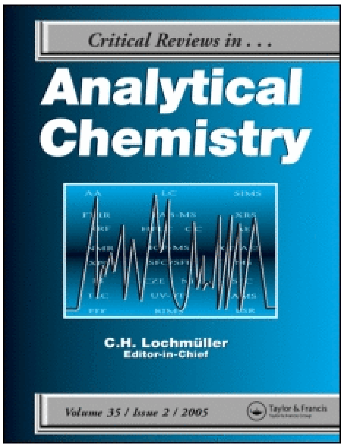

Critical Reviews in Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400837

Determination and Fractionation of Metals in Honey

Pawel Pohl[a]; Iwona Sergiel[b]; Helena Stecka[a]
[a] Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland [b] Faculty of Biotechnology, University of Zielona Gora, Zielona Gora, Poland

To cite this Article Pohl, Pawel , Sergiel, Iwona and Stecka, Helena(2009) 'Determination and Fractionation of Metals in Honey', Critical Reviews in Analytical Chemistry, 39: 4, 276 — 288
To link to this Article: DOI: 10.1080/10408340903001250
URL: http://dx.doi.org/10.1080/10408340903001250

Critical Reviews in Analytical Chemistry, 39:276–288, 2009

ISSN: 1040-8347 print / 1547-6510 online
DOI: 10.1080/10408340903001250

Determination and Fractionation of Metals in Honey

Pawel Pohl,[1] **Iwona Sergiel,**[2] **and Helena Stecka**[1]
[1]*Faculty of Chemistry, Wroclaw University of Technology, Wroclaw, Poland*
[2]*Faculty of Biotechnology, University of Zielona Gora, Zielona Gora, Poland*

The composition of metals in honey is correlated with the type of plant species from which resources (nectar, pollen, propolis, honeydew) are collected and ripened by honey bees into honey. However, the botanical origin is strictly attributed to the geographical locality of an apiary, because the soil composition and the climatic conditions determine the presence of certain melliferous flowers and trees harvested by bees. The environmental pollution or other anthropogenic processes and effects should also be considered as an additional source of metals in honey, namely Cd, Cr, Cu, Fe, Ni, Pb and Zn. These metals may condemn the quality and food safety of honey, and exhibit a potential hazard to human health.

The present literature survey reviews original works of the last decade which relate to the atomic and mass spectrometric analysis of honey on the presence of major, minor and trace metals. Different aspects of such analysis are treated in detail, including the methods of choice, calibration strategies, sample treatments, and preparation procedures applied. The suitability of the metals content for the classification of honey according to the botanical and geographical origins or indication and control of the environmental pollution are discussed.

Keywords Honey, metals, determination, fractionation, atomic spectrometry, mass spectrometry

INTRODUCTION

Honey is a viscous substance elaborated by domesticated honey bees (*Apis mellifera* and *Apis cerana*) mostly from nectar of honey plants. Additional forage sources for the honey bees can be honeydew excreted by plant sap sucking insects or sugary deposits of plants and trees. Normally, honey is stored by bees in honeycombs of a beehive where it ripens and matures and then remains an essential food supply for the bee colony development and activity (1). Owing to its special flavor and sweet taste, honey has been long appreciated as a desired natural sweetener consumed by humans without its previous processing (2). Being a valuable constituent of a rough daily diet, honey is also an important ingredient of other food products, beverages and pharmaceuticals (3, 4).

All the properties of honey, including nourishing and healing effects, result from its unique chemical composition and a specific processing subjected by bees to the collected forage sources (3, 5). The main parts of honey are simple sugars and water (see Fig. 1). With respect to the carbohydrates, the average content of fructose and glucose in honey is 38.2 and 31.3%, respectively (4). Other carbohydrates are maltose (7.3% on average), saccharose (2.4% on average), and few high sugars (1.5% on average). The average moisture content is 17.2%. Honey additionally contains proteins and enzymes (diastase, invertase, glucose oxidase, and catalase), amino acids, organic acids (acetic, butyric, citric, succinic, lactic, malic, gluconic, and ascorbic), vitamins (riboflavin, niacin, folic acid, panthothenic acid, and B6), flavonoids, and minerals (1, 4). Major, minor, and trace metals are the minor constituents of honey. Their composition and content are characteristic to the floral type of honey, geochemical and climatic conditions attributed to the forage area, and locality of an apiary. Usually, the total content of all metals in flora honeys contribute to about 0.1–0.2% of their total composition. In the case of honeydew honeys it may exceed more than 1% of the total composition (6).

The knowledge referring to the specific composition of metals in honey appears to be of a primary interest (7). The evaluation of the total metal concentration enables one to assess the nutritive quality of honey originated from different regions, and also helps in tracking and judging its authenticity according to certain geographical and biological origins. Furthermore, the content of metals is recognized to be useful for the purpose of honey classification and categorization because metals are stable honey components and highly distinctive for the certain conditions and surroundings. Their presence in honey largely depends on the mix of nectars of blossoming plants and secretions available to the bees during their forage in a given territory.

Address correspondence to Pawel Pohl, Analytical Chemistry Division, Faculty of Chemistry, Wroclaw University of Technology, Wybrzeze Stanislawa Wyspianskiego 27, Wroclaw 50-370, Poland. E-mail: pawel.pohl@pwr.wroc.pl

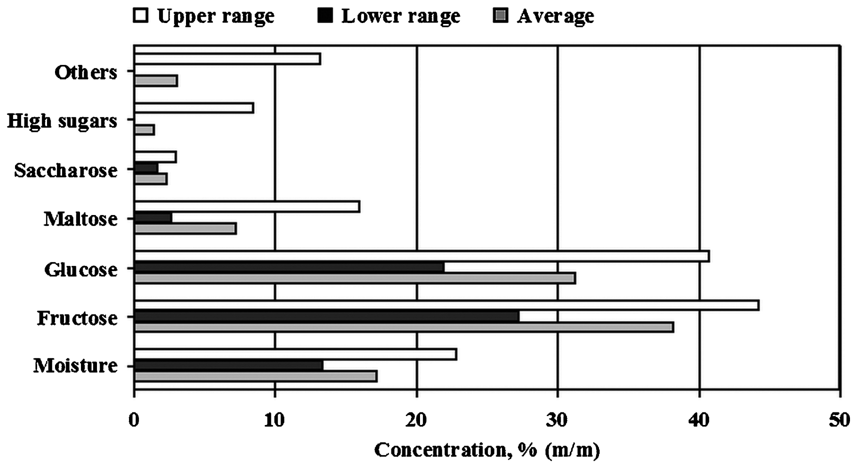

FIG. 1. Percentage contributions of honey constituents.

Reviewing research works devoted to the analysis of honey according to the metal content in the last decade points out that metals are primarily determined to assess the wholesomeness of honey in terms of the presence of essential metals and to assure human health safety due to the regulations or directives ordering admissible levels for undesirable and toxic metals. The measurements of metals in honey are also relevant for the environmental monitoring purposes as honey is regarded as a suitable biomarker. Finally, the data matrix related to the concentration of metals in honey is useful for the classification of honey due to botanical and geographical origins and its genuineness.

Among different instrumental techniques used for the determination of metals in honey, atomic and mass spectrometry are regarded as the most sensitive, accurate, and robust (8–12); therefore, these methods are routinely and customarily applied. In the case of major and some minor metals present in honey at concentrations ranging from thousands to several μg/L, atomic absorption and emission spectrometry with flames (FAAS, FAES) are preferred. To control the quality of honey in terms of the adulteration or contamination made by heavy metals, many sensitive techniques are required, including electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS). The analysis of honey by atomic and mass spectrometry methods is, however, a difficult and challenging task. Honey is a very complex organic matter matrix that poses problems related to sample heterogeneity, selection of proper sample treatment, and decomposition as well chemical interferences during measurements (12).

SOURCES OF METALS IN HONEY

The main natural sources of metals in honey are the environment of a beehive and an apiary, honey plants, and a bee population. Particularly, the soil composition related to different geochemical and geological features (geographical origin), i.e., volcanic and hydrothermal activity, regional conditions, and climatic changes occurred in the forage area of bees, affect the composition and content of major and minor metals in honey (7, 13–17). Certain metals are transported through the root system to honey plants and their composition in nectar reflects the soil characteristics. However, it should be noted that metal uptake by plants is dependent on their nutrient requirements. For example, acacia has very low nutrient requirements and can grow in very unfavorable nutrient conditions; hence, its nectar is scarce in minerals (18). Another source of metals in honey of geographical origin can be the vicinity of sea or ocean and a consequence of marine sprays. Their proximity leads to an elevated presence of K and Na in honeys from costal regions or islands (6, 19).

The main food source of bees during their foraging activity consists of nectar and pollen of flowering plants which are within a flight range and can cover the area of about 7 or 50 km^2 as stated by some authors (17). The floral type of honey plants, floral density, and the chemical composition of nectar, pollen, and other forage sources extensively differ with the location of the apiary, regional conditions, as well as season and type of vegetation. As a result, the botanical origin of honey has a remarkably effect on the content of certain metals in honey (8, 12–15, 17, 19–24).

Beside the geographical and botanical origins of metals in honey, the content of some heavy metals can result from different anthropogenic sources (7, 18, 25). As mentioned before, the bee forage area is relatively large and includes variety of the environments and feedings. The honey bees come into a contact with different parts of the surroundings and are exposed to potential pollutants when they forage for nectar, pollen, honeydew, or other exudates within such a territory (5). They touch flowers, branches, and leaves of plants, drink water from ponds and collect different kind of soil particles, dusts, and other deposits from the flowers, and aerosols and particles dispersed in the air (7, 18, 24, 26). If the area around the beehive is contaminated, honey bees become contaminated as well and carry the pollutants into the hive by themselves or with the raw materials collected (24, 25, 27–29). In this way, the contaminants of air, water, and soil can reach the honey and change its composition and quality (9, 30–32). Usually, the neighborhood of mines and steelworks, industrialized and urban areas, or highways in the forage area can result in the contamination of the apiary locality and is reflected by higher levels of certain metals in honey, particularly Al, Ba, Ca, Cd, Cr, Cu, Mg, Mn, Ni, Pb, Pd or, Zn, due to the chemical wastes, exhausts, or other pollutants emitted (5, 13, 16–18, 30, 32–34). Honey originated from rural areas, where there is a relatively low density of automotive traffic, typically presents low levels of heavy metals, e.g., Cd and Pb (5). The extent of the contamination can additionally be dependent on the floral origin of honey plants visited by bees (8). Accordingly, honeys elaborated from nectar of aromatic plants are characterized by extremely high concentrations of heavy metals since they tend to concentrate pollutants more easily than herbaceous plants.

Honey can be also polluted with certain metals during its harvesting and processing and this is primarily related to the apiculture technology used by the beekeepers (see Table 1). Different conditions of honey processing can result in significant differences in the content of Al, Cd, Co, Cr, Cu, Fe, Pb, Ni, and Zn. These metals can be released from materials (stainless steel, galvanized steel, and aluminum) of tools and equipment used for honey crop, extraction, centrifugation, or ripening. The materials of the containers applied for honey processing, shipping, and storage of the extracted and ripened honey or lids may also contribute to an increase of the mentioned metals in honey (13, 16, 22, 23, 25, 27, 30, 32, 35–37). The extent of this process is facilitated by the honey acidity (pH 3.5–4.8) and its corrosive effect on the surface of tools and containers (14, 16, 23, 27, 38). This metal source may be, however, reduced by proper selection of materials and careful following of admissible apicultural procedures and standards.

TABLE 1
Possible beekeeping related sources of contamination in honey

Apicultural practice or processing stage	Contamination source
Super and frames removal	Hive paints and preservatives, soil, plant materials, environment
Uncapping and extraction	De-capping equipment, extractors, containers (holding tanks), surrounding (moisture, dust)
Filtration	Equipment (ripeners, strainers, sieves, clarifiers, filters, heaters), containers, surrounding
Settling	Containers (buckets, ripeners, storage tanks), surrounding (moisture, dust, high temperature)
Bottling	Equipment (bottling and sealing machines), containers (jars, beers, bottles), dust, moisture, high temperature

Metals can be also be introduced with substances used for feeding the bees in the case of an absence of vigorous honey flows. Accordingly, syrup-feed honeys can exhibit elevated amounts of Cd, Co, Fe, K, Mg, Mn, Pb, and Na, which are contaminants of syrups derived from industrial processing (39).

ROLE OF METALS IN HONEY

In reference to major, minor, and trace metals, the mineral content of honey varies within a wide range as a result of differentiated geographical and floral provenience and, to a lesser degree, apicultural practices applied. The most important and abundant metal in honey is K. This mineral can account for 45 to 85% of the total mineral content quantified in honey (10, 14, 19, 21, 22, 30, 37, 38, 40–52). Other major metals of honey are Rb, Na, and Ca. Mg, Fe, Al, Zn, and Li are the constituents of honey with intermediate amounts. Finally, at levels below 100 and 1 μg/g, respectively, minor and trace metals are present in honey (see Table 2).

Concerning the nutritive quality and possible dietary supplementation, the presence of metals in honey is essential to human health (11, 17, 53). It can be regarded as a rich source of metals required by humans for well-being, but not merely the metal content but the bioavailability is of a prime importance when considering the nourishing value related to the honey consumption (54). Although major, minor, and trace metals participate in the nutritional value of honey, many factors affect their actual bioavailability from honey, namely the speciation forms (organic or inorganic) in which they are present in honey and the presence or absence of other substances and elements that have

TABLE 2
Concentrations (in μg/g) of major, minor and trace metals in honey[a]

Major metals	Minor and trace metals	
K 9.3–6785	Mn ND–82.0	Th ND–1.10
Rb ND–3193	Ti 0.10–71.0	In ND–1.10
Na 0.38–1221	Cu ND–34.6	Hg 0.0007–1.35
Ca 1.50–900	Sr ND–23.1	Mo ND–0.81
Mg ND–373	Ni ND–13.0	U 0.004–0.015
Fe ND–342	Pb ND–8.22	V ND–0.18
Al 0.004–132	Bi ND–7.63	Sn ND–0.008
Zn ND–113	Cd ND–5.78	Th 0.0001–0.003
Li ND–110	Cr ND–4.48	Be ND
	Ba ND–2.63	Pt ND
	Co ND–2.46	
	Ag 0.02–2.16	

[a] On the bases of all references cited
ND Not detected

an effect on their absorption in a intestinal tract (54). As a matter of fact, the total content of metals in honey may not directly correspond to their availability. Honey is additionally consumed in much lower amounts as other foodstuffs, and therefore, the actual intake of metals from honey is low. It usually contributes to not more than one to several percents or lower values, e.g., 0.005–0.5%, of recommended dietary allowances (RDAs) for many major, minor, and trace metals (K, Na, Ca, Mg, Fe, Mn, Zn, and Cu) (39, 40, 55). The content of certain metals in honey is of interest in terms of its wholesomeness and potential health risks (24, 51, 56). The excessive levels of some heavy metals are undesirable due to their supposed toxicity and a detrimental effect to the organism (11, 32, 57). Potentially toxic metals should not cause any concern about the consumer health. Therefore, the analysis of honey according to the concentrations of many metals is significant for its quality assessment and control. However, the admissible levels for contaminant metals in honey like Cd, Pb, Hg, Cu, and Zn are not settled or defined (5). The directive of the European Union Commission relating to honey includes some general and specific properties of honey composition but no guidelines about the content of heavy metals are given (58). No specific regulation about the presence of heavy metals, except for a statement that honey shall be free from them in amounts which may result in a hazard to the human health, is included either in the Codex Alimentarius (59). In consequence, the tolerable levels of individual metals that exhibit a potential threat or latent toxicity are regulated by national legislation (18, 46, 60) or standards for other food products (17, 18).

It is established that the mineral composition of honey is strictly correlated with its color, ash content, and electrical conductivity (7, 10, 11, 14, 21, 37, 45, 48, 49, 52, 53, 61). As compared to pale and light blossom honeys (orange, linden, and acacia), heavy-bodied dark and amber honeys (avocado, chestnut, and heather) contain higher amounts of certain major, minor, and trace metals, including Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, and Ni, and Zn, and usually produce higher ash content and electrical conductivity measured. Particularly, honeydew honeys are characterized by significantly higher metal concentrations as compared to nectar honeys (5, 7, 14, 17, 19, 30, 35, 46, 48, 53, 61, 62). Amber light and medium amber honeys (eucalyptus and thyme) exhibit medium quantities of the total mineral content. Interestingly, the color of dark honeys is mostly linked with the concentrations of Cd, Fe, and Pb, while the color of light and brown honeys is associated in a high degree with only Al and Mg content (48). Pb and Cr are found to be the only metals which are not strongly correlated with the electrical conductivity of honey (7). Likely, these metals are present in honey in the form of metal particles or not-dissociating metal species. It is also reasonable that they are introduced to honey through the pollution of air and bees or the pollutants of natural resources (7).

Because honey results from a bi-accumulation of different forage resources which are ripened by bees in the beehive, it is well acknowledged to accumulatively gather the information related to the changes in the environment within the forage area of bees (24, 51, 56, 63). Hence, the investigation of honey on the presence of trace metals, particularly Cd, Cu, Hg, Ni, and Pb, can be useful for the assessment and indication of the quality of the environment in different regions and the level of air, water, plant, and soil pollution with potentially hazardous contaminants over a large terrain within the foraging activity of bees (1, 7, 9, 14–16, 28–31, 33, 34, 39, 45, 51, 55, 56, 60, 64, 65). However, when applying honey as an indicator of the environmental quality in the settlement and industrialized regions, where the pollution with heavy metals is higher, the effect of the apicultural practices and processing made by beekeepers should be also considered (66).

Contrary, the susceptibility of honey for the environmental pollution monitoring is questioned because the evidence of the bi-accumulation of metals in honey is rather weak (64). It is postulated that the use of honey as a reliable bio-indicator could be restricted only to a very serious contamination. The low concentrations of trace metals and a great variability of their content as a result of botanical origin, seasonal, and climatic changes and different beekeeping practices also argue that honey may not be a very sensitive biological matrix for tracing the anthropogenic anomalies and heavy metal loads into the environment (32, 57). Sometimes, the differences between the concentrations of contaminants, for example Cd and Pb, in honeys originated from polluted and unpolluted areas are statistically insignificant or even the highest values are found in the samples from villages and country sides (7, 18). Hence, the botanic and geographic origins should also be considered in this kind of investigation since they have the greatest influence on the trace metal content in honey (7).

TAXONOMETRY OF HONEY

The demand for honey discrimination according to its geographical and botanical origins is of special significance since it ensures the stated quality and the provenience of honey (10, 67–69). Despite the national legislation or local regulations concerning proper honey production and labeling, for example, in countries of the European Union floral origin, source, organoleptic characteristic, physicochemical properties, as well as regional, territorial, or topographical conditions have to be specified (58, 67); falsification and adulteration of honey by mixing high-value original honeys with those of much lower grade and quality are very common (21, 70). A dilution with water or an addition of syrup are other manipulations that are frequently subjected to honey causing its deception (21). To combat the consumer and economic fraud, the authenticity of honey is identified and intends to differentiate natural and original honeys manufactured in a certain region by traditional procedures from those which are mislabeled, artificial, highly processed, or adulterated.

A traditional method of evidence of the geographical location and genus of honey plants is melissopalynology aimed on the

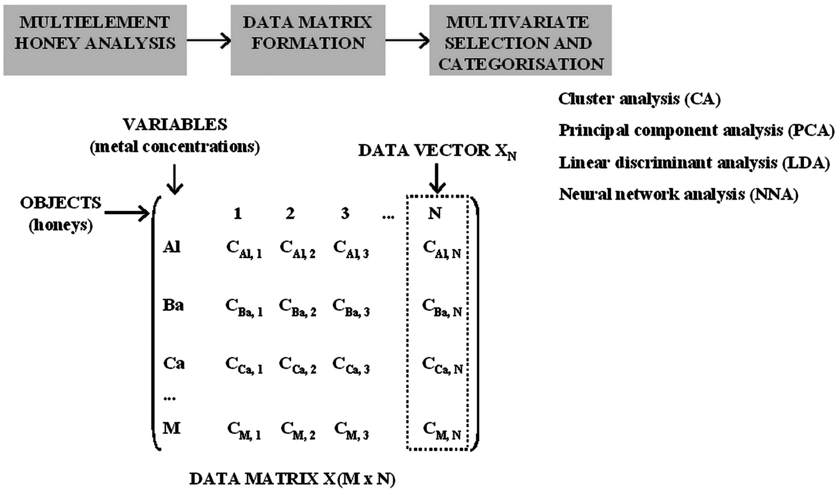

FIG. 2. Steps of honey taxonometry according to metals content.

study of pollen loads and components within honey and the recognition their sources (14).

The taxonometric examination of minor components of honey, particularly the metals, by means of multivariate statistical analysis of their concentrations (see Fig. 2) can be an alternative way of the recognition and discrimination of honey, in addition to the detection of potential honey fraud and deception (6–8, 10, 14, 15, 17, 19, 21, 22, 31, 35, 39, 43, 46, 47, 57, 62, 68, 70). This practice guarantees the quality of honey in the market to comply with the information stated on the label and certifies its strict properties and origin (10, 21, 27, 30). In general, metals are good descriptors of the floral and geographical origins. They are stable over a very long period because honey is resistant to microbial growth and spoilage, their concentrations are influenced by the type of soil in which the nectar producing plants are growing, the vegetation conditions in the forage area, and the type of blooming trees and plants visited by bees (10, 19, 35, 68, 70). Hence, the metal content is highly indicative for the characterization and identification of honey from a given area (6, 10, 15, 35). Usually, an excess or a deficiency of certain metals in soil and water is reflected by the mineral composition of the raw materials collected by bees for the honey production, i.e., nectar, pollen, and honeydew. For the same reason, mineral composition analysis can be useful for detecting the subjected adulterations, i.e., dilution with water dilution, addition of sugar and syrup, and honey blending (14, 39). Usually, the changes in the metal composition and content of honey due to different natural and external resources as well as possible adulterations is also very well reflected by other physicochemical parameters, i.e., the ash content and the electrical conductivity (17, 60).

However, metals derived from other sources, especially of anthropogenic origin, should also be considered when analyzing and interpreting the results of the multivariate statistical analysis of the metal content of honey (7).

METHODS OF ANALYSIS

Flame and Electrothermal Atomic Absorption Spectrometry

FAAS is a very common method of honey analysis applied mostly for the determination of alkali and alkaline earth metals that are the most abundant mineral constituents of honey (see Table 3). The advantages of FAAS are low operational costs and reasonably good analytical performance; however, linear response ranges are relatively narrow that imposes an adequate dilution of samples before measurements in order to match the linear ranges of the calibration curves (9, 71). The concentrations of K, Na, Ca, and Mg can be so high that the prepared sample solutions have to be diluted by 50 to 500 times. On the other hand, the dilution of the sample solutions results in a minimization of chemical interferences in commonly used acetylene-air flames. The NO_2-acetylene flame is applied for the determination of Ca and Sr (6).

The concentrations of minor and some trace metals (Mn, Fe, Zn, Sr, Cu, Ni, Co, Pb, and Cd) can also be measured using FAAS. These metals are frequently determined in undiluted sample solutions. Occasionally, metals in the sample solutions have to be initially pre-concentrated. For instance, Cd and Pb are complexed by APDC (ammonium pyrrolidine dithiocarbamate) and extracted in this form into IBMK (methyl isobuthyl keton), and then, the organic phase is analyzed (6). Hg is measured using cold vapor generation (CV-AAS) (26, 65).

Due to possible matrix effects, the samples of honey for the FAAS analysis are frequently mineralized (11, 15, 16, 23, 30, 34–36, 39, 40, 42, 45, 51, 56, 60, 62, 68, 70–72, 75, 80). The concentrations of metals can be determined in the resulting sample solutions using aqueous external standard solutions and simple linear regression for the calibration (6, 11, 15, 30, 36, 40, 42, 45, 47, 56, 62, 71). Direct analysis of sample solutions prepared by dissolution of respective honey portions in water containing small amounts of HNO_3 is very uncommon (6, 15,

TABLE 3
Determinations of metals in honey by means of atomic absorption spectrometry with flame and electrothermal atomizers (FAAS, ETAAS)

FAAS	ETAAS
Cd (11, 18, 30, 34, 36, 39, 65)	Al (13, 23, 32, 35)
Ca (6, 15, 16, 18, 23, 30, 40, 42, 45–47, 51, 62, 71–74)	Ca (54)
Co (39, 65, 70, 75)	Cd (1, 12, 16, 23, 31–33, 35, 51, 53, 55, 60, 64, 76–78)
Cu (6, 11, 15, 18, 30, 36, 39, 42, 45, 46, 53, 62, 65, 68, 70, 72, 75)	Co (13, 23, 35, 76)
Fe (6, 11, 15, 18, 23, 30, 32, 36, 39, 42, 45–47, 53, 65, 68, 70, 72, 74, 75, 79)	Cr (1, 12, 13, 23, 31, 32, 35, 64, 78)
K (16, 18, 23, 30, 39, 40, 42, 45, 47, 62)	Cu (1, 12, 13, 16, 23, 31, 32, 35, 40, 47, 51, 54, 60, 76, 78)
Mg (6, 11, 15, 16, 18, 23, 30, 36, 39, 40, 42, 45–47, 51, 62, 68, 70–75)	Fe (13, 16, 35, 40, 51, 54, 76, 78)
Mn (11, 15, 18, 23, 30, 36, 39, 45, 53, 62, 65, 68, 70, 72, 75)	Mn (1, 13, 16, 32, 35, 40, 47, 51, 78)
Na (16, 39, 40, 42, 45, 47)	Ni (1, 13, 23, 31, 32, 35, 54, 76, 78)
Ni (36, 39, 65, 70, 75)	Pb (1, 12, 23, 29, 31–33, 51, 53–55, 60, 64, 76, 78)
Pb (11, 18, 30, 34, 39)	Ti (54)
Sr (6, 39)	V (78)
Zn (6, 11, 15, 16, 18, 23, 30, 32, 34, 35, 39, 42, 45, 46, 53, 60, 62, 68, 70–72, 74, 75, 79)	Zn (1, 13, 33, 40, 51, 76, 78)

71, 73, 74, 79). In this case, a detrimental effect of the organic matter is alleviated by means of an on-line automatic sample dilution (71) or a flow injection manifold (FI) (6). The ionization of Ca and Mg is reduced by adding a NaCl solution to the sample solutions (30) or employing reducing conditions in the flame (6). Negative effects caused by the presence of phosphates and other anions in the determinations of Ca, Mg, Fe, Cu, Zn, and Mn are eliminated through the addition of $La(NO_3)_3$ or $LaCl_3$ solutions to the sample solutions (30, 45, 62, 71). The lanthanum modifiers can also be present in a carrier solution of the FI manifold (6) or a diluting solution of the automatic dilution system (71).

ETAAS is a very suitable analytical technique for the determination of trace metals of honey, i.e., Al, Cd, Co, Cr, Cu, Ni, Pb, Zn, and V. Some minor metals (Fe, Mn) can also be measured using this technique (Table 3). However, the analysis of honey without its previous digestion is troublesome because of the carbohydrate-rich matrix of honey which results in an accumulation of carbonaceous residues in graphite tubes and a deterioration of sensitivity and precision of measurements. Therefore, the honey samples are usually decomposed before analysis (13, 16, 23, 32, 33, 35, 40, 47, 51, 55, 60, 64, 76, 78, 80). Direct sample introduction into the graphite tube without an initial sample manipulation, except for water dissolution, is highly restricted (12, 29, 31, 63, 77). In such cases, the destruction of the organic matter during ashing at 650–1200°C is performed in the presence of added HNO_3 and H_2O_2 that enhances the efficiency of honey destruction and prevents from the formation of the carbonaceous residues inside the tubes (77). Both oxidizing reagents alleviate the disadvantages related to an absorption signal deterioration and prolong the lifetime of the atomizers. They can be directly added to the sample solutions at the sample pretreatment stage (1, 12) or mixed with the sample solutions in autosampler cups (29, 31, 63, 77). In the latter case, Triton X-100 is only added to the sample solutions in order to reduce the solution tension and an inappropriate dispensation of the solution in the graphite tubes. Usually, the solutions containing 4–6% of honey are recommended to be prepared for the analysis because at such honey dilution the pyrolysis products do not remain in the graphite tubes (12).

Metals are quantified in the analyzed sample solutions using the calibration with external aqueous standard solutions (1, 12, 13, 29, 31, 47, 77) or applying the standard additions method (29, 31, 64, 76–78). Due to short linearity ranges, the adequate dilution of the analyzed sample solutions is required (12, 76). Due to matrix interferences at ashing and atomization stages, different chemical modifiers are extensively used when ETAAS is applied for the determination of trace metals in honey. The role of these substances is to prevent metal losses, reduce the matrix-related effects, and supply reliable isothermal conditions for the pyrolysis and atomization processes in the graphite tubes. The solutions of modifiers assayed, i.e., $NH_4H_2PO_4$ in cases of Cd, Pb, and Zn (1, 29, 31, 32, 77), $Mg(NO_3)_2$ in the cases of Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, and Zn (32, 76, 78), $Pd(NO_3)_2$ in the cases of Cu, Cr, and Ni (31, 63), or mixtures of $NH_4H_2PO_4$ with $Mg(NO_3)_2$ and $Pd(NO_3)_2$ with $Mg(NO_3)_2$ in the cases of Cd, Cr, Cu, and Pb (12, 29, 31, 32, 55, 76, 77) are premixed with the sample solution portions before the injection or, more commonly, they are directly introduced into the atomizer together with the sample solution aliquots.

Flame and Inductively Coupled Plasma Optical Emission Spectrometry

Using the same equipment as for FAAS or separate photometers, flame can be used as a suitable excitation source for the determinations of common alkali and alkaline earth metals of honey, counting K (6, 10, 15, 30, 35, 46, 51, 56, 65, 68, 70, 72, 75), Na (6, 10, 15, 35, 46, 51, 56, 65, 68, 70, 72, 75), Li (6, 65, 68, 70, 75), Rb (6, 68, 70), and Ca (56, 75). Because the measurements may be accompanied by serious ionization interferences, the FI manifold is used to decrease the sample amounts introduced to the flame and ionization suppressors

TABLE 4
Determination of metals by means of inductively coupled plasma optical emission and mass spectrometry (ICP-OES, ICP-MS)

ICP-OES	ICP-MS
Ag (2, 8, 22)	Al (46)
Al (2, 8, 10, 20, 22, 43, 44, 48, 57, 62)	Cd (5, 7, 14, 26, 27, 81)
Ba (2, 21, 22, 43, 44, 48)	Cr (7, 26, 27, 46, 81)
Be (48)	Cu (7, 14, 26, 27, 81, 82)
Bi (22)	Co (14)
Ca (2, 8, 10, 14, 17, 21, 22, 31, 38, 41, 43, 44, 48, 49, 62, 69)	Ba (14, 82)
Cd (2, 8–10, 17, 20, 24, 43, 44, 48, 57, 66, 69)	Fe (26, 27, 81)
Co (2, 8–10, 43, 44, 57, 69)	Mn (7, 26, 46, 81)
Cr (2, 8–10, 24, 28, 43, 44, 57, 66)	Ni (7, 14, 26, 27, 46, 81)
Cu (2, 8–10, 17, 19–21, 24, 27, 37, 43, 44, 48–50, 52, 55, 57, 62, 66, 69, 81)	Pb (5, 7, 14, 26, 27, 81)
Fe (2, 8, 10, 14, 17, 19, 20, 27, 28, 31, 37, 43, 44, 48–50, 52, 55, 57, 66, 69, 81)	Pt (27)
Hg (8)	Sn (27, 81)
In (22)	Th (14)
K (2, 14, 17, 19, 21–22, 31, 37, 38, 41, 43, 44, 48–50, 52, 69)	U (14)
Li (2, 8, 31, 43, 44, 48)	V (27, 81)
Mg (2, 8, 10, 14, 17, 19, 21, 31, 37, 38, 41, 43, 44, 48–50, 52, 62, 69)	Zn (7, 26, 81, 82)
Mn (2, 8–10, 14, 17, 19–21, 24, 27, 28, 31, 37, 43, 44, 48, 50, 52, 57, 62, 66, 69, 81)	
Mo (2, 8, 43, 44)	
Na (2, 14, 20, 21, 31, 38, 41, 43, 44, 48, 49, 69)	
Ni (2, 8–10, 24, 28, 43, 44, 57, 62, 66, 69)	
Pb (2, 8–10, 17, 20, 24, 43, 44, 48, 49, 57, 69)	
Sr (2, 14, 21, 43, 44, 57)	
Ti (2)	
V (2, 43, 44)	
Zn (2, 8–10, 14, 17, 19–21, 24, 27, 31, 37, 43, 44, 48–50, 52, 55, 57, 62, 66, 69, 81)	

are also added to the carrier solution, i.e., KCl in the cases of Na, Li and Rb, or NaCl in the case of K (6). A solution of CsCl is added to the sample solutions when determining Na and K (46).

ICP-OES is extensively used for multielemental honey analyses (see Table 4). This technique offers wide dynamic ranges, high sensitivity, and detection power, in addition to a much lower risk of the appearance of matrix effects during the sample introduction and atomization as compared to other atomic absorption and emission methods. The samples of honey are usually decomposed by means of dry ashing (19, 24, 31, 37, 38, 41, 43, 44, 48, 50, 52, 55, 57, 61, 62, 66) or wet acid digestion in open or closed vessel systems (2, 8, 14, 17, 20, 27, 39, 49, 57, 81). The samples can be dissolved and diluted only with water, but such direct analysis of the resulting solutions is less common (9, 10, 28, 69).

When the sample matrix is eliminated by its decomposition or the samples are diluted in such a way that the matrix has no significant effect on the performance of ICP-OES, the calibration with the external aqueous solutions is commonly applied (8, 9, 19, 27, 28, 31, 38, 41, 44, 48, 57, 62, 66). The matching matrix standard solutions containing glucose and fructose, sucrose, or honey can also be used (9). In some cases, standard additions (21, 81) or internal standards (In or Y) (69, 81) are prepared and result in a relevant improvement of the results by increasing the precision of measurements.

Inductively Coupled Plasma Mass Spectrometry

ICP-MS is a powerful technique for determination of trace and ultra-trace metals in honey; however, it is rather infrequently used for that purpose (5, 7, 26, 27, 46, 81, 82). With a low resolution quadrupole mass filters-based instrument (Q-MS), the polyatomic interferences rising from the recombination of sample constituents with Ar and other matrix components (O, N, H, C, Cl, and S) can hinder the quantification of several trace metals, i.e., ^{35}Mn,^{52}Cr, ^{56}Fe, ^{63}Cu, ^{66}Zn, and ^{69}Ni (7, 26, 27, 81). Usually, the use of a high resolution magnetic sector field mass spectrometer (HR-SF-MS), with the resolution m/Δm of 300 units or higher (400, 3000, or 7500), results in keeping this kind of interferences under control (5, 7, 26, 27, 81). Additionally, the HR-SF-MSs have much improved ion transmission efficiencies and lower noise levels, and for that reason, they are able to enhance the detection power by two orders of magnitude as compared to the Q-MSs.

The samples of honey can be mineralized before analysis (5, 27, 46) or just dissolved in water or acidic solutions (7, 26, 81, 82). Nevertheless, to overcome the problems related to the high content of carbohydrates or mineral acids in the honey solutions, the standard additions method is privileged for the determination of metals in the respective sample solutions (5, 26, 27, 81). Aqueous external standard solutions (46) or matching matrix solutions (with fructose) are also applied for the calibration and quantification (7). Additionally the internal standards, including Sc (46), Y (46), Tb (46), Bi (46), In (5, 26, 81), and Rh (7, 27), are added to the sample and calibration solutions and it leads to the compensation of random fluctuations or drift of the recorded signals. The quantification of metals using the isotope dilution is performed and recognized to eliminate the errors referred to as the short-term and long-term signal drifts (82).

SAMPLE PREPARATION BEFORE ANALYSIS

Honeys for the analysis are kept in original glass shipping jars, bottles, or buckets (8, 30, 70, 73, 74, 79) or, in case of honeys collected from beekeepers, they are stored in glass or plastic vessels, containers, bottles, jars, and holders (5, 11–16, 22, 24, 27, 31, 32, 36, 38–41, 43–49, 52, 53, 56, 61, 63, 68, 70, 77, 80). The decontaminated plastic bottles or polyethylene tubes can be used for the storage to avoid the contamination from glass or metal (7, 19, 54, 55, 64, 76). The respective samples are stored in the laboratory in a dark place (12, 15, 31, 36, 39, 46, 53, 63, 73, 74, 76, 77, 79), at room temperature (5, 12, 13, 15, 27, 30, 36, 45, 49, 53, 56, 73, 74, 79, 80), 3–5°C (11, 19, 22, 24, 31, 32, 38–41, 46, 47, 52, 61, 63, 68, 70, 77), 0°C (43, 44, 48), or even −18°C (64). Before sampling, the containers with honey can be gently heated in water baths to 40–50°C (5, 9, 12, 14, 27, 62, 76) and stirred using glass sticks, or additionally sonicated for several minutes (5, 9, 14, 27). This causes a decrease of viscosity of honey and enhances its homogeneity.

The small portions of honey taken for the analysis may result in a high inconsistency of the results because the heterogeneity of a raw honey is significant (27). A solution to this problem can be the preparation of a homogeneous fluid that is a very dense solution of a huge amount of honey (10 or 20 g) dissolved in water (mL) at a ratio of 1:1 (69) or 2:1 (27, 78). The sample fluid achieved can be subsequently sonicated or heated at 50 °C and then digested or diluted before analysis.

Decomposition

Although honey is easily soluble in water and metals can be determined directly in the respective water or acidic solutions, the samples of honey are commonly decomposed using high temperature dry ashing or wet digestion procedures in order to destroy the carbohydrate-rich sample matrix and minimize the matrix-based interferences (5). The corresponding ashes or digests are subsequently dissolved in water or acid solutions to transfer the mineral components into the solution. This sample treatment is certainly time-consuming and includes the stages that may be potential sources of losses of metals due to a sample overheating or gains because of various contaminations. However, the digestion of honey facilitates the extraction of metals from the organic sample matrix and prevents numerous physical and chemical interferences accompanying the atomic and mass spectrometric measurements, or accumulation and deposition of carbonaceous residues, for example, in burners and nebulizers (9, 71).

High Temperature Dry Ashing

Dry ashing is a procedure for honey destruction in which the organic matter is removed by its thermal decomposition, while the non-volatile metals are pre-concentrated in the form of a non-combustible ash left. This method is very suitable for the determination of metals that are present in honey in large quantities and are stable at high temperatures, i.e., Ca, Fe, K, Mg, and Na. The disadvantage of this sample treatment is that possible losses can appear due to the volatilization (Cd, Cu, Hg, Pb, Zn, and Sn) or retention of metals on the walls of the ashing containers. On the other hand, the ashing procedure is performed in the open systems and for long periods of time; hence, a possible contamination of the ash from muffle furnaces and ashing containers should be considered. When high amounts of honey are taken for ashing, the risk associated with sample heterogeneity is much lower; however, the whole procedure is rather prolonged. When low portions of honey are ashed, the sample foaming is negligible and the preparation time is shortened to few hours (71).

The procedure recommended by the Association of Official Analytical Chemists (AOAC, Official Methods of Analysis, 1984, 1990, 2000) is commonly in use. In this method, relatively large samples of honey, i.e., 20 g (70, 75) or 5 g (11, 19, 38, 41, 43, 45, 48, 57), are placed in suitable crucibles made of silica or platinum, initially dried at 100°C, and finally ashed at 550 or 600°C in muffle furnaces. The samples can additionally be exposed to the IR lamp radiation to prevent foaming (70, 75). The ashes left are merged with small amounts (5 mL) of 0.1 mol/L HCl (11, 19, 24, 45, 50, 52, 61, 70, 75) or HNO_3 (38, 41, 43, 44, 48) and the resulting mixtures are evaporated to near dryness. The residues obtained are dissolved in 10 mL of the same acid solutions and made up with water to 100 mL (70, 75), 50 mL (19, 45, 50, 52, 61) or 25 mL (24, 38, 41, 43, 44, 48). If the final volume of the ash solution is 10 mL, the second portion of acid solution added to the residue is only 2 mL (11).

Other ashing procedures are also applied; however, as compared to the procedure quoted above, the differences lie in the sample masses taken for the analysis, conditions of calcination, and means of the dissolution of the resulting ash (6, 10, 13, 15, 18, 30–36, 51, 53, 55, 60, 62, 64, 66, 71). For example, before heating in the muffle furnace small amounts of concentrated HCl (62) and HNO_3 (71) can be added to the samples or the samples are mixed with $Mg(CH_3COO)_2$ (13) or $Mg(NO_3)_2$ (30) as combustion and ashing aids. Higher and lower temperatures, i.e., 700°C (34), 500°C (13, 36, 71), 450°C (18, 30, 32, 33, 55, 64, 66), or special temperature programs (6, 62) are employed. The ashes can be dissolved in concentrated HNO_3 (6, 33, 62, 64, 66, 71), 4.5 mol/L HNO_3 (32), 2 mol/L HNO_3 (13, 34), 0.8 mol/L HNO_3 (55), concentrated HCl (15), 6 mol/L HCl (10), 1 mol/L HCl (31), 0.2 mol/L HCl (53), the mixture of concentrated HNO_3 and HCl (30), the mixture of 2 mol/L HNO_3 and HCl (35), or concentrated $HClO_4$ (36).

Open Vessel Wet Acid Digestion

The open vessel system wet acid digestion of honey is usually performed in glass or PTFE beakers, Kjeldahl flasks, or porcelain crucibles. The sample sputtering and foaming is avoided using heating at 60–90°C (8, 51, 54, 78). When honey is initially dried, the higher digestion temperature can be used (39). For the efficient honey incineration and mineralization of the carbohydrate matrix, various oxidizing reagents or mixtures are used, including concentrated HNO_3, (2, 8), concentrated HNO_3 with the admixture of 30% H_2O_2 (32, 51, 54, 78), concentrated HNO_3 with the admixture of concentrated $HClO_4$ (21, 39, 82), or the mixture of concentrated HNO_3 and H_2SO_4 (42). The samples can also be heated on a hot plate with low concentrated HNO_3 solutions to almost complete dryness (17). If the samples are heated to near dryness, the resulting digests or residues left are normally taken up directly with water. A 1 mol/L HCl (39) or 0.1 mol/L HNO_3 (17) solution can also be used for that aim, or concentrated HNO_3 is added at first and then water is used for diluting (21). Typically, samples of 0.5 g (2, 54), 1–2 g (8, 17, 21, 32, 51, 57, 82), 3 g (78), or even 5 g (42) are digested. The final volume of the solutions is usually 25 or 50 mL. The microwave ovens are also used for the open vessel decomposition of honey in special burning cups and using concentrated HNO_3 (22, 56).

Closed Vessel Wet Acid Digestion

Close vessel wet acid digestions are carried out using special sealed PTFE bombs, autoclaves, or high temperature and pressure resistant containers of the commercial microwave ovens and systems. Considering the volatility of some metals, i.e., Cd, Pb, Ni, and Zn in high temperatures, closed vessel digestions facilitated by microwave action are recognized to be very reliable methods of honey decomposition as compared to dry ashing or ashing with $Mg(NO_3)_2$ and NH_4NO_3 additives (32). When using microwave assisted digestion procedures, the losses of mentioned volatile metal species and chances of sample contamination from the laboratory surroundings are negligible. Additionally, the quantities of the reagents used for the decomposition, the preparation time, and the number of manipulations are considerably reduced (5). As compared to the open vessel system digestions, relatively small sample portions, i.e., 0.2–0.3 g (20), 0.5 g (16, 49, 76), 0.6–0.7 g (40, 47, 63), 1 g (12, 14, 23, 27, 32, 46, 69, 76), 1.5 g (5), or 2 g (81), are mineralized with this method. Typically, the mixtures of concentrated HNO_3 and 30% H_2O_2 at ratios of 1:1 (16, 20, 63, 69), 3:1 (23, 32), 4:1 (14, 27, 47, 81), 5:1 (12), and 6:1 (5), or concentrated HNO_3 only (46, 49, 53, 60, 76) are used as the digestion reagents. The resulting digests are taken up and diluted with water to the required volume, e.g., 5 mL (32, 76), 10 mL (12, 20, 23), 25 mL (27, 49, 63, 69, 81), or 50 mL (16, 47).

Water Dissolution

From the standpoint of a much lower risk of the contamination and/or loss of metals, the plain dilution of honey and direct analysis of the resulting solutions by atomic and mass spectrometric methods is in particular very desirable (81). A prolonged sample manipulation or incomplete digestion and extraction of metals from honey is avoided. Shorter time of the sample treatment and reduced amount of the reagents is also important. The prepared samples have to be analyzed very quickly; otherwise, suspensions and precipitates are formed due to a denaturation of proteins and a microbial activity (26, 81).

Frequently honey samples are dissolved and diluted using water to obtain the solutions containing from 1 to 5% of honey; however, 20% solutions are analyzed as well when using ETAAS and ICP-OES (9, 26, 28, 29, 31, 63, 71, 73, 74, 77, 79, 81). The samples can be also diluted at first in small volumes of concentrated HNO_3 (15), concentrated HNO_3 with addition of H_2O_2 (12), and finally in water, or directly in low concentrated HNO_3 solutions (6, 7, 82). Honey can be also dissolved and diluted with water and acidified with HNO_3 before topping to a final volume (73, 74, 79). For better homogeneity, the prepared solutions are shaken (71) or sonicated (12, 15) before measurements. They can be also filtered in order to avoid impurities by beeswax (69).

QUALITY ASSURANCE AND CONTROL

Unfortunately, the validation of methods and procedures used for the analysis of honey is difficult because no certified reference material (CRM) of honey is available. The lack of such CRM can be ascribed to practical problems that posses honey. It is very difficult to obtain a long-term stable and homogeneous mass of honey ready for certification (26, 81). Three CRM candidates for trace elements in honey prepared by the Institute of Reference Materials and Measurements of Joint Research Center of the European Union (EC-JRC-IRMM) have been tested so far (26, 81). The mentioned CRM candidates were dense aqueous solutions of eucalyptus (70%), robinia (80%), and acacia (80%) ripened honeys placed in sealed ampoules purged with Ar as inert gas.

Instead, the trueness of the methods and procedures applied is commonly checked by the analysis of other CRMs containing high levels of carbohydrates, e.g., Antarctic krill (MURST-ISS-A2) (47), apple leaves (NBS 1515) (23, 32, 40, 47, 55, 64), brown bread (BCR 191) (26, 81), corn (NBS 8413) (14), mixed Polish herbs (INCT-MPH-2) (18), natural water (NIST 1640) (7), tea leaves (INCT-TL-1) (18), wheat (IPE 684) (62), or whole meal flour (BCR 189) (78). For the same purpose, the recovery tests are carried out on the chosen samples fortified with known amounts of metals under consideration (5, 9, 12, 16, 21, 23, 24, 29, 32, 51, 53, 60, 69, 73, 74, 77–79). In order to control the magnitude of possible matrix effects, the results obtained with the external aqueous standard solutions are compared to those achieved using the standard additions (29, 71, 77, 78). If they are in an agreement, the effect of matrix on the measurements is negligible, and the method of analysis used, including sample preparation treatment and a calibration strategy, is regarded to be valid and reliable. In the case of the direct analysis of honey without any sample manipulation or treatment, the results are compared with those based on the conventional sample calcination and ash dissolution (71, 82) or obtained using another dependable method of analysis, i.e., ion selective electrodes (ISE) (39) or HR-SF-ICP-MS (81).

FRACTIONATION ANALYSIS

Although the characteristics of honey through the total metal concentration assessment has received increasing attention due to the possibility of its discrimination, classification, and indication of a potential product fraud, a more comprehensive understanding of the honey quality requires new analytical tools enabling the measurements of a much wider variety of its properties. It is especially beneficial to take nourishment of the honey into consideration. Not only is the total metal content important but metal bioavailability is of a prime importance in the evaluation of the quality and the dietary value of food and beverages.

Unfortunately, a direct experimental evidence on the classification of the metal species in honeys is occasional. Correspondingly, a single column (73, 79) and a tandem column (74) solid phase extraction approach using strong cation and anion exchangers have been employed to distinguish the cationic, anionic, and residual species fractions of Ca, Fe, Mg, and Zn in different kind of honeys.

The fractionation patterns of Ca and Mg exhibit very great similarity (73, 74). Both metals are predominantly present in the form of the cationic metal species. The share of the cationic fraction in relation to the total contents of Ca and Mg are very high and vary from 97 to 100% in the case of Ca, and from 98 to 99% for Mg. Regarding the organic and inorganic composition of honeys, and the capability of different food constituents to complex the metals, it can be presumed that the distinguished cationic fraction likely contains free metal cations and stable cationic complexes of Ca and Mg with inorganic species or low molecular mass organic ligands, mostly monovalent anions of some organic acids (citric, malic, oxalic, and tartaric) that willingly bind Ca and Mg at the pH of honey. This fraction may be considered as the most bioavailable to humans and directly absorbed through the gastrointestinal tract.

In the cases of Fe and Zn, the distribution of the metal species in the analyzed honey samples among the fractions distinguished is more differentiated (74, 79). For Fe, the amount of the cationic metal fraction, relative to the total quantity of the metal, spans the range from 23 to 40%. The same class of the metal groupings for Zn is greater and changes from 73 to 100%. Remaining groups of the Fe species distinguished through the operationally defined fractionation procedures, i.e., the anionic and the residual fractions, are comparable. The abundance of the anionic metal species ranges from 31 to 40%, while in the case of the residual metal species it is from 29 to 45%. The contributions of the respective fractions of Zn to the total metal concentrations were from 2 to 10% in the case of the anionic metal species and from 5 to 20% for the residual metal species. Taking into account the capability of the endogenous ligands occurring in honeys to bind the metals and the potential bioavailability of different natural food components, it can be presumed that the cationic fraction of Fe and Zn, possibly containing free cations, hydroxycations, and a group of labile metal complexes, is the most bioavailable due to ease in migration through the cell membranes. The fraction of the anionic species of Fe and Zn is presumably attributed to the stable complexes of these metals with low molecular mass organic acids of honey, e.g., tartaric, citric, oxalic, or malic acids. This group of the metal species is also highly absorbable to the humans through the intestinal system. Finally, the residual fraction is expected to include the less bioavailable metal species or those that are inert to the human body, likely belonging to the complexes of Fe and Zn with different macromolecular ligands having a strong affinity to bind the metals via donor oxygen atoms from hydroxyl, carboxyl, or sulfate groups, i.e., mostly phenolic acids and other polyphenolic compounds and/or polysaccharides. Accordingly, these organic compounds of honey are presumed to substantially inhibit the absorbability of Fe and Zn.

FINAL REMARKS

The evaluation of the total content of major, minor, and trace metals enables the assessment of the nutritive quality of honeys originated from different regions. It also helps in tracking and judging the product authenticity according to the geographical and biological provenience. In this case, metals and their concentrations are useful descriptors of such honey classification, since they are relatively stable constituents of honey and characteristic for a certain forage area and localities. However, additional sources of metals, mostly of the anthropogenic origin, should also be considered when the mineral composition of honey is investigated.

The type of the sample preparation and the most suitable analytical technique are determined by metals of interest to be measured. However, potential matrix effects, interactions with solvents, the stability of metal species, and validation parameters of the selected method have to be taken into account. Usually, due to matrix-dependent effects, the calibration with standard additions or internal standards for the analysis is strongly advisable to be applied. Adequate fortified blanks when quantifying traces of metals in honey should be respected as well.

Unfortunately, the overwhelming studies devoted to the investigation of metals in honey are focused on the determination of their total amounts. However, it is well recognized that the bioavailability of metals and their impact on human health are not regulated by the total concentrations but the individual physicochemical forms. Apparently, metals can be present in honey matrix as simple ions and complexing ions of different stability with low and high molecular weight inorganic and organic compounds which are natural, endogenous bioligands of honey. The extent of this complexation and the equilibrium between the existing forms affect the availability and toxicity of each metal. Although recently attempted works have identified the operationally defined groups of metal species in honey, considering mutual properties of the metal species like lability, charge, inertness, and affinity to certain solid ionic sorbents, it can be expected that the speciation studies of metals in honey using different separation techniques, i.e., ultrafiltration, capillary electrophoresis, and size exclusion chromatography, would lie in the scope of future research interest for the analytical and food chemists. On the other hand, the use of gastrointestinal enzymatic assay would be helpful for measuring availability of metals from honey.

REFERENCES

1. O. M. Atrouse, S. A. Oran, and S. Y. Al-Abbadi, Chemical analysis and identification of pollen grains from different Jordanian honey samples. *International Journal of Food Science and Technology* 39 (2004):413–417.
2. U. Merin, S. Bernstein, and I. Rosenthal, A parameter for quality of honey. *Food Chemistry* 63 (1998):241–242.
3. I. S. Arvanitoyannis, C. Chalhoub, P. Gotsiou, N. Lydakis-Simantiris, and P. Kefalas, Novel quality control methods in conjunction with chemometrics (multivariate analysis) for detecting honey authenticity. *Critical Reviews in Food Science and Nutrition* 45 (2005):193–203.
4. H. D. Belitz, W. Grosch, and P. Schieberle, *Food Chemistry* (Springer Verlag, Germany, 2004).
5. C. Frazzoli, S. D'Ilio, and B. Bocca, Determination of Cd and Pb in honey by SF-ICP-MS. Validation figures and uncertainty of results. *Analytical Letters* 40 (2007):1992–2004.
6. O. M. Hernandez, J. M. G. Fraga, A. I. Jimenez, F. Jimenez, and J. J. Arias, Characterization of honey from the Canary Islands: Determination of the mineral content by atomic absorption spectrometry. *Food Chemistry* 93 (2005):449–458.
7. S. Bogdanov, M. Haldimann, W. Luginbuhl, and P. Gallmann, Minerals in honey: Environmental, geographical and botanical aspects. *Journal of Apicultural Research and Bee World* 46 (2007):269–275.
8. J. Devillers, J. C. Dore, M. Marenco, F. Poirier-Duchene, N. Galand, and C. Viel, Chemometrical analysis of 18 metallic and nonmetallic elements found in honeys sold in France. *Journal of Agricultural and Food Chemistry* 50 (2002):5998–6007.
9. M. D. Ioannidou, G. A. Zachariadis, A. N. Anthemidis, and J. A. Stratis, Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. *Talanta* 65 (2005):92–97.
10. M. J. Nozal Nalda, J. L. Bernal Yague, J. C. Diego Calva, and M. T. Martin Gomez, Classifying honeys from the Soria Province of Spain via multivariate analysis. *Analytical and Bioanalytical Chemistry* 382 (2005):311–319.
11. K. A. Osman, M. A. Al-Doghairi, S. Al-Rehiayani, and M. I. D. Helal, Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia. *Journal of Food, Agriculture & Environment* 5 (2007):142–146.
12. Z. Ajtony, L. Bencs, R. Haraszi, J. Szigeti, and N. Szoboszlai, Study on the simultaneous determination of some essential and toxic trace elements in honey by multi-element graphite furnace atomic absorption spectrometry. *Talanta* 71 (2007):683–690.
13. E. Yarsan, F. Karacal, I. G. Ibrahim, B. Dikmen, A. Koksal, and Y. K. Das, Contents of some metals in honeys from different regions in Turkey. *Bulletin of Environmental Contamination and Toxicology* 79 (2007):255–258.
14. A. Pisani, G. Protano, and F. Riccobono, Minor and trace elements in different honey types produced in Siena County (Italy). *Food Chemistry* 107 (2008):1553–1560.
15. J. Soares dos Santos, N. Soares dos Santos, M. L. Pires dos Santos, S. Novaes dos Santos, and J. J. de Jesus Lacerda, Honey classification from semi-arid, Atlantic and Transitional Forest zones in Bahia, Brazil. *Journal of Brazilian Chemistry Society* 19 (2008):502–508.
16. E. Stankovska, T. Stafilov, and R. Sajn, Monitoring of trace elements in honey from the Republic of Macedonia by atomic absorption spectrometry. *Environmental Monitoring and Assessment* 142 (2008):117–126.
17. H. Belouali, M. Bouaka, and A. Hakkou, Determination of some major and minor elements in the east of Morocco honeys through inductively coupled plasma optical emission spectrometry. *Apiacta* 43 (2008):17–24.
18. J. Wieczorek, Z. Wieczorek, and W. Mozolewski, Can bee honey serve as an environmental marker? *Polish Journal of Environmental Studies* 15 (2006):203–207.
19. A. Terrab, A. G. Gonzalez, M. J. Diez, and F. J. Heredia, Mineral content and electrical conductivity of the honeys produced in Northwest Morocco and their contribution to the characterisation of unifloral honeys. *Journal of the Science of Food and Agriculture* 83 (2003):637–643.
20. A. S. Al-Khalifa and I. A. Al-Arify, Physicochemical characteristics and pollen spectrum of some Saudi honeys. *Food Chemistry* 67 (1999):21–25.
21. R. Fernandez Torres, J. L. Perez Bernal, M. A. Bello Lopez, M. Callejon Mochon, J. C. Jimenez Sanchez, and A. Guiraum Perez, Mineral content and botanical origin of Spanish honeys. *Talanta* 65 (2005):686–691.
22. Y. Bagci, D. Arslan, M. M. Ozcan, and N. Dursun, Determination of the mineral content of bee honeys produced in Middle Anatolia. *International Journal of Food Sciences and Nutrition* 58 (2007):567–575.
23. S. Silici, O. D. Uluozlu, M. Tuzen, and M. Soylak, Assessment of trace element levels in Rhododendron honeys of Black Sea region, Turkey. *Journal of Hazardous Materials* 156 (2008):612–618.
24. M. W. Ashraf and S. Akram, Physicochemical characteristics and heavy metal contents of Saudi Arabian floral honeys. *Fresenius' Environmental Bulletin* 17 (2008):877–881.
25. S. Bogdanov, Contaminants of bee products. *Apidologie* 37 (2006):1–18.
26. G. Forte, S. D'Ilio, and S. Caroli, Honey as a candidate reference material for trace elements. *Journal of AOAC International* 84 (2001):1972–1975.
27. S. Caroli, G. Forte, A. L. Iamiceli, and B. Galoppi, Determination of essential and potentially toxic trace elements in

honey by inductively coupled plasma-based techniques. Talanta 50 (1999):327–336.

28. N. Matei, S. Birghila, S. Dobrinas, and P. Capota, Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products. *Acta Chimica Slovenica* 51 (2004):169–175.
29. J. C. Rodriguez Garcia, J. Barciela Garcia, C. Herrero Latorre, S. Garcia Martin, and R. M. Pena Crecente, Comparison of palladium-magnesium nitrate and ammonium dihydrogenphosphate modifiers for lead determination in honey by electrothermal atomic absorption spectrometry. *Food Chemistry* 91 (2005):435–442.
30. A. Uren, A. Serifoglu, and Y. Sarikahya, Distribution of elements in honeys and effect of a thermoelectric power plant on the element contents. *Food Chemistry* 61 (1998):185–190.
31. J. C. Rodriguez Garcia, R. Iglesias Rodriguez, R. M. Pena Crecente, J. Barciela Garcia, S. Garcia Martin, and C. Herrero Latorre, Preliminary chemometric study on the use of honey as an environmental marker in Galicia (Northwestern Spain). *Journal of Agricultural and Food Chemistry* 54 (2006):7206–7212.
32. M. Tuzen, S. Silici, D. Mendil, and M. Soylak, Trace element levels in honeys from different regions of Turkey. *Food Chemistry* 103 (2007):325–330.
33. P. Przybylowski and A. Wilczynska, Honey as an environmental marker. *Food Chemistry* 74 (2001):289–291.
34. I. Bratu and C. Georgescu, Chemical contamination of bee honey—identifying sensor of the environmental pollution. *Journal of Central European Agriculture* 6 (2005):467–470.
35. A. M. Gonzalez Paramas, J. A. Gomez Barez, R. J. Garcia Villanova, T. Rivas Pala, R. Ardanuy Albajar, and J. Sanchez Sanchez, Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *Journal of the Science of Food and Agriculture* 80 (2000):157–165.
36. F. Erbilir and O. Erdogrul, Determination of heavy metals in honey in Kahramanmaras city, Turkey. *Environmental Monitoring and Assessment* 109 (2005):181–187.
37. A. Terrab, M. J. Diaz, and F. J. Heredia, Palynological, physicochemical and colour characterization of Moroccan honeys. II. Orange (Citrus sp.) honey. *International Journal of Food Science and Technology* 38 (2003):387–394.
38. A. Terrab, A. F. Recamales, D. Hernanz, and F. J. Heredia, Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. *Food Chemistry* 88 (2004):537–542.
39. M. N. Rashed and M. E. Soltan, Major and trace elements in different types of Egyptian mono-floral and non-floral bee honeys. *Journal of Food Composition and Analysis* 17 (2004):725–735.
40. M. E. Conti, Lazio region (central Italy) honeys: A survey of mineral content and typical quality parameters. *Food Control* 11 (2000):459–463.
41. A. Terrab and F. J. Heredia, Characterisation of avocado (Persea americana Mill) honeys by their physicochemical characteristics. *Journal of the Science of Food and Agriculture* 84 (2004):1801–1805.
42. V. Nanda, B. C. Sarkar, H. K. Sharma, and A. S. Bawa, Physicochemical properties and estimation of mineral content in honey produced from different plants in Northern India. *Journal of Food Composition and Analysis* 16 (2003):613–619.
43. A. Terrab, D. Hernanz, and F. J. Heredia, Inductively coupled plasma optical emission spectrometric determination of minerals in thyme honeys and their contribution to geographical discrimination. *Journal of Agricultural and Food Chemistry* 52 (2004):3441–3445.
44. A. Terrab, A. F. Recamales, M. L. Gonzalez-Miret, and F. J. Heredia, Contribution to the study of avocado honeys by their mineral contents using inductively coupled plasma optical emission spectrometry. *Food Chemistry* 92 (2005):305–309.
45. G. Downey, K. Hussey, J. D. Kelly, T. F. Walshe, and P. G. Martin, Preliminary contribution to the characterisation of artisanal honey produced on the island of Ireland by palynological and physicochemical data. *Food Chemistry* 91 (2005):347–354.
46. M. Madejczyk and D. Baralkiewicz, Characterization of Polish rape and honeydew honey according to their mineral contents using ICP-MS and F-AAS/AES. *Analytica Chimica Acta* 617 (2008):11–17.
47. M. E. Conti, J. Stripeikis, L. Campanella, D. Cucina, and M. B. Tudino, Characterization of Italian honeys (Marche region) on the basis of their mineral content and some typical quality parameters. *Chemistry Central Journal* I (2007):14.
48. M. L. Gonzalez Miret, A. Terrab, D. Hernanz, M. A. Fernandez Recamales, and F. J. Heredia, Multivariate correlation between color and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry* 53 (2005):2574–2580.
49. A. Dag, O. Afik, Y. Yeselson, A. Schaffer, and S. Shafir, Physical, chemical and palynological characterization of avocado (Persea americana Mill.) honey in Israel. *International Journal of Food Science and Technology* 41 (2006):387–394.
50. A. Terrab, M. J. Diez, and F. J. Heredia, Palynological, physicochemical and colour characterization of Moroccan honeys. I. River red gum (Eucalyptus camaldulensis Dehnh) honey. *International Journal of Food Science and Technology* 38 (2003):379–386.
51. M. Tuzen, Determination of some metals in honey samples for monitoring environmental pollution. *Fresenius' Environmental Bulletin* 11 (2002):366–370.
52. A. Terrab, M. J. Diaz, and F. J. Heredia, Palynological, physicochemical and colour characterization of Moroccan honeys. III. Other unifloral honey types. *International Journal of Food Science and Technology* 38 (2003):395–402.
53. L. Vorlova and O. Celechovska, Activity of enzymes and trace element content in bee honey. *Acta Veterinaria Brno* 71 (2002):375–378.
54. P. E. Omode and S. A. Ademukola, Determination of trace metals in Southern Nigerian honey by use of atomic absorption spectroscopy. *Spectroscopy Letters* 41 (2008):328–331.
55. I. Frias, C. Rubio, T. Gonzalez Iglesias, A. J. Gutierrez, D. Gonzalez Weller, and A. Hardisson, Metals in fresh honeys from Tenerife Island, Spain. *Bulletin of Environmental Contamination and Toxicology* 80 (2008):30–33.
56. K. Turhan, Chemical contents and some trace metals of honeys produced in the middle Anatolia region of Turkey. *Fresenius' Environmental Bulletin* 16 (2007):459–464.
57. C. Fredes and G. Montenegro, Heavy metals and other trace elements contents in Chilean honey. *Ciencia e Investigación Agraria* 33 (2006):50–58.

58. Council of European Union, Brussels, Council Directive 2001/110/EC of 20 December 2001 relating to honey, *Official Journal of European Communities* L10 (2002):47–52.
59. Food and Agriculture Organization of the United Nations, World Health Organization, Codex Alimentarius Commission, Rome, Draft revised for honey at step 8 of the Codex Procedure, Alinorm 01/25, 19–27.
60. O. Celechovska and L. Vorlova, Groups of honey—Physicochemical properties and heavy metals. *Acta Veterinaria Brno* 70 (2001):91–95.
61. M. J. Diez, C. Andres, and A. Terrab, Physicochemical parameters and pollen analysis of Maroccan honeydew honeys. *International Journal of Food Science and Technology* 39 (2004):167–176.
62. J. Lachman, D. Kolihova, D. Miholova, J. Kosata, D. Titera, and K. Kult, Analysis of minority honey components: Possible use for the evaluation of honey quality. *Food Chemistry* 101 (2007):973–979.
63. J. C. Rodriguez Garcia, J. Barciela Garcia, C. Herrero Latorre, S. Garcia Martin, and R. M. Pena Crecente, Direct and combined methods for the determination of chromium, copper and nickel in honey by electrothermal atomic absorption spectroscopy. *Journal of Agricultural and Food Chemistry* 53 (2005):6616–6623.
64. M. E. Conti and F. Botre, Honeybees and their products as potential bioindicators of heavy metals contamination. *Environmental Monitoring Assessment* 69 (2001):267–282.
65. M. Nasiruddin Khan, M. Qaiser, S. Mubashir Raza, and M. Rehman, Physicochemical properties and pollen spectrum of imported and local samples of blossom honey from the Pakistani market. *International Journal of Food Science and Technology* 41 (2006):775–781.
66. Z. Leblebici and A. Aksoy, Determination of heavy metals in honey samples from central anatolia using plasma optical emission spectrofotometry (ICP-OES). *Polish Journal of Environmental Studies* 17 (2008):549–555.
67. E. Anklam, A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry* 63 (1998):549–562.
68. M. J. Latorre, R. Pena, S. Garcia, and C. Herrero, Authentication of Galician (N.W. Spain) honeys by multivariate techniques based on metal content data. *Analyst* 125 (2000):307–312.
69. T. M. F. F. Mendes, S. Nivaldo Baccan, and S. Cadore, Sample treatment procedures for the determination of mineral constituents in honey by inductively coupled plasma optical emission spectrometry. *Journal of Brazilian Chemistry Society* 17 (2006):168–176.
70. M. J. Latorre, R. Pena, C. Pita, A. Botana, S. Garcia, and C. Herrero, Chemometric classification of honeys according to their type. II. Metal content data. *Food Chemistry* 66 (1999):263–268.
71. I. Lopez Garcia, P. Vinas, C. Blanco, and M. Hernandez Cordoba, Fast determination of calcium, magnesium and zinc in honey using continuous flow flame atomic absorption spectrometry. *Talanta* 49 (1999):597–602.
72. M. Kucuk, S. Kolayli, S. Karaoglu, E. Ulusoy, C. Baltaci, and F. Candan, Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry* 100 (2007):526–534.
73. P. Pohl and B. Prusisz, Fractionation of calcium and magnesium in honeys, juices and tea infusions by ion exchange and flame atomic spectrometry. *Talanta* 69 (2006):1227–1233.
74. P. Pohl and B. Prusisz, Fractionation of calcium, iron, magnesium and zinc in bee honeys by means of tandem column ion exchange and flame atomic absorption spectrometry. *Canadian Journal of Analytical Sciences and Spectroscopy* 52 (2007):207–214.
75. H. Yilmaz and O. Yavuz, Content of some trace metals in honey from south-eastern Anatolia. *Food Chemistry* 65 (1999):475–476.
76. P. L. Buldini, S. Cavalli, A. Mevoli, and J. Lal Sharma, Ion chromatographic and voltammetric determination of honey and transition metals in honey. *Food Chemistry* 73 (2001):487–495.
77. J. C. Rodriguez Garcia, J. Barciela Garcia, C. Herrero Latorre, M. Freire Rodriguez, S. Garcia Martin, and R. M. Pena Crecente, Comparison of palladium-magnesium nitrate and ammonium dihydrogenphosphate modifiers for cadmium determination in honey samples by electrothermal atomic absorption spectrometry. *Talanta* 61 (2003):509–517.
78. M. Taddia, A. Musiani, and S. Schiavi, Determination of heavy metals in honey by zeeman electrothermal atomic absorption spectrometry. *Annali di Chimica* 94 (2004):107–111.
79. P. Pohl and B. Prusisz, Simple and versatile operational fractionation of Fe and Zn in dietary products by solid phase extraction on ion exchange resins. *Talanta* 71 (2007):411–418.
80. M. Tuzen and M. Duran, Physicochemical analysis of Tokat region (Turkey) honeys. *Advances in Food Sciences* 24 (2002):125–127.
81. S. Caroli, G. Forte, M. Alessandrelli, R. Cresti, M. Spagnoli, S. D'Llio, J. Pauwels, and G. N. Kramer, A pilot study for the production of a certified reference material for trace elements in honey. *Microchemical Journal* 67 (2000):227–233.
82. A. P. Packer and M. F. Gine, Analysis of undigested honey samples by isotope dilution inductively coupled plasma mass spectrometry with direct injection nebulization (ID-ICP-MS). *Spectrochimica Acta Part B* 56 (2001):69–75.