Mineral Profile of Croatian Honey and Differences Due to its Geographical Origin

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Abstract


The proportions of twelve minerals were determined in 200 samples of the black locust honey from five Croatian regions during two seasons. The average proportions were dominated by that of K (205.57–428.05 mg/kg), followed by Ca (33.53–329.00 mg/kg) and Na (23.34–218.04 mg/kg), which was in percentages as follows: K 31.69–81.34%, Ca 6.51–35.56%, and Na 7.36–23.65%. Melissopalynological and physico-chemical analysis of honey confirmed its general quality requirements and botanical origin. One-factor analysis of variance showed a significant differences between the regions in each season based on the average proportions of macro and micro elements except for Mn and Cd in season 2 (P < 0.05). t-Test enabled an insight into the significance of differences between the seasons within each region based on the average proportions of macro and micro elements. Principal components analysis (PCA) showed that Al (Bjelovar-Bilogora), Fe (Bjelovar-Bilogora and Istria), Cu (Eastern Croatia), and K (Istria) could present mineral substances typical for the black locust honey of each region.

Keywords: honey; Croatia; minerals; geographical origin

Honey has been used throughout the history of mankind without any supplements as a nutritional substitute, a natural food, and in medical therapy. It is certainly one of the most complex types of food derived from nature (Azeredo et al. 2003; Iglesias et al. 2004). The composition of honey depends on the plant species, climatic conditions, environmental conditions, and good beekeeping practices. For the most part, it consists of carbohydrates, water, protein, free amino acids, enzymes, vitamins and minerals, flavour and aroma substances, and phenolic compounds present in much smaller amounts along with 200 other compounds (Silva et al. 2009).

Melissopalynological analysis and physicochemical analysis are used to prove the botanical origin of honey and its meeting the general quality requirements set by the Regulations on the quality of monofloral honey and Honey Regulations.

According to some papers, botanical origin of honey can be proven by the combined determination of the electrical conductivity, pH value, free acids, and proportions of fructose, glucose, and raffinose (Mateo & Bosch-Reig 1998; Bogdanov 2009). During the honey labelling, the name of the product can be complemented with the data regarding its floral or herbal origin if the product comes entirely or mainly from the indicated plant source, and the data regarding its regional, territorial, or topographical origin if the product is entirely of this origin (European Commission 2002; MPRRR 2009a). The latter presents a very effective way of raising the value or price of the product, but also a method of counterfeiting. Consumers are willing to pay more for a product from certain areas, while they do not want to buy products coming from some other areas. Therefore, many analytical techniques and parameters combined with statistical techniques are used in determining the geographical origin of honey. Macro- and micro-elements (expressed in percentages in ash) can be found in nectar honey in the amounts...
of about 0.2% and in honeydew honey even above 1%. Honey contains mainly the following mineral substances: potassium, which is the most common (amounting to ¼ to ½ of the total amount of mineral substances), calcium, sodium, magnesium, chlorine, phosphorus, iron, manganese, zinc, copper, chromium, and others. The proportions of mineral substances primarily depend on the botanical origin, climatic conditions, but also significantly on the type of soil in which the plant that honey is produced from grows (Przybylowski & Wilczyńska 2001).

Darker types of honey are generally richer in mineral substances. Black locust honey and sunflower honey are characterised by small proportions of ash, while its higher proportions can be noticed in the meadow, chestnut, and honeydew honeys (Muñoz & Palmero 2005).

The proportions of mineral substances are also good indicator of the environmental pollution (Añklam 1998).

The determination of the macro and micro profiles of the black locust honey and pointing to the differences in the mineral composition due to its geographical origin are not a novelty in the international scientific literature (Latorre et al. 1999; Čelechovská & Vorlová 2001; Nanda et al. 2003; Fernández-Torres et al. 2005; Conti et al. 2007; Osman et al. 2007; Belouali et al. 2008; Madejczyk & Baralkiewicz 2008). Kolaylı and her coworkers believe that the results obtained for the minerals in the sample of three types of honey produced and used in the Black Sea region of Turkey are highly variable and depend on their geographical and botanical origins (Kolaylı et al. 2008).

There are not many studies of this kind conducted on the Croatian honey, which makes this paper a significant contribution to all previous studies of honey in our regions, supplementing the existing data which characterise the Croatian black locust honey (Bubalo et al. 2006; Kenjerić et al. 2007; Šarić et al. 2008).

**MATERIAL AND METHODS**

This research included in total 200 black locust honey samples collected by the beekeepers during two seasons (season 1 and season 2) in 5 regions of...
Croatia, with 20 samples from each region: Varaždin (VŽ), Krapina-Zagorje (KZ), Bjelovar-Bilogora (BB), Istočna Hrvatska (IH) – Eastern Croatia), and Istria (I) (Figure 1).

After conducting the melissopalynological analysis of the black locust honey, physico-chemical parameters were determined (water, free acids, electrical conductivity, reducing sugars, sucrose, diastasis, and hydroxymethylfurfural (HMF), and mineral content (Ca, Na, K, Mg, Zn, Fe, Cu, Mn, Al, Ni, Pb, and Cd). Statistical data analysis was conducted – one-factor analysis of variance (ANOVA), t-test and principal components analysis (PCA).

Melissopalynological analysis was conducted in accordance with the standard (DIN 10760:2002-05). The determination of water, free acid, electrical conductivity, reducing sugars and sucrose in honey was performed using the method prescribed by the International Honey Commission, and determined also the activity of diastase and HMF (IHC 2009; DIN 10750:1990; SLMB 1995), which was based on certain standards (HRN EN ISO 17294-1:2008, HRN EN 15763:2010). The sample preparation was carried out by wet combustion (concentrated nitric acid and hydrogen peroxide) in a microwave oven (Anton Paar Multiwave 3000; Anton Paar, Graz, Austria). Mass spectrometry method for determining metals in honey is based on inductively coupled plasma (ICP-MS) which leads to the ionisation process. Accordingly, mass spectrometer was used to detect and then identify ions on the basis of their mass-to-charge ratio (ELAN ICP-MS 2008; THOMAS 2008). The mean values of the results were calculated in samples of black locust honey for all the regions and the two seasons, based on physicochemical parameters, minerals contents in honey expressed in mg/kg, and in percentages of all the mineral substances in the total mass.

The limits of detection (LOD) and quantification (LOQ) were defined based on 21 measurements (n = 21) of analytes in the low concentration area.

The statistical data analysis included one-factor analysis of variance (ANOVA), t-test, and PCA using the software package STATISTICA 9.1 (StatSoft, 2010).

RESULTS AND DISCUSSION

Melissopalynological and physico-chemical analyses. The confirmation of the botanical origin of honey, which was declared by the manufacturer, was conducted using the melissopalynological analysis (the most common are those of the family Robinia – the black locust family > 21%, Rosaceae – rose family, Brassicaceae – cabbage family, and Fabaceae – bean family) and by determining the compliance of physico-chemical parameters (water, HMF, electrical conductivity, diastase activity, pH, sugar such as fructose, glucose, sucrose) with literature data and with the requirements of the Regulations on the quality of monofloral honey (Table 1) (MPRRR 2009b).

Recently, a distinguishing combination of quality parameters was applied which, besides the pollen number and sensory analysis, includes the determination of the physico-chemical parameters (Přidal et al. 2002; Bogdanov et al. 2004; Persano Oddo & Bogdanov 2004; Persano Oddo et al. 2004; Bogdanov et al. 2007; Primorac et al. 2011; Uršulin-Trstenjak et al. 2013)

Micro and micro elements proportions in honey. The proportions of Ca, Na, K, Mg, Zn, Fe, Cu, Mn, Al, Ni, Pb, and Cd in the black locust honey in both seasons and all the five regions studied varied in wide ranges, both between the regions and inside the regions (Table 2). The results obtained were compared with the literature data presenting the highest and lowest mean values of each mineral (mg/kg), as well as the percentage of each macro and micro element in relation to other minerals. The dominant mineral was K (205.571–428.050 mg/kg) followed by Ca (33.526–329.00 mg/kg) and Na

<table>
<thead>
<tr>
<th>Regulations</th>
<th>Water (%)</th>
<th>Free acids (mEq/1000 g)</th>
<th>Electrical conductivity (mS/cm)</th>
<th>Reducing sugars (g/100 g)</th>
<th>Sucrose (g/100 g)</th>
<th>Diastasis (DN)</th>
<th>HMF (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowest 8 (lowest 3)</td>
<td>9.07–15.14</td>
<td>0.10–2.90</td>
<td>66.94–70.68</td>
<td>0.10–2.90</td>
<td>9.07–15.14</td>
<td>0.50–18.99</td>
<td></td>
</tr>
<tr>
<td>lowest (10)</td>
<td>15.99–18.03</td>
<td>0.12–0.22</td>
<td>8.16–12.94</td>
<td>0.10–2.90</td>
<td>9.07–15.14</td>
<td>0.50–18.99</td>
<td></td>
</tr>
<tr>
<td>highest (0.8)</td>
<td>6.64–7.16</td>
<td>0.10–2.90</td>
<td>15.99–18.03</td>
<td>0.10–2.90</td>
<td>9.07–15.14</td>
<td>0.50–18.99</td>
<td></td>
</tr>
<tr>
<td>highest (50)</td>
<td>15.99–18.03</td>
<td>0.12–0.22</td>
<td>8.16–12.94</td>
<td>0.10–2.90</td>
<td>9.07–15.14</td>
<td>0.50–18.99</td>
<td></td>
</tr>
<tr>
<td>highest (20)</td>
<td>15.99–18.03</td>
<td>0.12–0.22</td>
<td>8.16–12.94</td>
<td>0.10–2.90</td>
<td>9.07–15.14</td>
<td>0.50–18.99</td>
<td></td>
</tr>
</tbody>
</table>

HMF < 15 mg/kg

\( \bar{x} \) – mean value

Table 1. Physico-chemical parameters of black locust honey (the range of mean values in all the regions during both seasons)
Table 2. Average proportions and the ranges of the average proportions \((\bar{x} \pm \sigma)\) of macro and micro elements of black locust honey for each region respectively and during both seasons (in mg/kg)

<table>
<thead>
<tr>
<th>Minerals range</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Ni</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>VŽ</td>
<td>1</td>
<td>126.19 ± 81.85</td>
<td>87.32 ± 46.68</td>
<td>336.87 ± 106.61</td>
<td>0.98 ± 0.43</td>
<td>128.18 ± 83.78</td>
<td>1.01 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>87.70 ± 56.71</td>
<td>128.18 ± 87.78</td>
<td>205.05 ± 31.34</td>
<td>18.09 ± 2.60</td>
<td>17.99 ± 10.94</td>
<td>210.60 ± 70.22</td>
</tr>
<tr>
<td>KZ</td>
<td>1</td>
<td>94.87 ± 25.20</td>
<td>43.00 ± 33.24</td>
<td>3.38 ± 2.73</td>
<td>8.58 ± 0.36</td>
<td>9.40 ± 3.14</td>
<td>0.86 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>61.29 ± 49.09</td>
<td>72.02 ± 20.77</td>
<td>3.38 ± 2.73</td>
<td>8.58 ± 0.36</td>
<td>9.40 ± 3.14</td>
<td>0.86 ± 0.31</td>
</tr>
<tr>
<td>BB</td>
<td>1</td>
<td>115.22 ± 97.60</td>
<td>57.40 ± 39.47</td>
<td>292.54 ± 80.19</td>
<td>26.16 ± 2.19</td>
<td>128.18 ± 83.78</td>
<td>1.01 ± 0.82</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>329.33 ± 199.52</td>
<td>218.042 ± 106.50</td>
<td>336.87 ± 106.61</td>
<td>0.98 ± 0.43</td>
<td>128.18 ± 83.78</td>
<td>1.01 ± 0.82</td>
</tr>
<tr>
<td>IH</td>
<td>1</td>
<td>39.44 ± 9.92</td>
<td>119.68 ± 86.64</td>
<td>428.05 ± 9.92</td>
<td>92.50 ± 24.78</td>
<td>26.16 ± 2.19</td>
<td>115.22 ± 97.60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33.53 ± 14.43</td>
<td>43.00 ± 33.24</td>
<td>43.00 ± 33.24</td>
<td>43.00 ± 33.24</td>
<td>43.00 ± 33.24</td>
<td>43.00 ± 33.24</td>
</tr>
</tbody>
</table>

The percentage of Mn ranged from 0.02% to 0.07% with the average amount from 0.10 mg/kg to 0.23 mg/kg, the values being approximately equal to those obtained with the Romanian (0.16 mg/kg), Swiss (0.45 mg/kg), and two Italian (0.10 mg/kg; 0.09 mg/kg) black locust honeys (CAROLI et al. 2000; BODGANOV et al. 2007). The tested samples contained Al in the proportion from 0.13% to 1.13%, with the average amount from 0.86 mg/kg to 3.38 mg/kg. Ni was present in the samples in the proportion from 0.02% to 0.20% with the average amount from 0.09 mg/kg to 1.86 mg/kg, while the average amounts in the Swiss honeys were from 0.09 mg/kg to 0.86 mg/kg, being higher than those obtained in the Slovenian (0.10 mg/kg) and Romanian black locust honey (0.09 mg/kg) (GOLOB et al. 2005; MARGHITAS et al. 2010). The amount of Na ranged from 7.36% to 23.65% with the range of mean value from 23.34 mg/kg to 218.04 mg/kg and was higher than that in the Romanian honey (13.02 mg/kg) (MARGHITAS et al. 2010). The average proportion of K ranged from 31.69% to 81.34%, the average amount being from 205.57 mg/kg to 428.05 mg/kg. The available literature data obtained with the Slovenian (390.00 mg/kg) and Romanian samples (187.10 mg/kg) are approximately equal to those from this research (GOLOB et al. 2005; MARGHITAS et al. 2010).

Mg ranged from 2.03% to 5.60%, with the average amount from 12.03 mg/kg to 49.07 mg/kg being higher than that obtained by MARGHITAS et al. (2010) (5.7 mg/kg). The portion of Zn ranged from 0.19% to 3.33% with the average amount from 0.94 mg/kg to 30.88 mg/kg, which was higher than those in the Italian (0.18 mg/kg) and the Swiss (0.22 mg/kg) black locust honeys (CAROLI et al. 2000; BODGANOV et al). The proportion of Fe ranged from 0.10% to 0.72% with the amount from 0.50 mg/kg to 2.27 mg/kg, which is equal (= 1.30 mg/kg) or higher compared to the European honey (> 0.28, 0.26, and 0.21 mg/kg) (CAROLI et al. 2000; FORTE et al. 2001; BODGANOV et al. 2007; MARGHITAS et al. 2010). The value from 0.01% to 0.10% demonstrates the proportion of Cu. The average amount from 0.07 mg/kg to 0.95 mg/kg was equal to the results in the literature (0.52, 0.18, 0.17, 0.08, 0.06 mg/kg) (FORTE et al. 2001; AJTNY et al. 2006; BODGANOV et al. 2007; CAROLI et al. 2007; MARGHITAS et al. 2010).
 (> 0.06 mg/kg) and the Italian samples (> 0.03 mg/kg and 0.02 mg/kg) present lower values (Caroli et al. 2000; Forte et al. 2001; Bogdanov et al. 2007). The proportion of Pb ranged from 0.01% with the average amount from 0.02 mg/kg to 0.11 mg/kg, which resembles that in the Swiss (0.01 mg/kg) and the Italian black locust honey (0.02 mg/kg) (Forte 2001; Bogdanov et al. 2007). The proportion of Cd ranged from 0.00058% to 0.00152%. The average amount ranged from 0.003 mg/kg to 0.011 mg/kg. The results obtained by Italian researchers are of lower values (> 0.0004 mg/kg and 0.0006 mg/kg) (Caroli et al. 2000; Forte 2001) (Table 2).

**Statistical analysis**. Statistical analysis of the results obtained was used to point out the differences between the mineral compositions of black locust honeys due to their geographical origins.

One-factor analysis of variance showed significant differences between the regions in each season given by the average proportions of all the macro and micro elements except Mn and Cd in the second season ($P < 0.05$) (Table 5).

The use of the $t$-test gave an overview of the significance of the differences between the seasons within each region given the average proportion of macro and micro elements. A significant difference in the proportion of Ca between the seasons was reflected in the KZ and IH, while with Na was it noted only in the IH region. With K, a significant difference was found between the seasons in KZ and I regions, and the proportion of Mg showed significant differences between the seasons in VŽ and IH regions. Mineral Zn showed a difference between the seasons in four regions (except for BB), while with Fe was it reflected in BB and I regions. In two regions, IH and I, the difference between the seasons was observed with Cu, while Mn showed a significant difference between the seasons in VŽ region. As regards Al, Ni, and Cd, significant differences between the seasons were observed in three regions (Al-VŽ, KZ and BB; Ni-KZ, and IH, I; Cd-VŽ, IH and I). IH and I regions are the regions in which a significant difference occurred between the seasons considering the proportion of Pb ($P < 0.05$) (Table 6).

The principal components analysis (PCA) was conducted using the proportions of 12 minerals from all the regions and both seasons in order to assess whether the geographical origin of honey can be determined on the basis of the determined proportions of macro and micro elements. By comparing Figures 2

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>2</td>
<td>0.08</td>
<td>0.3</td>
<td>0.2</td>
<td>0.03</td>
<td>0.06</td>
<td>0.005</td>
<td>0.008</td>
<td>0.008</td>
<td>0.005</td>
<td>0.03</td>
<td>0.0003</td>
</tr>
<tr>
<td>LOQ</td>
<td>5</td>
<td>0.3</td>
<td>1</td>
<td>0.7</td>
<td>0.1</td>
<td>0.2</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 2. The projection of macro and micro elements of the first two factors plan (PC1 vs. PC 2)

Figure 3. The projection of honey samples from all the regions during both seasons of the first two factors plan (PC1 vs. PC2)
Table 4. The ranges (in mg/kg) of the average proportions of micro and micro elements of black locust honey for all the regions during both seasons

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range x</td>
<td>33.53–329.33</td>
<td>23.34–218.04</td>
<td>12.30–49.07</td>
<td>0.94–30.88</td>
<td>0.50–2.27</td>
<td>0.07–0.95</td>
<td>0.09–0.23</td>
<td>0.86–3.38</td>
<td>0.09–1.86</td>
<td>0.02–0.11</td>
<td>0.003–0.011</td>
<td></td>
</tr>
</tbody>
</table>

x – mean value

Table 5. The significance of differences (P-value) between the regions in each season based on the average proportions of macro and micro elements (one-factor analysis of data variance)

<table>
<thead>
<tr>
<th>Season</th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.2 × 10^{-12}</td>
<td>1.26 × 10^{-18}</td>
<td>1.72 × 10^{-7}</td>
<td>7.06 × 10^{-14}</td>
<td>2.65 × 10^{-16}</td>
<td>1.12 × 10^{-5}</td>
<td>2.35 × 10^{-11}</td>
<td>2 × 10^{-4}</td>
<td>3.5 × 10^{-3}</td>
<td>2.49 × 10^{-16}</td>
<td>1.76 × 10^{-6}</td>
<td>1 × 10^{-3}</td>
</tr>
<tr>
<td>2</td>
<td>2.28 × 10^{-5}</td>
<td>1.89 × 10^{-6}</td>
<td>1.21 × 10^{-6}</td>
<td>4.2 × 10^{-3}</td>
<td>9.43 × 10^{-14}</td>
<td>1.25 × 10^{-7}</td>
<td>1.29 × 10^{-6}</td>
<td>6.9 × 10^{-2}</td>
<td>1 × 10^{-3}</td>
<td>6.7 × 10^{-3}</td>
<td>3.72 × 10^{-2}</td>
<td>4.05 × 10^{-2}</td>
</tr>
</tbody>
</table>

Table 6. The significance of differences (P-value) between the seasons within each region based on the average proportions of macro and micro elements (t-test)

<table>
<thead>
<tr>
<th>Regions</th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Cu</th>
<th>Mn</th>
<th>Al</th>
<th>Ni</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>VŽ</td>
<td>9.3 × 10^{-2}</td>
<td>6.02 × 10^{-2}</td>
<td>1.46 × 10^{-1}</td>
<td>7.8 × 10^{-2}</td>
<td>1.82 × 10^{-5}</td>
<td>7.27 × 10^{-1}</td>
<td>1.2 × 10^{-1}</td>
<td>2.68 × 10^{-2}</td>
<td>1.96 × 10^{-2}</td>
<td>1.85 × 10^{-1}</td>
<td>2.12 × 10^{-1}</td>
<td>2.91 × 10^{-2}</td>
</tr>
<tr>
<td>KZ</td>
<td>9.9 × 10^{-3}</td>
<td>6.54 × 10^{-1}</td>
<td>1.3 × 10^{-3}</td>
<td>9.68 × 10^{-1}</td>
<td>9.65 × 10^{-39}</td>
<td>6.6 × 10^{-1}</td>
<td>1.87 × 10^{-3}</td>
<td>3.48 × 10^{-1}</td>
<td>2 × 10^{-4}</td>
<td>1.83 × 10^{-12}</td>
<td>2.59 × 10^{-1}</td>
<td>1.03 × 10^{-1}</td>
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<tr>
<td>BB</td>
<td>4.27 × 10^{-1}</td>
<td>8.87 × 10^{-2}</td>
<td>1.72 × 10^{-6}</td>
<td>6.39 × 10^{-2}</td>
<td>4.91 × 10^{-1}</td>
<td>7 × 10^{-4}</td>
<td>4.05 × 10^{-1}</td>
<td>3.11 × 10^{-1}</td>
<td>2.2 × 10^{-3}</td>
<td>7.35 × 10^{-2}</td>
<td>4.37 × 10^{-1}</td>
<td>5.33 × 10^{-1}</td>
</tr>
<tr>
<td>IH</td>
<td>5.86 × 10^{-6}</td>
<td>7.1 × 10^{-3}</td>
<td>7.1 × 10^{-2}</td>
<td>2.45 × 10^{-7}</td>
<td>3.64 × 10^{-5}</td>
<td>4.56 × 10^{-2}</td>
<td>1.48 × 10^{-5}</td>
<td>1.05 × 10^{-1}</td>
<td>7.41 × 10^{-1}</td>
<td>8.61 × 10^{-6}</td>
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<td>2.44 × 10^{-2}</td>
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<tr>
<td>I</td>
<td>1.4 × 10^{-3}</td>
<td>2.14 × 10^{-1}</td>
<td>4 × 10^{-4}</td>
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<td>7.48 × 10^{-2}</td>
<td>6.7 × 10^{-3}</td>
<td>2.81 × 10^{-2}</td>
<td>8.9 × 10^{-3}</td>
</tr>
</tbody>
</table>

VŽ – Varaždin; KZ – Krapina-Zagorje; BB – Bjelovar-Bilogora; IH – Istočna Hrvatska (Eastern Croatia); I – Istria
and 3, Al is positioned in the same coordinate system as well as BB-10 (BB region, season 2). The position of Fe in Figure 2 would characterise the samples BB-10 (BB region, season 2) and I-9 (I region, season 1) in Figure 3. When all the regions are compared, IH-9 stands out most (IH region, season 1). If this is compared with Figures 2 and 4, it can be seen that Ca lies in this point in the coordinate system. In Figures 4 and 5 can be seen that K is positioned on the right side of the coordinate system as IH-10 (IH region, season 2). The following mineral substances typical for the black locust honey of each region could be potentially taken: Al (BB region), Fe (BB and I regions), K (IH region), and Ca (Figures 2–5).

**CONCLUSIONS**

The results of melissopalynological and physicochemical analyses of honey confirmed the given botanical origin of honey in all the samples and met the general quality requirements. The proportions of most of the 12 macro and micro elements in the analysed black locust honey samples demonstrated wide ranges. K appeared in the highest proportion out of the macro elements, followed by Ca and Na. One-factor analysis of variance showed significant differences between the regions either of the two seasons followed as given by the average proportions of all the macro and micro elements except Mn and Cd in season 2 ($P < 0.05$). The application of the $t$-test gave an overview of the significance of differences between the seasons within each region given by the average proportions of macro and micro elements. Based on the conducted principal components analysis (PCA), the following mineral substances typical for the black locust honey of a particular region could be potentially considered: Al (BB region), Fe (BB and I regions), and K (I region).

**References**


HRN EN 15763 (2010): Namirnice – Određivanje elemenata u tragovima – Određivanje arsena, kadmiija, žive i olova u hrani induktivno spregnutom plazmom sa spektrometrijom masa (ICP-MS) nakon razgradnje pod tlakom. Hrvatski zavod za norme


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