**, 51–59. doi:10.2478/jas-2013-0006
- Jang, E.S., Kim, I.S., Lee, E.J., Seo, H.S., Lee, H.J., Kim, E., Kim, K.T., & Kim, J.B. (2016) *Korean J. Food Sci. Technol.* **48**, 1–8. doi:10.9721/KJFST.2016.48.1.1
- Kropf, U., Jamnik, M., Bertonec, J., & Golob, T. (2008) *Food Technol. Biotechnol.* **46**, 335–340
- Silva, L.R., Videira, R., Monteiro, A.P., Valentão, P., & Andrade, P.B. (2009) *Microchem. J.* **93**, 73–77. doi:10.1016/j.microc.2009.05.005

- (39) Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsi, F., Ferrari, G., & Hamdi, S. (2014) *Arab. J. Chem.* In press, <http://dx.doi.org/10.1016/j.arabjc.2014.08.011>
- (40) Yadata, D. (2014) *Food Sci. Technol. (Campinas)* **2**, 59–63. doi:10.13189/fst.2014.020501
- (41) Kivrak, S., Kivrak, I., & Karababa, E. (2016) *Food Sci. Technol. (Campinas)* Epub July 28, 2016, <http://dx.doi.org/10.1590/1678-457X.07916>
- (42) Pohl, P. (2009) *Trends Analyt. Chem.* **28**, 117–128. doi:10.1016/j.trac.2008.09.015
- (43) Bogdanov, S., Haldimann, M., Luginbühl, W., & Gallmann, P. (2007) *J. Apic. Res. Bee World* **46**, 269–275. doi:10.1080/00218839.2007.11101407
- (44) Stankovska, E., Stafilov, T., & Šajn, R. (2008) *Environ. Monit. Assess.* **142**, 117–126. doi:10.1007/s10661-007-9913-x
- (45) Rashed, M.N., El-Haty, M.T.A., & Mohamed, S.M. (2009) *Toxicol. Environ. Chem.* **91**, 389–403. doi:10.1080/02772240802294870
- (46) Berinde, Z.M., & Michnea, A.M. (2013) *J. Sci. Arts* **2**, 173–180
- (47) Sitarz-Palczak, E., Kalembkiewicz, J., & Galas, D. (2015) *J. Ecol. Eng.* **16**, 130–138. doi:10.12911/22998993/2946
- (48) Golob, T., Doberšek, U., Kump, P., Nečemer, M., (2005) *Food Chem.* **91**, 593–600. doi:10.1016/j.foodchem.2004.04.043
- (49) Nečemer, M., Košir, I.J., Kump, P., Kropf, U., Jamnik, M., Bertoneclj, J., Ogrinc, N., & Golob, T. (2009) *J. Agric. Food Chem.* **57**, 4409–4414. doi:10.1021/jf900930b
- (50) Conti, M.E., Stripeikis, J., Campanella, L., Cucina, D., & Tudino, M.B. (2007) *Chem. Cent. J.* **1**, 14. doi:10.1186/1752-153X-1-14
- (51) Madejczyk, M., & Baralkiewicz, D. (2008) *Anal. Chim. Acta* **617**, 11–17. doi:10.1016/j.aca.2008.01.038
- (52) Fermo, P., Beretta, G., Facino, R.M., Gelmini, F., & Piazzalunga, A. (2013) *Environ. Pollut.* **178**, 173–181. doi:10.1016/j.envpol.2013.03.029
- (53) Bilandžić, N., Gačić, M., Đokić, M., Sedak, M., Ivanec Šipušić, Đ., Končurat, A., & Tlak Gajger, I. (2014) *J. Food Compos. Anal.* **33**, 132–138. doi:10.1016/j.jfca.2013.12.002
- (54) Czipa, N., Andrasi, D., & Kovacs, B. (2015) *Food Chem.* **175**, 536–542. doi:10.1016/j.foodchem.2014.12.018
- (55) Oroian, M., Amariei, S., Leahu, A., & Gutt, G. (2015) *Pol. J. Food Nutr. Sci.*, **65**, 93–100. doi:10.1515/pjfn-2015-0018
- (56) Kropf, U., Korošec, M., Bertoneclj, J., Ogrinc, N., Nečemer, M., Kump, P., & Golob, T. (2010) *Food Chem.* **121**, 839–846. doi:10.1016/j.foodchem.2009.12.094
- (57) Uršulin-Trstenjak, N., Levanić, D., Primorac, Lj., Bošnjir, J., Vahčić, N., & Šarić, G. (2015) *Czech J. Food Sci.* **33**, 156–164. doi:10.17221/502/2014-CJFS
- (58) Di Bella, G., Lo Turco, V., Potorti, A.G., Bua, G.D., Fede, M.R., & Dugo, G. (2015) *J. Food Compos. Anal.* **44**, 25–35. doi:10.1016/j.jfca.2015.05.003
- (59) Kolayli, S., Kongur, N., Gundogdu, A., Kemer, B., Duran, C., & Aliyazicioglu, R. (2008) *Asian J. Chem.* **20**, 2421–2425
- (60) Atanassova, J., Yurkova, L., & Lazarova, M. (2012) *Czech J. Food Sci.* **30**, 520–526
- (61) Perna, A., Simonetti, A., Intaglietta, I., Sofo, A., & Gambacorta, E. (2012) *Int. J. Food Sci. Technol.* **47**, 1909–1917. doi:10.1111/j.1365-2621.2012.03050.x
- (62) Grembecka, M., & Szefer, P. (2013) *Environ. Monit. Assess.* **185**, 4033–4047. doi:10.1007/s10661-012-2847-y
- (63) Pătruică, S., Hărmănescu, M., Bura, M., Jivan, A., & Ciobănas, C. (2008) *Sci. Pap. Anim. Sci. Biotechnol.* **41**, 325–327
- (64) Pătruică, S., Hărmănescu, M., Jivan, A., Cioabă, C., Simiz, E., & Călar, C.D. (2009) *Sci. Pap. Anim. Sci. Biotechnol.* **42**, 178–181
- (65) Nowak, L., Dzieżyc, H., & Piotrowski, M. (2011) *J. Elem.* **16**, 437–444. doi:10.5601/jelem.2011.16.3.08
- (66) Czipa, N., Burján, Z., Andrasi, D., & Kovács, B. (2012) *Eur. Chem. Bull.* **1**, 446–448
- (67) Stihl, C., Chelarescu, E.D., Duliu, O.G., & Toma, L.G. (2016) *Rom. Rep. Phys.* **68**, 362–369
- (68) Atanassova, J., Lazarova, M., & Yurkova, L. (2016) *J. Cent. Eur. Agric.* **17**, 640–651. doi:10.5513/JCEA01/17.3.1756
- (69) Rodríguez-Flores, S., Escuredo, O., & Carmen, S.-C.M. (2016) *J. Apic. Sci.* **60**, 19–30. doi:10.1515/JAS-2016-0002
- (70) Pehlivan, T., & Gül, A. (2015) *J. Appl. Pharm. Sci.* **5**, 042–045. doi:10.7324/JAPS.2015.50807
- (71) Alqarni, A.S., Owayss, A.A., Mahmoud, A.A., & Hannan, M.A. (2014) *J. Saudi Chem. Soc.* **18**, 618–625. doi:10.1016/j.jscs.2012.11.009