DETERMINATION OF GEOGRAPHICAL ORIGIN OF BLACK LOCUST HONEY OF FIVE CROATIAN REGIONS BY APPLYING THE PCA METHOD

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Abstract

The composition of honey depends on the plant species, climate conditions, environmental conditions, and good beekeeping practice. Honey mainly consists of: carbohydrates, water, proteins, free amino acids, enzymes, vitamins and minerals, flavorings and fragrances, and the phenol compounds alongside 200 other compounds. During the process of honey labelling, the product name may be supplemented with information referring product origin.

In order to determine the geographical origin of honey, a variety of analytical techniques and control parameters in combination with statistical techniques are used. Therefore, the purpose of this paper is to describe the application of the statistical method of Principal Component Analysis - PCA to determine the geographical origin of honey for five Croatian regions (Varaždin, Krapina-Zagorje, Bjelovar-Bilogora, Eastern Croatia and Istria) based on the defined share of micro and macro elements and physicochemical parameters obtained from 200 samples during two seasons. Upon conducting the melissopalynological analysis to confirm the botanical origin of honey declared by the manufacturer, the congruence of physicochemical parameters (electrical conductivity, free acids, diastase activity, water content, reducing sugars, sucrose and HMF) has been checked with literature data and the requirements of regulations. Twelve elements (Ca, Na, K, Mg, Zn, Fe, Cu, Mn, Al, Ni, Pb and Cd) have been determined and compared with literature data.

Obtained results have been used in the application of PCA method for determining the geographical origin of honey for five Croatian regions. The graphic overview is made for the first two factors, where each variable is shown by the current combination of factors: the projection of the physicochemical parameters and macro and micro elements.

As conclusion after the PCA analysis we could talk about the characteristic parameters: electrical conductivity, free acids, water and sucrose, as well as the share of Al, K, Fe, but only Cu could characterize the region of east Croatia, season I.

Key words: Honey, Geographical origin, PCA.

1. Introduction

The composition of honey depends on the plant species, climate conditions, environmental conditions, and good beekeeping practice. Honey mainly consists of: carbohydrates, water, proteins, free amino acids, enzymes, vitamins and minerals, flavourings and fragrances, and the phenol compounds present in a far smaller proportion alongside 200 other compounds [31]. Leading experts dealing with these issues alongside the melissopalynological determination of the pollen type and count, combine sensory analyses and physicochemical analyses such as determination of the: water content, HMF (hydroxymethylfurfural), electrical conductivity, diastase activity, pH values, and sugar content (fructose, glucose, sucrose, erlose, raffinose, melezitose) ([3], [23]). Since the distinctive composition of the soil of a given region is reflected in the mineral composition of honey producing plants, i.e. their nectar and produced honey, the share and composition of minerals is often used in determining

the geographical and/or botanical origin of honey [30]. Consequently, Lachman *et al.,* [15], tried to find a connection between the composition and the origin or type of honey by determining minerals in 24 samples of authentic Czech nectar honey and honeydew honey. Just like the Slovakian and Polish, samples of Czech honey samples had higher levels of nickel than the honey originating from other parts of the world [15]. In the international scientific literature this kind of characterization of honey is not a novelty ([1], [6], [7], [9], [14], [16], [18], and [22]), but there have not been many papers of this kind regarding the Croatian honey. Therefore, this paper represents a significant contribution to all previous research on honey of our region and complements the existing data that characterize the Croatian black locust honey ([4], [13], [27], [28], [29], [30], and [31]). Black locust honey is one of the most valuable types of honey on the European market. Its features are very valued by the consumers ([20], [23]). In Croatia, the largest production pertains to the production of black locust honey. During the process of honey labelling, the product name may be supplemented with information referring to its floral or vegetable origin, if the product is entirely or mostly of that plant source. It can also be supplemented with the data that refer to the regional, territorial or topographical origin, if the product is entirely of that origin ([8], [20]). Consumers are ready to pay more for a product from specific area, while they do not wish to buy products from some other areas. Therefore, many analytical techniques and parameters combined with statistical techniques are used to determine the geographical origin of honey.

2. Materials and Methods

This research included 200 honey samples in total. They were collected by the beekeepers (during two seasons: season I: KZ-9, VZ-9, BB-9, IH-9, I-9 and season II: KZ-10, VZ-10, BB-10, IH-10, I-10) (Figures 2, 3, 4, 5) in 5 regions of Croatia: Varaždin, Krapina-Zagorje, Bjelovar-Bilogora, Eastern Croatia, and Istria, marked with appropriate labels: Varaždin (VZ), Krapina-Zagorje (KZ), Bjelovar-Bilogora (BB), Eastern Croatia (IH), and Istria (I) (Figure 1).

In order to prove the botanical origin of honey, and whether samples meet the general quality requirements stipulated under the Croatian Regulations, melissopalynological and physicochemical analyses were carried out (water content, free acids, electrical conductivity, reducing sugars, diastase activity, sucrose, and HMF - [20], [21]). Additionally, the share of 12 micro and macro elements - calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), aluminium (Al), nickel (Ni), lead (Pb) and cadmium (Cd) - were determined. The values obtained were compared with literature data.
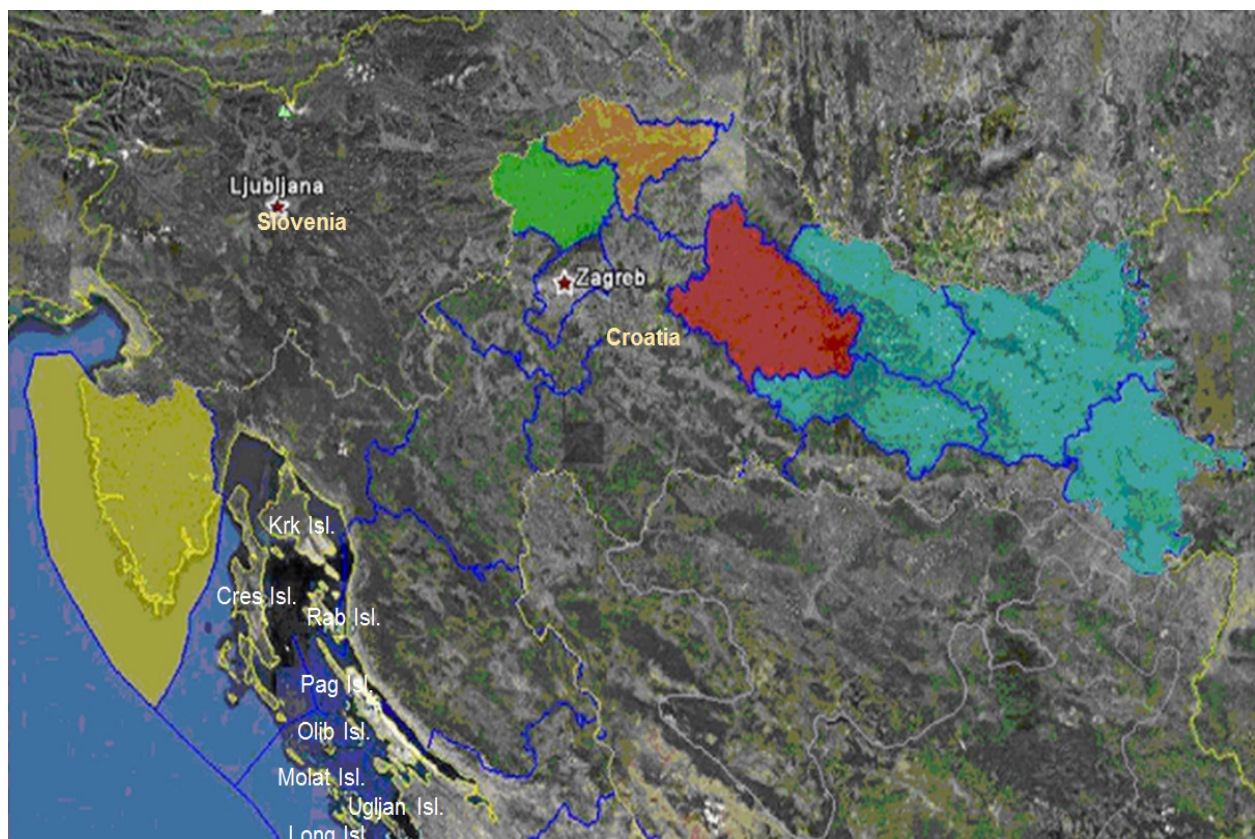

Figure 1. The map of geographical origin of honey

A statistical method Principal Component Analysis - PCA was applied to determine the geographical origin of honey for five Croatian regions based on the physicochemical parameters and micro and macro elements. The number of corresponding factors that is interpreted has been obtained by including all factors with eigenvalues greater than 1 and which comprise 84.26% of the initial data variability (Kaiser-Guttman criterion). The first three factors comprise a large percentage of the cumulative variance ([12], [19]). The first three factors are chosen for interpretation, as the first two factors explained 68.36% of the total variability of the original data. The third factor contributes with additional 9.78% [26].

3. Results and Discussion

By conducting pollen analysis of the black locust honey samples over both seasons from all the five regions, pollen grains of 36 plant species were detected. The most common are the ones from the families of *Robinia* (black locust family), *Rosaceae* (rose family), *Brassicaceae* (cabbage family) and *Fabaceae* (bean family). In the tested samples, *Rosaceae* pollen grains are more present in the Varaždin and Krapina-Zagorje region. The already mentioned pollen grains of *Brassicaceae* and *Fabaceae* are the most present in Varaždin and Eastern Croatia region - this has been expected since these are agricultural regions.

The obtained values of tested physicochemical parameters were compared with the literature data and meet the criteria stipulated by the Regulations [20] (Table 1).

Water share according to the mean values ranges from 15.99% to 18.03%, and thus are in accordance with the results obtained by other researchers on the samples of black locust honey from Croatia (from 15.40% to 16.30%) and Romania (17.90%) ([17], [27]). The mean values are shown to be in accordance with the permitted values of free acids ranging from 8.16 to 12.94 mEq/1000 g. Literature data show various values that are the same, higher or lower than the studied ones (7.3 - 8.4 mEq/1,000 g; 6.45 mEq/1,000 g, 11.2 mEq/1,000 g) ([10], [13], [17]). The mean values of electrical conductivity range from 0.12 to 0.22 mS/cm, which makes these results the same as the results obtained on 513 samples of the European black locust honey (0.16 mS/cm), but lower than the mean values of the Slovenian black locust with 0.26 mS/cm ([10], and [23]). The sum of the glucose and fructose, or reducing sugars, at the level of the European research on 454 samples of black locust honey gives the mean value of 69.2 g/100 g [23], in the range of which are the values obtained in this study, from 70.55 to 70.68 g/100g. All the samples tested show the sucrose share is from 0.10 to 2.90 g/100 g and is equal to the obtained range in the samples of black locust honey tested by other researchers from Romania (1.55 g/100 g), Croatia (2.8 g/100 g) and Italy (2.1 g/100 g) ([17], [23], and [24]). The obtained average for mean values of diastase activity range from 9.07 to 15.14 DN and are in accordance with the European (10.5 DN) and other Croatian results for black locust honey ([23], [27]).The share of HMF is low - a very large percentage of honey samples had less than 10 mg/kg (calculated as the upper limit for a first-class honey), and are in the range of 2.26 to 8.10 mg/kg and are below the mean values obtained by Šarić *et al.,* also on the samples of the Croatian black locust honey (4.7 - 36.5 mg/kg) [27].

Table 1. The range of mean values of physicochemical parameters in black locust honey in five Croatian regions over two seasons

	ANALYSIS	Water (%)	Free acids (mEq/1000g)	Electrical conductivity (mS/cm)	Reducing sugar (g/100g)	Sucrose (g/100g)	Diastase (DN)	HMF (mg/kg)
	REGULATIONS	max. 20%	max. 50 mEq/100g	max. 0.8 mS/cm	min. 60 g/100g	max. 10g/100g	min. 8 (min. 3 HMF < 15mg/kg)	max. 40 mg/kg
RESULTS	RANGE $\overline{x}$	15.99 – 18.03	8.16 – 12.94	0.12 – 0.22	66.94 – 70.68	0.10 – 2.90	9.07 – 15.14	0.50 – 18.99
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Legend:
Values according literature references:
= equally; < less; > larger.

The share of mineral substances in honey depends mostly on its botanical origin, but also on the climate conditions and soil composition [25]. Table 2 shows the results of the share of 12 micro and macro elements, expressed in mg/kg of all the five regions over season I and season II and are compared with the literature data. The dominant mineral in samples of black locust honey analysed in this study is K (31.69 - 81.34%), followed by Ca (6.51 - 35.56%) and Na (7.36 - 23.65%).

The lowest average share of Ca expressed in mg/kg is 33.526 mg/kg, while the highest is 329.327 mg/kg. These results are higher than the average results obtained in the Slovenian (9.40 mg/kg) and the Romanian honeys (3.05 mg/kg) ([11], and [17]). The highest mean value of the Na share amounts to 218.042 mg/kg, and the lowest value is 23.343. The obtained mean value in samples of the Romanian black locust honey is lower (13.02 mg/kg) [17]. The maximum average share of K is 428.050 mg/kg and the minimum is 205.571 mg/kg. The available literature data on the samples of the Slovenian (390.00 mg/kg) and the Romanian black locust honeys (187.10 mg/kg) are approximate to the values of this study ([11], [17]). The largest average share of Mg in the analysed samples is 49.072 mg/kg while the lowest is 12.295 mg/kg. Lower mean value of 5.7 mg/kg of the ones obtained in this study on the samples of black locust honey were measured the Marghitas *et al.,* [17]. The maximum value of 30.875 mg/kg of the average share of Zn is very high, while 0.937 mg/kg is the lowest. The average share of Zn in the European honeys ranges as follows: in the Italian 0.178 mg/kg and the Romanian 1.85 mg/kg, while in the samples of the Swiss black locust honey (0.217 mg/kg) the values are slightly lower than the interval of this study ([2], [5], and [17]). The lowest 0.495 mg/kg and the highest 2.268 mg/kg mean values of Feare equal to the values obtained with the samples of the Romanian black locust honey (1.30 mg/kg) [17]. However, the mean values of the shares of Fe in the Swiss (0.278 mg/kg) and

in the sample of the Italian black locust (0.209 mg/kg) were lower than the values obtained in this study ([2], [5]). The largest average share of Cu stands out with 0.951 mg/kg, and the lowest with 0.068 mg/kg. Within these values are the values of 0.52 mg/kg obtained by: Marghitas *et al.,* 0.18 mg/kg Bogdanov *et al.,* and 0.0576 Caroli ([17], [2], [5]).

The average share of Mn by regions and seasons is of the same approximate values of 0.229 mg/kg to 0.090 mg/kg, as well as the value obtained in the studies of the Romanian (0.16 mg/kg), the Swiss (0.453 mg/kg) and the Italian black locust (0.09 mg/kg), while in the Slovenian study the share is bigger (1.5 mg/kg) ([2], [5], [11], and [17]). The study records the lowest mean value of Al with 0,859 while the highest is 3.376 mg/kg. The value for Ni of 1.861 mg/kg stands out as the highest, and 0.086 mg/kg as the lowest. Average share for Ni of 0.056 mg/kg was recorded in the samples of the Swiss black locust honey whereas 0.018 mg/kg in the samples of the Italian black locust honey, which are lower values than the values obtained in this study ([2], [5]). The mean value of the share of Pb in all the regions over both seasons is of equal values and ranges from the lowest of 0.024 mg/kg to 0.107 mg/kg. Compliance with these results is reflected in the Swiss (0.013 mg/kg), but not in the Italian black locust honey (0.0028 mg/kg) ([2], and [5]). The lowest and the highest mean values of Cd are from 0.003 to 0.011 mg/kg. The results of the Italian researchers show lower values in relation to this study (0.0006 mg/kg) [5].

Graphic presentation is made for the first two factors, where each variable is shown by the current combination of factors: the projection of the physicochemical parameters and micro and macro elements.

Physicochemical parameters and macro and micro elements are grouped on the right side of the unit circle, which means that they are positively correlated with PC1 (minerals - K, Fe and Al; physicochemical

Table 2. Mean value of the mineral content in black locust honey in five Croatian regions over two seasons

MINERAL SUBSTANCES	Ca (mg/kg)	Na (mg/kg)	K (mg/kg)	Mg (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Al (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Cd (mg/kg)
AVERAGE SHARE mg/kg	33.526 - 329.327	23.343 - 218.042	205.571 - 428.050	12.295 - 49.072	0.937 - 30.875	0.495 - 2.268	0.068 - 0.951	0.090 - 0.229	0.859 - 3.376	0.086 - 1.861	0.024 - 0.107	0.003 - 0.011
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Values according literature references:
= equally; < less; > larger.

parameters - electrical conductivity, free acids, water and sucrose), but also show a negative correlation with PC1 on the left side and (all other minerals and physicochemical parameters) (Figure 2).

Figure 3 shows the results of grouping of honey samples from all the regions and both seasons according to the mean values of physicochemical parameters and macro and micro elements. It is noticeable that all of the regions of season II are grouped on the right side and that with PC1 they show high positive values, as well as in Varaždin and Bjelovar-Bilogora region of season I. One can talk about the characteristic electrical conductivity, free acids, water and sucrose, as well as share of Al, K, Fe, but only Cu may characterize IH-9 (Eastern Croatia region, season I).

By comparing Figures 4 and 5 it can be noticed that Fe is positioned in the coordinate system like the Bjelovar-Bilogora region of season I, and Al in the coordinate system as the Bjelovar-Bilogora region of season II. Potassium is on the same right side of the coordinate system like different regions/season so we cannot talk

about K as an element typical of a particular region and the season, perhaps for I-9 (Istria region - season I).

4. Conclusions

- The conducted pollen analysis shows that the samples meet the requirements to be classified as unifloral black locust honey.

- The analysed samples met the physicochemical parameters of the quality criteria stipulated by the Honey Regulations [20].

- The share of most of the 12 macro and micro elements in the analysed black locust honey samples has wide ranges and the obtained results are in accordance with the ranges mentioned in the literature data. K appears in the highest share regarding the macro elements, followed by Ca and Na.

- In order to gain an insight into whether physicochemical parameters and the share of micro and macro elements can characterize a specific region, the first three
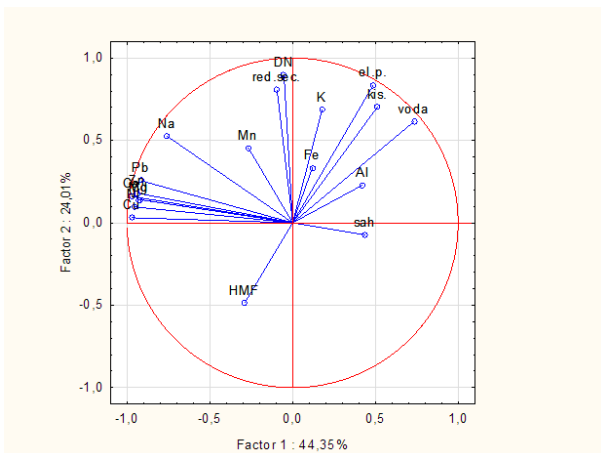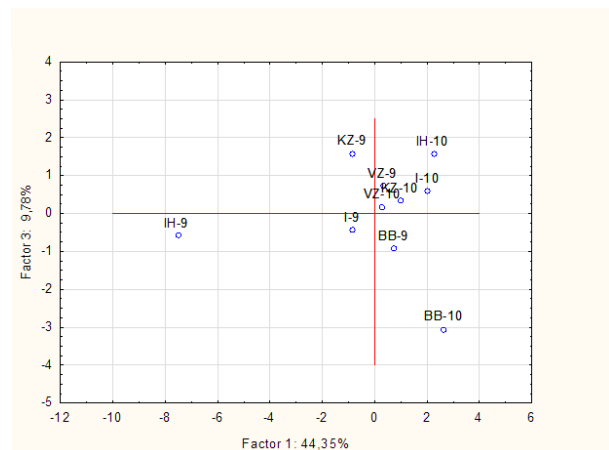

Figure 2. The projection of physicochemical parameters and macro and micro elements in the areaof the first two factors (PC1 *vs*. PC2)
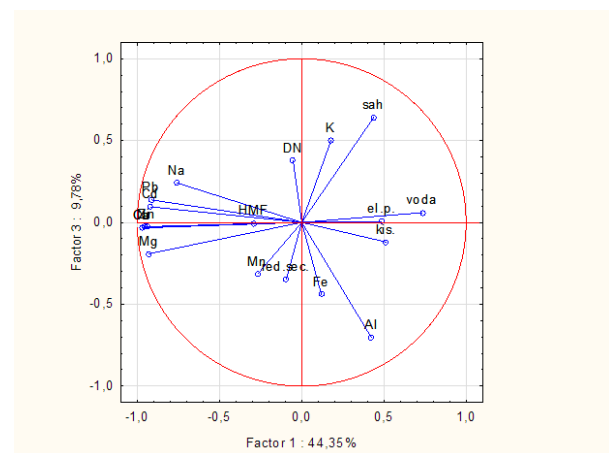
Figure 4. The projection of physicochemical parameters and macro and micro elements in the area of the first and the third factors (PC1 *vs*. PC3)
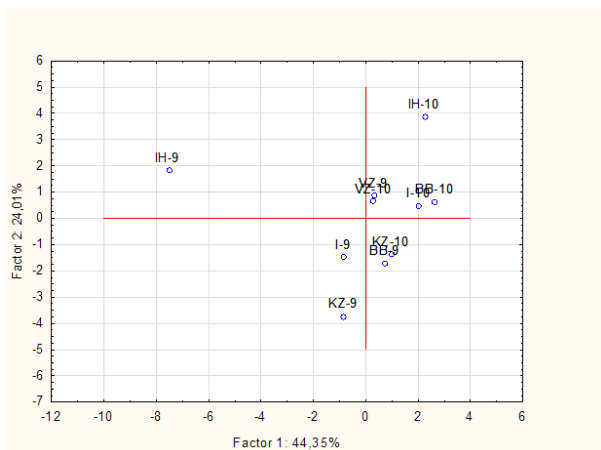
Figure 3. The projection of samples of honey from all the regions over both seasons in the area of the first two factors (PC1 *vs*. PC2)

Figure 5. The projection of samples of honey from all the regions over both seasons in the area of the first and the third factors (PC1 *vs*. PC3)

factors were taken into consideration in the interpretation. One can speak of the characteristic electrical conductivity, free acids, water and sucrose, as well as the share of Al, K, Fe, but only Cu may characterize IH-9 (Eastern Croatia region, season I).

- One can speak of the characteristic physicochemical parameters: electrical conductivity, free acids, water and sucrose, as well as the share of macro and micro elements AL, K and Fe, but only Cu may characterize a specific region which would be Eastern Croatia region, season I.

5. References

[1] Belouali H., Bouaka M., Hakkou A. (2008). *Determination of some major and minor elements in the east of Morocco honeys through Inductively Coupled Plasma optical Emission Spectrometry*. Apiacta, 43, pp.17-24.

[2] Bogdanov S., Haldimann M., Luginbühl W., Gallmann P. (2007). *Minerals in honey: environmental, geographical and botanical aspects*. Journal of Apicultural Research and Bee World, 46, pp. 269-275.

[3] Bogdanov S., Ruoff K., Persano Oddo L. (2004). *Physico-chemical methods for the characterisation of unifloral honeys: a review*. Apidologie, 35 (special issue) 4-17.

[4] Bubalo D., Dražić M., Mandić M. L., Kenjerić D., Kezić N. (2006). *Botanical origin of honey from the Adriatic region* (in Croatian). 41 Croatian and 1 International Agronomy Scientific Symposia Proceedings, Faculty of Agronomy, Osijek, Croatia, pp. 549-550.

[5] Caroli S., Forte G., Alessandrelli M., Cresti R., Spagnoli M., D'Ilio S., Pauwels J., Kramer G. N. (2000). *A pilot study for the production of a certified reference material for trace element in honey*. Microchemical Journal, 67, pp. 227-233.

[6] Conti M. E., Stripeikis J., Campanella L., Cucina D., Tudino M. B. (2007). *Characterization of Italian honeys (Marche region) on the basis of their mineral content and some typical quality parameters*. Chemistry Central Journal, 1, pp.1-17.

[7] Čelechovská O., Vorlová L. (2001). *Groups of honey - physicochemical properties and heavy metals*. Acta Veterinaria, 70, pp. 91-95.

[8] EU Council. (2001). *Directive 2001/110/EC relating to honey*. Official Journal of the European Communities L 10:47-52.

[9] Fernandez-Torres R., Perez-Bernal J. L., Bello-Lopez M. A., Callejon-Mochon M., Jimenez-Sanchez J. C., Guiraum-Perez A. (2005). *Mineral content and botanical origin of Spanish honeys*. Talanta, 65, pp. 686-691.

[10] Golob T., Plestenjak A. (1999). *Quality of Slovene honey*. Food Technology and Biotechnology, 37, pp. 195-201.

[11] Golob T., Doberšek U., Kump P., Nečemer M. (2005). *Determination of trace and minor elements in Slovenian honey by total reflection X-ray fluorescence spectroscopy*. Food Chemistry, 91, pp. 593-600.

[12] Halami A. (2003). *Multivariate analysis in the social sciences* (in Croatian). Alinea, Zagreb, Croatia.

[13] Kenjerić D., Mandić M. L., Primorac Lj., Bubola D., Perl A. (2007). *Flavonoid profile of Robiniahoneys produced in Croatia*. Food Chemistry, 102, pp. 683-690.

[14] Khaled A. O., Mohamed A. A., Suloiman A., Mohamed I. D. H. (2007). *Mineral contents and physicochemical properties of natural honey produced in Al-Qassim region, Saudi Arabia*. Journal of Food & Environment, 5, pp. 142-146.

[15] Lachman J., Kolihova D., Miholova D., Kosata J., Titera D., Kult K. (2007). *Analysis of minority honey components: Possible use for the evaluation of honey quality*. Food Chemistry, 101, pp. 973-979.

[16] Latorre M. J., Pena R., Pita C., Botana A., Garcia S., Herrero C. (1999). *Chemometric classification of honeys according to their type. II. Metal content data*. Food Chemistry, 66, pp. 263-268.

[17] Marghitas L. A., Dezmirean D. S., Pocol C. B., Ilea M., Bobis O., Gergen I. (2010). *The Development of a Biochemical Profile of Acacia Honey by Identifying Biochemical Determinants of its Quality*. Notulae Botanicae Horti Agrobotanici, Cluj - Napoca, Romania, Special Issue 38, pp. 84-90.

[18] Madejczyk M., Baralkiewicz D. (2008). *Characterization of Polish rape and honeydew honey according to their mineral contens using ICP-MS and F-AAS/AES*. Analytica Chimica Acta, 617, pp. 11-17.

[19] Mejovšek M. (2008). *Methods of scientific research in the social sciences and humanities* (in Croatian). Naklada Slap, Zagreb, Croatia.

[20] Republic of Croatia Ministry of Agriculture, Fisheries and Rural Development. (2009). *Regulation on honey* (in Croatian). Narodne novine 93/09.

[21] Republic of Croatia Ministry of Agriculture, Fisheries and Rural Development. (2009). *Regulation on the quality of unifloral honey*. Narodne novine, 122/09.

[22] Nanda V., Sarkar B. C., Sharma H. K., Bawa A. S. (2003). *Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India*. Journal of Food Composition and Analysis, 16, pp. 613-619.

[23] Persano Oddo L., Bogdanov S. (2004). *Determination of Honey Botanical Origin*. Apidologie, 35, S2-S3.

[24] Primorac Lj., Flanjak I., Kenjerić D., Bubola D., Topolnjak Z. (2011). *Specific Rotation and Carbohydrate Profile of Croatian Unifloral Honeys*. Czech Journal of Food Sciences, 29, pp. 515-519.

[25] Przybylowski P., Wilczyńska A. (2001). *Honey as an environmental marker*. Food Chemistry, 74, pp. 289-291.

[26] StatSoft Inc. (2010). *Statistica 9.1*. Single user version, University of Zagreb, Zagreb, Croatia.

[27] Šarić G, Marković K., Major N., Krpan M., Uršulin-Trstenjak N., Hruškar M, Vahčić N: Changes of Antioxidant Activity and Phenolic Content in Acacia and Multifloral Honey During Storage. Food Technology and Biotechnology, 50, 4:434-441, 2012.

[28] Šarić G., Matković D., Hruškar M., Vahčić N. (2008). *Characterization and Classification of Croatian Honey by Physicochemical Parameters*. Food Technology and Biotechnology, 46, pp. 355-367.

[29] Uršulin-Trstenjak N., Levanić D., Galić A., Šušnić S. (2015). *Profile and diversity of physicochemichal parameters of the black locust honey from Eastern Croatia region given the testing seasons*. Journal of Hygienic Engineering and Design, (9), 1, pp. 26-34.

[30] Uršulin-Trstenjak N., Levanić D., Šušnić V., Barušić L., Jurica K., Vahčić N. (2014). *Confirming the botanical origin of the Croatian black locust honey (Istria region) using physicochemichal parameters during two seasons*. Journal of Hygienic Engineering and Design, (8), 3, pp. 124-128.

[31] Uršulin-Trstenjak N., Levanić D., Primorac Lj., Bošnir J. Vahčić N., Šarić G. (2015). *Mineral Profile of Croatian Honey and Differences Due to its Geographical Origin*. Czech Journal of food sciences, (2), 33, pp. 156-164.