Influence of the Botanical and Geographical Origin on the Mineral Composition of Honey

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Summary

Elemental composition was analysed to determine the effect of the botanical and geographical origin on the mineral content in black locust (*Robinia pseudoacacia* L.), chestnut (*Castanea sativa* Mill.), and lime (*Tilia spp.*) honey. The study included 174 samples from three different geographical and climatic regions in Croatia. A total of 15 elements were analysed using inductively coupled plasma mass spectrometry (ICP-MS). The highest total mineral content was determined in chestnut honey samples, followed by the lime and black locust honey samples, in descending order of presence: K, Ca, Na, Mg, Mn and Fe. A good potential of marking the botanical origin was confirmed on the basis of increased contents of Mn and Sr in chestnut honey. Increased concentrations of Pb and Zn determined in honey samples from the Pannonian Region showed to be a good basis for demarcation of the geographical origin. The content of some heavy metals in honey were low (Median: Cd <0.005 mg/kg; Pb 0.015 mg/kg; Ni 0.083 mg/kg; Zn 1.362 mg/kg; with no harmful health effects).

Key words

honey, ICP-MS, mineral composition, botanical origin, geographical origin

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Introduction

In order to achieve better placement and competitiveness on the market, food producers are trying to profile authentic products whose characteristics and qualities mostly arise from the value of their composition, method of production and the region where they were produced. To ensure placement on the desired market, it is necessary to conduct extensive research aimed at the confirmation and protection (designation) of geographical origin of certain bee products. In case of honey, the authors are usually focused on the analysis of components, such as volatile compounds, phenolic acids, flavonoids, carbohydrates, amino acids, stable isotopes, as well as many other accompanying substances (da Silva et al., 2016; Thrasyvoulou et al., 2018). In a series of papers, the authors described the difference in chemical composition, primarily physicochemical, melissopalynological and sensory properties of honey, depending on the botanical and geographical origin, climatic conditions, the length of ripening in the honeycomb, as well as the processing and storage conditions (Anklam, 1998; Radović et al., 2001; Bogdanov et al., 2007; Persano Oddo and Piro, 2004; Lachman et al., 2007; Madejczyk and Baralkiewicz, 2008). In the Republic of Croatia and neighbouring countries, several papers have been published on the subject of botanical characterization of honey (Golob et al., 2005; Bubalo et al., 2006; Kenjerić et al., 2006; Jerković et al., 2009; Gašić et al., 2015; Svečnjak et al., 2015; Rajs et al., 2017), where some authors gave good guidelines and described the differentiation potential of regional characteristics of honey.

Valuable data based on 11 common physicochemical parameters on different types of Croatian honey were made by Šarić et al. (2008) based on comprehensive analysis of more than 250 honey samples from Croatia. However, this investigation did not include one of the most important features for botanical determination: pollen analysis. Authors stated that the pollen analysis was not performed because the samples were already classified by the beekeepers. This fact implies that the botanical origin has not been controlled in accordance with Croatian regulation and consequently some misclassifications and anomalous values are noted. Furthermore, regarding the *Aesculus hippocastanum* honey, specified as chestnut honey in that study, it must be stressed that this species cannot give unifloral honey, since only *Castanea sativa* can.

One of the approaches that provide great potential for determination of geographical markers of honey is the share and mutual ratios of minerals, among which the macro-and microelements play an important role. The determination of their ratio in honey is also important because of the possible presence of heavy metals and their toxic effects on human health. The mineral composition of the soil, via mineral composition of plants, is ultimately reflected in the mineral composition of honey, because of which the composition and quantity greatly depend on the botanical origin of honey (Porrini et al., 2003; Hernandez et al., 2005). Darker honey types are richer in minerals compared to lighter ones, so a greater proportion of minerals was found in chestnut honey and honeydew, compared to black locust and sunflower honey (Gonzalez-Miret et al., 2005). It was previously noticed that various prevalent salts and minerals are: potassium, sodium, calcium, magnesium, iron, zinc, phosphorus, selenium, copper and manganese (Bogdanov et al., 2007). A good correlation between the mineral content and characteristics of the botanical and geographical origin was found by examining Turkish black locust honey (Tuzen et al., 2007). Similar conclusions have been reported by Pisani et al. (2008), who studied the mineral composition of different types of Italian honey. Many other studies also confirmed that mineral composition and botanical and/or geographical origin of honey are directly connected (Latorre et al., 1999; Fernandez-Torres et al., 2005; Hernandez et al., 2005; Nozal Nalda et al., 2005; Corbella and Cozzolino, 2006; Lachman et al., 2007; Bordean et al., 2007; Louppis et al., 2017; Pohl et al., 2017; Purcarea et al., 2017; Lemos et al., 2018). A good tool for assessing the geographical origin of honey may also be the anthropogenic origin of certain heavy metals. Honey plants of the brassica family (*Brassicaceae*) are, therefore, very good indicators of pollution with metals such as Pb, Cd, Fe, Zn and As, as proved on honey samples in some studies (Porrini et al., 2003; Bilandžić et al., 2011; Oroian et al., 2016; Altun et al., 2017). Some authors have also included the creation of a mathematical model for environmental heavy metal contamination evaluation (Bordean et al., 2007; Bordean et al., 2010). Likewise, the regional origin of honey was well established through the analysis of stable carbon isotopes (Anklam, 1998; Ghidini et al., 2006) showing that isotope-ratio mass spectrometry (LC/EA IRMS) is the most promising and accurate technique. The problem is that this technique is not available to many laboratories, which includes a specially trained analytical crew, and the fact that they should make their unique database of honey samples and isotope data. Consequently, the price of analysis is high and still not practical for the routine determination of the botanical and/or geographical origin of honey. Some authors published promising results of rare elements found in very low proportions in the soil, nectar flow plants and also in honey (Fredes and Montenegro, 2006; Staniškiene et al., 2006).

The research objectives of this study were to determine: a) the impact of the botanical and geographical origin on the mineral composition of investigated types of honey; b) the proportion of potential contaminants (Cd, Ni, Pb and Zn) in analysed honey samples.

Materials and methods

Honey samples were collected according to the sampling instructions given by the International Honey Commission (IHC, 2009) — directly from the beekeepers, and stored at room temperature in the dark until the analysis. All samples of honey were well homogenized before each analysis and prepared in accordance with the provisions of the AOAC 920.180-1920 methods. A total of 174 samples of black locust (N=74), lime (N=50) and chestnut honey (N=50) were analysed, from three pedologically and climatically different areas of Croatia: the Pannonian (*PR*), Mountainous (*MR*) and mostly Adriatic Region: Istria (*AR*), collected over three years (2009-2011). The botanical origin was confirmed based on a pollen analysis, as well as an analysis of sensory and several physicochemical properties by an accredited laboratory.

Figure 1 shows the approximate locations of honey bee forages and samples used in this study.

The organic substance of honey was decomposed with microwave digestion (Multiwave 3000, Anton Paar, Austria) together with concentrated nitric acid and hydrogen peroxide. In the resulting clear solution, upon dilution, the content of elements was measured by inductively coupled plasma mass spectrometry (ICP-MS) technique (*Elan DRC E; Perkin Elmer*, USA). All analyses were performed by applying a validated in-house protocol (SOP-231-053) in an accredited laboratory (ISO 17025:2007).

To ensure the quality of results and prevention of cross-contamination after analysis, all laboratory glassware and utensils were in contact with 10% (v/v) HNO_3 for 24 hours and rinsed with ultra-pure distilled water several times. Before measuring element

Table 1. Method performance: Limit of detection (LOD) and Limit of quantification (LOQ) [mg/kg]

	Ca	Na	K	Mg	Zn	Fe	Cu	Mn	Al	Ni	Pb	Cd
LOD	2	0.08	0.3	0.2	0.03	0.06	0.005	0.008	0.008	0.005	0.03	0.0003
LOQ	5	0.3	1	0.7	0.1	0.2	0.03	0.03	0.03	0.03	0.1	0.001

Figure 1. Honey sampling locations: Pannonian Region (PR) - upper right corner; Mountainous Region (MR) - centre; Adriatic Region: Istria (AR) – on the left

concentrations in samples, a calibration was performed. Multi-element standard solutions (*IV CertiPUR,* Germany) for calibration were prepared at concentrations of 0; 0.1; 0.2; 0.5; 1; 1.5 and 2 mg/L, where 2% nitric acid was used for dilutions. After device calibration, the concentration of selected elements was measured. All measurements were performed in three replicates, and each sample was averaged in terms of content of each element. In order to check the accuracy of the sample preparation and measurements, results were verified by analysing certified reference material (*NIST SRM*), which was dissolved in a solution of a matrix similar to honey (fructose and nitric acid). The default values closely matched the resulting concentrations within a 95% confidence interval. The measurement of the utilization of elements was carried out by inoculation of samples of chestnut and black locust honey by external standard (spike). The utilization of the analysed elements ranged from 91% to 98%, indicating good accuracy, precision and validity of the method used. Table 1 shows method performance parameters, with limit of detection (*LOD*) and limit of quantification (LOQ).

Univariate statistical analysis of a group of means and medians was based on the analysis of variance (*ANOVA*) and the Kruskal-Wallis non-parametric analysis. For the subsequent analysis of measures of the degree of linear dependence between two variables, the Spearman product-moment correlation coefficient was used, which represents a measure of linear correlation between the two variables X and Y, giving a value between 1 and -1 (where 1 is the total positive correlation, 0 no correlation, and -1 is the total negative correlation).

Results

The descriptive multi-element analysis is given in Table 2, showing the measured values of certain elements in relation to the type of honey. It is notable that the content of macroelement types is similar in all three analysed honey types (in descending order): K, Ca, Na, Mg, Mn and Fe. Very similar results of honey samples from Croatia are given by Bilandžić et al. (2014), where significant differences in Ca, Fe, K, Mg, Zn, As and Hg levels were observed between honey types.

Table 2. Descriptive statistics for analysed samples of the three types of Croatian honey [mg/kg]

Element		Black locust (N=74)	Chestnut (N=50)	Lime (N=50)
Al	Median	1.323	1.489	1.904
	Mean ±SD	1.473±0.620	1.574±0.578	2.387±1.862
	Range	0.039-3.563	0.139-3.061	0.721-12.226
Cr	Median	0.029	0.022	0.022
	Mean ±SD	0.049±0.077	0.036±0.052	0.029±0.027
	Range	0-0.541	0-0.296	0.003-0.181
Mn	Median	0.157	19.614	1.390
	Mean ±SD	0.164±0.081	22.056±10.299	1.580±1.262
	Range	0.010-0.475	0.069-48.357	0.307-8.341
Ni	Median	0.052	0.065	0.131
	Mean ±SD	0.076±0.087	0.093±0.129	0.153±0.159
	Range	0.003-0.603	0.007-0.870	0.000-0.993
Cu	Median	0.105	0.198	0.185
	Mean ±SD	0.134±0.090	0.198±0.051	0.267±0.248
	Range	0.008-0.603	0.069-0.318	0.012-1.440
Zn	Median	0.858	1.523	1.705
	Mean ±SD	0.958±0.790	1.906±1.598	1.888±1.077
	Range	0.027-5.830	0.359-9.739	0.478-7.274
Cd	Median	0.000	0.000	0.000
	Mean ±SD	0.001±0.003	0.001±0.003	0.001±0.003
	Range	0.000-0.023	0.000-0.016	0.000-0.011
Pb	Median	0.013	0.015	0.017
	Mean ±SD	0.019±0.026	0.032±0.085	0.023±0.031
	Range	0.000-0.193	0.000-0.599	0.000-0.145
Fe	Median	2.434	3.764	3.509
	Mean ±SD	2.723±0.959	3.959±0.936	3.545±1.054
	Range	1.308-6.031	2.317-6.433	1.521-7.710
Na	Median	28.886	31.150	31.108
	Mean ±SD	29.545±8.858	32.038±7.501	32.365±5.862
	Range	10.309-71.211	18.363-48.550	18.000-48.950
Mg	Median	12.340	36.527	32.400
	Mean ±SD	12.958±4.876	38.632±12.191	33.543±11.816
	Range	3.337-25.283	7.326-68.320	14.542-68.880
K	Median	230.735	1941.629	937.008
	Mean ±SD	225.589±45.746	1972.826±797.716	1055.963±322.750
	Range	29.700-301.008	179.776-3806.335	508.700-2085.687
Ca	Median	28.068	129.810	84.371
	Mean ±SD	29.225±11.063	132.861±41.448	92.946±29.642
	Range	0.662-57.749	27.040-250.310	58.650-186.421
Rb	Median	0.343	7.595	4.615
	Mean ±SD	0.367±0.132	8.990±6.140	4.840±3.067
	Range	0.113-0.940	2.915-33.206	0.623-15.280
Sr	Median	0.055	0.204	0.096
	Mean ±SD	0.059±0.028	0.209±0.084	0.112±0.056
	Range	0.001-0.126	0.042-0.434	0.007-0.255

Table 3. The Kruskal-Wallis test for the mineral composition differences between honey types

Element	Kruskal-Wallis test	df	P
Al	17.998	2	<0.001
Cr	5.397	2	0.067
Mn	143.290	2	<0.001
Ni	15.244	2	<0.001
Cu	39.382	2	<0.001
Zn	48.185	2	<0.001
Cd	3.608	2	0.165
Pb	.247	2	0.884
Fe	47.582	2	<0.001
Na	7.113	2	0.029
Mg	117.943	2	<0.001
K	134.223	2	<0.001
Ca	133.849	2	<0.001
Rb	135.531	2	<0.001
Sr	97.885	2	<0.001

The highest proportion of the elements in this study was determined in samples of chestnut honey (2215.4 mg/kg), followed by the lime honey samples (1229.7 mg/kg), while the lowest values were determined in the black locust honey samples (303.3 mg/kg). The data were not normally distributed, which was confirmed by the difference in ranges between means and medians. These observations suggest that, for further processing of the results, only non-parametric statistical comparisons between groups are adequate, and therefore, the interpretation of results was primarily based on observed medians. The Kruskal-Wallis test confirmed that honey types differ significantly in 12 of the 15 analysed elements (Table 3). The content of Mn and Sr stands out due to their high proportion in chestnut honey, compared to the other two types of honey. The content of Na, Mg, Fe and Zn in black locust honey in relation to the other two types was significantly lower than that of other elements, while other elements (K, Ca, Rb) were statistically significantly different for all types of honey. This is evident from mean values of K (the dominant element in all types of honey), where the highest median was recorded in chestnut honey (1941 mg/kg), followed by lime honey (937 mg/kg), while the lowest values were determined in black locust honey (230.7 mg/kg).

The correlation coefficients between all measured parameters are shown in Table 4. It is notable that six elements have a positive statistically significant correlation factor (above 0.6) among them: Mn, Mg, K, Ca, Rb and Sr. The multi-element analysis is given in Table 5, showing the measured values (in mg/kg) of the individual elements according to the geographical origin; in honey samples from three climatologically and geologically different regions: Mountainous, Pannonian and Adriatic Region. By observing mean and median values of the individual elements by regions, it can be concluded that the data are not normally distributed. Given the above mentioned, the non-parametric Kruskal-Wallis test was performed for the further processing of results (Table 6). As presented in Table 6, most of the elements were distributed as statistically significant for regional origin, where the content of Pb

Table 4. Spearman's coefficients of measured correlations in analysed honey samples (N=174)

		Al	Cr	Mn	Ni	Cu	Zn	Cd	Pb	Fe	Na	Mg	K	Ca	Rb	Sr
Al	rho	1.000	−.180	.192	.181	.340	.224	.085	−.012	.162	.335	.313	.219	.278	.296	.152
	P	–	.017	.011	.017	.000	.003	.263	.871	.033	.000	.000	.004	.000	.000	.045
Cr	rho		1.000	−.094	.033	−.103	−.008	−.020	.040	−.089	−.019	−.120	−.112	−.129	−.185	−.088
	P		–	.219	.662	.177	.917	.792	.596	.242	.802	.113	.141	.089	.014	.246
Mn	rho			1.000	.164	.507	.506	.177	.035	.518	.246	.824	.862	.870	.855	.736
	P			–	.030	.000	.000	.020	.646	.000	.001	.000	.000	.000	.000	.000
Ni	rho				1.000	.248	.228	−.023	.037	.258	.104	.150	.145	.110	.228	−.003
	P				–	.001	.003	.760	.632	.001	.172	.048	.056	.148	.002	.966
Cu	rho					1.000	.368	.133	.092	.341	.205	.503	.491	.430	.498	.256
	P					–	.000	.080	.227	.000	.007	.000	.000	.000	.000	.001
Zn	rho						1.000	.013	.144	.381	.292	.514	.491	.492	.476	.410
	P						–	.868	.057	.000	.000	.000	.000	.000	.000	.000
Cd	rho							1.000	.008	.077	.046	.165	.126	.125	.196	.147
	P							–	.916	.313	.550	.030	.098	.100	.009	.053
Pb	rho								1.000	.139	−.127	−.155	.042	.039	.096	.056
	P								–	.067	.094	.041	.580	.605	.209	.460
Fe	rho									1.000	.135	.419	.461	.473	.483	.371
	P									–	.076	.000	.000	.000	.000	.000
Na	rho										1.000	.336	.232	.249	.174	.298
	P										–	.000	.002	.001	.022	.000
Mg	rho											1.000	.813	.820	.767	.699
	P											–	.000	.000	.000	.000
K	rho												1.000	.854	.819	.735
	P												–	.000	.000	.000
Ca	rho													1.000	.814	.798
	P													–	.000	.000
Rb	rho														1.000	.605
	P														–	.000
Sr	rho															1.000
	P															–

Correlations marked in bold represent significance level >0.6.

Table 5: Descriptive statistics for analysed honey samples collected from three Croatian regions [mg/kg]

Element		Pannonian (N=77)	Mountainous (N=52)	Adriatic (N=45)
Al	Median	1.462	1.472	1.771
	Mean ±SD	1.826±1.608	1.593±0.643	1.857±0.708
	Range	0.039-12.226	0.721-3.563	1.052-4.258
Cr	Median	0.024	0.030	0.023
	Mean ±SD	0.042±0.074	0.036±0.029	0.039±0.060
	Range	0-0.541	0-0.181	0-0.31
Mn	Median	1.833	0.197	1.049
	Mean ±SD	9.938±12.679	0.608±0.847	8.822±12.009
	Range	0.01-48.357	0.045-4.354	0.064-43.804
Ni	Median	0.064	0.061	0.066
	Mean ±SD	0.099±0.124	0.101±0.107	0.111±0.153
	Range	0-0.87	0-0.603	0.007-0.993
Cu	Median	0.176	0.134	0.171
	Mean ±SD	0.221±0.212	0.159±0.100	0.174±0.069
	Range	0.008-1.44	0.012-0.603	0.047-0.387
Zn	Median	1.470	1.144	1.073
	Mean ±SD	1.814±1.648	1.186±0.663	1.316±0.706
	Range	0.027-9.739	0.17-3.149	0.277-3.115
Cd	Median	0.000	0.000	0.000
	Mean ±SD	0.002±0.004	0.000±0.002	0.000±0.001
	Range	0-0.023	0-0.011	0-0.01
Pb	Median	0.023	0.014	0.000
	Mean ±SD	0.033±0.070	0.023±0.036	0.009±0.013
	Range	0-0.599	0-0.193	0-0.044
Fe	Median	3.473	3.015	3.178
	Mean ±SD	3.463±1.213	3.076±0.897	3.334±1.132
	Range	1.353-6.433	1.557-6.031	1.308-7.71
Na	Median	30.070	30.795	30.640
	Mean ±SD	30.308±8.514	31.867±8.222	31.460±5.698
	Range	10.309-48.95	18-71.211	19.7-46.43
Mg	Median	27.373	16.205	31.470
	Mean ±SD	26.990±14.442	21.600±15.509	30.360±14.472
	Range	3.337-60.18	6.592-68.88	9.68-68.32
K	Median	942.389	251.910	921.940
	Mean ±SD	1054.504±849.873	506.855±458.408	1346.218±1004.690
	Range	29.7-3806.335	155.54-1866.92	187.17-3620.086
Ca	Median	87.380	38.260	80.700
	Mean ±SD	88.574±60.221	55.254±41.312	83.544±41.095
	Range	0.662-250.31	13.114-176.272	18.27-186.421
Rb	Median	5.701	0.407	5.026
	Mean ±SD	5.813±6.299	0.985±1.156	4.885±4.050
	Range	0.113-33.206	0.187-5.999	0.221-22.655
Sr	Median	0.092	0.082	0.095
	Mean ±SD	0.133±0.104	0.100±0.061	0.111±0.065
	Range	0.001-0.434	0.02-0.255	0.007-0.254

stands out with its high proportion in honey originating from the Mountainous region with a mean value of 0.023 mg/kg. Moreover, the highest identified value in this study was recorded for Pb (0.559 mg/kg). Zn content in this region had the highest value recorded in this study (9.739 mg/kg). Table 5 shows that other analysed elements (Mn, K, Cd, Ca, Rb and Mg) had significantly lower values of mass fraction in honey originating from the Mountainous Region, while results of the mineral composition from the Adriatic Region revealed the lowest proportions in Zn content. On the other hand, the Mountainous Region showed the significantly highest results in some heavy metals content (Pb, Cd and Cr), while the Adriatic Region showed the highest content of Al, and finally, the Pannonian Region had the highest median in Mn content.

Table 6: The Kruskal-Wallis test for the mineral composition differences within the three Croatian regions

Element	Kruskal-Wallis test	df	P
Al	4.892	2	0.087
Cr	2.192	2	0.334
Mn	27.280	2	<0.001
Ni	.659	2	0.719
Cu	3.883	2	0.143
Zn	6.315	2	0.043
Cd	12.061	2	0.002
Pb	22.664	2	<0.001
Fe	3.418	2	0.181
Na	1.046	2	0.593
Mg	11.858	2	0.003
K	20.827	2	<0.001
Ca	12.343	2	0.002
Rb	35.021	2	<0.001
Sr	1.351	2	0.509

Discussion

Botanical origin factors. This research confirmed that darker types of honey are richer in minerals, compared to the lighter ones, so the highest proportions were established in chestnut honey, followed by lime honey (which often contains nectar and chestnut pollen, due to secondary pollen contamination), while black locust honey contained the lowest proportion of minerals. Similar results were obtained by other authors (Ruhnke, 1993; Dobrzancki, 1994; Gonzalez-Miret et al., 2005; Bogdanov et al., 2007; Bilandžić, et al., 2014; Rajs et al., 2017). Uniflorality of certain honey types might be jeopardized by other floral contamination, such as in the case of lime honey. Lime and chestnut flowering do not overlap. However, beekeepers often do not extract honey after each honey bee forage. Consequently, sensory and chemical properties and the pollen profile can often contain multiple characteristics.

Golob et al. (2005) found insignificantly higher values of K content in Slovenian honey samples of black locust (390 mg/kg) than in this research (230.7 mg/kg). Similarly, higher values are found in the results of K content for chestnut honey (3500 mg/kg in Slovenian honey compared to 1941.6 mg/kg in this research).

The second most present element was Ca, whose mass fractions in all analysed honey types were statistically significantly different; the highest values were observed in samples of chestnut, followed by lime and black locust honey. The determined values of Ca content are comparable to the values reported by Polish (Madejczyk and Baralkiewicz, 2008) and Slovenian authors (Golob et al., 2005). In comparison with the Slovenian types of honey, the values of Ca in black locust honey obtained in this study were three times higher, while values obtained for lime and chestnut honey were slightly lower. It is also interesting to mention the results given by Conti et al. (2007) who found the Ca content of 32.7 mg/kg in samples of Italian black locust honey, which is very close to the average values observed in this study. The same authors have reported similar Na content, with ~ 3% of the mass fraction in the total composition of minerals. In the mentioned study, the mean value of Na content was lower (24.5 µg/g of dry weight) compared to the values established by Bordean et al. (2007) who found values of 80.0 µg/g and 75.7 µg/g of dry weight of honey.

Mg mass fractions determined in this study were statistically significantly different among the three types of honey (Table 2) as the highest concentrations were determined in samples of chestnut honey, followed by lime and locust honey. Madejczyk and Baralkiewicz (2008) reported significantly lower Mg content ranging from 0.07 to 19.83 mg/kg compared to Mg content results obtained in this study (3.3 to 68.88 mg/kg). Lachman et al. (2007) determined values of Mg that ranged from 18.4 to 62.4 mg/kg (mean 38.1 mg/kg), which indicates similar concentrations to those found in this research.

The results on the Fe content revealed that the samples of black locust honey are segregating with significantly lower proportion (median 2.43 mg/kg) compared to the samples of chestnut (median 3.76 mg/kg) and lime honey (3.51 mg/kg). By comparing the concentration range of Fe from 1.31 mg/kg in samples of black locust honey to 7.70 mg/kg in lime honey samples as obtained in this study, it can be concluded that the observed shares are similar or moderately higher compared to the results reported by Madejczyk and Baralkiewicz (2008), which ranged from 1.0 to 16.1 mg/kg. The share of Fe in samples of black locust honey determined in this study showed half the values (4.51 mg/kg) determined in the research conducted by Conti et al. (2007).

A significantly higher proportion of Al was found (1.90 mg/kg) in samples of lime honey compared to the other two types of honey, among which there was no statistically significant difference. The proportion of Al determined in this study was slightly lower (1.88 mg/kg) compared to the results obtained for lime honey by Lachman et al. (2007), with an average of 2.9 mg/kg.

No statistically significant difference among the samples of studied honey types was determined neither for the mean value of the share of Cr, nor among regions (Tables 5 and 6). The content of Cr in the analysed honey samples was lower compared to the study of Bogdanov et al. (2007), where an average of 0.005 mg/kg and a maximum value up to 0.039 mg/kg were recorded. The highest concentration found in that study was as high as 0.541 mg/kg in one sample, but it has to be taken into account that this was a sporadic high concentration of this metal in one sample.

By observing mass fraction values of Mn, several times higher concentrations were determined, and a statistically significant difference was recorded in samples of chestnut honey (median 19.61 mg/kg) compared to the samples of black locust (median 0.157 mg/kg) and lime honey (median 1.39 mg/kg). Golob et al. (2005) found several times higher mass fractions of Mn in honey samples of black locust from Slovenia (1.5 kg/kg), as well as higher values in samples of lime (2.8 mg/kg) and chestnut honey (28.0 mg/kg).

The results on Cu content revealed statistically significant differences due to the low value noticeable in black locust honey samples compared to lime honey samples. In this study, Cu concentrations ranged from 0.008 to 1.882 mg/kg, which is very similar to values ranging from 0.26 to 1.82 mg/kg determined by Madejczyk and Baralkiewicz (2008). Values similar to the average concentrations obtained in this study (0.20 mg/kg Cu) were obtained by Staniškiene et al. (2006), who reported an average Cu value of 0.21 mg/kg, while Lachman et al. (2007) recorded twice the average value of 0.40 mg/kg. The higher Cu levels can be attributed to the soil origin, and nectar in areas with traditional and heavy agricultural practice.

Lime honey samples were singled out with significantly higher Ni content values (median 0.131 mg/kg) compared to the samples of black locust and chestnut honey. Statistically lower content of Zn (Table 2) was determined in samples of black locust honey (median 0.858 mg/kg) in comparison to chestnut and lime honey, with concentrations ranging from 0.027 in samples of black locust honey up to 9.74 mg/kg in samples of chestnut honey. Lachman et al. (2007) determined the values of Zn in the range of 0.40 to 3.42 mg/kg, with a mean value of 1.69 mg/kg, which corresponds to the mass portion determined in this study, but with a wider range of concentrations. Similar ranges were observed in Italian honey types studied by Persano Oddo and Piro (2004) where the amounts ranged from 0.004 to 3.23 mg/kg, while other authors, such as Celli and Maccagnani (2003), found lower concentrations in French honey ranging from 0.09 to 0.34 mg/kg.

Mass fractions of Cd indicate very low concentrations in all of the analysed honey samples which did not statistically significantly differ among studied honey types (Table 2). The ranges from 0.001 to 0.023 mg/kg were recorded, although the most frequently recorded concentrations were below the LOQ. Based on the weekly intake, observing all types of honey through the prism of consumption, it can be concluded that not even an increased intake of honey containing these proportions should endanger the health of consumers. Specifically, the input of Cd would not even nearly exceed the safe weekly dose of 2.5 mg/kg of body weight, which is prescribed by the European Food Safety Authority (EFSA, 2009). A similar conclusion was also reached by Staniškiene et al. (2006) who analysed the content of Cd in honey samples in accordance with the German regulations. The Cd concentrations in the mentioned study were similar to the results of this study (4.1 to 14.6 μg/kg), with slightly wider ranges. Similar range values were determined in the articles by authors from other regions (Rashed and Soltan, 2004; Bogdanov et al., 2007). The research by Bilandžić et al. (2014), with an average of 2.14 μg/kg in Croatian lime honey, delivered similar results to this study.

However, in several studies authors (Dobrzancki, 1994; Leita et al., 1996) have reported higher average values than those determined in this study (12-63 μg/kg).

Mass fraction of Pb in this study revealed no statistically significant differences among individual types of honey, with a median of 0.015 mg/kg. Given the positive trend which indicates a decrease in the level of lead in the environment over the last decades, the same trend is noticeable in food, including honey, as reported by Leita et al. (1996). Some authors stated that a relatively low level of honey contamination with heavy metals is explained by the fact that honey bees partially "filter" heavy metals from honey (Leita et al., 1996; Celli and Maccagnani, 2003). However, there are exceptions, such as those describing high proportions of Pb in Croatian honey samples, where Bilandžić et al. (2012) recorded high mean values in honey from the central region of Croatia (0.131 mg/kg) with an average value of 0.065 mg/kg determined based on all investigated samples. Leita et al. (1996) found even higher Pb concentration ranges (from 1.7 to 1.8 mg/kg) in honey samples. The results obtained in this study did not confirm such high values of Pb in honey from different Croatian areas. However, Biladžić studies (Bilandžić et al., 2011; Bilandžić et al., 2012) are important because they have accentuated the lead content in Croatian honeys as problem of particular concern. Negative connotation from those studies regarding high values should not be ignored, even other Croatian studies (Ursulin-Trstenjak et al, 2015; Ursulin-Trstenjak et al, 2017), including our study, did not confirm such high values. In Ursulin-Trstenjak et al. (2015) study the proportion of Pb ranged with the average amount from 0.02 mg/kg to

0.11 mg/kg. In another study Ursulin-Trstenjak et al. (2017) stated that potential contaminants (Pb and Cd) were accounted for a total average of insignificant value of ≤0.06 mg/kg. The results mentioned are also similar with foundings in the Swiss (0.01 mg/kg) and the Italian black locust honey (0.02 mg/kg) (Forte et al. 2001; Bogdanov et al. 2007), and also in honey samples from eight subregions of Bosnia and Herzegovina Pb content was 0.03–0.66 mg/kg (Alibabić et al., 2015). Nevertheless, recommendation regarding setting position for honey production hives in areas distant from highways generally can be reasonable, even high results need critical evaluation. In our study it can be assumed that all samples were in line with the requirements of EU Regulation No 2015/1005 of 25 June 2015, amending Regulation (EC) No. 1881/2006 setting maximum levels of lead in certain foodstuffs (max. 0.10 mg/kg in honey). Accordingly, the permissible weekly dose (PTWI) of 0.025 mg/kg of body weight (or 1.75 mg Pb/week for people weighing 70 kg) proposed by the World Health Organization (WHO) will not be reached. Based on the aforementioned, it can be concluded that these concentrations of Pb in honey samples from different areas of Croatia do not harm consumers' health, and that even an increased consumption of honey will not significantly increase the weekly intake of this contaminant.

The mean concentrations of Rb (4.732 mg/kg) in the samples of chestnut honey were several times higher (median 7.595 mg/kg) compared to the samples of black locust honey (median 0.343 mg/kg), as well as compared to the samples of lime honey (4.615 mg/kg). Considering the statistically significant difference between the analysed types of honey (Table 3), it can be concluded that the content of Rb can be used as a good discriminating potential for determining the botanical origin.

Golob et al. (2005) determined approximately twice the value of Rb concentration in Slovenian black locust honey samples (0.7 mg/kg), and also twice the value in the lime honey samples (9.5 mg/kg) compared to those determined by this research.

When concentrations of Sr were analysed, statistically significant differences were noticed between the studied types of honey. The highest average values were recorded in chestnut honey, where the highest values of the minimum and maximum content of Sr in the three types of honey (Table 2) were also recorded. Staniškiene et al. (2006) determined the range of Sr mass fraction from 19.5 to 240.9 µg/kg, with a mean of 106.5 µg/kg, in different types of Lithuanian honey, which is similar to the results shown in this study.

Geographical origin factors. Looking at the mineral composition by region, 8 of 15 examined elements showed statistically significant differences: Mn, Zn, Cd, Pb, Mg, K, Ca and Rb (Table 6). Comparing the values with the research carried out by Fredes and Montenegro (2006), who found a very high Al content (22.6 mg/kg) in honey samples originating from Chile, the values established in this study (1.88 mg/kg) are significantly lower. As possible reasons for the high concentrations of Al in those samples, authors have mentioned pollution caused by poor quality beekeeping equipment or by volcanic activity which are common phenomena in that country. Only the content of Pb and Zn was significantly higher in honey samples originating from the Pannonian Region, and it is a good basis for demarcation of the geographical origin. Partially, the Cd content also suggests a possible confirmation of the regional origin of honey from the same region, due to sporadic elevated levels (max. 0.023 mg/kg) and consequently the highest average values (0.002 mg/kg). In support of this thesis, the results are presented for the purpose of discrimination by botanical origin (Table 3). It is apparent that some heavy metals (Pb, Cd) had no statistically significant positive correlation factor in darker types of honey because they typically occur occasionally and often have local (regional) character. The same was described in a series of scientific papers with the conclusion of good demarcating potential of these metals, just like in honey from the Croatian Pannonian Region. These results might be confirmed by future studies, with the inclusion of additional analytical parameters.

Conclusions

The ICP-MS technique proved to be very useful as an analytical tool for the routine determination of the botanical and geographical origin of honey, especially as a good complement to the pollen analysis, because it was found that each honey type has its own characteristic multi-elemental profile. The presence of Mn and Sr in analysed chestnut honey samples clearly indicates the marking of the botanical origin for that type of honey. The other elements also statistically suggest the marking of three tested types of honey, with K, Ca and Rb being the most prominent, showing an increasing content trend from black locust, through lime to the highest content in chestnut honey. Croatia is a small country by area, but due to its distinctive geological and climatological diversity, the content of some elements and their ratios indicate the possibility of recognition of particular honey types according to the regional origin of the samples. This is indicated by unusually high concentrations of Pb (median 0.023 mg/kg) and Zn (median 1.47 mg/kg) in the samples from the Pannonian Region, compared to the samples from the Adriatic Region (Pb median 0.000 mg/kg; Zn median 1.073 mg/kg) or the Mountainous Region (Pb median 0.014 mg/kg; Zn median 1.144 mg/kg).

Determined concentrations of Pb, Cd, Ni and Zn, as potential contaminants in the studied samples, are low and do not harm consumers' health, and even an increased consumption of honey will not significantly increase their weekly intake. On the basis of the obtained results, this preliminary study is the foundation for future analysis in order to prove both the botanical and geographical origin of Croatian types of honey.

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