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Food Chemistry 91 (2005) 593–600
Food Chemistry
www.elsevier.com/locate/foodchem

Determination of trace and minor elements in Slovenian honey by total reflection X-ray fluorescence spectroscopy

T. Golob [a], U. Doberšek [a,*], P. Kump [b], M. Nečemer [b]

[a] Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[b] Department for Low and Medium energy Physics, Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia

Received 2 February 2004; accepted 8 April 2004

Abstract

Trace and minor elements in Slovenian honey were analysed by total reflection X-ray fluorescence spectroscopy. Upto 16 elements (K, Cl, S, P, Ca, Mn, Rb, Cu, Fe, Ni, Cr, Br, Ti, Pb, Sr and As) were detected, in a range of average content from 1.24 mg kg^{-1} for Sr to 2590 mg kg^{-1} for K. Statistically significant differences were established between different types of honey (acacia, floral, lime, chestnut, spruce, fir, forest and *Metcalfa pruinosa* honeydew honey). The highest content of elements was determined in forest honey and the lowest in acacia honey. Honeys were also separated according to their botanical origin as nectar honey or honeydew honey. Total elemental content and the content of S, Cl, K and Rb in honeydew honey was statistically significantly higher than in nectar honey. Chestnut honey differed in statistically its significantly higher contents of Rb and Ca from nectar and honeydew honeys. The year of honey production proved to have no statistically significant influence on elemental content. A comparison of our data with the literature data showed a relatively large diversity.

Keywords: Honey; Elements; Total reflection X-ray fluorescence spectroscopy; Botanical origin; Geographical origin

1. Introduction

Honey is the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature. The main types of honey according to botanical origin are blossom honey or nectar honey, obtained from the nectar of plants, and honeydew honey, obtained mainly from excretions of plant sucking insects on the living parts of plants or secretions of living parts of plants (Council directive, 2001).

Honey contains many different substances, mainly sugars such as fructose, glucose and sucrose. Water can be present in general in an amount lower than 20% (Council directive, 2001) or lower than 18.6% according to Slovenian legislation for honey of topmost quality (Regulation on Honey, 1999). In honey, there are also present various organic and inorganic acids, proteins, amino acids, enzymes, vitamins, hormones, flavonoides and elements (Golob & Plestenjak, 1999). The total content of elements or ash must be lower than 0.6% for nectar honey and lower than 1.0% for honeydew honey (Regulation on Honey, 1999). The presence of individual elements in Slovenian honey has not yet been determined. Different researchers determined only the content of ash in the main types of Slovenian honey (Božnar & Senegačnik, 1998; Golob & Plestenjak, 1999).

* Corresponding author. Tel.: +386 01 423 11 61; fax: +386 01 256 62 96.
E-mail address: urska.dobersek@bf.uni-lj.si (U. Doberšek).

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doi:10.1016/j.foodchem.2004.04.043

The elemental composition of a particular honey sample greatly depends on the composition of the nectar or honeydew and pollen from which it originates. This enables determination of the botanical and possibly also the geographical origin of honey (Latorre et al., 1999; Paramás et al., 2000; Sanz, Perez, Herrera, Sanz, & Juan, 1995).

The elemental content of honey has been determined by many authors all over the world (Conti, 2000; Devillers et al., 2002; Przybyłowski & Wilczyñska, 2001; Rodriguez-Otero, Paseiro, Simal, Terradillos, & Capeda, 1995; Trstenjak-Petrović, Mandić, Grgić, & Grgić, 1994; Vorlová & Čelechovská, 2002; Yılmaz & Yavuz, 1999). Different researchers used various methods such as flame atomic absorption spectrometry (FAAS) (Conti, 2000; Trstenjak-Petrović et al., 1994; Vorlová & Čelechovská, 2002), electrothermal atomic absorption spectrometry (ETAAS) (Przybyłowski & Wilczyñska, 2001; Vorlová & Čelechovská, 2002), inductively coupled plasma atomic emission spectrometry (ICP-AES) (Devillers et al., 2002), ion chromatography and voltammetry (Buldini, Cavalli, Mevoli, & Sharma, 2001). The elemental contents of elements in all of the main types of honey produced in one country have not been determined until now.

Slovenia produces many types of unifloral (acacia, lime, chestnut, spruce, fir and *Metcalfa pruinosa* honeydew honey) and multifloral (floral and forest) commercially available honey. Until now there has been no research that would determine the content of elements in Slovene honey, except one of Kump, Nečemer, and Šnajder (1996), who analysed honey, pollen and bee tissue in order to determine the status of the environment. The method they used (total reflection X-ray fluorescence spectrometry) proved to be appropriate, because of its simplicity, the possibility of multielemental analysis, fast elemental determination, avoidance of contamination and low impact on the environment. We decided to expand that research and to try to determine both the botanical and geographical origin of Slovene honey through their elemental profiles.

2. Materials and methods

2.1. Sample preparation

Sampling was done by beekeepers in Slovenia. All 79 samples of honey were classified according to their botanical origin using the method of Crane (1979). The following eight types of honey were identified: acacia, floral, lime, chestnut, spruce, fir, forest and honeydew honey from *M. pruinosa*. A botanical classification was achieved when the pollen spectrum contained >40% (>80% for chestnut honey) of the corresponding dominant pollen. Only the *M. pruinosa* honey was not classified using this method because this is a honey produced from the honeydew secreted by the cicada *M. pruinosa* which is found only in the south western region of Slovenia, Primorska. All samples were analysed for water, proline, free and total acids, lactones and HMF content, ash, specific electrolytic conductivity and pH value and were found adequate according to the Council directive (2001).

Table 1
Samples of honey analysed

Type of honey	Number of samples
Nectar	
Acacia	9
Lime	7
Floral	9
Nectar and honeydew	
Chestnut	25
Honeydew	
Forest	7
Spruce	7
Fir	8
Metcalfa pruinosa	7

The number of samples analysed is presented in Table 1. The number of samples of different types were not equal, because honey samples were chosen randomly. The types of honey chosen are those produced in the highest amounts in Slovenia.

2.2. Elemental analysis

Total reflection X-ray fluorescence spectroscopy (TXRF) was used to determine the content of a number of elements present in honey. TXRF has several advantages: it is a rapid, non-destructive and multielemental method; it allows analyses of upto 10 samples per hour. The sample is measured directly, just diluted with doubly distilled water, and therefore any sample contamination during preparation is avoided. The analysis gives information about almost the complete range of minerals in the sample, which means that only one measurement of a sample is needed to determine the presence and quantity of all elements with atomic number equal or higher than 13. TXRF was also shown to have a good sensitivity when analysing organic samples such as honey (Klockenkämper, 1997; Kump et al., 1996).

The spectrum of a floral honey sample is shown in Fig. 1. The elements present in the sample are shown by their names above the respective fluorescent peaks. The quantity of an element depends on the net peak area and on the efficiency of excitation and detection of fluorescent radiation.

The TXRF analytical system was assembled by P. Kump at the Jožef Stefan Institute. For excitation a focused X-ray beam from a fine focus X-ray tube (Seifert,

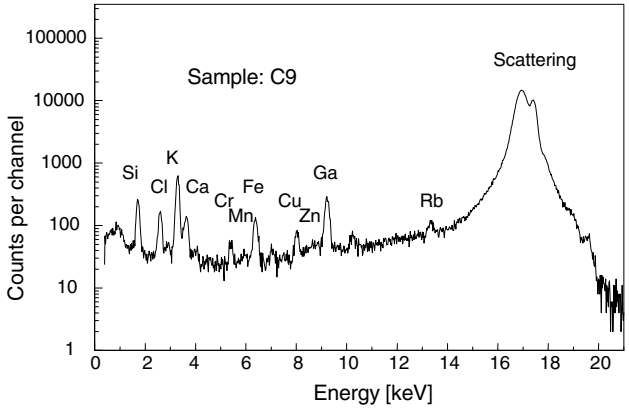

Fig. 1. Spectrum of sample C9 (floral honey).

Germany) with a Mo anode operating at 40 kV and 30 mA, and monochromatised using a carbon–tungsten (C/W) multilayer to the energy of the Mo Kα line (17.4 keV) was used. The sample deposit on the optically flat quartz substrate was irradiated at an incident angle lower than the critical angle of the substrate material (⩽1.8 mrad). The beam was totally reflected from the substrate but excited fluorescence and scattering practically only from the sample deposit. Because of the strongly reduced scattering and efficient excitation of the sample, the sensitivity of elemental determination was increased by several orders of magnitude relative to the standard X-ray fluorescence (XRF) technique.

The X-ray spectrometer attached to the excitation system was based on a Si(Li) semiconductor detector (Princeton Gamma Tech Co., USA, FWHM 140 eV at 5.9 keV) with the appropriate preamplifier, amplifier and HV bias supply and PC-based Multi Channel Analyzer (MCA) from Canberra, USA.

A solution of 0.3 g of honey and 10 ml of doubly distilled water was made. To this solution 1 ml of gallium with a concentration 0.01 g/l as an internal standard was added. The solution was stirred and 10 μl of the solution was transferred to the quartz (reflector) substrate where it was dried under an IR lamp. Then the measurement of whole mineral spectrum of a sample took place. The measurement lasted 5 min. In order to determine the background of the system the elemental content of a blank sample from pure glucose dissolved in doubly distilled water was measured.

The combined uncertainty of elemental analysis by TXRF was estimated by considering the standard uncertainty in all the steps of the measurement and spectrum analysis. Typical uncertainties of the results were 5–15% depending on the element and the concentration.

In order to assess repeatability, six measurements on samples prepared independently from the same sample material were analysed. For most of the elements the repeatability was within the estimated uncertainty.

A homogeneity test of the honey solution was performed by analysing six samples prepared from the same solution. The results of this test showed that the homogeneity of the solution is the main factor contributing to the repeatability of the results.

For all the measured elements the limit of detection (LOD) was estimated and results which were close to or lower than the respective LODs were excluded from any statistical data analysis.

2.3. Statistical analysis

After choosing the data which well exceeded the uncertainty and/or repeatability of the TXRF method statistical treatment of these results was made. The following statistical parameters were calculated; average value, standard deviation and coefficient of variability, correlation analysis and Duncan's test. Correlations were obtained by Pearson's correlation coefficient in bivariate linear correlations. Differences between means at the 5% ($P < 0.05$) level were considered significant.

3. Results and discussion

3.1. Testing the method

The first step of testing the TXRF method was made on seven samples of acacia honey, which were analysed in six replicates. The results of this test are shown in Table 2. Elements with the most repeatable content were S, Cl, K, Ca, Mn, Br and Rb. These elements were used in further analysis where comparison between types of honey was made.

The LOD was set separately for each sample and also for each element. It depended on the quantity of element that was analysed and mostly on the quantity of sugars present in the sample. LODs were practically the same in samples of different types of honey and the values for each element are presented in Table 2.

The results of the analyses of one sample of each type (except chestnut) of Slovenian honey (acacia, floral, lime, forest, spruce, fir and *M. pruinosa* honeydew honey) are presented in Table 3. The analyses were performed in six replicates from the same solution. Coefficients of variability are high due to the non-homogeneous distribution of some elements, which are probably not in ionic form but are bound to different organic molecules such as acids, proteins, enzymes etc. and therefore distributed in the form of clusters in the honey solution. The result of the non-homogeneity of honey solutions is a low repeatability of the method. That is why only data with differences much higher than the repeatability were used for further analysis.

Elements with the highest coefficient of variability (CV) were Ca and Mn and those with the lowest CV

Table 2
Content of elements (mg kg^{-1}) detected in acacia honey analysed in six replicates and limit of detection (LOD) for each element

Sample (n = 6)		Content of elements in acacia honeys (mg kg^{-1})														
		P	S	Cl	K	Ca	Cr	Mn	Fe	Ni	Cu	Zn	As	Pb	Br	Rb
1	$\bar{x}$	[a]	[a]	119	435	10.4	0.78	1.1	2.2	0.70	1.4	4.55	1.47	3.07	0.81	1.98
	SD			15	0	11.7	0.13	0.06	2.2	0.12	0.15	1.1	0.45	0.22	0.12	0.59
2	$\bar{x}$	70.3	37	59	183	6.79	2.82	0.30	2.91	0.60	1.76	3.8	[a]	1.86	0.77	0.85
	SD	19	16	18	2.8	0.11	0.05	0.12	0.23	0.56	0.18	1.2		0.51	0.19	0.21
3	$\bar{x}$	119	54	193	573	10.6	3.55	2.02	3.93	0.92	1.85	3.10	1.49	1.90	0.76	2.49
	SD	23	21	25	18	1.2	0.32	0.01	0.35	0.94	0.27	0.56	0.68	0.46	0.11	0.39
4	$\bar{x}$	98.8	52.1	116	300	10.3	3.13	1.08	2.61	0.21	1.94	4.73	1.45	3.45	0.97	0.79
	SD	18	17	34	2	8.0	0.11	0.34	0.03	0.28	0.51	0.30	0.23	0.48	0.15	0.08
5	$\bar{x}$	104	56.3	92.3	269	3.4	1.6	2.30	2.82	1.7	2.70	3.5	1.41	2.1	0.69	1.60
	SD	20	13	8.7	9	4.1	2.0	0.74	0.69	1.1	0.18	1.2	0.36	1.3	0.09	0.23
6	$\bar{x}$	117	65	123	417	9.16	2.58	1.91	1.98	0.91	2.26	4.15	1.48	4.3	0.56	1.01
	SD	28	25	4	0	10.2	0.44	0.50	1.55	0.03	0.51	0.08	0.59	1.5	0.08	0.15
7	$\bar{x}$	97.7	54	174	779	12.9	1.6	1.97	2.88	1.15	1.60	2.92	1.24	2.31	1.03	2.36
	SD	19	23	26	35	2.0	1.2	0.06	0.76	0.32	0.08	0.36	0.21	0.95	0.18	0.42
LOD		20.3	14.2	8.17	3.29	2.56	0.71	0.60	0.51	0.37	0.35	0.31	0.36	0.44	0.50	0.58

[a] Concentrations were lower than LOD.

Table 3
Mean value, SD and coefficient of variability (CV) in one sample of each type of honey analysed in six replicates

Type of honey		Content of elements (mg kg^{-1})						
		S	Cl	K	Ca	Mn	Br	Rb
Acacia	$\bar{x} \pm$ SD	41 ± 5	51 ± 6.1	200 ± 7.2	12 ± 3.7	1.6 ± 0.5	0.9 ± 0.1	0.7 ± 0.1
	CV (%)	12.0	11.9	3.6	31.5	28.4	12.6	14.2
Floral	$\bar{x} \pm$ SD	54 ± 5.9	135 ± 16	564 ± 20	38 ± 3.4	1.4 ± 0.1	0.7 ± 0.1	2.3 ± 0.2
	CV (%)	10.8	11.9	3.6	9.0	8.5	11.7	10.2
Linden	$\bar{x} \pm$ SD	87 ± 7.1	323 ± 37	2245 ± 122	110 ± 12	8.8 ± 1.3	1.5 ± 0.4	9.5 ± 0.4
	CV (%)	8.1	11.5	5.5	10.7	14.6	26.3	4.0
Spruce	$\bar{x} \pm$ SD	54.6 ± 5.3	280 ± 20	1720 ± 25	25.2 ± 2.7	3.5 ± 0.6	15 ± 0.3	8.8 ± 0.3
	CV (%)	9.7	7.2	1.5	10.9	16.9	19.3	3.4
Fir	$\bar{x} \pm$ SD	72 ± 10	300 ± 27	2350 ± 58	24 ± 5.7	2.5 ± 0.7	0.7 ± 0.1	15.6 ± 0.5
	CV (%)	14.3	9.1	2.5	23.8	29.4	15.9	3.0
Forest	$\bar{x} \pm$ SD	69 ± 6.3	439 ± 29	1580 ± 51	116 ± 22	7.0 ± 1.2	1.0 ± 0.1	6.5 ± 0.3
	CV (%)	9.1	6.6	3.2	19.3	17.0	14.2	5.2
Metcalfa pruinosa	$\bar{x} \pm$ SD	136 ± 22	329 ± 35	5080 ± 87	38.2 ± 8.0	7.1 ± 0.7	0.9 ± 0.1	8.2 ± 0.9
	CV (%)	16.5	10.6	1.7	21.0	9.9	5.6	10.6

were K and Rb. In general the CV for minerals is higher in nectar honey than in honeydew honey.

3.2. Analytical results for honey samples

The elemental content of different types of Slovenian honey are summarised in Table 4. This table shows that K was the element present in all types of honey in the highest concentrations. The concentrations of Cl, S and Ca were also high, while concentrations of other elements were low. Only Mn in chestnut honey and Rb in chestnut and *M. pruinosa* honeydew honey were present in relatively high concentrations. Total mineral content (ash) was the highest in forest honey and the lowest in acacia honey.

From Duncan's test of the data on mineral content in Slovenian honey of different botanical origin it was concluded that nectar honeys statistically significantly differed from honeydew honeys, as shown in Table 5. A further conclusion was that chestnut honey differed from honeydew and nectar honeys.

Table 4
Summary of the results for elemental content (mg kg^{-1}) in different types of Slovenian honey

Type of honey	Number of samples	Basic statistics	Average content of elements (mg kg^{-1})							Total elemental content (ash)
			S	Cl	K	Ca	Mn	Br	Rb	
Acacia	9	$\bar{x}$	51^{a}	110^{a}	390^{a}	9.4^{a}	1.5^{a}	0.8^{a}	1.2^{a}	680^{a}
		Interval	37–65	59–190	180–780	3.4–13	0.3–2.3	0.77–081	0.4–2.5	
Lime	7	$\bar{x}$	50^{a}	290^{b}	780^{a}	43^{b}	2.8^{a}	1.0^{a}	2.3^{a}	1180^{a}
		Interval	38–68	110–460	470–1200	20–78	1.3–4.9	0.6–1.7	1.2–5.0	
Floral	9	$\bar{x}$	66^{a}	340^{b}	2000^{b}	49^{b}	3.2^{a}	1.1^{a}	11^{b}	2470^{b}
		Interval	42–120	300–420	1600–3300	0.0–110	0.6–9.1	0.6–1.5	3.6–27	
Chestnut	25	$\bar{x}$	140^{a}	200^{b}	3500^{b}	150^{c}	28^{b}	0.9^{a}	22^{c}	4300^{b}
		Interval	57–440	130–310	350–4800	81–270	12–66	0.9–0.9	14–35	
Forest	7	$\bar{x}$	140^{a}	240^{b}	5100^{c}	63^{b}	4.3^{a}	1.0^{a}	8.1^{b}	5530^{c}
		Interval	130–160	150–320	4400–5800	30–100	2.2–6.9	1.0–1.0	5.7–14	
Spruce	7	$\bar{x}$	88^{a}	260^{b}	2400^{b}	41^{b}	6.6^{a}	1.0^{a}	15^{b}	2830^{b}
		Interval	49–180	4.0–400	1700–3000	8.4–110	4.1–9.0	0.7–1.5	9.0–19	
Fir	8	$\bar{x}$	59^{a}	360^{b}	2100^{b}	39^{b}	3.2^{a}	0.8^{a}	13^{b}	3800^{b}
		Interval	46–72	130–560	560–3000	0.9–110	0.1–6.3	0.3–1.4	0.6–23	
Metcalfa pruinosa	7	$\bar{x}$	150^{a}	350^{b}	3000^{b}	19^{b}	4.1^{a}	0.7^{a}	22^{c}	3570^{b}
		Interval	68–260	290–410	2700–3300	3.8–37	0.8–13	0.6–0.8	19–23	

^{a,b,c} Data with different mark in column statistically significantly differ from each other.

Table 5
Average content of main elements and total elemental content (mg kg^{-1}) in honey of different botanical origin

Type of honey	Average content of elements							Total elemental content (ash)
	S	Cl	K	Ca	Mn	Br	Rb	
Nectar honey	56^{a}	243^{a}	1100^{a}	33^{a}	2.5^{a}	1.0^{a}	5.0^{a}	1410^{a}
Honeydew honey	115^{b}	300^{b}	3100^{b}	40^{a}	4.5^{a}	0.8^{a}	14^{b}	3680^{b}
Chestnut	140^{b}	200^{a}	3500^{b}	150^{b}	28^{b}	0.9^{a}	22^{c}	4300^{b}

^{a,b,c} Data with different mark in column statistically significantly differ from each other.

We also investigated whether the elemental content could help in determining botanical origin. The logarithmic values of the content of the seven main elements in nectar types of honey and in chestnut honey are shown in Fig. 2, and in Fig. 3 the corresponding logarithmic values in honeydew types of honey and in chestnut honey. The results for chestnut honey are presented in both figures. According to the statistical treatment of results, chestnut honey statistically significantly differed from nectar and honeydew honeys. From Figs. 2 and 3 it can be seen that the shapes of the plots for different types of honey are different. The results of Duncan's test of differences between the contents of specific element in different types of honey are presented in Table 4. The final conclusion of this test is that all types of Slovenian honey differ from each other in elemental content; every type has its specific profile or "fingerprint". For example, chestnut honey contained statistically significantly more Ca and Mn than other types of Slovene honey. Forest honey contained statistically significantly more K and the total elemental content was higher than in other types of Slovenian honey. Spruce, fir and floral honey differed from each other in the contents of trace elements. For example, Ti was present only in floral and *M. pruinosa* honeydew honey. And further, fir honey contained significantly more Fe than other types of honey.

In order to examine whether the year of honey production has any influence on its elemental content or not, a comparison between two samples of the same type, of the same geographical origin but different year of production was made. The main elemental contents in each analysed pair of samples are shown in Table 6. Statistical treatment of these data (Duncan's test at $\alpha = 0.05$) showed that the year of production did not significantly influence the content of different elements.

The numbers of positive responses for 16 studied elements with their corresponding mean, lowest and highest content (mg kg^{-1}) in Slovene honey are given in Table 7. It can be seen that only Cr, Fe, Cu, Cl, K, Ca, Mn and Rb were found in all 79 samples analysed. The rarest elements were As and Sr. The mean content of elements differed

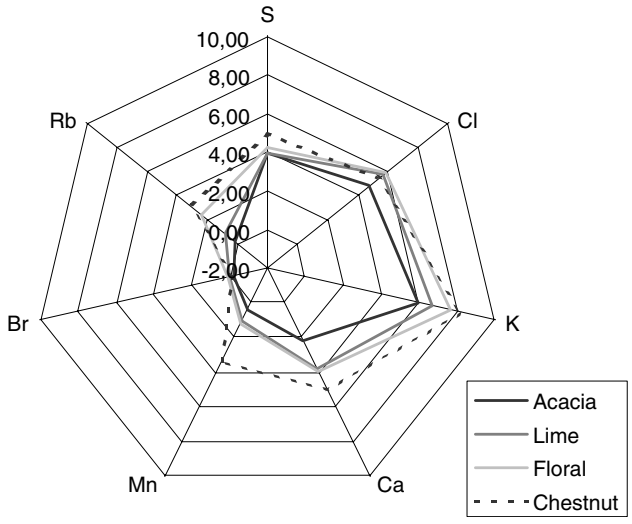

Fig. 2. Comparison of logarithmic values of contents of main elements in nectar types of honey and in chestnut honey.

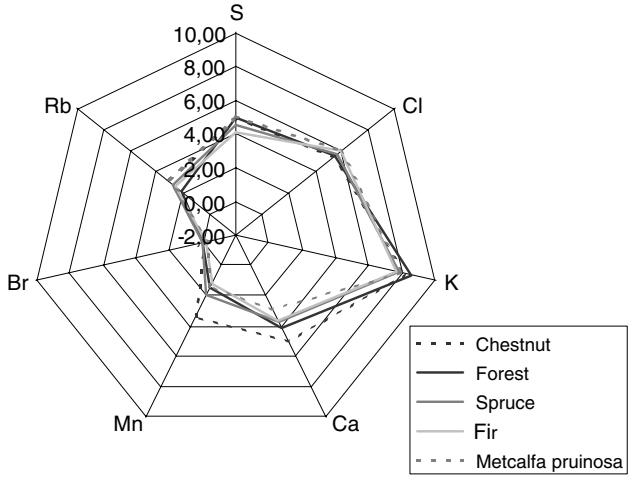

Fig. 3. Comparison of logarithmic values of contents of main elements in honeydew types of honey and in chestnut honey.

from 1.24 mg kg^{-1} for Sr to 2590 mg kg^{-1} for K. Nine elements were found in Slovenian honey at a mean content lower than 10 mg kg^{-1}. Another three elements were present with a mean content lower than 15 mg kg^{-1}. Only four elements (S, Cl, K and P) were present in mean contents higher than 100 mg kg^{-1}.

Correlation analysis showed that there were two strong positive correlations; between the content of K and the total elemental content (ash) in honey ($r = 0.996$), as well as between the content of Ca and the content of Mn ($r = 0.857$). The relationship between the contents of Ca and Mn is not understood, but for the relationship between content of K and total elemental content it can be said that this relationship is simply due to the fact that K represents the overwhelmingly greatest part by mass of the total elemental content.

Table 6
Content of elements (mg kg^{-1}) in paired honey samples produced in different years at the same location

Sample		Content of elements (mg kg^{-1})						
Type	Year	S	Cl	K	Ca	Mn	Br	Rb
Acacia 1	2001	40.9	119	435	10.4	1.07	0.00	0.99
	2000	43.3	58.8	200	11.9	1.03	0.81	0.69
Acacia 2	2001	37.4	58.6	183	6.79	0.30	0.00	0.43
	2000	44.0	74.4	351	8.69	2.01	0.77	0.76
Floral 1	2001	37.5	456	743	20.2	1.26	1.22	2.09
	2000	45.3	325	883	58.5	3.35	1.15	1.98
Floral 2	2001	68.5	111	470	27.6	3.32	0.75	1.83
	2000	56.2	130	564	37.1	2.71	0.78	2.58
Floral 3	2001	52.4	447	1200	78.4	4.91	1.66	2.75
	2000	45.9	558	985	13.0	0.12	0.31	0.63
Fir 1	2001	185	321	3070	3.77	3.63	0.80	22.8
	2000	84.1	299	2390	27.3	2.39	0.67	15.9

Table 7
Number of positive responses (Nb/79) for 16 studied elements with their corresponding mean, lowest and highest content (in mg kg^{-1})

Element	Nb/79	Mean (mg kg^{-1})	Interval (mg kg^{-1})
S	71	102	37.4–445
Cl	79	251	3.97–558
K	79	2590	183–5810
Ca	79	73.5	0.03–274
Mn	79	11.3	0.12–66.4
Br	39	0.92	0.31–1.66
Rb	79	13.8	0.04–34.8
P	12	155	70.3–398
Ti	12	2.85	0.23–4.97
Cr	79	7.22	0.11–33.8
Fe	79	12.5	0.30–70.4
Ni	77	1.69	0.00–12.7
As	6	1.42	1.24–1.49
Cu	79	3.22	0.37–15.5
Zn	79	3.61	0.55–11.2
Pb	28	5.94	0.21–79.1
Sr	8	1.24	0.73–1.76

3.3. Comparison with the results of other authors

The mean concentrations of K, Mn and Cl found in Slovenian honey were much higher than the values given in Tables of Foods Composition in Germany (Souci, Fachmann, Kraut, Scherz, & Senser, 2000) and in Great Britain (Holland et al., 1992). The concentration of K was five times higher and the concentration of Mn twice as high as in the Tables from Great Britain and 20 times higher than in Tables from Germany. A comparison between concentrations of Ca, Fe, and P in Slovenian honey and concentrations of these elements in the British and German Tables were similar. The analytical methods and the nature of the samples (botanical and geographical origin) were not specified so it is hard to compare

the results of this research with the data from different food composition tables.

Previously the content of Cr in Slovenian floral honey was found to be 12 times higher than in Croatian floral honey and 18 times higher in Slovenian acacia honey than in Croatian acacia honey (Trstenjak-Petrović et al., 1994). The content of Cl and S was twice as high in Slovenian floral honey as in Spanish floral honey according to results of Rodriguez-Otero et al. (1995). It is hard to find data about the mineral content in specific types of honey, because not all types of honey are present in all countries and most authors do not classify honey according to botanical origin into different types. Authors usually analyse samples from one country and give results as the mean content of analysed elements in all samples regardless of the botanical origin of samples.

The results of this research are also difficult to compare with other available data due to the different methods used to analyse honey. Most authors also restricted their researches to analysis of only a few elements present in honey (Buldini et al., 2001; Conti, 2000; Przybyłowski & Wilczyñska, 2001; Trstenjak-Petrović et al., 1994; Vorlová & Čelechovská, 2002; Yılmaz & Yavuz, 1999). The mean content of Pb in Slovenian honey was higher than in French honey according to Devillers et al. (2002) and than in Czech honey according to Vorlová and Čelechovská (2002).

The great differences in the mean concentration of K in Slovenian honey and other analysed honeys (Conti, 2000; Yılmaz & Yavuz, 1999) could be due to the higher number of chestnut honey samples in this study and the presence of chestnut pollen in almost every sample of Slovenian honey. Bernard and Poklukar (2001) found that the presence of chestnut pollen is typical of Slovenian honey and that chestnut pollen was present in 94.6% of all samples of Slovenian honey analysed.

4. Conclusions

TXRF proved to be very useful for determination of the botanical origin of honey, because it was shown that every type of honey has its own characteristic profile in the content of elements that can be detected with this method. As stated above, the method has several advantages: it is quick and therefore appropriate for analysing numerous samples; further, contamination of samples is eliminated due to absence of chemical pre-treatment.

Sampling in further research should be done systematically and by qualified personnel rather than beekeepers. In this research samples of honey that were collected by beekeepers were used and it is not known where, how and for how long the honey was stored before being analysed. Contamination with metals may have occurred before pouring the honey into glass containers where it was stored prior to analysis.

This preliminary research provided the groundwork for further analyses that will determine the geographical origin of Slovenian honey. In order to properly determine geographical origin the number of samples of each type of Slovenian honey from each geographical region of Slovenia should be enlarged. Also more sophisticated statistical tests (approaches) for data analysis, such as neural networks, should be used. The finding of characteristic elemental profiles for different types of honey allows the monitoring of the quality of honey, by detection of adulteration, dilution and mixing of honeys.

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