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Analysis of minority honey components: Possible use for the evaluation of honey quality

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Abstract

The minerals (Al, B, Ca, Cu, Mg, Mn, Ni, Zn) of 24 authentic Czech nectar and honeydew honey samples (2003 and 2004 harvests) were determined, to find the relationship between their content and the origin or type of honey. The concentrations of analytes in the digests, obtained via optimised dry decomposition of honey samples, were measured by flame atomic absorption spectrometry (F-AAS), as well as optical emission spectrometry with inductively coupled plasma (ICP-OES). With regard to its speed of analysis and cost, ICP-OES was the more favourable method. By combining the analytical data and electrolytic conductivities of samples, the honey samples could be divided into two groups – honeydew honeys and nectar honeys. Like Slovak and Polish honeys, samples of Czech honeys had higher nickel levels than honeys originating from other parts of the world.
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1. Introduction

The chemical composition of honey is dependent on its origin and thus the composition of nectar and honeydew honeys differ. The concentration of mineral compounds ranges from 0.1% to 1.0 %. In comparison with nectar honeys, honeydew honeys are higher in minerals, resulting in higher electrolytic conductivity. Potassium is the major metal, followed by calcium, magnesium, sodium, sulphur and phosphorus. Trace elements include iron, copper, zinc and manganese.

Golob, Doberšek, Kump, and Nečemer (2005) detected up to 16 elements in Slovenian honey by total X-ray fluorescence spectroscopy and established statistically significant differences between different types of honey

originating from acacia, flowers, lime, chestnut, spruce, fir and forest honeydew. Anklam (1998) published a review of the analytical methods used to determine the geographical and botanical origins of honey. Such methods include the determination of amino acids and proteins, aroma compounds, sugars, enzyme activity, fermentation products, flavonoids, organic acids, phenolic compounds, pollen analysis, minerals and trace elements, and specific stable isotopic ratios.

With increasing international interest in honey characterization, various studies have been carried out in relation to physicochemical parameters (Felsner et al., 2004); many major and trace elements in different types of bee honeys, have been analysed (Rashed & Soltan, 2004) and new methods for their determination have been developed (Ioannidou, Zacharidis, Anthemidis, & Stratis, 2005; Taddia, Musiani, & Schiavi, 2004). González-Miret, Terrab, Hernanz, Fernández-Recamales, and Heredia (2005), by

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multiple linear regression analysis, found and established significant correlations and equations relating honey lightness and colour to the mineral content. The combination of methods based on multi-component analysis with the support of statistical data evaluation seems to be a useful approach for determining the authenticity of honeys.

The aim of this study was to determine differences between 24 Czech nectar and honeydew honeys, related to their mineral composition and electrolytic conductivity. The other task was to evaluate F-AAS and ICP-OES methods for honey mineral analyses.

2. Materials and methods

2.1. Honey samples

Honey samples originating from 24 regions of the Czech Republic (from the years 2003 and 2004) were obtained from the Bee Research Institute at Dol, Libčice nad Vltavou, Czech Republic (Table 1).

2.2. Chemical and laboratory material and equipment

For dry decomposition, the following were used: nitric acid (HNO_3, 65%, p.p., Lachema Neratovice CZ and Suprapur® Merck, Germany), hydrochloric acid (HCl, 37%, p.a, Analytika, CZ), methyl-oleate, lanthanum nitrate [$La(NO_3)_3 \cdot 6H_2O$, p.a., Lachema, CZ], demineralised water (17.5–18 $M\Omega \, 10^{-2}$ m, adjusted with PCS Sybron Barnstedad for the preparation of samples and 18.2 $M\Omega \, 10^{-2}$ m adjusted with Millipore: Elix 3+ MilliQ gradient, corresponding to quality degree 1, according to ČSN EN ISO 3696 – Czech State Standard for the calibration of ICP-OES).

For the calibration of AAS and ICP-OES and testing of the method of modified dry decomposition, the following were used:

- for AAS-CRM water calibration solutions with one element (Analytika, Ltd., CZ) – Ca (1.00 ± 0.002 g l^{-1}) in 2% HCl, Cu (1.00 ± 0.002 g l^{-1}) in 2% HNO_3, K (1.00 ± 0.002 g l^{-1}) in 2% HNO_3, Mg (1.00 ± 0.002 g l^{-1}) in 2% HNO_3, Mn (1.00 ± 0.002 g l^{-1}) in 2% HNO_3, Zn (1.00 ± 0.002 g l^{-1}) in 2% HNO_3;
- for ICP-OES multielemental CRM CZ 9090 (Analytika, Ltd., CZ) 100 ± 0.2 mg l^{-1} Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V, Zn in 5% HNO_3 and internal reference material (IRM) from International Plant Analytical Exchange (IPE), RM Sample 3, Wheat 684, Quarterly Report 2000.3;
- for decomposition and preparation of sample beakers (50 ml, Simax, CZ), graduated test tubes (20 ml, graduation 0.2 ml, Simax, CZ), pipettes with adjustable volume (Finnpipette, Labsystems 4500), plastic white laboratory spoon (Huhtamaké, Italy), laboratory parafilm, centrifuge JOAN C312, muffle oven (LM 112.10, MLW electro VEB Elektro Bad, Germany), ALTEC® JRT 350 heating plate with temperature regulation, 10 °C, Elma Transonic T660/H ultrasonic bath;
- for measurements, a Varian SpectrAA 110 atomic spectrometer with possibility of measuring emission spectra and a THERMO IRIS Intrepid II XSP atomic emission spectrometer (duo) ICP-OES Series were used.

Table 1
Honey samples provided by Bee Research Institute at Dol

Sample labelling	Region	District	Year	Electrolytic conductivity (mS m^{-1})
1	Nový Bor–Hřebenka	Česká Lípa	2003[n]	75.8
2	Nový Bor–Sloup	Česká Lípa	2003[n]	60.8
3	Nový Bor–Okrouhlá	Česká Lípa	2003[n]	67.8
4	Nový Bor–Chot. kopec	Česká Lípa	2003[n]	54.5
5	Nový Bor–Č.L. letiště	Česká Lípa	2003[n]	60.1
6	Nový Bor–Sloup Chotovický kopec	Česká Lípa	2003[n]	69.5
7	Velká Bukovina, Žandov	Česká Lípa	2003[n]	77.0
8	Markvartice–Jablonné v Podještědí	Česká Lípa	2003[n]	69.9
9	Na Nivách–Jablonné v Podještědí	Česká Lípa	2003[n]	59.1
10	Sosnová Peklo	Česká Lípa	2003[hd]	85.8
11	Pískovec–Kamenický Šenov	Česká Lípa	2003[n]	65.0
12	Zbyny	Česká Lípa	2003[n]	35.3
13	Svojkov–Nové Domky	Česká Lípa	2003[n]	44.8
14	Svojkov	Česká Lípa	2003[n]	49.0
65	Velká Bukovina	Děčín	2004[hd]	113.5
66	Rožnov pod Radhoštěm	Vsetín	2004[hd]	106.1
83	Švihov–Nedaničky	Klatovy	2004[hd]	103.4
91	Litoměřice	Litoměřice	2004[hd]	114.9
100	Kyjov	Hodonín	2004[hd]	119.1
101	Konice–Ochoz	Prostějov	2004[hd]	111.8
123	Kralovice–Ondřejov	Plzeň-sever	2004[hd]	119.5
132	Horní Lánov	Trutnov	2004[hd]	101.2
1A	Máslovice	Praha-východ	2003[n]	58.1
2A	Dobříš	Příbram	2003[n]	57.1

[n]Nectar honey, [hd]Honeydew honey (according to electrolytic conductivity).

2.3. Electrolytic conductivity determination

Electrolytic conductivity of a honey solution at 20% (dry matter basis) in CO_2-free deionised distilled water was measured at 20 °C (AOAC, 1990) with an Inolab Cond Level 1 WTW conductometer (Germany). Results were expressed as $mS\,m^{-1}$.

2.4. Dry decomposition procedure

Since atomic absorption measurement, as well as ICP atomic emission spectrometry, require liquid samples, decomposition of the honey samples was necessary. Dry decomposition was used as a method suitable for this purpose. All laboratory glassware was washed before use for 3 days in 5% nitric acid and then rinsed in twice-distilled water. Mean concentration of analytes in blanks was used for the correction of the content of analytes in the honey samples.

Before weighing, all samples were homogenised in an ultrasonic water bath at 50 °C for 2 h with periodic agitation. For dry decomposition, the method published by Mader, Száková, and Miholová (1998), together with the method for ash determination (ČSN 570190 – Czech State Standard), were used. All samples were decomposed in three replicates. Then this procedure was modified using 3 g of honey, sonicated after addition of 1 ml 37% HCl p.a.+, 0.5 ml 65% HNO_3 p.p. and 5 ml 1.5% HNO_3. Digests were transferred to the probes and after centrifugation at 2000 revs min^{-1}, filled to 20 ml with demineralised water and closed with PARAFILM.

2.5. F-AAS determination (flame atomic absorption spectrometry)

With F-AAS Ca, Cu, K, Mg, Mn and Zn in 1A and 2A samples prepared with dry decomposition without HCl addition were determined in the Trace Laboratory of the Department of Chemistry of the Czech University of Agriculture in Prague. For Ca determination, the samples (2 ml of digest) were adjusted with the addition of 3 ml $La(NO_3)_3$ solution in 1.5% HNO_3 (conc. 5000 ppm La^{3+} – a modifier for the elimination of phosphate effect). From this solution, the solution for Mg determination was prepared by addition of 1 ml of $La(NO_3)_3$ solution and 8 ml 1.5% HNO_3 to 1 ml of the initial solution. Parameters are given in Table 2 and results in Table 6. All the analytical data were corrected according to content of analytes in blanks as mentioned above.

2.6. Determination with ICP-OES (inductively coupled plasma-optical emission spectrometry)

By means of emission spectrometry with inductively coupled plasma, Al, B, Ca, Cu, Mg, Mn, Ni and Zn were determined in the Customs Technical Laboratory of the Customs Directory in Prague. The following parameters were used for the determination in all honey samples prepared with modified dry decomposition with HCl addition: glass cyclone spray chamber, sample flow 3.70 ml min^{-1} for 30 s during washing, sample flow 1.85 ml min^{-1} during analysis, relax pump time 5 s, Tyron-Orange pump tubing, pressure in diffuser 220.6 kPa. Integrating time for low wavelength range in 30 s axially, as well as radially, and for high length range, axially and radially 5 s. Wavelengths for the determination of individual elements are given in Table 3. The calibration curve was prepared by pipetting 5 and 10 ml multielement CRM calibration solution into 100 ml volume flasks with the addition of 5 ml HNO_3 Suprapur® and adjusting with demineralised water. All the analytical data were corrected according to contents of analytes in blanks.

2.7. Analytical quality assurance

According to ISO Guide 30, the trueness of the method was determined as closeness of agreement between the result of analysis and reference value agreement (ČSN ISO 5725 – Czech State Standard) – International Plant Experiment RM Sample 3, Wheat 684, IPE 2000.3, obtained from four series of determinations. Outlying

Table 3
Wavelength for individual elements for ICP-OES determination

Element	Wavelength (nm)
Al	396.152
B	208.959
Ca	315.887
Cu	324.754
K	766.491
Mg	285.213
Mn	257.610
Na	589.592
Ni	231.604
Zn	206.200

Table 2
Parameters of F-AAS determination

Parameter	Ca	Cu	K	Mg	Mn	Zn
Wavelength (nm)	422.7	324.8	404.4	285.2	279.5	213.9
Spectral interval width (nm)	0.5	0.5	0.5	0.5	0.2	1.0
Supply current of discharge lamp (mA)	7	4	5	7	5	5
Background correction	N	N	N	D_2	D_2	D_2

N – without background correction; D_2 – background correction with deuterium discharge lamp.

extreme values were excluded by Dixon Q test, trueness of average was determined by z-score test, and the limit of detection, limit of determination, repeatability of the method and additional verification of obtained results were determined.

2.8. Statistical evaluation

Obtained data were evaluated in the Microsoft Excel programme and statistical cluster analysis with Statistica 5.0 at the level of significance $p = 0.05$.

3. Results and discussion

Determined levels of elements in samples of Czech honeys from the honey harvest from the years 2003 and 2004 are given in Tables 4–6. The F-AAS method was used for the evaluation of statistical parameters (accuracy, precision, robustness, confidence interval) and more detailed evaluation of statistical parameters will be given in the next report. Concentrations of Al, B, Mg, Mn, Ni, and Zn were higher in honeydew honeys than in nectar honeys. The concentrations of Cu in honeydew samples were similar to the values found in nectar honey samples while, for Ca, higher concentrations were found mainly in nectar honeys and the lowest concentrations were found in honeydew samples. The levels of concentrations found are in accordance with values reported in the literature (Crane, 1979), but trace element Ni concentrations were higher and comparable with honeys from Slovakia and Poland. Content of Ni did not reach the allowable amount of 6.0 mg kg^{-1} in any sample (Regulation of Ministry of Health of the Czech Republic 53/2002). Also Cu, Zn, and Al did not reach the upper allowed limits in any case.

The results of cluster analysis (Figs. 1 and 2) confirm the optimal usefulness of simultaneous application of both values of electrolytic conductivity and concentrations of determined elements for the evaluation of honey

Table 4
Average element levels and range between minimal and maximal value in analysed Czech honey samples [mg kg^{-1}]

Element	Nectar honeys		Honeydew honeys		All analysed honey samples	
	Range	Average	Range	Average	Range	Average
Al	0.61–5.49	2.9	5.19–20.2	14.3	0.61–20.2	7.2
B	9.4–24.7	15.3	13.6–54.9	30.4	9.4–54.9	21.0
Ca	39.1–142	82.9	11.2–66.7	34.9	11.2–142	64.9
Cu	0.11–0.88	0.40	0.27–0.70	0.45	0.11–0.88	0.42
Mg	18.4–62.4	38.1	48.9–89.0	70.1	18.4–89.0	50.1
Mn	1.16–4.92	2.87	4.83–8.99	7.01	1.16–8.99	4.43
Ni	0.06–0.40	0.20	0.32–1.53	0.82	0.06–1.53	0.43
Zn	0.40–2.42	1.20	1.86–3.42	2.51	0.40–3.42	1.69

Table 5
Contents of determined elements in individual honey samples [mg kg^{-1}] found by ICP OES method after dry decomposition

Sample	Al	B	Ca	Cu	Mg	Mn	Ni	Zn
1	5.5 ± 3.7	24.7 ± 11.5	85.1 ± 6.7	0.88 ± 0.72	44.9 ± 0.9	3.68 ± 0.09	0.25 ± 0.01	1.66 ± 0.28
2	3.5 ± 0.03	20.2 ± 7.7	99.7 ± 4.5	0.30 ± 0.15	35.7 ± 1.7	2.85 ± 0.07	0.20 ± 0.02	1.27 ± 0.09
3	3.9 ± 1.9	18.8 ± 16.1	120 ± 3.0	0.48 ± 0.16	33.5 ± 2.4	3.56 ± 0.20	0.18 ± 0.04	1.24 ± 0.05
4	1.1 ± 1.2	18.1 ± 11.0	142 ± 1.2	0.53 ± 0.09	31.9 ± 1.1	1.77 ± 0.10	0.12 ± 0.01	0.94 ± 0.01
5	1.6 ± 0.9	16.7 ± 4.1	125 ± 1.8	0.56 ± 0.12	40.1 ± 0.4	2.68 ± 0.02	0.15 ± 0.003	1.02 ± 0.06
6	2.6 ± 1.3	14.9 ± 11.7	66.0 ± 1.2	0.24 ± 0.13	42.0 ± 0.1	1.64 ± 0.01	0.17 ± 0.01	1.41 ± 0.09
7	4.9 ± 2.5	15.7 ± 9.0	68.2 ± 6.5	0.46 ± 0.13	55.3 ± 1.4	3.68 ± 0.39	0.40 ± 0.07	1.41 ± 0.05
8	3.9 ± 0.6	14.2 ± 3.5	81.1 ± 13.0	0.55 ± 0.18	52.0 ± 6.0	4.92 ± 0.63	0.30 ± 0.04	1.50 ± 0.22
9	4.5 ± 0.8	16.2 ± 9.4	52.8 ± 2.7	0.19 ± 0.03	40.4 ± 0.9	4.56 ± 0.02	0.22 ± 0.01	1.08 ± 0.10
10	5.2 ± 0.5	16.7 ± 9.4	66.7 ± 0.8	0.53 ± 0.07	89.0 ± 1.0	7.95 ± 0.34	0.50 ± 0.02	1.96 ± 0.03
11	2.8 ± 0.4	13.9 ± 0.9	97.5 ± 0.2	0.46 ± 0.08	43.1 ± 1.2	2.83 ± 0.02	0.32 ± 0.004	2.42 ± 0.14
12	1.9 ± 0.1	9.5 ± 3.0	39.1 ± 3.4	0.11 ± 0.04	20.5 ± 1.5	1.16 ± 0.07	0.08 ± 0.01	0.68 ± 0.19
13	0.6 ± 0.2	9.7 ± 1.5	94.0 ± 4.9	0.29 ± 0.05	24.6 ± 0.7	1.48 ± 0.05	0.08 ± 0.04	0.55 ± 0.02
14	1.3 ± 0.03	12.8 ± 4.1	65.3 ± 4.1	0.39 ± 0.23	27.1 ± 1.6	2.09 ± 0.10	0.08 ± 0.03	0.67 ± 0.27
65	13.6 ± 3.4	13.6 ± 2.4	11.2 ± 1.2	0.70 ± 0.05	85.2 ± 1.7	8.84 ± 0.08	1.35 ± 0.03	3.05 ± 0.09
66	13.5 ± 6.0	29.2 ± 22.1	22.3 ± 0.1	0.27 ± 0.10	53.0 ± 11.1	4.89 ± 0.99	0.32 ± 0.08	2.02 ± 0.35
83	14.1 ± 8.6	22.2 ± 28.1	21.2 ± 1.0	0.29 ± 0.19	67.5 ± 5.9	7.14 ± 0.12	1.10 ± 0.03	2.35 ± 0.07
91	19.1 ± 13.6	48.3 ± 51.8	45.3 ± 0.2	0.41 ± 0.21	61.4 ± 11.3	6.33 ± 1.24	0.59 ± 0.08	2.37 ± 0.48
100	20.2 ± 10.2	39.8 ± 46.8	33.0 ± 3.5	0.66 ± 0.46	81.6 ± 9.4	8.99 ± 4.41	1.05 ± 0.18	3.42 ± 0.41
101	18.5 ± 3.0	54.9 ± 7.6	50.7 ± 3.0	0.30 ± 0.01	57.9 ± 5.0	5.48 ± 0.62	0.52 ± 0.06	2.16 ± 0.25
123	12.8 ± 0.1	20.4 ± 9.5	22.4 ± 1.8	0.41 ± 0.005	86.3 ± 0.5	8.69 ± 0.06	1.53 ± 0.002	3.40 ± 0.13
132	11.7 ± 8.8	28.7 ± 34.3	41.6 ± 1.2	0.45 ± 0.37	48.9 ± 13.2	4.83 ± 1.41	0.38 ± 0.15	1.86 ± 0.46
1A	1.0 ± 0.6	9.9 ± 4.8	66.8 ± 4.8	0.21 ± 0.10	18.4 ± 2.0	2.53 ± 0.23	0.06 ± 0.005	0.40 ± 0.07
2A	4.9 ± 0.2	14.8 ± 1.4	40.7 ± 0.7	0.31 ± 0.03	62.0 ± 1.0	3.67 ± 0.06	0.35 ± 0.01	1.70 ± 0.03

Table 6
Contents of determined elements in honey samples [mg kg^{-1}] found by F-AAS method after dry decomposition

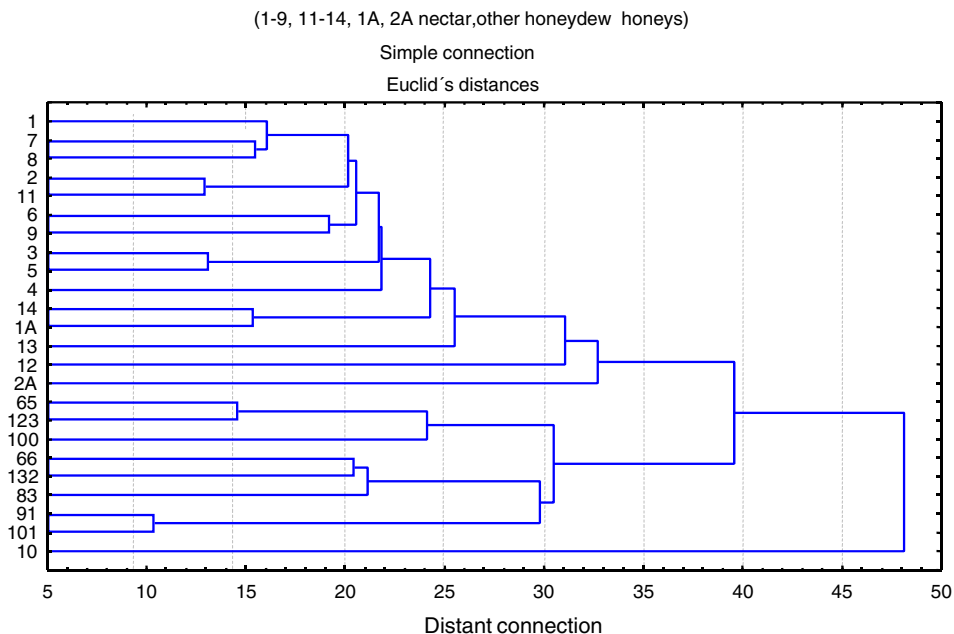| Sample	Mg	Mn	Cu	Ca	Zn
1A	16.6 ± 0.74	2.40 ± 0.40	0.09 ± 0.03	69.3 ± 3.40	0.39 ± 0.07
2A	57.9 ± 0.80	2.51 ± 0.20	0.34 ± 0.05	47.9 ± 0.91	1.49 ± 0.09

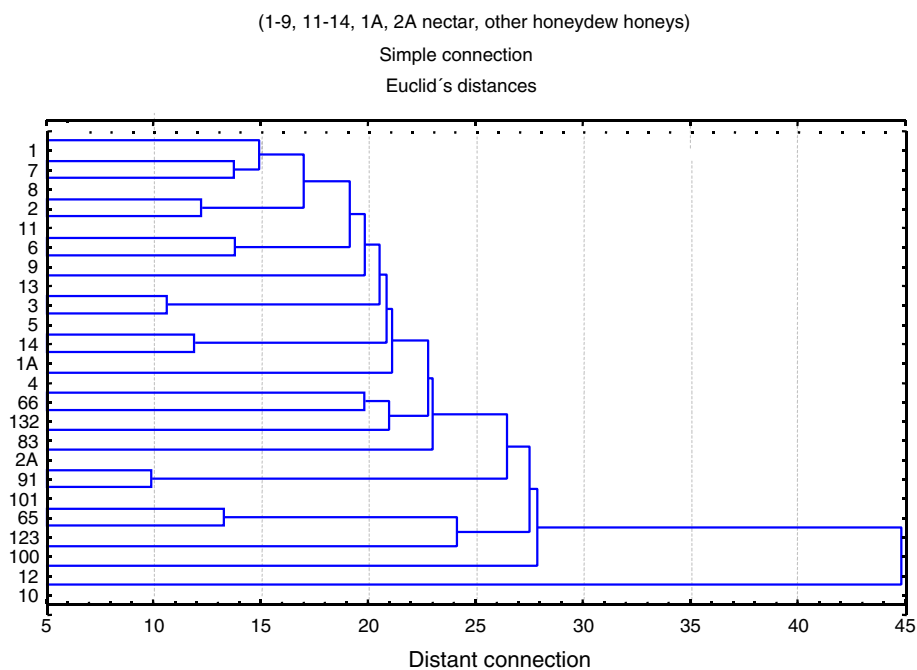Fig. 1. Diagram of honey samples clustered according to combination of element composition and conductivity.

Fig. 2. Diagram of honey samples clustered according to element composition.

samples and their division into groups according to their origin. On the basis of obtained results, the honey samples could be divided into two different groups – nectar and honeydew honeys. Sample 10 was evaluated as an individual after cluster analysis. Thus, concentrations of analysed elements and electrolytic conductivity could contribute to the differentiation between nectar honey and honeydew honey and their origin, as also did pH,

acidity (free, lactonic and total), ash content, polyphenol content, net absorbance and other parameters as shown by Sanz, Gonzalez, de Lorenzo, Sanz, and Martínez-Castro (2005) and Terrab and Heredia (2004). Some parameters, such as electrolytic conductivity, colour, ash content and pH or acidity, have been considered useful characteristics for the differentiation of the two types of honey (Campos, della Modesta, da Silva, & Raslan, 2001). Using the determination of Al, B, Ca, Cu, Mg, Mn, Ni and Zn with the values of electrolytic conductivity could complete other useful characteristics for the differentiation of the two types of honey, such as proline (Sánchez, Huidobro, Mateo, Muniategui, & Sancho, 2001), quercitol (Sanz et al., 2005) and cyclitol contents (Sanz, Sanz, & Martínez-Castro, 2004).

Satisfactory results were obtained in the majority of samples processed via modified dry decomposition; however, for Ca, Zn and Mn, questionable results were obtained and, for K and Na, unsatisfactory results were obtained according to z-score values. Unsatisfactory results for Na and K could be explained by the fact that glass etching of beaker walls was observed in the dry decomposition of honey samples, and this type of contamination cannot be reflected in blanks, in which glass etching does not take place. This means that use of glassware is not suitable for dry ashing of honey. For this reason, K and Na data are not recorded in this report. For speed of analysis and cost reasons, the method of optical emission spectrometry with inductively coupled plasma (ICP-OES) could be recommended as more favourable for such a broad scale of elements. Terrab, Hernanz, and Heredia (2004) Terrab, Recamales, Hernanz, and Heredia (2004) also recommended inductively coupled plasma optical emission spectrometry for the determination of minerals and characterization of Spanish thyme honeys. According to these authors, Ca, K, Mg, and Na were very abundant elements, and Al, Cu, Mn and Zn were present to a lesser extent. ICP-OES determination with ultrasonic nebulization, for preconcentration of Pb^{2+} and its determination in honey samples were successfully applied by Cerutti, Orsi, Gasquez, Olsina, and Martínez (2003) for determination of trace amounts, also confirming the advantage of this method.

4. Conclusions

Twenty-four authentic honey samples of Czech origin (nectar and honeydew types) were analysed by means of the ICP-OES method with the use of dry decomposition for eight elements (Al, B, Ca, Cu, Mg, Mn, Ni and Zn). The ICP-OES method was compared with the F-AAS method after analysis of some samples. The method of optical emission spectrometry with inductively coupled plasma (ICP-OES) could be recommended as a more favourable method for such a broad scale of elements for speed of analysis and cost reasons. Obtained data were evaluated by statistical and chemometric methods and basic metrological properties of methods were determined. Results (after exclusion of outlaying results) were analysed by cluster analysis. By combination of element content and electrolytic conductivity, together, it was possible to divide the samples into two main groups according to their origin, namely honeydew honeys and nectar honeys. Since the content of elements in honey depends not only on its nature, but reflects the environmental contamination of the locality as well, knowledge of element contents without conductivity does not allow separation of honey samples according to their origin, as mentioned above. Czech honeys had higher nickel levels.

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