Examination of the nutrient content and color characteristics of honey and pollen samples

Keywords: honey, pollen, nutrient content, botanical origin, moisture content, sugar content, ash content, amino acid composition, HMF, color characteristics

1. SUMMARY

Due to its nutritional value, physiological effects and unique aroma, honey is one of our widely consumed foods, used for sweetening. There are several regulations concerning the composition and analysis of honey, of which the specifications and guidelines of the Hungarian Food Codex are authoritative in Hungary. In the present study, the color characteristics and nutrient composition of domestic and foreign honeys are examined. Our intention was to review the physical and chemical characteristics of honeys of different origin marketed in Hungary. As a point of interest, a honey obtained from a foreign market was also examined. Pollen is a less widely consumed apiculture product, mostly a dietary supplement known to health-conscious consumers. There is also much less knowledge is available about its composition than in the case of honey. With our work, we intended to fill this gap. In addition, the nutrient content and color characteristics of pollen samples from some plant species that also occur in Hungary are described.
2. Introduction

Honey is one of our oldest foods and is still a popular sweetener around the world. According to the definition of the Hungarian Food Codex, „Honey is a natural sweet substance collected by Apis mellifera bees from plant nectar or the sap of live plant parts, or by insects that suck plant sap from the secreted material of live plant parts, which is collected by the bees, converted by the addition of their own substances, then stored, dehydrated and matured in honeycombs” [1]. Its energy content is provided by easily absorbed carbohydrates, but it also contains many other nutrients such as minerals, phenolic compounds and amino acids. Thanks to its natural aroma substances, honey has pleasant organoleptic properties, so it can be characterized by a high level of enjoyment [2]. Honey is also used for medicinal purposes, mainly due to its anti-inflammatory and antibacterial effects [3].

The pollen cluster is a little-known apiculture product that is of growing interest, especially among health-conscious consumers. The pollen cluster is formed by bees moistening the pollen adhering to their bodies with nectar and their glandular secretions, then compacting it into spherical pellets and transporting them to their hives in their “baskets” on their hind legs. This product can be collected by the beekeeper using a perforated device mounted in front of the hive entrance [4]. The product is usually preserved by drying or freezing. Pollen contains relatively high concentrations of nutrients essential for the body and can therefore be used as a dietary supplement [5] or a functional food raw materials [6]. According to some research, pollen has immunostimulatory and antioxidant effects, and thus plays an important role in apitherapy [3]. As the demand for apiculture products (honey, pollen, bee bread, propolis, wax) has increased, so has the number of scientific studies on honey. The number of studies on honey and pollen has increased exponentially since the 1990s [7]. From a food safety point of view, apiculture products have been the focus of research, as they may contain a number of risk factors, including pesticides, toxic metals, molds, mycotoxins, pyrrolizidine alkaloids, allergens, genetically modified organisms, and so on. The food safety risks of pollens are presented in detail in the review article of Végh et al. [8].

There is a tradition of beekeeping in Hungary, as the climatic and landscape conditions of the Carpathian Basin allow the production of high quality honey. Bees visit more than 800 plant species, several of which are suitable for the production of single flower honey [9]. The two main products of the domestic honey market are mixed flower honey and acacia honey. The latter is considered a hungaricum, as there are large acacia forests in Hungary, and acacia honey is a high quality, sought-after product both at home and abroad [10]. The production of rapeseed and sunflower honey is widespread throughout the country, but smaller amounts of other single flower honeys such as chestnut, linden, phacelia, hawthorn, goldenrod, lavender, buckwheat and milkwreod honey are also produced by Hungarian beekeepers. In addition to honey, other apiculture product also add color to the product range of beekeepers, of which one of the most popular is pollen cluster.

Examining export and import data, Mucha et al. proved that Hungary has a comparative advantage in the European Union in terms of honey production [11]. A significant part of the total honey production of the EU comes from Hungary, which, in addition to environmental conditions, is due to the relatively high bee density of the Carpathian Basin. The number of bee colonies is constantly increasing, which also indicates the effectiveness of the National Beekeeping Programs. Nevertheless, it is a serious challenge for the sector that Hungarian honey is behind world competitors in the price competition, especially compared to lower quality honey from China [11, 12]. According to the in-depth interviews of Oravecz and Kovács with consumers, Hungarian honey buyers can be divided into two distinct groups based on where they get their product: some consumers buy only from primary producers, while other look for readily available, cheaper products online or on store shelves [13]. According to the majority of the consumers surveyed, honey from Hungarian producers is not only more reliable, but also tastes better and is healthier than imported honey.

The regulation of honey quality is dealt with in the Hungarian Food Codex (Codex Alimentarius Hungaricus): specification 1-3-2001/110 contains the definitions and compositional requirements of honeys, guideline 2-100 the requirements and characteristics of honey types with a distinctive quality mark, while guideline 3-2-2009/1 the sampling and analytical methods of honey [1, 14, 15]. The Hungarian Food Codex does not cover the quality requirements of other apiculture products. There are currently no specific regulations for pollen clusters at the international level, however, product standardization was initiated by one of the working groups of the International Organization for Standardization, Technical Committee Food Products, Subcommittee Bee Products (ISO/TC34/SC19/WG 3) in 2018 [16].

The nutritional value and organoleptic properties of honeys and pollen are mainly determined by the botanical origin, but are also influenced by the geographical origin, the climatic conditions of the collection area, the bee species producing the product, as well as the processing and storage conditions [2, 3, 5, 9, 17]. In our research, domestic and foreign honeys of different plant origin were compared, based on their moisture, reducing sugar, ash, free amino acid and hydroxymethylfurfural (HMF) contents and pH. Our work also included the study of the macronutrient composition of pollen clusters from plants typical of the Hungarian
flora. The color of honey and pollen samples was also investigated, as this property plays an extremely important role in the consumer perception of foods and in consumer decisions [6, 18].

3. Materials and methods

3.1. Samples examined

The products involved in the study included eight honey from Hungary and eight honeys from abroad. The plants indicated as the nectar sources of the Hungarian honeys were acacia, linden, chestnut, goldenrod, rapeseed and phacelia, and a forest (honeydew) honey and a mixed flower honey were also included in the study. The foreign samples included products that are considered specialties in Hungary such as thyme (Spain), wild lavender (Portugal), coriander (Bulgaria), buckwheat (EU), larch (Czech Republic), coffee flower (Guatemala) and orange blossom honey (Mexico), and a mixed flower honey from Ghana. These products were purchased in a specialty store in Budapest, while the mixed flower honey from Ghana was obtained from the market in the country of origin. The pollen clusters used in the study were purchased from Hungarian beekeepers and stores. The products were dried at 38±2 °C for 20 hours, and then ten subsamples were formed by color sorting and their botanical composition was determined. Honey and pollen samples were stored at room temperature (20±2 °C) in the dark.

3.2. Methods used

An Abbe refractometer was used to determine the moisture content of the honeys [19]. Reducing sugar content was determined by the Schoorl-Regenbogen method [20], while ash content was determined by incineration [21]. The determination of the free amino acid content was carried out with an INGOS AAA 400 amino acid analyzer. HMF content was measured by the method of White [22, 23]. To determine the pH value of the honeys, a Radelkis universal pH meter (OP-204/1) was used [24]. The botanical origin of the pollen clusters was determined by microscopic pollen analysis. Moisture content of the samples was analyzed by the vacuum drying method [25]. To determine the protein content, the classical Kjeldahl method was used. Crude fat content was determined by Soxhlet extraction [25]. Ash content was determined by incineration [26], while the following formula was used to calculate the carbohydrate content:

\[
\text{Carbohydrate(\%) = 100 - Moisture(\%) - Protein(\%) - Raw fat(\%) - Ash(\%)}
\]

Color characteristics of the honeys and pollen were examined with a Minolta CR-100 instrument. The results are expressed with the coordinates of the CIE-Lab color space, where „L” is the perceptual lightness, while „a” and „b” are values for red-green and blue-yellow colors respectively. Each analysis was carried out in three parallel measurements.

4. Results and evaluation

4.1. Honey test results

4.1.1. Moisture content

Moisture content is one of the most basic parameters determining the quality of honey, which affects the viscosity, color, taste and crystallization of the product, as well as significantly affecting its shelf life. The moisture content of honeys generally varies between 15 and 21%, depending on the species of the source plant, the dehydration processes taking place in the hive and the way the honey is processed and stored [17]. Honeys produced in a dry, warm environment generally have a lower moisture content than those coming from countries with cool, humid climates [27]. According to specification 1-3-2001/110 of the Hungarian Food Codex, the moisture content of honeys must not exceed 20% [1].

The moisture content of the honey samples examined by us ranged from 17.5 to 21.8% (Figure 1). Of the honeys originating from Hungary, the moisture content of rapeseed honey and mixed flower honey, and of the foreign honeys, the moisture content of buckwheat honey exceeded the current limit value in Hungary. According to Czipa et al., a moisture content above the permissible limit indicates that the bees were not able to thicken honey properly due to heavy carrying, so these honeys should be considered immature [28]. However, the water absorption capacity of the honeys is also influenced by their botanical origin, so the high moisture content of buckwheat honey may be traced back to this.
4.1.2. Reducing sugar content

Approximately 95% of the dry matter content of honey consists of carbohydrates, of which simple reducing sugars are present in high concentrations: fructose accounts for 32-44% of the weight of honey, while glucose accounts for 23-38% [29]. The fructose and glucose present in honey are derived from the sucrose content of the nectar through the action of the enzyme invertase produced by the bees [2, 9]. According to the Hungarian Food Codex, flower honeys must have a fructose and glucose content of at least 60%, while forest (honeydew) honeys at least 45% [1]. Smaller amounts of various disaccharides, oligosaccharides and polysaccharides may also be present in the products. Lower reducing sugar and higher sucrose contents may be characteristic of the plant, but may also indicate the immaturity of the honey or the feeding of bees with sugar syrup [28, 29].

The reducing sugar content of the samples examined by us ranged from 64.50 to 75.25% (Figure 2). Foreign samples contained 3% more reducing sugars than Hungarian honeys on average. The highest value was obtained for wild lavender honey, while the lowest was obtained for forest (honeydew) honey. According to literature data, it is a special feature of honeydew honeys that they contain higher proportions of complex sugars, mainly raffinose and melezitose, than honeys made from flower nectar [30].

![Figure 1. Moisture content of the honey samples](image-url)

*Figure 1. Moisture content of the honey samples*

*Mixed flower M: mixed flower honey, Hungary; mixed flower G: mixed flower honey, Ghana*
4.1.3. Ash content

According to literature data, the ash content of honeys of nectar origin is generally between 0.02 and 0.3%, while forest (honeydew) honeys contain inorganic substances in a concentration of about 1% [29]. The amount of minerals depends on the geographical and botanical origin of the honey, the composition of the soil and the extent of contamination in the vicinity of the source plant, so honey can also be considered an environmental bioindicator [31]. According to research, the ash content of dark-colored honeys is generally higher than that of lighter honeys [17, 32]. Our results (Figure 3), in line with literature data, showed that forest honey contains an outstanding amount (0.97%) of minerals. Of honeys of nectar origin, larch, goldenrod and linden had an ash content of more than 0.3%. Acacia, rapeseed, phacelia, mixed flower from Ghana, thyme and orange blossom honeys on the other hand had relatively low levels of inorganic matter, less than 0.1%. No close correlation was observed between the color and ash content of the products. The forest, larch and goldenrod honeys with the highest ash content were dark in color, but linden and chestnut honeys, despite their high mineral content, were characterized by a light color. Buckwheat honey and the mixed flower honey from Ghana had a very dark color and a low ash content.
4.1.4. Amino acid composition

Some of the amino acid content of honeys comes from the nectar or the pollen, according to which the amino acid composition may be an indicator of botanical origin [29, 33, 34]. Nevertheless, free amino acids also enter honey as a result of bee secretion processes, which increases the variability in the amino acid content of honeys from the same source plant [35]. The amino acid composition of nectar, and thus of honey, is also affected by the time of the year it is collected by the bees: in spring, when the trees are budding, and in autumn, when the color of the leaves changes, the concentration of amino acids and nitrogen containing compounds in the phloem increases significantly [36]. The variability of the amino acid content of the same type of honey is also increased by the fact that their amount decreases during storage [37] and upon heat treatment [38].

Most amino acids are present in honey in bound form. The free amino acid content accounts for approximately one-fifth of the total amino acid content [29]. Proline makes up 50-85% of the amino acids present, the amount of which decreases continuously during storage, so it can also be an indicator of the aging of honey [39]. Some of the proline enters the honey due to secretion processes in the bees [9], while another part is of plant origin, as both nectar [40] and pollen [5] have a high proline content. There is no clear regulation of its amount in Hungary, so the minimum limit value of 180 mg/kg in force in Germany is generally taken into account [39].

The average free amino acid concentration in the honeys studied by us was 663.3 mg/kg. Foreign honeys had a slightly higher average amino acid content (787.6 mg/kg) than products from Hungary (539.0 mg/kg). The concentration of free amino acids in coriander, wild lavender and goldenrod honey exceeded 1,000 mg/kg, while in acacia honey only 162.2 mg/kg was detected (Table 1). Research has shown that acacia honeys are generally characterized by a relatively low amino acid content [33, 41]. The high amino acid content of goldenrod honey can be traced to the fact that the flowering period of the plant can last from August to the end of October.

The amount of proline was remarkably high in all samples. In addition, most honeys had relatively high levels of aspartic acid, glutamic acid, asparagine, glutamine and phenylalanine. Relatively high levels of serine, alanine, valine and tyrosine were observed in some samples. Buckwheat honey had extremely high methionine, threonine and valine contents, while wild lavender honey had outstanding amounts of phenylalanine, tyrosine and arginine.
**Table 1. Free amino acid composition of honey samples**

<table>
<thead>
<tr>
<th>Honey sample</th>
<th>Asp</th>
<th>Thr</th>
<th>Ser</th>
<th>Asn</th>
<th>Glu</th>
<th>Gln</th>
<th>Pro</th>
<th>Gly</th>
<th>Ala</th>
<th>Val</th>
<th>Met</th>
<th>Ile</th>
<th>Leu</th>
<th>Tyr</th>
<th>Phe</th>
<th>Lys</th>
<th>His</th>
<th>Arg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>9.8</td>
<td>1.0</td>
<td>1.9</td>
<td>10.0</td>
<td>4.0</td>
<td>2.6</td>
<td>114.8</td>
<td>0.4</td>
<td>1.4</td>
<td>1.6</td>
<td>0.6</td>
<td>0.9</td>
<td>0.9</td>
<td>0.4</td>
<td>1.4</td>
<td>3.4</td>
<td>1.6</td>
<td>1.3</td>
<td>162.2</td>
</tr>
<tr>
<td>Linden</td>
<td>19.0</td>
<td>0.9</td>
<td>2.4</td>
<td>10.0</td>
<td>6.8</td>
<td>4.6</td>
<td>247.2</td>
<td>0.8</td>
<td>3.0</td>
<td>0.8</td>
<td>2.9</td>
<td>2.5</td>
<td>2.3</td>
<td>1.1</td>
<td>4.0</td>
<td>9.6</td>
<td>1.4</td>
<td>6.4</td>
<td>318.6</td>
</tr>
<tr>
<td>Chestnut</td>
<td>24.8</td>
<td>6.0</td>
<td>9.5</td>
<td>11.6</td>
<td>31.5</td>
<td>10.4</td>
<td>457.7</td>
<td>2.2</td>
<td>10.5</td>
<td>7.8</td>
<td>1.7</td>
<td>2.7</td>
<td>2.5</td>
<td>2.8</td>
<td>6.5</td>
<td>3.0</td>
<td>1.1</td>
<td>1.5</td>
<td>606.6</td>
</tr>
<tr>
<td>Goldenrod</td>
<td>61.9</td>
<td>8.8</td>
<td>16.6</td>
<td>25.4</td>
<td>55.8</td>
<td>2.0</td>
<td>789.1</td>
<td>4.1</td>
<td>25.8</td>
<td>12.5</td>
<td>5.5</td>
<td>4.2</td>
<td>8.3</td>
<td>8.0</td>
<td>43.0</td>
<td>2.2</td>
<td>3.0</td>
<td>4.0</td>
<td>1083.2</td>
</tr>
<tr>
<td>Rapseed</td>
<td>19.3</td>
<td>3.2</td>
<td>4.0</td>
<td>0.0</td>
<td>9.8</td>
<td>8.8</td>
<td>253.0</td>
<td>0.7</td>
<td>3.1</td>
<td>1.2</td>
<td>0.7</td>
<td>3.0</td>
<td>2.8</td>
<td>1.8</td>
<td>4.7</td>
<td>9.9</td>
<td>1.8</td>
<td>7.4</td>
<td>336.6</td>
</tr>
<tr>
<td>Phacelia</td>
<td>14.1</td>
<td>3.6</td>
<td>4.7</td>
<td>5.9</td>
<td>10.2</td>
<td>4.2</td>
<td>395.1</td>
<td>2.4</td>
<td>3.9</td>
<td>4.3</td>
<td>0.2</td>
<td>1.9</td>
<td>2.3</td>
<td>2.7</td>
<td>7.1</td>
<td>10.5</td>
<td>2.8</td>
<td>2.3</td>
<td>483.0</td>
</tr>
<tr>
<td>Forest</td>
<td>60.6</td>
<td>8.2</td>
<td>4.9</td>
<td>4.3</td>
<td>35.8</td>
<td>2.9</td>
<td>335.9</td>
<td>1.0</td>
<td>10.7</td>
<td>2.6</td>
<td>2.1</td>
<td>1.8</td>
<td>6.2</td>
<td>0.9</td>
<td>2.4</td>
<td>0.0</td>
<td>1.3</td>
<td>0.3</td>
<td>493.9</td>
</tr>
<tr>
<td>Mixed flower, M</td>
<td>50.1</td>
<td>8.8</td>
<td>24.7</td>
<td>12.6</td>
<td>145.2</td>
<td>36.6</td>
<td>465.1</td>
<td>1.3</td>
<td>20.8</td>
<td>20.6</td>
<td>0.8</td>
<td>5.2</td>
<td>7.2</td>
<td>2.5</td>
<td>8.2</td>
<td>7.4</td>
<td>5.4</td>
<td>1.7</td>
<td>828.2</td>
</tr>
<tr>
<td>Mixed flower, G</td>
<td>16.9</td>
<td>7.7</td>
<td>3.9</td>
<td>20.6</td>
<td>13.3</td>
<td>12.6</td>
<td>173.7</td>
<td>3.4</td>
<td>9.8</td>
<td>8.4</td>
<td>7.0</td>
<td>3.2</td>
<td>14.9</td>
<td>40.1</td>
<td>59.7</td>
<td>3.7</td>
<td>2.3</td>
<td>4.4</td>
<td>407.2</td>
</tr>
<tr>
<td>Thyme</td>
<td>12.7</td>
<td>5.6</td>
<td>5.9</td>
<td>31.5</td>
<td>17.6</td>
<td>13.9</td>
<td>343.1</td>
<td>3.6</td>
<td>9.8</td>
<td>5.1</td>
<td>3.4</td>
<td>1.5</td>
<td>7.8</td>
<td>31.2</td>
<td>96.2</td>
<td>11.8</td>
<td>3.7</td>
<td>0.0</td>
<td>607.6</td>
</tr>
<tr>
<td>Wild lavender</td>
<td>19.0</td>
<td>5.8</td>
<td>8.0</td>
<td>41.2</td>
<td>46.9</td>
<td>33.3</td>
<td>681.7</td>
<td>6.0</td>
<td>11.8</td>
<td>9.4</td>
<td>6.7</td>
<td>5.8</td>
<td>18.4</td>
<td>127.4</td>
<td>227.3</td>
<td>19.1</td>
<td>4.7</td>
<td>10.9</td>
<td>1288.1</td>
</tr>
<tr>
<td>Coriander</td>
<td>39.7</td>
<td>9.9</td>
<td>24.5</td>
<td>16.8</td>
<td>96.0</td>
<td>23.8</td>
<td>943.8</td>
<td>9.3</td>
<td>29.0</td>
<td>16.6</td>
<td>6.7</td>
<td>11.1</td>
<td>14.4</td>
<td>23.2</td>
<td>49.7</td>
<td>5.4</td>
<td>2.7</td>
<td>3.0</td>
<td>1349.5</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>12.6</td>
<td>30.0</td>
<td>33.8</td>
<td>45.9</td>
<td>32.0</td>
<td>46.4</td>
<td>495.3</td>
<td>13.3</td>
<td>26.7</td>
<td>21.5</td>
<td>24.4</td>
<td>9.0</td>
<td>28.0</td>
<td>17.5</td>
<td>53.0</td>
<td>10.0</td>
<td>2.2</td>
<td>1.5</td>
<td>955.0</td>
</tr>
<tr>
<td>Larch</td>
<td>20.9</td>
<td>7.3</td>
<td>9.0</td>
<td>39.9</td>
<td>49.4</td>
<td>27.0</td>
<td>516.3</td>
<td>6.1</td>
<td>12.3</td>
<td>8.6</td>
<td>3.9</td>
<td>6.8</td>
<td>9.1</td>
<td>24.7</td>
<td>97.0</td>
<td>0.8</td>
<td>4.4</td>
<td>0.0</td>
<td>845.0</td>
</tr>
<tr>
<td>Coffee flower</td>
<td>20.8</td>
<td>15.8</td>
<td>8.8</td>
<td>19.6</td>
<td>30.1</td>
<td>22.2</td>
<td>170.9</td>
<td>4.3</td>
<td>11.2</td>
<td>14.7</td>
<td>5.8</td>
<td>3.8</td>
<td>6.9</td>
<td>14.0</td>
<td>29.0</td>
<td>1.7</td>
<td>1.8</td>
<td>2.2</td>
<td>385.3</td>
</tr>
<tr>
<td>Orange blossom</td>
<td>9.3</td>
<td>4.1</td>
<td>5.0</td>
<td>13.3</td>
<td>13.1</td>
<td>13.0</td>
<td>275.4</td>
<td>4.4</td>
<td>17.4</td>
<td>7.3</td>
<td>2.1</td>
<td>4.0</td>
<td>5.1</td>
<td>27.2</td>
<td>70.0</td>
<td>4.7</td>
<td>1.9</td>
<td>3.2</td>
<td>483.1</td>
</tr>
</tbody>
</table>

* Mixed flower honey, Hungary
** Mixed flower honey, Ghana

Figure 4 shows the ratio of proline to the amount of total free amino acids. According to Hermosín, proline accounts for at least two-thirds of the amino acid content of fresh honey [34]. Half of the honeys examined by us showed a lower proline ratio. Of domestic honeys, the proline ratio was 56% in mixed flower honey, while it was less than 66% in all foreign honeys, with the exception of coriander honey. Average proline content values were determined by Kaskonienė and Venskutonis for single-flower honeys of great economic importance in Europe, taking into account hundreds of test results per variety. Based on their results, thyme (Thymus spp.) honeys have an outstanding proline content (956±196 mg/kg), however, the thyme honey examined by us contained relatively little proline [33]. The average proline content of acacia (Robinia pseudacacia L) honeys was approximately twice the value detected by us. Linden (Tilia spp.), chestnut (Castanea sativa Miller) and forest (honeydew) honeys had on average 20-30% proline contents than the samples examined by us. Concentrations similar to those reported by the authors were obtained by us for rapeseed (Brassica napus L.) honey. Of the honey samples, coriander honey had the highest proline content (943.8 mg/kg), but this was significantly lower than the value (2,283 mg/kg) reported by Czipa [9]. The differences are presumably due to the longer storage time. With the exception of the acacia honey, the mixed flower honey from Ghana and the coffee flower honey, all products accounted for at least two-thirds of the amino acid composition.
4.1.5. Hydroxymethylfurfural content

Hydroxymethylfurfural (HMF) is formed in an acidic medium by the decomposition of hexoses. The maturity of honey can be inferred from its concentration, since this compound is present in minimal amounts in fresh honey. HMF content increases during the heating and storage of honey, but high acid, moisture and sugar contents also accelerate its formation \[9, 29\]. Its concentration also depends on the type of honey: tropical and subtropical honeys from warm environments have inherently high HMF content \[27\]. Specification 1-3-2001/110 of the Hungarian Food Codex prescribes a limit of 40 mg/kg for honeys in general, while the limit value is 80 mg/kg for honeys of tropical origin \[1\].

The HMF content of the honeys examined by us varied widely (Figure 5): its concentration was only 3.98 mg/kg in acacia honey, while the mixed flower honey from Ghana had an extremely high HMF content (140.42 mg/kg). All honeys from Hungary complied with the limit value in force. Of foreign honeys, the mixed flower honey from Ghana significantly exceeded the limit set for tropical honeys. According to its tropical origin, the coffee flower honey from Guatemala can also be characterized by a high HMF content (64.41 mg/kg).

![Figure 5. Hydroxymethylfurfural content of honey samples](image)

*Figure 5. Hydroxymethylfurfural content of honey samples*

Mixed flower M: mixed flower honey, Hungary; mixed flower G: mixed flower honey, Ghana

4.1.6. pH

The pH of honeys is usually below 6, mainly due to the organic acids found in them. The amount of organic acids is less than 0.5%, but they significantly affect the color, aroma and shelf life of the product. Certain acids (e.g., citric acid, malic acid, oxalic acid) come from nectar and honeydew, while others (e.g., formic acid) are formed by enzymatic processes during maturation and storage \[29\]. A significant proportion of the organic acids in honey is gluconic acid, which is formed from glucose by the enzyme glucose oxidase. The pH of honey does not depend directly on the amount of organic acids, which is mainly due to the honey components with buffer capacity \[9\].

The pH of the honey samples examined by us varied between 2.85±0.02 and 4.60±0.04 (Figure 6). The lowest value was obtained for phacelia honey, while the highest value was measured for forest (honeydew) honey. Our results support the finding of Tischer Seraglio et al. that the pH of honeydew honeys is relatively high, generally between 3.8 and 4.6 \[30\]. This is due to the fact that the minerals and amino acids in them buffer the acidic pH \[9\].
4.1.7. Color characteristics

The color of honey is an important organoleptic parameter, as it significantly influences consumer decisions. In most countries, high quality is associated with light honey, but in Germany, Switzerland and Greece, for example, darker products are more popular. Honey ranges in color from colorless to dark amber, sometimes with a greenish or reddish tinge. The color of honey is influenced, for example, by the plant and geographical origin, climatic conditions, soil condition of the source plant, storage time, exposure to light, possible heat treatment, certain enzymatic reactions and crystallization processes [17, 29]. This property is related to, among other things, moisture content and the concentrations of minerals, carotenoids, phenolic compounds and sugars [18].

The values of L (brightness), a* (green-red color) and b* (blue-yellow color) obtained for the honeys examined by us were plotted on a three-dimensional diagram (Figure 7). The darkest samples were the buckwheat honey and the mixed flower honey from Ghana, while the lightest were the acacia, linden and phacelia honeys. Based on the a* value, most of the honeys were more or less reddish in color, but the acacia, linden and phacelia honeys exhibited a very slight greenish hue. In several cases, an inverse relationship was observed between the lightness value and HMF content of the honeys: goldenrod honey, forest honey and the mixed flower honey from Ghana were characterized by relatively low L values and high HMF content, while the lightest honeys had low HMF content. The reason for this is that some of the HMF is formed during the Maillard reaction [9, 17].

![Figure 6. pH of the honey samples](image)

**Figure 6. pH of the honey samples**

*Mixed flower M: mixed flower honey, Hungary; mixed flower G: mixed flower honey, Ghana*

![Figure 7. L, a* and b* values of the honey samples](image)

**Figure 7. L, a* and b* values of the honey samples**

*Mixed flower M: mixed flower honey, Hungary; mixed flower G: mixed flower honey, Ghana*
4.2. Test results of pollen clusters

4.2.1. Botanical origin

The results of the microscopic pollen analysis confirmed that the pollen clusters used in the research had a lead pollen content of more than 80%, i.e. they could be considered monofloral [42]. The pollen cluster samples are shown in Figure 8, and their pollen composition is summarized in Table 2.

![Figure 8. Monofloral pollen cluster samples](image)

Table 2. Botanical composition of the pollen cluster samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lead pollen Species</th>
<th>Lead pollen %</th>
<th>Other pollens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>Brassica napus</td>
<td>96</td>
<td>Frangula, Tilia, Leucanthemum vulgare, Ambrosia artemisiifolia, Tragopogon orientalis</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Helianthus annuus</td>
<td>97</td>
<td>Taraxacum officinale</td>
</tr>
<tr>
<td>Phacelia</td>
<td>Phacelia tanacetifolia</td>
<td>100</td>
<td>No data</td>
</tr>
<tr>
<td>Cherry</td>
<td>Prunus avium</td>
<td>96</td>
<td>Salix</td>
</tr>
<tr>
<td>Blackberry</td>
<td>Rubus fruticosus</td>
<td>98</td>
<td>No data</td>
</tr>
<tr>
<td>Dandelion</td>
<td>Taraxacum officinale</td>
<td>84</td>
<td>Salix, Brassica napus, fruits</td>
</tr>
<tr>
<td>Common poppy</td>
<td>Papaver rhoesas</td>
<td>94</td>
<td>Phacelia tanacetifolia, Ligustrum, Tilia, Convolvulus arvensis</td>
</tr>
<tr>
<td>Musk thistle</td>
<td>Carduus nutans</td>
<td>94</td>
<td>Helianthus annuus, Calluna vulgaris, Impatiens, Cyanus segetum</td>
</tr>
<tr>
<td>Old man’s beard</td>
<td>Clematis vitalba</td>
<td>89</td>
<td>Plantago, Tilia, Taraxacum officinale, Trifolium repens</td>
</tr>
<tr>
<td>Rockrose (Cistus)</td>
<td>Cistus incanus</td>
<td>97</td>
<td>Calluna vulgaris, Taraxacum officinale, Thymus</td>
</tr>
</tbody>
</table>

4.2.2. Macronutrient composition

The nutritional value of the pollen clusters showed great heterogeneity, as the proportion of nutrients is significantly influenced by the botanical origin. Summarizing the results of more than one hundred scientific studies, Thakur and Nanda concluded that the products contained an average of 54.2% (18.5-84.3%) carbohydrates, 21.3% (4.5-40.7%) protein, 5.3% (0.4-13.5%) lipid and 2.9% (0.5-7.8%) ash [5]. Their moisture content in the fresh state was between 20 and 30%. Dried products, in an optimal case, contained 4-8% water, as this range is suitable from both a food safety and organoleptic point of view [42].

The pollen clusters analyzed had a moisture content between 4.9 and 8.2%, which ensures adequate microbiological stability. The carbohydrate content of our samples was on average 12% higher than the
average value reported by Thakur and Nanda [5]. The difference is mainly due to the fact that, when examining the average concentration, the authors took into account the results obtained not only for dried but also fresh pollen. The protein content of the samples ranged from 14.5 to 26.7%. The most protein-rich pollen clusters came from phacelia and rapeseed, which are strong attractants for bees [43]. In terms of crude fat content, dandelion pollen, also preferred by bees, exhibited outstanding concentrations, but rapeseed pollen was also found to be rich in lipids. The ash content of the pollen clusters ranged from 1.0 to 3.2%. The most minerals were contained in the samples from musk thistle and cherry. Our results (Table 3) are consistent with literature data [5, 42].

Table 3. Macronutrient composition of the pollen cluster samples

<table>
<thead>
<tr>
<th>Pollen sample</th>
<th>Moisture (%)</th>
<th>Carbohydrate (%)</th>
<th>Protein (%)</th>
<th>Crude fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapeseed</td>
<td>6.2±0.1</td>
<td>57.9±0.5</td>
<td>26.0±0.2</td>
<td>7.1±0.3</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>Sunflower</td>
<td>4.9±0.3</td>
<td>74.0±0.6</td>
<td>15.8±0.4</td>
<td>3.8±0.6</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>Phacelia</td>
<td>7.9±0.1</td>
<td>61.4±0.9</td>
<td>26.7±0.6</td>
<td>1.4±0.3</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Cherry</td>
<td>6.5±0.1</td>
<td>63.6±0.3</td>
<td>24.4±0.2</td>
<td>1.9±0.3</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>Blackberry</td>
<td>6.0±0.1</td>
<td>69.0±0.5</td>
<td>20.0±0.2</td>
<td>2.8±0.4</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>Dandelion</td>
<td>5.4±0.2</td>
<td>66.9±0.5</td>
<td>15.7±0.1</td>
<td>10.5±0.3</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Common poppy</td>
<td>7.3±0.1</td>
<td>63.1±0.2</td>
<td>24.2±0.1</td>
<td>2.9±0.2</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>Musk thistle</td>
<td>8.2±0.6</td>
<td>66.8±0.8</td>
<td>17.1±0.6</td>
<td>4.6±0.3</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>Old man’s beard</td>
<td>5.2±0.3</td>
<td>68.8±0.7</td>
<td>18.6±0.2</td>
<td>5.6±0.7</td>
<td>1.8±0.0</td>
</tr>
<tr>
<td>Rockrose (Cistus)</td>
<td>6.4±0.2</td>
<td>73.4±0.3</td>
<td>14.5±0.3</td>
<td>4.6±0.6</td>
<td>1.0±0.1</td>
</tr>
</tbody>
</table>

4.2.3. Color characteristics

The color of pollen clusters from different plants varies widely: they are most often yellowish and orange in color, but there are also blue, green, red, black, brown and white pollens [44]. The color of pollens is primarily determined by their botanical origin. Since bees usually collect pollen from a single plant species at a given time, each pollen cluster can be characterized by a homogeneous color [4]. The color characteristics of the product are also affected by the geographical origin, climatic conditions, the time of collection, the age and nutrient supply of the source plant, the preservation method of the pollen, as well as the duration and conditions of storage [6].

The values of L (brightness), a* (green-red hue) and b* (blue-yellow hue) obtained for the pollen clusters are shown in Figure 9. The darkest samples were musk thistle, phacelia and common poppy, the other samples had relatively high L values. The light samples can be divided into three groups based on their a* values: rapeseed, cherry and blackberry pollens had a slight greenish tinge, old man’s beard was slightly reddish, while rockrose, sunflower and dandelion exhibited a stronger reddish hue. The value of b* was positive in all cases, indicating that the yellow color dominated in the samples. Phacelia pollen, which is relatively common in the domestic market, is strikingly dark in color. This pollen is characterized by a lighter shade of yellow compared to the also dark common poppy, and a weaker shade of red compared to the musk thistle.

Figure 9. L, a* and b* values of the pollen cluster samples
5. Summary

In the course of our research, domestic and foreign honeys were compared on the basis of the parameters determining their quality, and the macronutrient composition and color characteristics of several pollen clusters from the plants characteristic of the flora of the Carpathian Basin were also determined. Of the honeys examined, the moisture content of two Hungarian and one foreign sample exceeded the limit value in force in Hungary. The reducing sugar content of the honeys ranged from 64.5 to 75.3%. Our results support the observation that honeydew honeys have a lower reducing sugar content, a higher ash content and pH, and can be characterized by a darker color than nectar-derived honeys. Proline was the dominant amino acid in the honeys, but its proportion was lower in several cases than the values reported in the literature. Of domestic honeys, the proline content of acacia honey and mixed flower honey did not reach the minimum limit of 180 mg/kg, while in the case of foreign honeys, the same was true for the coffee flower honey. In terms of the HMF content, large differences were observed. All of the domestic honeys met the requirements, but the mixed flower honey from Ghana contained an extremely high concentration of this compound. The color yellow dominated the honeys. Most of the products could be characterized by a reddish hue, but some of the honey samples had a slightly greenish tinge. In several cases, an inverse relationship was observed between the brightness value and the HMF content of the honeys.

By examining the botanical composition of the dried pollen clusters included in the study, it was confirmed that least 80% of the samples used were from the plant species named as the source plant. In line with literature data, the products contained 57.9-74.0% carbohydrates, 14.5-26.7% protein, 1.4-10.5% cruse fat and 1.0-3.2% ash. Their moisture content ranged from 4.9 to 8.2%, which meets the requirements from both an organoleptic and microbiological point of view. In terms of their color characteristics, the products exhibited great variation, but in most cases the yellow hue dominated their color.

6. Acknowledgment

Our research was realized with the help of the EFOP-3.6.3-VEKOP-16-2017-00005 project and the „OTKA” Young Researcher Excellence Program (FK_20, id. no. 135700). The authors would like to thank Rózséné dr. Etelka Büki for her help in determining the botanical origin of the pollen clusters.

7. References


