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Geographical Origin of Honey from Eight Sub-regions of Bosnia and Herzegovina

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Abstract

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Principal component analysis (PCA) and cluster analysis (CA) were used to define the geographical origin of three types of monofloral (chestnut, linden, and acacia), two types of multifloral (meadow and mixed), and forest honey produced over two consecutive harvest seasons in the Una-Sana Canton (Bosnia and Herzegovina), which is geographically divided into eight sub-regions. Statistical analysis was applied to the measurement of physico-chemical and sensory parameters, as well as micro- and macronutrient (K, Na, Mg, Zn, Fe, Mn, and Al) content, along with some heavy metals (Cd, Pb, and As). Using the PCA method the characteristic parameters for all eight sub-regions were determined, while the CA grouping method was used to determine the characteristic parameters for six sub-regions. Chestnut honey is predominantly found in Cazin, chestnut and linden honey in Bosanska Krupa, chestnut and acacia honey in Bužim, acacia honey in Sanski Most, mixed honey in Velika Kladuša and forest honey in Ključ.

Keywords: honey; cluster analysis; geographical characterisation; principal component analysis

The designation of the geographical origin of honey within an area is important for several reasons. Firstly, it is a very effective tool to raise the value of honey, and also to prevent counterfeiting and unfair competition, which can destabilise the market (Cordella *et al.* 2002). In addition, consumers today are willing to pay more for products from certain areas, because the area's characteristics ensure quality (Roman *et al.* 2013). The application of the methods for honey quality control prescribed by the Codex Alimentarius Commission (Codex STAN 2001), EU Directives (2001/110/EC, the EU Council 2002) and National Regulations (OGBH No. 37/09, 2009) can also be used to determine the geographical origin of honey; for example, different methods used in the routine checking of honey quality and which are in compliance with IHC and the CAC standards or European directives (Bogdanov *et al.* 2004). However, different methods are combined to determine the geographical and botanical origin of honey, because the composition of honey and its properties are directly dependent on the region of honey origin, environmental conditions, season, method of beekeeping and other factors as evidenced by the large number of scientific publications on this topic. For example, Makhloufi *et al.* (2010) combined palynological, physicochemical and sensory methods, and El Sohaimy *et al.* (2015) combined palynological and physico-chemical methods. Some authors base identification of geographical/botanical origin on the determination of additional mineral composition (Stankovska *et al.* 2008; Uršulin-Trstenjak *et al.* 2015). Markers of monofloral honey include phenolic acids, flavonoids, carbohydrates and other substances (Kaškonienė & Venskutonis 2010). The further processing of data for characterisation of honey includes multivariate statistical methods; e.g., Fangio *et al.* (2010) used the PCA method to characterise samples of honey from Argentina and reported that colour and water content were the most

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important parameters in distinguishing the studied samples. Based on physicochemical parameters, Devillers *et al*. (2004) classified seven types of monofloral honey. Nanda *et al*. (2009) succeeded in classifying samples of honey from Northern India with 90% accuracy through the analysis of proline, K content and free acidity. The results of the study of Karabagias *et al*. (2014) showed that using five phenolic compounds in combination with 11 conventional quality parameters and chemometrics may allow determination of the geographical origin of Greek thyme honey.

Northwest Bosnia and Herzegovina is traditionally known for the production of good quality honey with unique characteristics, especially chestnut honey, which represents an opportunity for development in the region. With this in mind, the present research was aimed at the characterisation of honey produced in eight sub-regions of the Una-Sana Canton (USC). For this purpose, pollen analysis was performed and a determination of physicochemical and sensory attributes was combined with mineral content analysis. Characterisation of the different honey types studied and determination of geographical origin was accomplished using multivariate analyses.

MATERIAL AND METHODS

The material used for this research consisted of 201 monofloral and multifloral honey samples, which were produced and sampled in USC during 2009 and 2010. Administratively, this area is divided into eight municipalities (sub-regions) and covers 4,125 km^2. Sampling was conducted directly from the hive sites using standard sampling methods described in the National Rulebook of Methods for the Control of Honey and Other Bee Products (OGBH 2009). Pollen analysis of honey samples was performed using an Olympus microscope, model Bx53F (Japan), according to national legislation (OGBH 2009). Pollen grains were compared with images from a pollen atlas (Von der Ohe & Von der Ohe 2002). Physicochemical parameters (water content, reducing sugars, sucrose, ash, electrical conductivity, diastase and invertase activity, hydroxymethylfurfural content, proline and optical rotation) were determined according to the International Honey Commission (IHC 2002) method. The sensory analysis was carried out according to previously published literature (Golob *et al*. 2008).

The mineral composition in honey and heavy metals was measured using an AAS 6800 (FAAS), equipped with a HGA graphite furnace and deuterium lamp. The analysis of K, Na, Mg, Zn, Fe, Mn, and Al was performed using a flame technique, 10-cm long slot flames and lighting. An air-acetylene flame was used. The analysis of Cd, Pb, and As were conducted using a graphite technique, with argon as the inert gas. Additionally, pyrolytic tubes and tubes with high density were used. Samples were injected into the graphite furnace using ASC-6100 Shimadzu auto sampler. The microwave oven was a Milston model START D, MPR-600/12S. The limits of detection (LOD) in mg/kg were 0.01 for Na, Fe, Mn, and Al; 0.03 for K; 0.001 for Zn and As; 0.004 for Mg; 0.0001 for Pb; 0.00001 for Cd.

In order to determine the possibility of distinguishing different types of honey according to geographical origin on the basis of the studied parameters, independent methods of multivariate statistics were used. PCA was used to determine interrelations between various parameters, while CA was used for data grouping. Data processing was accomplished using the XLSTAT 2011 computer program and Statistica v10.0 software.

RESULTS AND DISCUSSION

Pollen analysis of honey samples from USC showed a great variety of pollen grain types. Twenty-six pollen types were found, with a predominance of *Castanea sativa* pollen grains in chestnut and mixed honey and with a high proportion of these pollen grains in all other samples. The percentage of pollen grains in chestnut honey was 87.9 and 25.4% in acacia samples, 31.1% in meadow samples, 61.5% in mixed honey and 13.1% in forest honey. Only in linden honey samples were pollen grains of *Tilia* sp. found at a higher percentage (25.8%) than *Castanea sativa* pollen grains (23.6%). Comparison of these results with studies from northern Croatia (Sabo *et al*. 2013) or northern Spain (Rodríguez-Flores *et al***.** 2016), shows that they are quite similar. Pollen grains in those reports were found to range between 84.41 and 90.2%, which is explained by the chestnut woods in those areas. The dominant secondary pollens in the honey samples (except for chestnut) were *Tilia* sp. (3.0–25.8%), *Robinia pseudoacacia* (2.4–25.4%), and *Brasica napus oleif* L. (1.0–21.3%). Important minor pollens (3–15%) in all the samples (except for chestnut) were represented by the pollen of *Lamiacaea* (4.7–11.0%), *Salix* L. (4.0–5.3%),

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Rumex acetosella (3.1–5.2%) and *Planatago lancolate* (4.2–6.3%), while 19 other types of pollen grain were present in different proportions. According to the standards for monofloral honey (IHC 2002), six linden honey samples did not meet the demands for linden (> 25%) and two honey samples (> 20%) for acacia honey.

Values of physicochemical parameters, sensory scores, minerals and heavy metals for the 201 analysed honey samples are given in Table 1. According to the requirements of national regulations, which are aligned with the EU standards for the investigated honey samples, 21 samples (10.4%) showed a higher than permitted water content (< 20%). Šarić *et al.* (2008) reported that only two out of 254 samples of honey from Croatia did not meet the demands imposed by the regulations concerning the water content. In the present study, the average water content varied between 17.58–19.63%, while the range is slightly lower (15.4–17.5%) in Croatian honey. A higher water content in honey can occur due to fermentation during storage and generally depends on beekeeping practice, storage and environmental conditions (Bogdanov *et al.* 2004). Furthermore, one sample of honey showed a proline content that is higher than permitted under national rules (≥ 180 mg/kg). The proline content ranged between 445.51 mg/kg and 703.60 mg/kg, while in Croatian honey it ranged between 191.7–688.5 mg/kg. In all other parameters, honey samples met the quality requirements and the results are comparable (to a certain extent) to those of other researchers (Conti *et al.* 2007; Kropf *et al.* 2008; Gačić *et al.* 2015). It has been stated that electrical conductivity should be higher than 0.8 μS/cm for chestnut and forest honey, and lower than 0.8 μS/cm for other examined types. This difference is highlighted in Table 1. The sensory scores of the studied samples ranged between 47.21 and 51.53 out of the possible 58 (Table 1).

The mineral profile of honey revealed that first predominant mineral in the examined honey samples was K, with a total content ranging between 308.3–533.87 mg/kg. Mg (21.8–61.43 mg/kg) and Na (22.22–29.69 mg/kg) were the second and the third most common minerals in honey samples, which is similar to what has been reported for Italian (Conti *et al.* 2007), Romanian (Oroian *et al.* 2015) and Croatian honey (Uršulin-Trstenjak *et al.* 2015). The levels of all other examined minerals were comparable with previously reported studies (Bratu & Georgescu 2005; Conti *et al.* 2007; Pohl & Prusisz 2007; Uršulin-Trstenjak *et al.* 2015). The levels of the potential contaminant Mn were in the range of 2.09–3.37 mg/kg on average. This is very similar to that reported by Bogdanov *et al.* (2007) and Belouali *et al.* (2008) who reported levels of 0.13–12.4 and 0.08–9.76 mg/kg, respectively. Heavy metals (Cd, Pb, and As) were also detected in honey samples. Cd content ranged between 0.02–0.03 mg/kg, Pb content was 0.03–0.66 mg/kg, and As content was in the range of 0.38–2.25 mg/kg, respectively. The average measured content for As in five sub-regions was higher than permitted by national legislation (< 0.5 mg/kg) and much higher than in samples from the Czech Republic (Batelková *et al.* 2012) where the levels of As were between 0.001 and 0.004 mg/kg or from Chile (Bastías *et al.* 2013) where they ranged from 0.002 mg/kg to 0.172 mg/kg. The high concentration of As is worrying, and this issue may be the subject of future research. In contrast, in 38 samples of honey the levels of Mn were below the limit of detection, while Cd, Pb, and As were below the limits of detection in 101, 79, and 57 samples, respectively.

Analysis of variance (ANOVA) revealed significant differences ($P \leq 0.05$) in the sucrose content, electrical conductivity, invertase activity and Al content in chestnut honey samples from different sub-regions. Electrical conductivity, acidity, invertase activity and HMF, Al, and Pb levels were significantly different in linden honey, while reducing sugar, acidity, diastase activity, proline and K levels were significantly altered in acacia honey. The largest variation in the measured parameters across the region (water, reducing sugar, sucrose, ash content, electrical conductivity, acidity, invertase activity. K, Mg, and Fe content) was found in samples of meadow honey. Finally, a significant difference ($P \leq 0.05$) in the sucrose content, electrical conductivity, invertase and diastase activity and sensory scores were observed in forest honey samples.

Furthermore, honeys were identified within each type according to their geographical location (eight sub-regions) and PCA analysis was conducted. Coordinate factors for variables were calculated and are presented in the text while the coordinate factors for the geographical area are shown with the score-plot of the first two components (PC1 vs. PC2) for each type of honey. In addition, data grouping using CA was carried out according to geographical area within each type of honey and characteristic parameters specific to a particular area were found. The results of CA grouping are presented in dendrograms, also for each honey type. The studied sub-regions are

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Table 1. Physico-chemical parameters, sensory scores, and mineral content of honey samples from investigated sub-regions

Measured parameters	C (*n* = 41)	BU (*n* = 23)	BI (*n* = 19)	BK (*n* = 34)	VK (*n* = 28)	SM (*n* = 22)	K (*n* = 20)	BP (*n* = 14)
Water (%)	18.1 ± 0.48	17.87 ± 0.72	17.77 ± 0.18	17.58 ± 0.09	18.4 ± 0.03	17.72 ± 0.53	19.63 ± 0.76	17.92 ± 1.46
Reducing sugars (%)	69.9 ± 2.39	68.65 ± 2.32	70.1 ± 3.72	71.19 ± 2.22	69.5 ± 1.99	68.23 ± 2.52	70.98 ± 3.38	66.96 ± 1.52
Sucrose (%)	1.2 ± 0.30	0.79 ± 0.19	2.28 ± 0.33	0.81 ± 0.31	2.1 ± 0.18	1.19 ± 0.32	1.84 ± 0.4	1.53 ± 030
Ash (%)	0.4 ± 0.23	0.44 ± 0.25	0.47 ± 0.22	0.56 ± 0.27	0.6 ± 0.18	0.37 ± 0.26	0.63 ± 0.16	0.47 ± 0.04
El. cond. (μS/cm)*	0.53 ± 0.21	0.43 ± 0.23	0.58 ± 0.16	0.58 ± 0.09	0.71 ± 0.18	0.41 ± 0.22	0.63 ± 0.09	0.69 ± 0.11
El. cond. (μS/cm)**	0.96 ± 0.07	0.93 ± 0.05	0.99 ± 0.11	0.95 ± 0.05	0.94 ± 0.07	1.10 ± 0.16	1.13 ± 0.06	–
Acidity (mmol/kg)	21.4 ± 5.55	14.53 ± 3.15	21.11 ± 5.15	22.61 ± 4.19	19.6 ± 2.69	26.24 ± 4.68	24.4 ± 4.07	29.14 ± 359
Diastase activity (DN)	29.0 ± 5.34	19.61 ± 3.91	23.69 ± 5.44	27.08 ± 1.45	23.1 ± 0.02	22.75 ± 7.68	24.22 ± 4.48	28.96 ± 323
Invertase activity (IN)	18.6 ± 8.79	20.54 ± 6.83	18.28 ± 5.26	21.77 ± 1.78	23.5 ± 0.66	19.51 ± 6.11	30.31 ± 3.15	30.86 ± 4.32
Udio HMF-a (mg/kg)	7.8 ± 1.68	16.87 ± 5.61	16.44 ± 10.03	5.94 ± 2.96	20.8 ± 1.61	6.98 ± 3.64	4.91 ± 0.57	6.19 ± 4.98
Proline (mg/kg)	489.1 ± 127.9	481.89 ± 187.5	604.48 ± 72.1	533.4 ± 41.5	703.6 ± 54.7	445.51 ± 133.8	516.88 ± 106.3	589.48 ± 103.2
Optical rotation °(D)	−1.5 ± 0.27	−1.02 ± 1.10	−0.51 ± 0.46	−0.72 ± 0.45	−1.4 ± 0.18	−1.48 ± 0.48	−0.99 ± 0.00	−0.50 ± 0.77
Sensory scores	48.3 ± 1.36	50.14 ± 0.00	48.83 ± 0.83	47.21 ± 3.92	50.8 ± 0.00	51.53 ± 0.50	51.13 ± 2.19	48.93 ± 6.32
K (mg/kg)	308.3 ± 206.1	370.01 ± 221.9	379.48 ± 211.0	477.53 ± 257.5	488.1 ± 176.5	341.01 ± 223.4	533.87 ± 149.6	366.77 ± 171.3
Na (mg/kg)	24.5 ± 3.66	23.16 ± 5.83	28.70 ± 2.08	27.98 ± 2.10	29.1 ± 0.55	22.22 ± 4.38	26.96 ± 1.41	29.69 ± 3.85
Mg (mg/kg)	21.8 ± 10.37	22.69 ± 11.89	37.16 ± 10.64	32.17 ± 11.7	32.9 ± 1.17	23.69 ± 11.92	47.49 ± 14.2	61.43 ± 2.73
Zn (mg/kg)	0.8 ± 0.40	0.654 ± 0.53	1.91 ± 0.88	1.06 ± 0.92	1.2 ± 0.37	0.72 ± 0.16	1.33 ± 0.2	1.96 ± 1.30
Fe (mg/kg)	4.5 ± 3.05	6.39 ± 6.61	8.81 ± 3.52	5.20 ± 2.57	8.3 ± 1.90	5.14 ± 2.75	12.74 ± 0.56	13.43 ± 1.41
Mn (mg/kg)	2.6 ± 0.69	3.37 ± 1.52	2.92 ± 0.22	2.30 ± 0.68	3.2 ± 0.49	2.40 ± 0.79	3.11 ± 1.00	2.09 ± 1.69
Al (mg/kg)	2.7 ± 0.81	7.87 ± 6.59	9.58 ± 2.51	6.17 ± 2.06	5.0 ± 0.47	5.87 ± 3.41	6.312 ± 1.52	3.49 ± 0.23
Cd (mg/kg)	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.02
Pb (mg/kg)	0.06 ± 0.04	0.12 ± 0.13	0.66 ± 0.33	0.42 ± 0.42	0.13 ± 0.08	0.03 ± 0.02	0.06 ± 0.00	0.43 ± 0.42
As (mg/kg)	0.5 ± 0.15	0.49 ± 0.27	1.46 ± 0.93	2.25 ± 1.75	0.38 ± 0.03	0.73 ± 0.14	0.51 ± 0.37	1.89 ± 0.93

C – Cazin, BU – Bužim, BI – Bihać, BK – Bosanska Krupa, VK – Velika Kladuša, SM – Sanski Most, K – Ključ, BP – Bosanski Petrovac; electrical conductivity: *for linden, acacia, meadow, mixed samples, **for chestnut and forest samples

indicated in the following way: C-Cazin, BU-Bužim, BI-Bihać, BK-Bosanska Krupa, VK-Velika Kladuša, SM-Sanski Most, K-Ključ, and BP-Bosanski Petrovac. For chestnut honey, PC1 explained 19.32% of variability, and was positively correlated with K, Na, Mg, and Pb content. PC2 explained the additional 12.37% variability and had a positive correlation with Cd and Fe content, along with water and invertase activity. The PCA score-plot of the first two components (Figure 1A) showed that the three groups may be distinguished based on reducing sugars, HMF and diastase activity in samples from VK, on the basis of water content for samples from C and based on Al content and acidity for samples from BU. A specific dendogram was obtained using CA grouping (Figure 1B) in which the smaller groups from the areas of C, BU, and BK are shown. Chestnut honey was found to group separately according to areas or season of production also in other research (Šarić *et al.* 2008; Kivrak *et al.* 2017). For linden honey, PC1 explained 20.36% of variability and was positively correlated with HMF, Al, and Pb content and negatively correlated with K, acidity and diastase activity. PC2 explained the additional

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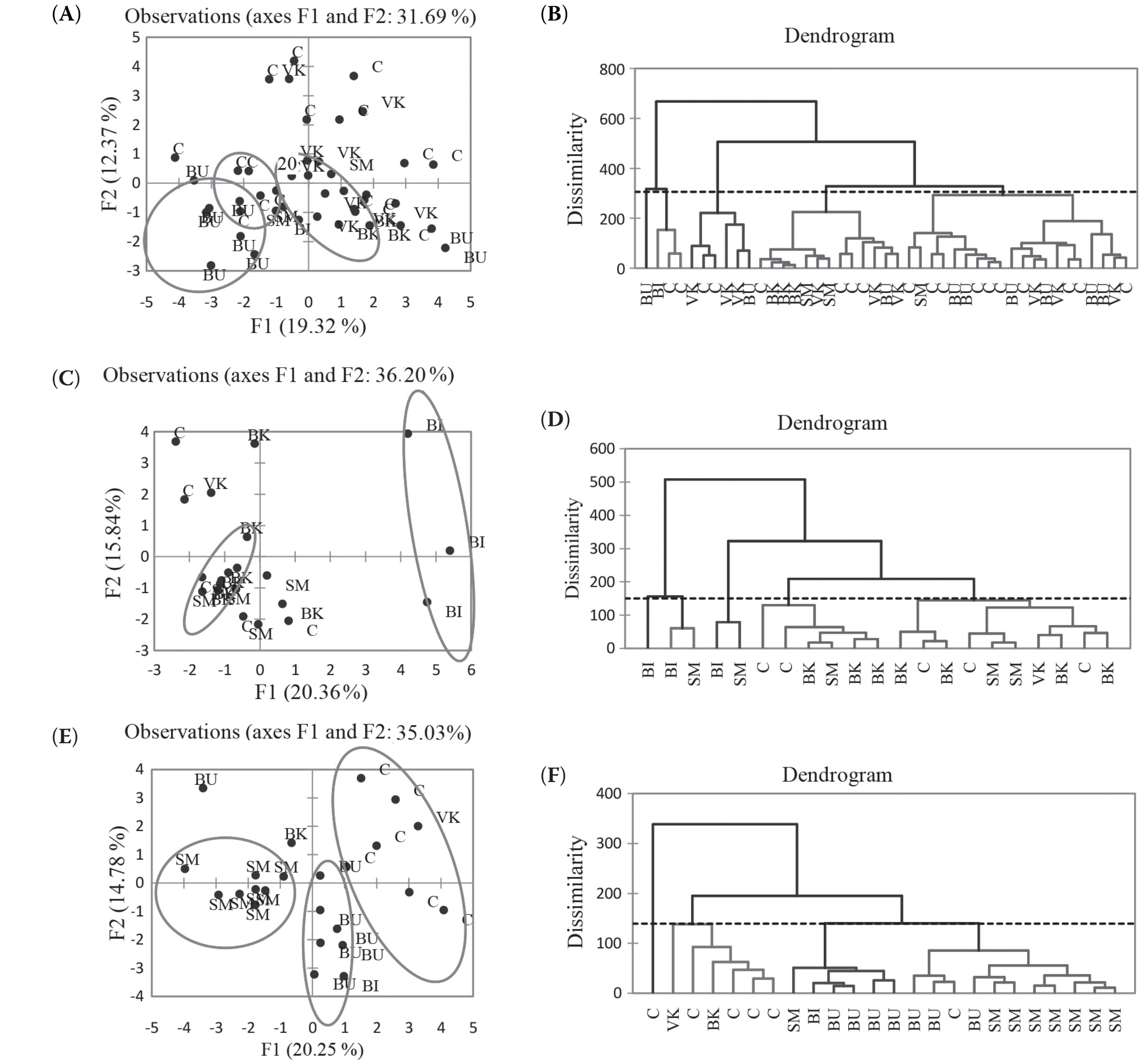

Figure 1. PC1 vs. PC2 and dendogram for monofloral honeys (chestnut **A**, **B**; linden **C**, **D**; and acacia **E**, **F**) according to geographical origin

C – Cazin, BU – Bužim, BI – Bihać, BK – Bosanska Krupa, VK – Velika Kladuša, SM – Sanski Most

15.84% variability and was positively correlated with ash, electrical conductivity, Mg, K, Na content and was negatively correlated with water. Two principal groups were found (Figure 1C): first, samples from the BI sub-region which could be distinguished by HMF content as a characteristic parameter; second samples from the BK sub-region which could be distinguished by diastase activity, acidity and K content. Using CA (Figure 1D), samples were divided into one group linked with the BK sub-region. PC1 for acacia honey explained 20.25% of variability and was positively correlated with K, Na, reduced sugars, ash and diastase activity, and negatively correlated with As content. PC2 explained 14.78% of variability and was positively correlated with water, acidity, invertase activity, and negatively correlated with the specific rotation angle. Figure 1E shows that acacia honey samples could be split into three groups. K, Na and sensory parameters influence the score-plot of the SM sub-region, diastase activity and acidity that of the C sub-region and content of water, HMF and invertase activity that of the BU sub-region. Figure 1F clearly shows that the samples from the BU sub-region separated into one group and the samples from SM into a second. Recent research from Croatia carried out on 200 samples of acacia honey

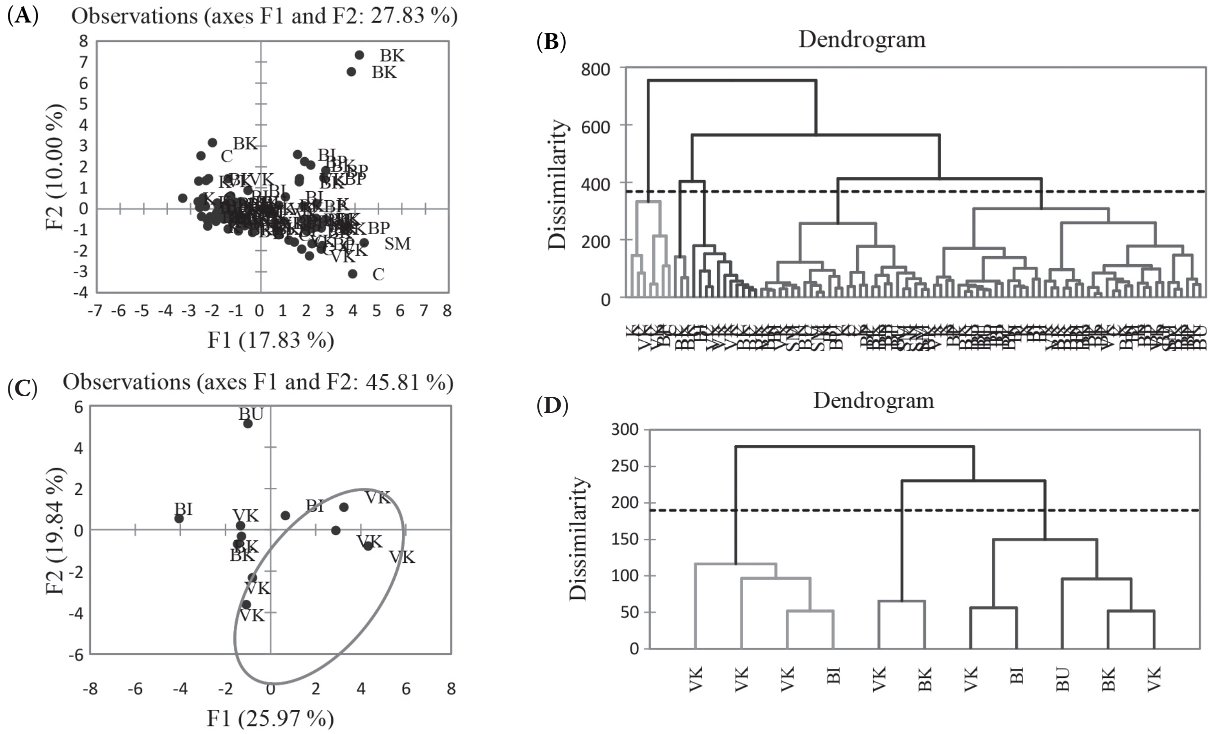

Figure 2. PC1 vs. PC2 and dendogram for multifloral (meadow **A**, **B** and mixed **C**, **D**) honeys according to geographical origin

C – Cazin, BU – Bužim, BI – Bihać, BK – Bosanska Krupa, VK – Velika Kladuša, SM – Sanski Most, K – Ključ

from five different production regions has identified minerals that are characteristic for specific regions: Al – Bjelovar-Bilogora, Fe – Bjelovar-Bilogora and Istria, Cu – Eastern Croatia, and K – Istria (Uršulin-Trstenjak *et al*. 2015).

The first principal component for meadow honey explained 17.83% of variability and was positively correlated with Mg, Na, electrical conductivity and specific rotation angle, and negatively correlated with K, water and ash content. PC2 explained 10.00% of the variability and was positively correlated with As and Pb content. Taking into consideration the coordinate factors of the geographical origin (Figure 2A), three groups can be distinguished, but these are not shown in the figure because they are barely visible. Samples from the BP sub-region are distinguished by Mg content and their electrical conductivity, those from the K sub-region by water, K and ash content and those from the BK sub-region by electrical conductivity. The results of the CA grouping of samples of meadow honey (Figure 2B) showed that the groups are heterogeneous and that there is no obvious separation. These results are comparable with those of Isopecu *et al*. (2014) who failed to achieve a good discrimination between different types of multifloral honey. In mixed honey, PC1 explained 25.97% of the variability and was positively correlated with Mn, water, reducing sugars, electrical conductivity, acidity and diastase activity, and negatively correlated with Na, HMF and specific rotation angle. PC2 explained the additional 19.84% of variability and was positively correlated with As, Zn, K, sucrose, and ash content, while a negative correlation was observed for Mg content and invertase activity. Typical parameters which characterised the samples of mixed honey from the VK sub-region and distinguished it from other sub-regions are water, acidity and diastase activity (Figure 2C). CA grouping (Figure 2D) revealed clear separations in this area.

PC1 explained 31.69% of the variability in forest honey and was positively correlated with Mn, Mg, water, sucrose, electrical conductivity, invertase activity and sensory grade. PC1 was negatively correlated with Pb, As and diastase activity. PC2 explained the additional 23.77% of variability and was negatively correlated with Na, HMF and proline, but positively correlated with K, ash and acidity. Samples of forest honey from the BK sub-region could be distinguished by Zn content and diastase activity and those from Ključ by their electrical conductivity, Mg, sucrose, invertase activity and sensory grade (Figure 4A). CA grouping (Figure 4B) showed the formation of an obvious group consisting of the samples from the

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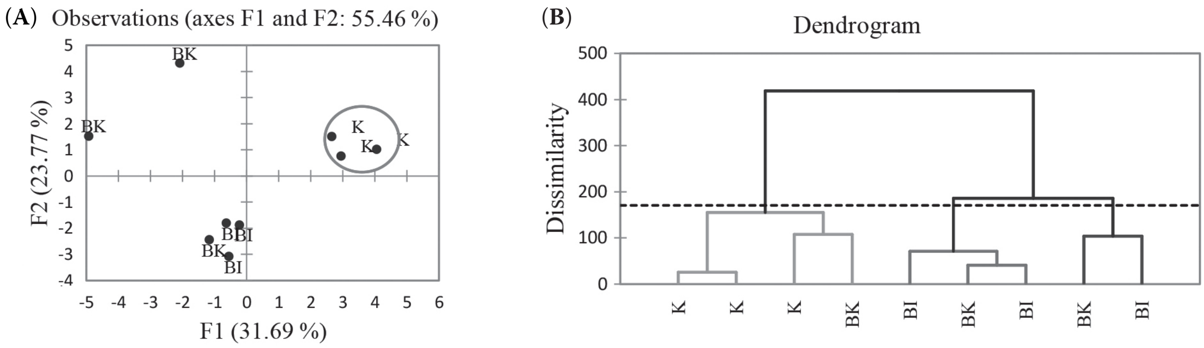

Figure 3. Forest honey PC1 vs. PC2 (**A**) and dendogram (**B**) for forest honey according to geographical origin

BI – Bihać, BK – Bosanska Krupa, K – Ključ

Ključ sub-region. Similarly, Belouali *et al.* (2008) successfully classified 42 honey samples from the area of eastern Morocco into two groups: forest and nectar honey. The present results are in good agreement with other similar experiments performed on samples of honey from different regions around the world (Santos *et al.* 2008; Uršulin-Trstenjak *et al.* 2015; Kivrak *et al.* 2017).

CONCLUSIONS

Honey samples from the eight municipalities of USC were characterised successfully with respect to geographical origin using PCA and CA methods based on measured physicochemical and sensory parameters, micro- and macroelements and some heavy metals. Samples were grouped into different types according to the production area. Using PCA analysis based on the first two factors (PC1 vs. PC), chestnut honey was divided into three groups (VK, C, and BU) according to the characteristic parameters, linden honey into two (BI and BK), acacia honey into three (SM, C, and BU), meadow honey into three (BP, K, and BK), mixed honey into two (VK and BK) and forest honey into two groups (BK and K). Applying the CA grouping method to all analysed parameters revealed that chestnut honey exhibited distinct properties in BK, C, and BU, linden honey in BK, acacia honey in BU and SM, mixed honey in VK, while forest honey showed distinct properties in K. Meadow honey from different regions were not clearly separated from each other. The present study shows that PCA and CA analysis can be useful tools for distinguishing different honey types, while the collected data allowed the geographical characterisation of honey from Bosnia and Herzegovina.

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