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CHARACTERIZATION OF ROMANIAN HONEY USING PHYSICO-CHEMICAL PARAMETERS AND THE ELEMENTAL CONTENT DETERMINED BY ANALYTICAL TECHNIQUES

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Abstract. The aim of this work was to characterise 36 samples of bee honeys from some geographical zone of Romania, which were collected two years consecutive 2012 and 2013, by physico-chemical characteristics together with content of chemical elements. For this purpose physico-chemical characteristics together with elemental content were evaluated. Physicochemical parameters: pH, electrical conductivity, ash content, refractive index and water content, were analysed using the Harmonised Methods of the International Honey Commission. Concentrations of K, Ca, Fe, Cu, Zn and Pb were determined by analytical techniques of high accuracy and sensitivity: Atomic Absorbtion Spectrometry (AAS) and Energy Dispersive X-Ray Fluorescence (EDXRF), combined with Internal Standard Technique. The obtained results indicate a good quality level, that demonstrates an adequate extracting and processing of honey.

Key words: honey, quality, AAS, EDXRF.

1. INTRODUCTION

Honey is defined as the natural sweet substance produced by *Apis mellifera*, dehydrate, store and leave in honeycombs to ripen and matbees 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 the bees collect, transform by combining with specific substances of their own, depositure [1]. The main constituents of honey are: water, glucose, fructose, maltose, sucrose, proteins, free amino acids, phenolic compounds and mineral elements [2,3]. The honey composition is influenced by the plant species (plant flowers), soil resources, environmental conditions, and extraction mode and processing.

Romania has a very long tradition of beekeeping. Its favourable climate, good geographical conditions and a variety of botanical species provide a great potential for the development of apiculture. Romanian honey could be very interesting for the EU market, so it is very important to verify its compliance with the quality specifications of the European Union. The European Council Directive [1], include general and specific characteristics of honey, which are important in the assessment of its authenticity, such as: *pH*, electrical conductivity, ash content, refractive index, water content and mineral content (K, Ca, Na, Mg, Fe, etc.).

The aim of this study was to characterise 36 honey samples, with different botanical origin, collected from different sites of Romania. Honey samples were collected from the same bee-keepers, 18 samples in summer of 2012 and 18 samples in summer of 2013, with the same botanical origin.

Physico-chemical parameters: pH, electrical conductivity, ash content, refractive index and water content, were analysed using the Harmonised Methods of the International Honey Commission [4]. Concentrations of K, Ca, Fe, Cu, Zn and Pb were determined by analytical techniques with high accuracy and sensitivity: Atomic Absorption Spectrometry (AAS - with flame and graphite furnace) and Energy Dispersive X-Ray Fluorescence (EDXRF) combined with Internal Standard Technique [5-8].

2. EXPERIMENTAL

Honey samples, with different botanical origin, collected from different sites of Romania during the 2012 and 2013, harvesting season were collected from individual bee-keepers. About 72 % of honey samples were mono-floral (rape, acacia, sunflower and linden) and about 28 % were multi-floral (table 1). Samples were transferred to the laboratory in the original packages, kept at 20-22 ^{o}C and analysed no longer than 48 h after extraction from the beehive by bee-keepers.

Physicochemical parameters were analysed using the Harmonised Methods of the International Honey Commission [4].

The pH solution of 10 g honey in 75 mL of CO_5 free distilled water was measured by a Consort P501 *pH*-meter. Electrical conductivity was measured at 20 ^{o}C with the conductivity meter HACH CO15 using a solution of 20 g dry matter of honey in 100 ml deionised water. Before the measurements the calibration of the conductivity meter was made using a 0.1 M KCl solution. Final data regarding the electrical conductivity were used to calculate the corresponding ash content.

The refractive index and water content was determined using the NOVEX ABBE refractometer, thermostated at 20 ^{o}C and calibrated with distilled water. Each honey sample was measured twice and the average value was considered. Concen-

Table 1

Botanical origin of honey samples

Code	Sample	Code	Sample
S1	*Brassica ol.* (rape)	S10	*Helianthus tb.* (sun flower)
S2	*Robinia pa.* (acacia)	S11	*Robinia pa.* (acacia)
S3	*Robinia pa.* (acacia)	S12	Bosoms (blend)
S4	*Robinia pa.* (acacia)	S13	Bosoms (blend)
S5	*Robini pa.* (acacia)	S14	Bosoms (blend)
S6	*Tilia tm.* (lime)	S15	*Tilia tm.* (lime)
S7	*Helianthus tb.* (sun flower)	S16	Bosoms (blend)
S8	*Tilia tm.* (lime)	S17	Bosoms (blend)
S9	*Robinia pa.* (acacia)	S18	*Robinia pa.* (acacia)

Table 2

Physico-chemical parameters values of honey samples collected in 2012 and 2013

Sample	pH		EC (μS/cm)		TDS (mg/l)		RI		Water content (%)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
s1	3.91	3.93	163	159	889	711	1.4875	1.4975	20.8	17.4
s2	3.74	3.65	98	98	457	441	1.4990	1.4990	16.8	16.8
s3	4.02	3.96	102	101	497	450	1.4940	1.4960	18.8	18.0
s4	3.95	3.87	97	100	436	446	1.4985	1.4985	17.0	17.0
s5	4.31	4.29	268	252	1177	1114	1.4920	1.4940	18.6	18.8
s6	3.97	3.84	202	202	9017	891	1.4975	1.4980	17.4	17.2
s7	3.72	3.67	192	188	868	830	1.4995	1.4985	16.4	17.0
s8	4.35	4.25	346	334	1496	1494	1.4920	1.4940	18.6	18.8
s9	4.62	4.72	115	109	566	439	1.4840	1.4835	21.8	22.8
s10	3.91	4.09	240	206	1164	920	1.4920	1.4950	18.6	18.4
s11	4.63	4.58	162	162	670	728	1.4920	1.4900	18.6	20.6
s12	4.08	4.12	264	259	1183	1163	1.4960	1.4950	18.0	18.4
s13	4.22	4.08	421	431	1749	1934	1.4965	1.4955	17.4	18.2
s14	3.81	3.61	309	114	372	464	1.4990	1.4995	16.7	16.4
s15	3.82	3.76	292	267	1412	1320	1.4950	1.4950	18.2	18.4
s16	4.64	4.45	336	324	1244	1574	1.4990	1.4930	16.8	19.2
s17	4.03	4.08	161	151	737	640	1.4960	1.4975	18	17.4
s18	4.33	4.28	161	161	776	681	1.4875	1.4990	19.8	16.7

trations of K, Ca, Fe, Cu, Zn and Pb were determined by analytical techniques with high accuracy and sensitivity: Energy Dispersive X-Ray Fluorescence (EDXRF) combined with Internal Standard Technique and Atomic Absorption Spectrometry (AAS).

EDXRF measurements were performed using an ElvaX spectrometer having a X-ray tube with Rh anode, which working, normally, at 45 kV. For the EDXRF experiments, honey samples were prepared thus: An amount of 3 g of honey was mixed with yttrium internal standard (100 μL, from Y_2O_3 nitric solution of 160.78 mg Y/l). Honey samples were placed in containers with thin mylar window (6 microns) and were mounted on the sample holder. The geometry of the excitation of characteristic X-rays was 90 degrees to the direction of primary X-rays of Rh. X-ray spectra characteristic of chemical elements contained in the sample were detected by ElvaX multichannel spectrometer based on a solid state Si-pin-diode X-ray detector with a thickness of 140 μm Be window and a energy resolution of 165 eV at 5.9 KeV of Fe. Measuring time per sample analysed was 1800 seconds. The characteristic X-rays spectra were detected and recorded by a multichannel spectrometer ElvaX. ElvaX software was used for data acquisition and for the EDXRF spectra processing. The accuracy of the measurements was checked by using NIST SRM 1085b Wear-Metals in Lubricating Oil and was obtained a recovery between 97.4 % and 103.5 %.

The AAS measurements were performed using two models of AAS spectrometer: flame atomic absorption spectrometer GBC Avanta model and atomic absorption spectrometer with graphite furnace model Avanta Ultra Z. The honey samples preparation for AAS measurements was following: honey sample was digested in an acid solution using a microwave digestion system and the clear solution volume was made up to 50 ml for each sample using deionised water.

3. RESULTS AND DISCUSSION

The results obtained for determined physico-chemical parameters for honey samples collected in 2012 and 2013 are presented in Table 2. The pH values ranged from 3.61 to 4.72 for honey samples collected in 2012 and values ranged from 3.72 to 4.64 for honey samples collected in 2013. These values are in concordance with the pH acceptable range for honey [9]. The observed variation of *pH* at honey can be given by different honey extraction conditions.

The electrical conductivity of honey depends on botanical origin and can give information about mineral salts and proteins content of honey [3]. The values obtained for honey samples are in range between 97.72 and 431.0 μScm^{-1}, for honey samples collected in 2012, and between 98.10 to 420.7, for honey samples collected in 2013. These values are under maximum values admitted by European honey directive [1, 2].

Through the electrical conductivity measurements, total dissolved solids (TDS) in honey samples were determined (table 2 and table 3). It resulted a linear relationship, between TDS and electrical conductivity at Pearson's correlation coefficient $R^2 = 0.9914$, for honey samples collected in 2012, and R^2 = 0.9596, for honey samples collected 2013 (fig. 1a).

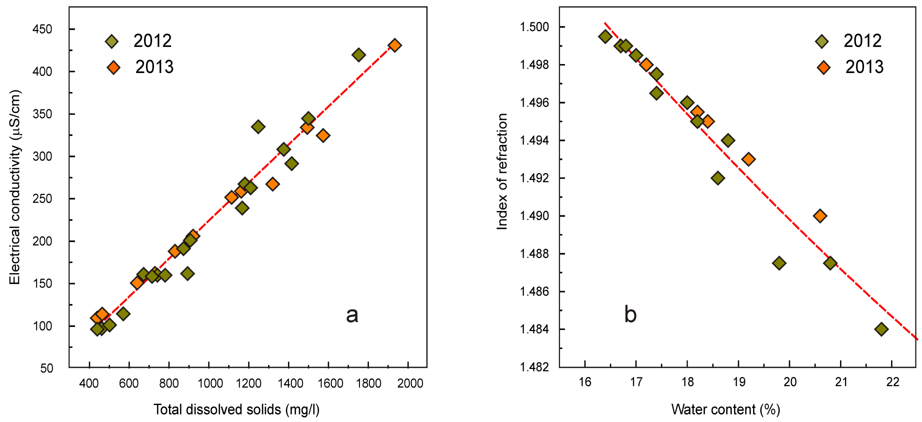

Fig. 1 – The linear relationship between some characteristic parameters of the honey collected in 2012 and 2013: electrical conductivity *vs.* TDS (a) and the index of refraction *vs.* water content (b).

Table 3

The elemental content (in mg/kg) of some natural and possible polluting elements in honey samples collected in 2012 and 2013. K and Ca content determined by XRF, Fe, Zn, Cu and Pb content determined by AAS.

Sample	K		Ca		Fe		Cu		Zn		Pb	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
s1	207	221	46	51	11.5	9.8	0.38	0.39	4.8	4.6	0.10	0.10
s2	258	267	64	61	5.6	5.9	0.22	0.19	1.6	0.9	0.11	0.12
s3	187	169	58	54	6.3	5.9	0.15	0.14	1.8	1.5	1.15	1.13
s4	193	202	63	60	6.5	7.2	0.44	0.40	2.4	2.3	0.10	0.11
s5	433	486	92	89	4.8	4.0	1.38	1.42	3.6	3.8	0.06	0.07
s6	305	339	38	36	6.9	6.2	0.27	0.27	2.7	1.8	0.23	0.11
s7	192	189	50	48	10.2	9.8	0.25	0.26	3.5	2.7	0.25	0.10
s8	327	298	34	35	7.2	6.8	0.20	0.23	3.0	2.8	0.12	0.10
s9	211	225	61	63	7.9	7.4	0.19	0.17	2.2	2.0	0.11	0.13
s10	172	163	49	51	11.0	10.8	0.18	0.17	4.1	3.9	0.07	0.09
s11	244	232	95	98	6.7	6.2	0.10	0.13	3.8	3.5	0.09	0.08
s12	370	368	90	93	5.7	5.0	1.41	1.42	3.1	2.9	0.12	0.14
s13	289	276	66	71	5.8	5.5	2.12	2.04	2.3	2.1	0.10	0.12
s14	368	359	87	90	5.0	4.7	0.28	0.24	1.7	1.0	0.07	0.09
s15	225	249	54	59	6.8	7.2	1.42	1.30	3.7	3.5	0.07	0.08
s16	329	310	32	36	3.2	4.0	0.83	0.94	2.5	2.2	0.09	0.10
s17	352	328	48	56	4.7	3.2	0.21	0.29	4.3	4.9	0.10	0.11
s18	280	278	45	46	1.8	1.3	0.30	0.25	4.0	4.3	0.05	0.06
average	275	279	60	61	6.8	6.2	0.58	0.57	3.1	2.9	0.17	0.16
t-test	-	0.97	-	0.83	-	0.65	-	0.98	-	0.50	-	0.92

The refractive index depends on solids content of honey and default on the water content. A linear relationship based on $R^2 = 0.997$, for honey samples collected in 2012) and $R^2 = 0.9628$, for honey samples collected in 2013 (fig.1b).

A higher water content in honey than the maximum permissible values (20%) by international legislations can be a proof of honey adulteration. For one honey sample in 2012 (S9) and three honey samples in 2013 (*i.e.* S1, S9 and S18) the water content exceeded the maximum admitted values. The concentrations of K, Ca, Fe, Cu, Zn and Pb determined in honey samples under this study are given in Tables 3 and 4.

The most abundant mineral was K with an average content of 269.86 mg/kg (2012) and 271.92 mg/kg (2013) followed by Ca with an average content of 56.31 mg/kg (2012) and 57.95 mg/kg (2013). Fe and Zn were present in moderate amounts in the honey samples, with average content of 6.46 mg/kg (2012) and 6.10 mg/kg (2013) respectively 3.08 mg/kg (2012) and 2.8 mg/kg (2013). Cu and Pb were determined in honey samples - according to the Revised Codex Standard for Honey, Codex STAN [9]: ”Honey shall be free from heavy metals in amounts which may represent a hazard to human health”.

A two-tailed t-test performed to evidence at which extent the content of the same element was coincident or not for bot 2012 and 2013 set of samples, showed that only the content of K, Cu and Pb could be considered coincident with a probability of 97, 98 and 92 % respectively. In the case of other elements, the same probabilities varied between 50 % and 83 % for Zn am respectively for Ca. In the case of Fe, the t-test had a value of 0.63, *i.e.*, the probability the Fe content to be the same for both set of samples was of 63 % (see Table 3).

To get more date regarding the homogeneity of honey samples with respect to the six considered elements chosen to characterize the degree of purity we have analysed their content in all samples (2013 as well as 2013) by means of the Principal Component Analysis (PCA). By considering the honey samples as cases and the six elements as variables, the PCA was performed in both Q (similitude and dissimilitudes between cases) and R (similitude and dissimilitudes between variables) mods. In this way it was possible to evidence the relative high degree of similarity between the honey collected in 2013 and 2013 from the point of view of the six elements considered in this study. Indeed, as the bi-plot reproduced in Fig. 2a shows, all samples, with the exception of the 2012 s3 sample, form a single cluster. On the other hand, the same kind of analysis performed in the R mode, showed that Ca, Cu and K forms a single cluster, faraway from the other three elements which form, each of them its own cluster. This finding could suggests that K, Ca and Cu are connected in a certain measure to the physiologic activity of both bees and plants, while the other thee elements, *i.e.* Fe, Zn and Pb enter in honey as environmental pollutants.

A comparison of the mineral and heavy metals, average concentrations (mg/kg), between Romania and other countries producing of natural honey is presented in table 4. In other studies, from other countries [10,11,12,13,14], Pb was not detected

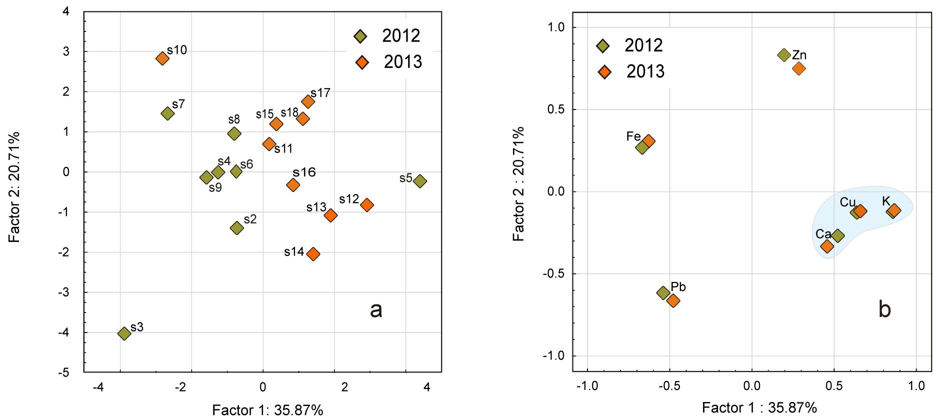

Fig. 2 – Two scatter-plots illustrating the results of PCA analysis performed in the Q mode (a) and R mode (b).

in honey samples (Argentina, Spain, Italy) or was higher than the Pb concentration detected in Romanian honey samples (Turkey, Egypt).

4. CONCLUSIONS

This study demonstrate that basic physico-chemical parameters and mineral and heavy content, determined by using rapid, cheaper but sensitive and reliable methods, give essential information about the honey quality.

Honey samples studied in this work, generally, present a good quality level, according to the European honey directive. Only three honey samples did not fit within European standards relative to water content, reflecting a poor honey adulteration by beekeepers. The most abundant mineral in honey samples is K, followed by Ca. Fe

Table 4

Comparative values of the investigated elements in honey produced in Romanian as well as in other countries.

Country	Element (mg/kg)					
	K	Ca	Fe	Cu	Zn	Pb
Romania	460	96	6.5	0.58	3.01	0.17
Argentina [10]	56	56	3.9	0.19	1.1	nd
Spain [11]	1283	92	4.6	0.60	2.1	nd
Italy [12]	472	48	4.5	0.31	3.1	nd
Turkey [13]	296	51	6.6	1.90	2.7	1.89
Egypt [14]	1500	192	113	1.70	7.2	12.5

and Zn were present in moderate amounts in the honey samples. The presence of heavy metals in large amount can be attributed to environmental pollution.

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