HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF SUGARS IN TRANSYLVANIAN HONEYDEW HONEY

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Abstract. 28 declared honeydew honey samples were collected from different Transylvanian areas. Origin of the samples was verified by physico-chemical parameters and pollen analysis. Their sugar profile was analysed by means of HPLC with refractive index detection. The results obtained showed that only 25% of the samples correspond to the requirements of honeydew honey. The rest of 75% samples were mixed blossom-honeydew or pure blossom honeys. Our principal objective was the determination of sugar profile for Transylvanian honeydew honey.

INTRODUCTION

The honeydew is a sweet substance, clear and viscous, sometimes solid, which in some period of the year is found on leaves, limbs or stalk plants. It can have of animal origin, when produced by insects, or vegetal, when secreted by leaves, buds or other parts of the plants (Marghitas, 2002). According to the EU honey standard, honeydew honey is obtained mainly from excretions of plant sucking insects on the living parts of plants or secretions of living parts of plants (European Commission, 2002).

The honeydew honeys is often mixed with various amounts of nectar honey. Bogdanov and Gfeller (2006) have studied the use of discriminant analysis for the classification of blossom, honeydew and mixed honeys by parameters which can be determined easily in routine honey control: the sugars, pH, free acidity, electrical conductivity and protein content. The honeys were classified by discriminant analysis using single and multiple variables. The best single variable for honey classification was melezitose with 96% correct classification, while the other variables had a weaker discriminatory power. Two canonical variable functions proved to be powerful for discrimination between blossom and honeydew honeys. The first used following variables: sum of fructose and glucose content, ratio of fructose and glucose content, pH and electrical conductivity. This function classified correctly 98.2% of the blossom and 81.2% of the honeydew honeys. The other function using in addition melezitose classified correctly 88.2% of the honeydew and all blossom honeys. The second discriminant function was calculated according to the following equation (Bogdanov & Gfeller, 2006).

\[ X = 2.49 \times (F/G) + 0.20 \times (F+G) - 0.30 \times \text{Mel} + 1.09 \times \text{pH} - 2.18 \times C - 19.78 \]

For a correct evaluation of botanical origin for honey is necessary to determine the physico-chemical parameters corroborated with microscopic analysis. International Commission for Plant-Bee relationships-ICPBR established that a honey is floral when the
honeydew elements/number of pollen grains from nectariferous species (HDE/NPGN) ratio is < 1; if this ratio is three or more, the honey is considered as a honeydew honey, while those honeys with a ratio between one and three are considered as mixed honeys. (Díez & al., 2004).

Comparative with blossom honey, honeydew honeys are characterized generally by their dark colour, weak fragrance and higher values of acidity, ash, mineral content and electrical conductivity. The sugar concentration in honeydew honey is different then in blossom honey. The honeydew contains enzyme secreted by the salivary glands and intestines of the insects, but the nectar does not contain these substances. For this reason in honeydew appear some sugars which in plants lymph are missing. In honeydew honey the oligosaccharide concentration is higher than in floral honey. Melezitose is a characteristic trisaccharide for honeydew honey and its concentration is different according to honey type (Sabatini & al., 2007).

MATERIAL AND METHODS

24 honey samples were obtained directly from beekeepers and 4 samples were purchased from the supermarket. 9 sugars were determined according to the method described in Harmonised Methods of the European Honey Commission. Sugar concentrations were expressed in g per 100g honey (%).

A Shimadzu Liquid Chromatograph with refractive index detector was used. HPLC separation was performed on an analytical column containing amino-modified silica gel using acetonitrile: water (85:15, v/v) as mobile phase. The flow rate was 1.3 ml/min.

The carbohydrate standards: glucose, fructose, sucrose, turanose, maltose, trehalose, erlose, melezitose and raffinose were purchased from Merck. The organic solvents used were of HPLC grade (Sigma).

A 5% honey solution was prepared in methanol-water 1-3. Each solution was filtered (0.45 µm) before chromatographic analysis.

The identification of honey sugars was obtained by comparison of their retention times with these of the standard sugars. For sugars quantification were establish calibration curves for each sugar.

RESULTS AND DISCUSSIONS

We were able to identify and quantify two monosaccharides (glucose and fructose), four disaccharides (maltose, trehalose, sucrose, turanose) and three trisaccharides (melezitose, erlose and raffinose). Comparing the sugars profile obtained with the rest of parameters we can conclude that all samples purchased from supermarket were indeed honeydew honey. Different results were obtained for honey samples acquired directly from beekeepers: 3 of them were honeydew honey, 9 of them were of mixed origin (blossom and honeydew) and 12 samples were blossom honey.

Certified honeydew honeys (7 samples) present the following sugar profile: fructose and glucose ranged between 33,15 – 37,03% and 24,65 – 30,19%, respectively. The sum of fructose and glucose had an average value of 62,59%. The content of maltose, the most abundant disaccharide, ranged between 2,04 and 3,14%. Turanose presented values between 1,47 and 2,89%. Trehalose was detected in 6 samples, with values ranging between 0,23 and 1,23%. The concentration of sucrose did not exceed 0,4% in any samples. Maximum content of melezitose was 6,76%. The percent of erlose was sub unitary in all the samples (max. 0,9%). Raffinose was identified only in 3 samples at low percentage (max. 0,67%).
The mean values of sugar concentrations from mix and blossom honey are showed in table 1, and they are compared with those obtained for honeydew honey.

Table 1
Sugar profile for honeydew, mix and blossom honeys

<table>
<thead>
<tr>
<th>Sugar (G/100g)</th>
<th>Type Of Honey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honeydew</td>
</tr>
<tr>
<td>Fructose</td>
<td>35.52</td>
</tr>
<tr>
<td>Glucose</td>
<td>27.07</td>
</tr>
<tr>
<td>Maltose</td>
<td>2.48</td>
</tr>
<tr>
<td>Turanose</td>
<td>2.1</td>
</tr>
<tr>
<td>Trehalose</td>
<td>0.74</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.19</td>
</tr>
<tr>
<td>Melezitose</td>
<td>4.23</td>
</tr>
<tr>
<td>Erlose</td>
<td>0.53</td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.35</td>
</tr>
</tbody>
</table>

According to the present EU honey standard, the G+F minimum value for blossom honeys should be 60g/100g; while for honeydew honeys it is 45g/100g (European Commission, 2002). The sum of glucose and fructose content (G+F) was smaller for honeydew honey samples (62.59g/100g) than mixed honeys (66.17g/100g) or blossom honeys (69.92g/100g).
CONCLUSIONS

Not all the samples were honeydew honey; a considerable percent of them had a mixed or floral origin. The correct classification of honeys by botanical origin was verified using the equation of discriminant function established by Bogdanov and Gfeller (2006). The intersection point between honeydew and blossom honey was −0.259. The values of the canonical variables for both honey types were classified as follows:

Honeydew honeys < -0.259 < blossom honeys
Honeys with values around the intersection point of −0.259 contain both honeydew and nectar. We obtained the values:

\[ z = -2.28 \pm 0.66 \text{(sd)} \] for honeydew honey

\[ z = +0.35 \pm 0.72 \text{(sd)} \] for blossom honey

The honeydew honeys presented smaller amounts of monosaccharides and higher amounts of di- and trisaccharides than the rest of honeys. Melezitose, an indicative of honeydew honey was present in variable quantities, while sucrose was found in lower quantities in all the samples. Erlose was present in every sample but the raffinose was identified in many honeydew and mix honeys but only in a blossom honey sample was presented.

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