NUTRITION MARKETING OF HONEY: CHEMICAL, MICROBIOLOGICAL, ANTIOXIDANT AND ANTIMICROBIAL PROFILE

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ABSTRACT
Honey and all bee products have very good biological and chemical properties. They have been used in medicine for a long time. In our study we evaluated twenty polyfloral honey samples, the first ten were commercial honeys from a selected supermarkets with country of origin indicated “blend of EU and non-EU honeys” and represented imported honey. The second ten honeys were from a local beekeepers (Nitra region) and represented the Slovak origin. The aim of the study were to analyze chemical, microbiological, antioxidant, antimicrobial profile of honey and recommend marketing strategies for honey producers by applying nutrition marketing. From chemical point of view, the study examined mineral profile of honeys, antioxidant properties as antioxidant activity, total polyphenols, flavonoid and phenolic acid content and from microbiological view the study evaluated a total count bacteria, coliform bacteria and microscopic filamentous fungi. Results of minerals showed that the most dominant element in commercial honeys is sodium (30 mg.100g⁻¹) followed by calcium, potassium, magnesium and phosphorus. Iron, arsenic and selenium are present only in trace amounts. In local honeys the most dominant element is potassium (84.181 mg.100g⁻¹) followed by calcium, phosphorus, sulfur and magnesium. The presence of hazardous heavy metals (cadmium, lead and chrome) was not detected in either of the samples. Moreover, antioxidant activity determined by the DPPH method was slightly higher in local polyfloral honeys and vice versa the content of total polyphenol, flavonoid and the phenolic acid content was slightly higher in commercial polyfloral honeys. From the microbiological point of view, the total count of bacteria was found only in commercial polyfloral honeys while local honeys were without detectable microorganisms. The best antimicrobial activity was found against gram-negative bacteria such as Escherichia coli in both concentrations of honeys, and the local honeys reached better antimicrobial activity. All in all, honey has very good biological properties and mineral composition which opens opportunities for beekeepers to apply nutrition marketing and target new segments of consumers, e.g. sportsmen, people in convalescence, consumers following healthier lifestyle or seeking functional food. Moreover, educating consumers from a nutritional point of view will foster daily intake of honey and will increase annual consumption of honey in the future.

Keywords: nutrition marketing; honey; microorganisms; polyphenolic content; mineral profile

INTRODUCTION
Nutrition marketing can be defined as a marketing which provides nutrition and health information about certain food or beverages as well as the health claims for these products. It may involve any form of marketing communication starting with advertisement in TV, radio, press or product labelling (Colby et al., 2010). According to Gulevska and Martinovski (2018) it is a concept of creating a platform for emphasizing product qualities and components which either enables consumers to improve or strengthen their health. Besides well-known marketing mix (product, price, place and promotion), the nutritive marketing recognises 5N principles (nutritive qualities, nutritive quality, nutritive benefits, nutritive strategy and nutritive integration). Furthermore, this type of marketing could attract consumers’ attention and influence their perception and preferences for certain product, its healthiness and nutritional qualities by applying health claims and nutrition facts (Dunay et al., 2015; Royo-Bordonada et al., 2016; Zou, Li, and Liu, 2018; Pulker, Scott and Pollard, 2018). Health claims are defined as statements which indicate a certain relationship of health related issues and substance in the product and include phrases like reducing the risk of heart attacks (Schaefer, Hooker and Stanton, 2016; Grunert, 2017). In general, nutrition marketing provides consumers the necessary information to make healthy food choices in the purchasing process (Schermer, 2013) and it opens new opportunities towards functional consumers and segment of health and well-being of consumers (Paluchová, 2017). Consumers usually perceive the environment in shops by all their senses and prefer to buy products with high quality (Berčík et al., 2016; Nagyová et
Scientific hypothesis
The scientific hypothesis of this study was to examine the differences between commercial honeys and honeys from local beekeepers in chemical composition, polyphenolic profile, microbiological spoilage of microorganisms and antibacterial activity.

MATERIAL AND METHODOLOGY

Samples
For microbiological properties, antimicrobial activity and chemical properties twenty honey samples were tested. First ten samples were commercial polyfloral honey purchased from selected supermarkets with country of origin marked as “blend of EU and non-EU honeys” and represent imported honey. The second ten samples were polyfloral honey directly from the local beekeepers (Nitra region) and represent the Slovak origin.

Sample preparation for antioxidant activity
An amount of 0.5 g of sample was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min., the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction was carried out in triplicate.

Radical scavenging activity – DPPH method
The radical scavenging activity of the extract was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchez-Moreno et al., 1998). The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (25 – 300 mg·L⁻¹; R² = 0.989) was used as the standard and the results were expressed in mg·g⁻¹ caffeic acid equivalents.

Total polyphenol content
The total polyphenol content extract was measured by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 mg·L⁻¹; R² = 0.998) was used as the standard and the results were expressed in mg·g⁻¹ gallic acid equivalents.

Total flavonoid content
The total flavonoids were determined using the modified method of Willett (2002). 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5 – 20 mg·L⁻¹; R² = 0.989) was used as the standard and the results were expressed in mg·g⁻¹ quercetin equivalents.

Total phenolic acid content
Total phenolic acid content was determined using method of Farmakopea Polska, (1999). A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% NaNO₂ +10% Na₂MoO₄), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg·L⁻¹; R² = 0.999) was used as a standard and the results were expressed in mg·g⁻¹ caffeic acid equivalents.

Mineral compounds
The samples of honey were subjected to mineralization under high pressure, in HNO₃ 65%, super pure. 0.2 gram samples were weighed and placed in Teflon vessels which were then filled with 8 mL of nitric acid and sealed tightly. For each group of nine samples, during the microwave dissolution process, the rotor of the digestion system was additionally filled with a blank sample comprising 8 mL of nitric acid alone. The samples were digested for one hour, with the applied algorithm of temperature increase as specified for biological samples, without exceeding 200 °C. This was carried out using Ethos One microwave digestion system from Milestone. The vessels were opened after the mineralization process had been completed and the samples with acid had been brought to room temperature. The samples were cooled down to room temperature and supplemented with water to the volume of 50 mL. The obtained detection threshold for each element was not lower than 0.01 mg·kg⁻¹ (with the assumed detection capacity of the measuring apparatus at a level exceeding 1 ppb). The measurements were performed by ICP-OES spectrometer, Thermo iCAP Dual 6500 with horizontal plasma, and the capacity of detection along and across the plasma flame (Radial and Axial). Before measuring each batch of 2 samples the method was calibrated with the use of certified Merck models, with concentrations of 10000 ppm for Ca, Fe, K, Mg, P and 1000 ppm for Al, Ba, Cd, Cu, Na, Pb.
measurement result for each element was compensated to account for the measurement of elements in the blank sample. In each case a 3-point calibration curve was used for each element, with optics correction applying the method of internal models, in the form of yttrium and ytterbium ions, at the concentrations of 2 mg.L⁻¹ and 5 mg.L⁻¹, respectively. The analytic methods were validated with two independent tests.

**Microbiology of honey**

**Determination of cfu counts**

The plate diluting method was applied for quantitative cfu count determination of the respective groups of microorganisms in 1 g of honey. Into Petri dishes, 1 mL of honey dilution was inoculated and poured by gelatinous nutritive substrate (Table 1).

**Dilution of the samples**

Basic dilution (10⁻¹) was prepared as follows: 5 g of honey content was added to the test tube containing 45 mL of distilled water.

**Antimicrobial activity of honey**

Honey solutions were prepared in two fractions: 50 and 25% (by mass per volume). The samples of each honey (10 g) and sterile water were stored at 37 °C for 30 min. before mixing, to facilitate homogenization. The 50% (by mass per volume) solutions thus prepared were diluted to 25%. The samples were assayed immediately after dilution. Six strains of microorganisms were tested in this study, including three Gram-negative bacteria (*Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subs. *enterica* CCM 3807), three Gram-positive bacteria (*Bacillus cereus* CCM 2010, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subs. *aureus* CCM 4223). All tested strains were collected from the Czech Collection of microorganisms (Brno, Czech Republic). The bacterial suspensions were cultured in the nutrient broth (Oxoid, Basingstoke, United Kingdom) at 37 °C. The antimicrobial effect of the natural honey was tested using the agar well diffusion method. Overnight microbial cultures were used for surface inoculation of Petri dishes containing 15 mL of Muller Hinton agar (MHA). Each Petri dish was spread on with 100 µL of strain inoculum streaked thoroughly all over the surface of the MHA. Subsequently, four equidistant wells 6 mm in diameter each were punched into the inoculated medium with sterile glass Pasteur pipettes and were filled up with 250 µL of honey using a precise eppendorph.

All plates were incubated at 37 °C and inhibition zones were measured after 24 hours. Six different strains of bacteria species were tested in sets of plates, which were simultaneously processed for each strain. All the experiments were repeated triplicate, including control with plain 40% phenol every time. After incubation the zones of inhibition of the growth of the bacteria around the disks were measured. The mean values of the three trials were calculated.

**Statistical analysis**

All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. In addition, t-test for Equality of Means was applied in SPSS Statistics 25 to examine differences among honey samples.

**RESULTS AND DISCUSSION**

**Antioxidant activity**

Antioxidant activity determined by the DPPH method was slightly higher in polyfloral honeys from local beekeepers in comparison to commercial honeys (Table 2). In antioxidant activity, statistically significant differences were found between commercial honey samples and local honey samples from beekeepers (p ≤0.001). Generally, bee honey is rich in compounds with antioxidant activity. According to Khalil et al. (2010) antioxidant properties have peroxides, glucose, vitamin C, glucose oxidase enzymes, phenol compounds and catalase. Moreover, carotenoids and flavonoids are present in honey as well. Antioxidants in honey are ensured by the high number of these indicators which depends on locality, type of honey and agro ecological condition. Wilczyńska (2010) determined antioxidant activity of different Polish honey and found the best activity by the DPPH method in heather (100%) and honeydew honey (83.51%), which was higher compared to polyfloral honey (42.53%). Mellen et al. (2015) found the best activity evaluated by the ABTS method in buckwheat honey from Poland (4.63 mmol TEAC.kg⁻¹) and also in frost honey from Serbia (2.71 mmol TEAC.kg⁻¹). These authors also measured polyfloral honey from Slovakia (1.47 mmol TEAC.kg⁻¹) which had higher activity compared to polyfloral honey from Poland (1.11 mmol TEAC.kg⁻¹). Their results confirmed that the antioxidant activity of honey is very strongly influenced by origin and locality.

In addition, Juszczak et al. (2015) determined significantly higher antioxidant activity in honey enriched with propolis, bee pollen and royal jelly with compared to pure polyfloral honey as well as Dzugan et al. (2017) tested honey enriched with dried herbs and determined significantly higher antioxidant activity in this honey compared to pure polyfloral honey. Nowadays, the attractivity of enriched honey is increasing among consumers and these facts can be an effective tool for

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**Table 1** Applied nutritive substrates.

<table>
<thead>
<tr>
<th>Microbial groups</th>
<th>Medium</th>
<th>Length of incubations (h)</th>
<th>Cultivation method</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total count of bacteria (TCB)</td>
<td>Plate count agar</td>
<td>48</td>
<td>aerobic</td>
<td>30</td>
</tr>
<tr>
<td>Coliform bacteria (CB)</td>
<td>Violet red bile agar</td>
<td>24</td>
<td>aerobic</td>
<td>37</td>
</tr>
<tr>
<td>Moulds (M)</td>
<td>Malt extract agar</td>
<td>120</td>
<td>aerobic</td>
<td>25</td>
</tr>
</tbody>
</table>
nutrition marketing to strengthen the position of these new products.

**Total polyphenol, flavonoid and phenol acid content**

The content of total polyphenol, flavonoid and phenolic acid content was slightly higher in commercial polyfloral honey in comparison to local polyfloral honey (Table 2). Statistically significant differences of total polyphenol, flavonoid and acid content were found between commercial honeys and local honeys of beekeepers ($p \leq 0.001$). *Sime et al. (2015)* tested several polyfloral honeys from Ethiopia and reported that honey is rich in biologically active compounds especially from phenolic groups. The sample had from 3.3 to 6.1 mg GAE·g$^{-1}$ of total polyphenol content. The results of total polyphenol content in the current study are comparable with results of *Al-Mamary et al. (2002)* which tested different types of honey and amount of total polyphenols ranged from 0.56 to 2.46 mg CE·g$^{-1}$ (catechin equivalent). Total polyphenol content was higher than flavonoid content. The similar results were reported *Sime et al. (2015)* where study determined the total flavonoid content in honey from Ethiopia was from 0.18 to 0.42 mg CE·g$^{-1}$ (catechin equivalent). These authors also reported that amount of flavonoids is attributable to the differences in the type of honey samples, floral origin and season of collection. Darker honeys use to have higher flavonoids in comparison to lighter ones. In general, honey contains approximately 0.02 mg·g$^{-1}$ of the flavonoid concentration (*Ayoub et al., 2009*). This value is in agreement with current samples. In *Sime et al. (2009)* tested several polyfloral honeys and local honeys of beekeepers. These authors compared the content of total flavonoid, phenolic acid and flavone in honeys from Argentina. The similar results were reported in honeys from different regions in Rio de Janeiro. In general, honey contains approximately 0.02 mg·g$^{-1}$ of the flavonoid concentration (*Ayoub et al., 2009*). This value is in agreement with current samples. In *Sime et al. (2009)* tested several polyfloral honeys and local honeys of beekeepers. These authors compared the content of total flavonoid, phenolic acid and flavone in honeys from Argentina. The similar results were reported in honeys from different regions in Rio de Janeiro.
while copper, zinc and selenium was present in trace amounts (Ribeiro et al., 2014).

Mineral composition in honey could be used for identification of its geographical and botanical origin. Other trace elements (Pb, Cd, Hg, Cu, Mn, Zn, Ag), belonging to the heavy metals, play important roles as bio-indicators for environmental pollution (Solayman et al., 2015). In our study was not detected presence of hazardous heavy metals. Statistically significant differences between commercial and local honeys were found in case of Ca ($p \leq 0.001$), K ($p \leq 0.001$), Na ($p \leq 0.001$), Mg ($p \leq 0.001$) and P ($p \leq 0.001$).

### Microbiology of honey

The number of microorganisms found in honey samples is shown in Table 4. Polyfloral honey from local beekeepers was without any detectable microorganisms. In commercial honey were found only total count of bacteria ($1.50 \pm 0.07$ log cfu.g$^{-1}$). Total viable count of aerobic bacteria did not exceed $2.00 \log$ cfu.g$^{-1}$ in any sample. Kňazovická et al. (2011) reported a mean value of $1.4 \times 10^2$ cfu.g$^{-1}$ of the bacteria. Several authors indicate, that total aerobic viable count in honey range between zero and tens of thousands per gram (Kačániová et al., 2009).

A good secondary manufacturing practise controls secondary sources of contamination. A usual microbiological examination may include several different assays. General information is provided by a standard plate count. Furthermore, the useful information is provided by count of yeasts and an assay for bacterial spore-formers. Bacteria are not able to replicate in honey, thus the high amount of vegetative bacteria may be the result of secondary contamination. Nevertheless, some vegetative microbes are able to survive at cool temperatures in honey for several years. Due to antimicrobial properties, the persistence and growth of many microorganisms are being discouraged. In general, the low number of microbes and its limited variation is expected in honey (Kačániová et al., 2007).

#### Antimicrobial activity

Honey has been found to possess antimicrobial activity which has been attributed to specific chemicals in the honey (Kačániová et al., 2008). The antibacterial activity of honey is shown in Table 5. The best antibacterial activity of commercial polyfloral honey was found against Gram negative bacteria Escherichia coli > Klebsiella pneumoniae > Salmonella enterica. The lower antimicrobial activity of commercial honey was found against Gram positive bacteria. Statistically significant differences were found between commercial honeys and local honeys from beekeepers in case of all microorganisms ($p \leq 0.001$, $p \leq 0.004$ and $p \leq 0.006$).

Polyfloral honeys from local beekeepers have the best antimicrobial activity similar to commercial honeys against E. coli. The bactericidal activity of the honeys on Pseudomonas aeruginosa, Salmonella typhi and E. coli was found to be between 50 and 100% concentration of honey sample from Dembia, Debark and Gondar Zuria (Ahmed et al., 2014).

The honeys showed bactericidal activities against the tested organisms up to the dilutions of 50%. This is similar to those reported by Nzeako and Handi (2000) who studied on six commercial honeys found inhibition in an agar diffusion of S. aureus, E. coli and P. aeruginosa. The well documented antibacterial properties of honey are mainly caused by the hydrogen peroxide which is a potent antimicrobial agent mostly produced during oxidation of glucose catalysed by bee enzymes added by the bees during nectar harvest. The rate of hydrogen peroxide production, glucose oxidase and its destruction by catalases determine its concentration in honey. The amount of hydrogen peroxide differs from honey to honey. A antimicrobial activity similar to those reported by Kňazovická et al. (2011) was detected in honey.

### CONCLUSION

The study evaluated chemical, biological, microbiological and antibacterial properties of twenty different polyfloral honey samples. The first ten were commercial honeys from a selected supermarket with country of origin indicated as "blend of EU and non-EU honeys" while second ten were honeys from local beekeepers (Nitra region) representing the domestic origin. Results showed that all samples have antioxidant activity, antimicrobial activity and possess polyphenol, flavonoid phenolic acid and mineral substances. Local honeys obtained better results in

### Table 4 Indicence of microorganisms in analyzed samples (in log cfu.g$^{-1}$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Commercial honeys</th>
<th>Local honeys from beekeepers</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCB</td>
<td>1.50 ±0.07</td>
<td>nd</td>
</tr>
<tr>
<td>CB</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>M</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Note: mean ($n=10$) ±standard deviation, nd – not detected.

### Table 5 Antimicrobial activity of analyzed samples (mm).

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Commercial honeys 50%</th>
<th>Commercial honeys 25%</th>
<th>Local honeys from beekeepers 50%</th>
<th>Local honeys from beekeepers 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>7.45 ±0.49</td>
<td>4.28 ±0.26</td>
<td>8.33 ±0.26</td>
<td>5.17 ±0.37</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>7.00 ±0.41</td>
<td>3.69 ±0.11</td>
<td>8.12 ±0.34</td>
<td>4.33 ±0.26</td>
</tr>
<tr>
<td>S. enterica</td>
<td>5.81 ±0.55</td>
<td>3.35 ±0.15</td>
<td>7.77 ±0.35</td>
<td>4.74 ±0.25</td>
</tr>
<tr>
<td>B. cereus</td>
<td>4.71 ±0.29</td>
<td>3.19 ±0.48</td>
<td>7.150 ±0.40</td>
<td>3.83 ±0.44</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>4.86 ±0.24</td>
<td>2.86 ±0.31</td>
<td>6.41 ±0.22</td>
<td>3.56 ±0.38</td>
</tr>
<tr>
<td>S. aureus</td>
<td>4.93 ±0.32</td>
<td>2.50 ±0.39</td>
<td>5.69 ±0.39</td>
<td>3.00 ±0.26</td>
</tr>
</tbody>
</table>

Note: mean ($n=10$) ±standard deviation.
antioxidant activity, antimicrobial activity and possess no detectable microorganisms. Commercial honeys have slightly higher content of total polyphenol, flavonoid, phenolic acid and few microorganisms. Regarding the mineral composition, the differences were in sodium and potassium content comparing of commercial and local honey. Commercial honeys contained sodium (30 mg.100g⁻¹) following by calcium, potassium, magnesium and phosphorus, iron and selenium. Local honeys contained higher amount of potassium (84.181 mg.100g⁻¹) followed by calcium, phosphorus, magnesium, sulfur, sodium and selenium. Heavy metals (cadmium, lead and chrome) were not detected in either of the samples.

In conclusion, honey has very good biological properties and mineral composition which offer many opportunities for applying nutrition marketing. Beekeepers should educate their consumers not only about positive healing effects of honey, but also include nutritional point of view. By emphasizing the benefits of consuming honey on a daily basis will increase consumers’ annual consumption in the future. Furthermore, there is a space for targeting new segments such as sportsmen, people in convalescence or consumers following the healthy lifestyle.

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