Physico-chemical indicators and identification of selected Slovak honeys based on colour measurement

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Abstract

The aim of study was to characterize 52 samples of Slovak honeys of six types (multifloral, acacia, rape, honeydew, forest and mixed). Physico-chemical analysis of honey included the water content, free acidity, pH, water activity, electrical conductivity, and hydroxymethylfurfural (HMF) content. In addition, the colour of honeys was measured using spectrophotometer and Commission Internationale de l’Eclairage method (CIE L*a*b*); four types of honey were used for identification: multifloral, acacia, rape, and honeydew. Mean values of physico-chemical indicators were as follows: water content 16–21%, acidity 6–42 mekv·kg\(^{-1}\), pH 3.68–5.15, water activity 0.505–0.667, electrical conductivity 0.155–1.585 mS·cm\(^{-1}\) and HMF content 0.17–78.5 mg·kg\(^{-1}\). The highest HMF content was found in forest honey with one sample above the limit established by Council Directive 2001/110/EC. The colour of Slovak honeys analysed in this study was very variable and ranged from pale yellow to dark brown. Differences of physico-chemical indicators and colour of honey samples were found to be significant (P < 0.05). The CIE L*a*b* methods can be used for identification of selected honey samples. This is the first similar study on Slovak honey.

Water content, acidity, HMF content, CIE L*a*b* method

Honey is 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 the bees collect and transform by combining it with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Codex Alimentarius 2004). Honey is variable in its composition due to contribution of the plant, climate, environmental conditions and the ability of the beekeeper (White 1978). The main types of honey are as follows according to origin: blossom or nectar honey and honeydew honey (Codex Alimentarius 2004).

The colour of honey, beside flavour and aroma, is one of the characteristics that serve to indicate the plant source. It ranges from very pale yellow through amber and dark reddish amber to nearly black (Terrab et al. 2004). It is related to the content of minerals, pollen and phenolics, and is characteristic of floral origin (Baltrusaitytė et al. 2007).

The Codex Alimentarius of the Slovak Republic (2004) includes several physical and chemical indicators comprising moisture content, acidity, sugar content, sucrose content, electrical conductivity, free acidity, diastatic activity and hydroxymethylfurfural (HMF) content. The aim of this study was to determine the water and sugar content, free acidity, pH, water activity, electrical conductivity, hydroxymethylfurfural and colour of Slovak honeys; and to apply results of colour measurement in an effort to use combination by the Commission Internationale de l’Eclairage (CIE) L\(^*\) a\(^*\) b\(^*\) methods to identify selected Slovak honey types.

Materials and Methods

Sample collection

Fifty-two honey samples from regions of Central, Southern and Eastern Slovakia were analysed. The samples were obtained from retailers and beekeepers. Samples included multifloral (n = 21), acacia honey (n = 6), rape honey (n = 5), honeydew honey (n = 7), forest honey (n = 7) and mixed honey (n = 6) originated from 2006 and

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2008. The honey samples were analysed for the following physico-chemical indicators: water and sugar content, free acidity, pH, water activity, electrical conductivity and HMF content.

Laboratory analyses

Water and sugar content was determined using the honey tester Meopta (Meopta Přerov, Czech Republic). The tester is a hand refractometer featuring automatic temperature compensation in the range of 10 to 30 °C. The apparatus scale enables to read directly the weight percentage of aqueous sugar solution (saccharose) degrees BRIX (°Bx) and water contents (%). The acidity was determined by the titrimetric method (with 0.1M sodium hydroxide solution) by IHC method (2002). The pH was measured with pH-meter (Wissenschaftlich-Technische Werkstätten, Germany) in a solution containing 10 g honey in 75 ml of CO₂ free distilled water. Water activity was determined by using a Labmaster-a (Novasina, Switzerland) at 25 °C. Electrical conductivity (mS·cm⁻¹) was measured at 20 °C in 20% solution of honey samples in deionised water, using a conductivity meter Vario Cond (Wissenschaftlich-Technische Werkstätten, Germany). Hydroxymethylfurfural content was determined using the HPLC method (Hewlett Packard, USA) with a UV detection (detector Agillent 1500). The samples were prepared according to an International Honey Commission (IHC) method (2002) and analysed using column Hypersil BDS 100 × 4 mm, 3µm, with some modification in the mobile phase composition when acetonitrile was used instead of methanol. Elution was applied with water – acetonitrile (90:10) isocratically, at the flow rate of 1 ml·min⁻¹. The HMF content was determined at 285 nm using the calibration curve of HMF standard.

Colour characteristics of 31 samples from the whole testing scope including multifloral (n = 12), acacia (n = 6), rape (n = 5), and honeydew (n = 8) samples of honey were assessed by the CIE L*a*b* method where lightness represents L* and a* and b* are two colour coordinates. The colour was estimated by Spectrophotometer CM-2600d (Konica Minolta, Japan). Values of L*a*b* represent hue, lightness and saturation of measured honey. Standard illuminating D65, 10° observer SCE measurements were used (in SCE mode the specular reflectance is excluded from the measurement). Also, colour difference based on measurement of reflectance in spectra with wavelengths 360–740 nm was determined. Honey samples were heated at 40 °C to dissolve sugar crystals and then placed into white plastic container (29 mm in diameter, height layer of honey 17 mm, volume of honey 10 ml) and covered with a glass plate without air bubbles on the surface. Origin of the honey was confirmed using mellisopalynology analysis.

Statistical analysis

All analyses were carried out in triplicate and the data were expressed as mean ± standard deviations (SD). GraphPad Prism 5 (2007) was used to perform statistical analyses. For each indicator, the differences among honeys were analysed using ANOVA (one-way of analysis of variance) followed by Tukey’s test. Differences among means at the 95% (P < 0.05) confidence level were considered significant.

Results

The results of physico-chemical indicators of selected Slovak honeys are shown in Table 1. The values of water content ranged from 16 to 21%; in two samples the water content exceeded 20% which is the maximum value stated by the Council Directive 2001/110/ES

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Multifloral (n = 21)</th>
<th>Acacia   (n = 6)</th>
<th>Rape (n = 5)</th>
<th>Honeydew (n = 7)</th>
<th>Forest (n = 7)</th>
<th>Mixed (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (%)</td>
<td>Mean ± SD</td>
<td>18.21 ± 1.26</td>
<td>17.83 ± 1.13</td>
<td>19.20 ± 1.61</td>
<td>17.86 ± 0.80</td>
<td>16.33 ± 1.53</td>
</tr>
<tr>
<td>Free acidity (mg·kg⁻¹)</td>
<td>Mean ± SD</td>
<td>22.33 ± 4.86</td>
<td>10.83 ± 2.42</td>
<td>19.0 ± 7.91</td>
<td>32.0 ± 7.91</td>
<td>24.67 ± 7.51</td>
</tr>
<tr>
<td>pH</td>
<td>Mean ± SD</td>
<td>4.18 ± 0.279</td>
<td>4.38 ± 0.457</td>
<td>3.83 ± 0.169</td>
<td>4.66 ± 0.370</td>
<td>4.36 ± 0.275</td>
</tr>
<tr>
<td>Water activity</td>
<td>Mean ± SD</td>
<td>0.079 ± 0.048</td>
<td>0.562 ± 0.018</td>
<td>0.583 ± 0.021</td>
<td>0.590 ± 0.023</td>
<td>0.592 ± 0.026</td>
</tr>
<tr>
<td>El. conductivity (mS·cm⁻¹)</td>
<td>Mean ± SD</td>
<td>0.461 ± 0.196</td>
<td>0.297 ± 0.251</td>
<td>0.516 ± 0.599</td>
<td>1.122 ± 0.111</td>
<td>0.977 ± 0.363</td>
</tr>
<tr>
<td>HMF (mg·kg⁻¹)</td>
<td>Mean ± SD</td>
<td>14.97 ± 17.200</td>
<td>11.08 ± 8.909</td>
<td>13.70 ± 11.08</td>
<td>11.42 ± 5.010</td>
<td>26.02 ± 21.500</td>
</tr>
</tbody>
</table>

HMF - hydroxymethylfurfural, SD - Standard deviation
The results of water content showed a significant difference \((P < 0.05)\) between water content of mixed honey and all other types of honey. Sugar content ranged from 78 to 83 Bx°. The values of free acidity ranged from 6 to 42 meq·kg⁻¹. The value of free acidity did not exceed the limit of 50 meq·kg⁻¹ permitted by Codex Alimentarius of the Slovak Republic (2004) in any of the samples. Significant differences of free acidity values were found among honeydew honey and multifloral or rape honeys \((P < 0.05)\), and acacia honeys \((P < 0.001)\). Significant differences were found also among acacia honey versus mixed \((P < 0.05)\), and multifloral or forest honeys \((P < 0.001)\).

The pH values for honey samples ranged from 3.68 to 5.15. Likewise differences in pH values, namely among multifloral honey and forest or honeydew honey \((P < 0.05)\) and rape honey versus forest and honeydew honey \((P < 0.01)\) were found.

The values of water activity ranged from 0.505 to 0.667. No differences were found among the analyzed samples of honeys \((P > 0.05)\).

Variation of honey samples in their conductivity depended on the origin of honey and ranged between 0.155–1.585 mS·cm⁻¹. Significant differences of electrical conductivity among acacia honey and forest \((P < 0.01)\), and honeydew honeys \((P < 0.001)\) were determined. Differences were determined also among multifloral and forest \((P < 0.01)\), honeydew honeys \((P < 0.001)\); and between rape and honeydew honeys \((P < 0.01)\).

The results of HMF content determination showed variability of values between 0.17–78.5 mg·kg⁻¹ with the mean value of 16.19 ± 16.38. Three samples exceeded the limit (40 mg·kg⁻¹) permitted by the Council Directive 2001/110/ES (2001).

<table>
<thead>
<tr>
<th>Type of honey</th>
<th>Specular component excluded (Mean ± SD)</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multifloral (n = 12)</td>
<td>8.230 ± 1.785</td>
<td>4.072 ± 1.665</td>
</tr>
<tr>
<td>Acacia (n = 6)</td>
<td>11.290 ± 1.236</td>
<td>4.072 ± 1.334</td>
</tr>
<tr>
<td>Rape (n = 5)</td>
<td>45.570 ± 6.990</td>
<td>2.508 ± 1.694</td>
</tr>
<tr>
<td>Honeydew (n = 8)</td>
<td>4.455 ± 1.175</td>
<td>1.304 ± 2.385</td>
</tr>
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</table>

SD - Standard deviation, L* - lightness, a* b*- two colour coordinates

The colour characteristics of honey are presented in Table 2 which summarizes the means, standard deviations and ranges of the indicators L*, a* and b* for the four types of Slovak honey. L* value indicates degree of lightness, positive a* indicates red, negative a* green component, positive b* indicates yellow, and negative b* blue component. Rape honey had significantly the highest average value of indicator L* (45.57) compared to other honeys \((P < 0.001)\). However, honeydew honey (4.455) formed significantly lower values \((P < 0.05)\) in comparison with acacia (11.29) honey. Green components (negative a* values) were determined only in samples of honeydew honey. Honeydew can be distinguished from all the other honey types because of its dark colour. Floral types of honey (multifloral, acacia and rape) with a* and b* values, in the range from 2.508 to 4.072 and 9.835 to 14.880, respectively, overlap extensively in the diagram SCE. The following results were calculated using the values of their b* indicators; honeydew was significantly different in comparison with all other types of honey, and multifloral versus acacia and rape honeys \((P < 0.001)\). By comparison the remission spectra in each type of honey were found to be different and their shapes typical for the respective types of honey.
Discussion

Water content is a very important quality indicator for practically every food product as well as for its ingredients. Regulations dealing with food quality usually refer to the water content. Officially, honey should contain no more than 20% of water (Abramovič et al. 2008). Our results correspond with the results of Bartáková et al. (2007). They found 15–20% water content in honey samples from the Czech Republic. Gomes et al. (2010) found 15.9–17.2% water content in commercial honeys from Portugal. Moisture and sugar content strictly correlate and anomalous values of Brix degrees may be a reliable index of adulteration (Terrab et al. 2004). Conti (2000) stated that the mean value of sugar content in Italian honey is 82.0 ± 1.1 °Bx with the range of 80–83.8 °Bx. Kirs et al. (2011) analyzed 14 honeys from Estonian beekeepers for physico-chemical and melissopalynological characterization of summer honeys. The sum of glucose and fructose was found to meet the European legislation with the values of over 60 g·100 g⁻¹ for all honey samples.

Honey acidity is mainly influenced by organic acids with a quantity lower than 0.5%. Acidity contributes to honey flavour, stability against microorganisms, enhancement of chemical reactions, and antibacterial and antioxidant activities (Bogdanov 1997; Gheldof et al. 2002). High acidity can be indicator of sugar fermentation converted into organic acids (Gomes et al. 2010). No sample with a value of free acidity higher than the limit permitted by Codex Alimentarius of the Slovak Republic (2004) was determined in the analysed samples.

The pH value has great importance during honey extraction and storage, due to influence on texture, stability and endurance (Terrab et al. 2002). Our results are comparable with the results of Bartáková et al. (2007). They found that pH values in honey samples from the Czech Republic varied from 3.64 to 4.95. Conti et al. (2000) determined a similar pH value of 4.3 ± 0.5 in Italian honey. Manzanares et al. (2011) applied multivariate analysis on physico-chemical indicators in blossom and suspected honeydew honeys. All the analyzed honeys were acidic with a pH in the range of 3.52–6.91, and free acidity contents below 50 mEq·kg⁻¹.

The water activity in food is an important property that can be used to predict the stability and safety of food with respect to microbial growth, rates of deteriorative reactions and chemical/physical properties (Fontana 1998). Osmophilic yeast are specialists which have an obligate need for high sugar concentrations and are able grow to a minimal water activity up to 0.6 and such osmophilic yeast are causing honey fermentation (Gleiter et al. 2006). The mean value of water activity of Slovak honey samples analysed in our study was 0.58 ± 0.03. Bartáková et al. (2007) found that value of water activity in honeys from the Czech Republic ranged from 0.485 to 0.607.

The conductivity of honey is the main quality indicator for this product, which is specified in the Codex Alimentarius of the Slovak Republic (2004) and the Council Directive 2001/110/ES (2001). The maximum permitted value for nectar and mixture honey is 0.8 mS·cm⁻¹ and permitted value for honeydew and chestnut honeys must be more than 0.8 mS·cm⁻¹. Our results are comparable with the values of 0.19–0.53 mS·cm⁻¹ described by Gomes et al. (2010) in samples of nectar honey.

The HMF content is indicative of honey freshness as it is absent in fresh honey and tends to increase during processing and/or ageing of the product (Terrab et al. 2002). Our results correspond, except for three samples which exceeded the permitted limit, with data presented by Bartáková et al. (2007) who described the values in the range of 0–15.51 mg·kg⁻¹.

The results of physico-chemical determinations showed that honey samples from the Slovak Republic were of good quality based on the results shown in Table 1 characterising the individual types of honey.
Bertoloncelj et al. (2007) analysed the colour of Slovenian honey samples from seven most common honey types by the CIE L* a*b* method and found that acacia and lime-tree honeys had the highest average values (64.40 and 63.24, respectively) of indicator L* (lightness). No significant difference ($P < 0.05$) was found between these two types of honey. The L* value decreased further in multifloral (53.87) and chestnut honey (48.11). Fir, spruce and forest honeys were the darkest, with similar L* values of 43.17, 43.48 and 42.12, respectively. The lightest acacia and lime-tree honeys were found to be clearly distinguished from all the other honey types. Honeydew types of honey (fir, spruce and forest) with a* and b* values in the range of 8.18–10.14 and 32.88–34.98, respectively, overlapped extensively in the (a*, b*) diagram. We found similar values L*, a*, b*. All types of honey had the typical L*, a*, b* values representing the colour of honey and could be used for identification of different kinds of honey.

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