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RESEARCH ARTICLE

# Physicochemical and bioactive characterization of beekeeper and market honeys

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## ABSTRACT

Honey is a popular sweetener that makes significant contributions to human nutrition. The aim of this study was to analyze and compare specific properties relating to honey quality and biological value such as total phenolic content, color, color intensity, proline and other quality parameters like moisture content, electrical conductivity, 5-hydroxymethylfurfural, diastase activity in various honeys directly from beekeepers and from markets. In total fiftysix honey samples directly from Czech and Slovak beekeepers (n = 25) and from markets in the Czech Republic (n = 31) were collected during 2018. The determined parameters varied depending on the botanical and geographical origin of honeys. The identified most significant differences ($p < 0.01$) between the analyzed beekeepers and market honeys in color, color intensity, total phenolic content, moisture content and 5-hydroxymethylfurfural point to the different properties and impact of honey processing on honey composition. In several parameters, like color, color intensity, total phenolic content, proline content and electrical conductivity a very good correlations with botanical origin of honey were observed and beside the melissopalynological analysis these parameters participated on characterization and authentication of unifloral honeys. Normally, there is a positive correlation between color and electrical conductivity, however we have also confirmed that the color of honey also depends on the proline content. The results of this study confirmed that the origin (beekeeper/market) and botanical source of honeys (unifloral/nectar/honeydew) had effect on their quality and biological value.

**Keywords:** hydroxymethylfurfural; proline; color intensity; phenolic content; correlation

## INTRODUCTION

Honey is a popular sweetener that makes significant contributions to human nutrition for its composition. The honey as natural bee product is variable in chemical composition mainly depends on the floral source and geographical origin. Honey predominantly composes from saccharides (65%-70%), water (14%-20%), and wide range of substances, like amino acids, enzymes, proteins, vitamins, minerals, Maillard reaction products, volatile compounds, pigments, and phenolic compounds (Boukraâ, 2016). Honey is one of the few foods which requires littlest number of technological steps before entering the market. On the other hand, commercial honey production, compared to honey directly from beekeepers, can involve several additional steps like dehumidification, liquefaction, mixture, and heating before final packaging steps. Finally, the composition of processed honey could be negatively affected (Baglio, 2018). The quality of produced honey in European Union is regulated by Council Directive of the EU 110/2001 (2014) related to honey, which specifies composition criteria for sugar content, sucrose content, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, and hydroxymethylfurfural (HMF) content in honey.

The moisture content of honeys, shortly after extraction, is a relatively stable parameter, which normally ranges from 15 % to 21 %, affects the stability of the honey, and the low content prevents the honey fermentation (da Silva et al., 2016). Amount of HMF and diastase activity serve as the indicators of honey freshness and heat treatment (Baglio, 2018). For its composition, honey is a favorable condition for the Maillard reaction products formation (Boukraâ, 2016). Fresh honeys, shortly after extraction, have low HMF concentration and relatively high diastase activity. Due to

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honey manufacturing, mainly heating and long-term storage, concentration of HMF is increasing, and diastase activity decreases (Baglio, 2018). Except enzymes, from nitrogenous substances main part represent free amino acids. Among them, proline is predominant amino acid and creates 50 %-85 % of the amino acid fraction (Boukraâ, 2016). Although the concentration of proline is not a parameter of honey stipulated by law, a concentration lower than 180 mg/kg may indicate an immaturity of honey or an adulteration of honey with sugar syrups (Bogdanov et al., 2009).

The chemical composition determines the physicochemical parameters, such as color, electrical conductivity, pH, and water activity. Electrical conductivity of honey mainly depends on the honey mineral content, which ranges from 0.04 % to 0.2 % (Boukraâ, 2016). Honeydew honeys have higher electrical conductivity due to higher mineral content, in comparison with blossom honeys (Ecem Bayram et al., 2020). Therefore, electrical conductivity is main determination parameter between honeydew and blossom honeys, according to the Council Directive of the EU 110/2001 (2014). Honey color and color intensity (Abs450) reflects the content of different pigments such as carotenoids, minerals, pollen, Maillard products, phenolic acids, flavonoids (Beretta et al., 2005; Moniruzzaman et al., 2013) and contaminating pigments arising from handling, processing, storage and from biochemical reactions during honey maturation (Beretta et al., 2005). Honey color and color intensity participate on typical properties of unifloral honeys (Karabagias et al., 2016). Bertoncelj et al. (2007) and Pontis et al. (2014) demonstrated positive correlation between honeys phenolic content and their color.

Honey´s phenolic compounds are secondary plant metabolites represented by flavonoids and phenolic acids (Ankalm, 1998), originating from flower nectar, propolis and pollen (Gašić et al., 2017). Honey contains approximately 0.1 % to 0.5 % of phenolic compounds (Boukraâ, 2016). Phenolic acids are derivates of cinnamic and benzoic acids. Besides the hydroxy derivates of benzoic acid, honey contains mainly p-hydroxybenzoic, protocatechuic, vanillic, gallic and syringic acids, and besides the hydroxy derivates of cinnamic acid, honey contains p-coumaric, caffeic, ferulic, and sinapic acids (Gašić et al., 2017). Several studies have been conducted to determine phenolic compounds and total phenolic content in honeys with different botanical and geographical origin (Meda et al., 2005; Silici et al., 2010; Pontis et al., 2014; Ecem Bayram et al., 2020). Phenolic compounds are considered to be one of the most important nutrition substances, responsible for its antioxidant, antimicrobial, antiviral, anticancer, anti-inflammatory, and anti-atherogenic properties. Due to the correlations between botanical origin and phenolic content of honey, phenolic acids and flavonoids can serve as important markers of botanical origin (Boukraâ, 2016; Gašić et al., 2017).

In recent years, some studies have been published which analyze the quality of honeys collected from markets (Bhuvaneswari et al., 2014; Makarewicz et al., 2017; Aljohar et al., 2018; Mondragón-Cortez et al., 2019) and which compare the quality of honeys from markets with honeys directly from beekeepers (Bušová and Kouřimská, 2018; Hoxha et al., 2019; Aypak et al., 2019). Published results of market honeys most often point to the detected higher or above-limit HMF content and low diastase activity (Makarewicz et al., 2017; Aypak et al., 2019; Hoxha et al., 2019; Mondragón-Cortez et al., 2019) or above-limit water content (Hoxha et al., 2019). Aypak et al. (2019) determined statistically most significant difference ($p < 0.01$) between HMF content and diastase activity between beekeeper and market honeys from Turkey. In contrast, Bušová and Kouřimská (2018) detected statistically significant difference ($p < 0.05$) only in titratable acidity between beekeeper and market honeys from Czech Republic.

Due to a many benefits of honey consumption for human health, it is very important to evaluate physical and chemical properties of honey. The benefits of honey are conditioned by the presence and concentration of wide range of substances. Some of these substances may be affected by the honey processing. However, for the consumers is important that honeys reach same quality regardless of the origin of the honey (beekeeper or market). There is not enough published data that comprehensively evaluates the properties of honeys directly from beekeepers compared to honeys from markets. Based on the above, the main objectives were to (1) analyze specific physicochemical and bioactive parameters relating to honey quality and biological value in different Czech and Slovak honeys directly from beekeepers and honeys collected from markets, and (2) to compare the quality of honey between beekeeper honeys and market honeys. Correlations between analyzed parameters were also evaluated.

## MATERIALS AND METHODS

### Honey samples from beekeepers

Twenty-five honey samples (5 black locust, *Robinia pseudoacacia* L.; 5 lime, *Tilia* spp.; 2 rape, *Brassica* spp.; 8 multifloral and 5 honeydew honeys) were obtained directly from beekeepers from various regions in the Czech Republic and Slovak Republic in 2018. The samples were stored in the dark at room temperature (21 ± 2 °C) in original packaging until the analysis. Unifloral honey samples were sensory and melissopalynologically analyzed

to confirm their botanical origin using the method of the International Commission of Bee Botany (Louveaux et al., 1978; Von Der Ohe et al., 2004). Pollen grains were counted and identified under the light microscope (Nikon Eclipse E200, Japan).

### Honey samples from markets

Thirty-one honeys of different botanical and geographical origin were purchased from Czech markets in 2018. The samples were classified by the region of their origin into multifloral honeys from the Czech Republic (n=10), blend of the (European Union) EU and the non-EU nectar honeys (n=12), blend of the EU and the non-EU honeydew honeys (n=3), blend of the EU nectar honeys (n=2), blend of the EU honeydew honeys (n=1), blend of the non-EU nectar honeys (n=2) and blend of the non-EU honeydew honeys (n=1). The samples were stored in the dark at room temperature (21 ± 2 °C) in their original packaging until the analysis.

### Legislative composition criteria and proline content

All standards and chemicals used in this study were of analytical grade. Diastase activity, 5-hydroxymethylfurfural content, moisture content, electrical conductivity, and proline content were determined according to Bogdanov et al. (2009):

Diastase activity in honey samples was determined based on the Phadebas method using Specord 200 Plus spectrophotometer (Analytic Jena AG, Germany). The diastase activity was expressed as the diastase number (DN) in Schade units.

5-hydroxymethylfurfural (HMF) content in honey samples was determined using the HPLC-UV method. The analysis was performed using an HPLC system (Alliance 2695, PDA detector 2996, Waters, Milford, Massachusetts, USA), a column (Zorbax Eclipse XDB-C18-5 μm, Agilent, Santa Clara, California, USA), with water-methanol (90:10) mobile phase. The analysis conditions were as follows: isocratic elution, flow rate 1 mL/min, sample injection 20 μL and column temperature 25 °C. HMF was detected and quantified in the UV at 285 nm using external standard and expressed in mg/kg of honey.

Moisture content in honey samples were determined using the refractometric method using the Abbé refractometer (AR 4, A.Krüss Optronic GmbH, Hamburg, Germany). Moisture content was expressed in %.

Electrical conductivity was determined using the conductometric method on an inoLab Cond 730 conductometer (WTW, Weilheim, Germany) and expressed in milli Siemens per meter (mS/m).

The content of proline in honey samples was determined using spectrophotometric method based on color reaction of ninhydrin with proline using Specord 200 Plus spectrophotometer (Analytic Jena AG, Germany). Proline content of honey was determined comparing with a proline standard and expressed in mg per kg.

### Color intensity

Honey color intensity was analyzed according to the method described by Beretta et al. (2005). The absorbance was measured at two different wavelengths (450 nm and 720 nm) using Specord 200 Plus spectrophotometer (Analytic Jena AG, Germany) and the difference in absorbance was expressed as mAU.

### Color

The color of the honey samples was measured according to the Ferreira et al. (2009). The absorbance of honey solution was measured at 635 nm using Specord 200 Plus spectrophotometer (Analytic Jena AG, Germany) and calculated honey color was expressed in millimetre (mm) Pfund. The analyzed honey samples were categorized using the United States Department of Agriculture (USDA) approved color standards (1985).

### Total phenolic content

The total phenolic content of all honey samples was determined by the Folin-Ciocalteu method described by Silici et al. (2010). The absorbance of reaction mixture was measured at 765 nm using a Specord 200 Plus spectrophotometer (Analytic Jena AG, Germany). Standard calibration solutions were diluted from a gallic acid (Penta, Czech Republic) stock solution at a concentration range of 0–900 mg/10 mL ($R^2 = 0.9990$). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of honey.

### Statistical analysis

All assays were performed in duplicate and the results were expressed as a mean value ± standard deviation (SD). The statistical analysis of the results was performed with the Unistat (6.0) software and the Microsoft Excel 2016. The significant differences were obtained by a Shapiro-Wilk Test followed by Tukey's Honestly Significant Difference (HSD) Test. The differences at a 95 % ($p < 0.05$) confidence level were considered statistically significant. The correlations were calculated using Pearson's correlation coefficient ($r$) in bivariate linear correlations ($p < 0.05$).

## RESULTS AND DISCUSSION

### Legislative composition criteria

#### *Diastase activity*

Diastase (*alpha*-amylase) activity and 5-hydroxymethylfurfural (HMF) content are used as the indicators of honey freshness

and heat treatment. These analytes are the main parameters distinguishing honeys directly from beekeepers and honeys from markets. Diastase activity in fresh unheated honeys is highly variable parameter, which depends on several pre-extraction (floral and geographical origin) and post-extraction factors (storage conditions). Da Silva et al. (2016) indicates in different honeys diastase activities ranging from 6.05 DN to 45.8 DN. The measured diastase activity values are summarized in Table 1 and Table 2.

Diastase activities of all tested honey samples from Czech and Slovak beekeepers fulfilled the limit (not less than 8 Schade scale) according to the Council Directive of the EU 110/2001 (2014). Among the beekeeper honeys the black locust honeys had the lowest diastase activities (DN), ranging from 8.0 ± 0.3 to 13.6 ± 0.4 with a mean activity (10.9 ± 2.0). The black locust honeys were followed by the rape honeys, with a mean activity (13.6 ± 3.1 DN) and the lime honeys (18.7 ± 4.0 DN). Diastase activities showed a statistically most significant difference ($p < 0.01$) between nectar and honeydew honeys. The measured diastase activities of the black locust and the lime honeys agreed with the finding for European black locust (10.5 ± 5.0 DN) and lime (16.8 ± 3.4 DN) honeys presented by Persano Oddo and Piro (2004). Pospiech et al. (2021) detected very similar diastase activity (DN) in lime honeys directly from Czech beekeepers (18.6 ± 7.2), and higher diastase activities for rape (16 ± 1.8) and black locust honeys (24.4 ± 1.5). Comparable results of the diastase activity were investigated by Tomczyk et al. (2019), which reported for Slovak black locust and rape honeys diastase activities 14.15 ± 5.11 and 12.38 ± 1.93, respectively.

The diastase activity of the Czech beekeeper nectar honeys (14.6 ± 3.3 DN) was higher as compared to the analyzed Czech nectar honeys from markets (9.8 ± 3.6 DN) and the most significant difference ($p < 0.01$) between nectar honeys from the Czech beekeepers and the Czech nectar honeys from markets was observed.

Among the analyzed market honeys diastase activity lower than limit was detected in 6 honey samples (19.4 %), specifically in two Czech honeys, two blends of the EU and the non-EU honeys, one blend of the nonEU honeys and finally one blend of the EU honeys. The average diastase activity of market nectar honeys (11.4 ± 4.0 DN) was lower than average diastase activity of beekeeper nectar honeys (14.5 ± 3.6 DN). In comparison, market honeydew honeys had higher diastase activity (19.2 ± 4.0 DN) than beekeeper honeydew honeys (14.8 ± 5.7 DN). Non-significant difference ($p > 0.05$) between the diastase activities of beekeeper and market honeys was observed. In contrast, Aypak et al. (2019) determined statistically most significant difference ($p < 0.01$) in diastase activity between

**Table 1: Physicochemical and bioactive parameters of the analyzed Czech and Slovak beekeeper honeys**

| | Black locust | | Rape | | Lime | | Multifloral | | Honeydew | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CZ | SK | CZ | SK | CZ | SK | CZ | SK | CZ | SK |
| | (*n* = 5) | | (*n* = 2) | | (*n* = 5) | | (*n* = 9) | | (*n* = 5) | |
| Moisture (%) | 16.5 ± 0.7 | 16.4 ± 1.3 | 17.3 ± 0.10 | 17.3 ± 0.1 | 16.4 ± 0.5 | 16.3 ± 0.1 | 15.8 ± 0.7 | 16.3 ± 0.1 | 15.4 ± 1.0 | 17.7 ± 0.1 |
| EC (mS/m) | 13 ± 1 | 18 ± 2 | 16 ± 0 | 19 ± 0 | 68 ± 23 | 52 ± 6 | 44 ± 21 | 40 ± 22 | 94 ± 49 | 105 ± 24 |
| HMF (mg/kg) | 8.1 ± 5.8 | 4.7 ± 0.8 | 4.1 ± 0.2 | 6.8 ± 0.4 | 2.9 ± 2.4 | 4.3 ± 1.6 | 7.3 ± 6.4 | 2.0 ± 2.0 | 7.2 ± 2.3 | 20.2 ± 2.1 |
| Diastase (DN) | 12.6 ± 1.4 | 9.8 ± 1.6 | 15.8 ± 0.3 | 11.5 ± 0.6 | 19.5 ± 5.2 | 17.6 ± 2.5 | 14.0 ± 3.4 | 16.9 ± 2.9 | 14.6 ± 6.9 | 15.0 ± 0.3 |
| Proline (mg/kg) | 224 ± 25 | 181 ± 27 | 212 ± 2 | 228 ± 20 | 436 ± 131 | 330 ± 79 | 401 ± 161 | 309 ± 84 | 443 ± 176 | 493 ± 192 |
| Color intensity (mAU) | 25 ± 10 | 27 ± 2 | 40 ± 1 | 55 ± 1 | 103 ± 44 | 79 ± 12 | 153 ± 65 | 105 ± 48 | 252 ± 128 | 697 ± 602 |
| Color (mm Pfund) | 14.2 ± 8.1 | 16.1 ± 4.5 | 27.0 ± 1.6 | 23.3 ± 0.5 | 67.4 ± 24.6 | 58.4 ± 5.0 | 77.1 ± 11.5 | 51.5 ± 16.7 | 100.4 ± 38.7 | 195.8 ± 124.2 |
| TPC (mg GAE/100 g) | 20.3 ± 1.8 | 23.1 ± 8.7 | 32.7 ± 0.8 | 29.9 ± 0.9 | 45.6 ± 10.2 | 41.8 ± 3.5 | 51.2 ± 7.0 | 39.3 ± 11.6 | 63.5 ± 17.5 | 73.8 ± 41.3 |

CZ – Czech Republic, SK – Slovak Republic, EC – electrical conductivity, HMF – 5-hydroxymethylfurfural, TPC – total phenolic content

**Table 2: Physicochemical and bioactive parameters of the analyzed market honeys**

| | CZ | blend of EU+non-EU honeys | | blend of EU honeys | | blend of non-EU honeys | |
|---|---|---|---|---|---|---|---|
| | N (*n* = 10) | N (*n* = 12) | HD (*n* = 3) | N (*n* = 2) | HD (*n* = 1) | N (*n* = 2) | HD (*n* = 1) |
| Moisture (%) | 16.8 ± 1.0 | 17.4 ± 0.7 | 16.6 ± 0.1 | 16.8 ± 0.0 | 15.5 ± 0.1 | 17.4 ± 0.4 | 16.2 ± 0.2 |
| EC (mS/m) | 37 ± 19 | 40 ± 11 | 99 ± 22 | 17 ± 4 | 151 ± 3 | 20 ± 3 | 118 ± 2 |
| HMF (mg/kg) | 16.0 ± 7.8 | 19.4 ± 5.9 | 13.1 ± 4.5 | 15.6 ± 7.2 | 11.0 ± 1.3 | 19.2 ± 1.3 | 11.5 ± 0.1 |
| Diastase (DN) | 9.8 ± 3.6 | 12.8 ± 3.6 | 19.8 ± 2.4 | 10.5 ± 4.0 | 23.3 ± 0.0 | 11.3 ± 8.2 | 13.3 ± 0.1 |
| Proline (mg/kg) | 214 ± 71 | 464 ± 167 | 943 ± 127 | 246 ± 53 | 863 ± 9 | 273 ± 142 | 350 ± 5 |
| Color intensity (mAU) | 285 ± 157 | 414 ± 238 | 1285 ± 226 | 101 ± 26 | 1190 ± 16 | 131 ± 7 | 634 ± 19 |
| Color (mm Pfund) | 86.7 ± 25.8 | 130.3 ± 52.4 | 265.7 ± 23.8 | 27.4 ± 13.7 | 267.3 ± 4.2 | 40.4 ± 37.3 | 296.3 ± 2.1 |
| TPC (mg GAE/100 g) | 49.4 ± 10.2 | 63.8 ± 17.8 | 130.4 ± 15.7 | 33.3 ± 4.1 | 125.2 ± 7.9 | 34.6 ± 3.4 | 92.5 ± 4.9 |

CZ – Czech Republic, EU – European Union, N – nectar honey, HD – honeydew honey, EC – electrical conductivity, HMF – 5-hydroxymethylfurfural, TPC – total phenolic content

beekeeper and market honeys from Turkey. Diastase activity of honey is mainly influenced by the freshness of the honey, initial value of diastase activity, storage conditions and possible heating of the honey. All determined statistical differences between each group of analyzed honeys are summarized in Table 3.

### Hydroxymethylfurfural content

Compared to diastase activity, concentration of HMF in honeys, shortly after extraction, is relative stabile parameter, with low values, which is also indicated by the concentrations of HMF (1.7 ± 1.3 to 4 ± 3.4 mg/kg) in honeys directly from Czech beekeepers reported by Pospiech et al. (2021). According to the Council Directive of the EU 110/2001 (2014) honey should not contain more than 40 mg per kg of the HMF. The HMF contents of all tested honey samples from Czech and Slovak beekeepers and all analyzed market honey samples did not exceed the established limit. The HMF contents (mg/kg) of honeys from Czech beekeepers ranged from 0.4 ± 0.1 to 15.4 ± 0.1 and the determined average HMF content (6.6 ± 5.2) was 2.4-times lower than the average HMF content of market Czech honeys (16.0 ± 7.8). In the same way, by comparing the average HMF concentration (mg/kg) of the analyzed honeys from beekeepers (6.6 ± 5.8) with the average HMF concentration of the analyzed market honeys (16.9 ± 6.5), was determined 2.6-times lower average HMF concentration of beekeeper honeys. Higher determined HMF contents in market honeys pointed to impact of honey processing or handling on honey quality. The most significant difference ($p < 0.01$) between the HMF concentrations of the beekeeper and market honeys was observed. In great agreement with our results, Aypak et al. (2019) determined statistically most significant difference ($p < 0.01$) in HMF contents between beekeeper and market honeys from Turkey. The average HMF contents of all analyzed honeys are presented in Table 1 and Table 2.

### Moisture content

The moisture content of honeys shortly after extraction is a relatively stable parameter, which normally ranges from 15 % to 21 %. Moisture content affects the stability of the honey, and influence physical properties of honey like viscosity, crystallization, color, flavor, taste, etc. (da Silva et al., 2016). The average moisture content (%) of the analyzed beekeeper honeys (16.3 ± 0.9) was comparable to the moisture content of the analyzed market honeys (17.0 ± 0.8). The average moisture contents of all analyzed honeys are presented in Table 1 and Table 2. Pospiech et al. (2021) detected very similar moisture content in honeys directly from Czech beekeepers, which varied from 16.2 ± 2.4 % to 17.8 ± 1.6 %, depending on floral source of honeys. In contrast, Tomczyk et al. (2019) determined higher moisture contents in honeys directly from Slovak beekeepers, from 17.45 ± 0.38 % to 18.53 ± 0.8 %, in comparison with our results. All tested honey samples from Czech and Slovak beekeepers and all analyzed market honey samples did not exceed the established limit for moisture content (not more than 20 %) according to the Council Directive of the EU 110/2001 (2014). Furthermore, average moisture content (%) of Czech beekeeper honeys (16.1 ± 0.8) was comparable with moisture content of analyzed market Czech honeys (16.8 ± 1.0). Non-significant difference ($p > 0.05$) between moisture contents of the Czech beekeeper nectar honeys and the market Czech nectar honeys was observed.

### Electrical conductivity

The electrical conductivity of honey is mainly related to ash content, which depends on the predominant source of honey – nectar or honeydew. Ash content is very variable parameter of honey ranging from 0.02 % to 1.03 %, and therefore no standard value is specified for this parameter (da Silva et al., 2016). The measured values of electrical conductivity (mS/m) for black locust (16 ± 3), rape (17 ± 2), lime (62 ± 18) and honeydew (98 ± 16) honeys from Czech and Slovak beekeepers were comparable with other authors' findings for Hungarian black locust honeys (14.1 ± 3.4) (Czipa et al., 2019) and European unifloral honeys black locust (16 ± 4), rape (19 ± 5), lime (62 ± 12), honeydew (120 ± 22) (Persano Oddo and Piro, 2004). The lowest electrical conductivities for black

**Table 3: Statistical differences between each group of analyzed honeys**

| | Beekeeper-market | | | Nectar-honeydew | Beekeeper-market |
|---|---|---|---|---|---|
| | all | nectar | honeydew | all | Czech nectar |
| TPC | ** | ** | ** | ** | no |
| Color int. | ** | ** | * | ** | ** |
| Color | ** | ** | ** | ** | * |
| Proline | no | no | no | ** | * |
| Diastase | no | ** | no | ** | ** |
| HMF | ** | ** | no | no | ** |
| EC | no | no | no | * | no |
| Moisture | ** | ** | no | no | no |

(**) most significant difference ($p < 0.01$), (*) significant difference ($p < 0.05$), non-significant difference ($p > 0.05$), TPC – total phenolic content, HMF – 5-hydroxymethylfurfural, EC –electrical conductivity

locust and rape honeys also corresponded with the results published by Bartáková et al. (2007), where black locust and rape honeys had the lowest electrical conductivities among the analyzed Czech honeys. Pospiech et al. (2021) also detected very similar electrical conductivities (mS/m) in Czech rape (24 ± 7), lime (70 ± 13.5), and honeydew honeys (110 ± 19.7). In contrast, Tomczyk et al. (2019) determined lower electrical conductivities in lime honeys directly from Slovak beekeepers 23 ± 9 mS/m. The highest electrical conductivities of analyzed honeydew honeys reflected higher mineral content in honeydew honeys compared to other honey types (Ecem Bayram et al., 2020). Non-significant difference ($p > 0.05$) between electrical conductivities of nectar honeys from Czech beekeepers and Czech nectar honeys from markets was observed. The average electrical conductivities of all analyzed honeys are presented in Table 1 and Table 2.

**Proline content**

Proline is added to honey by bees, and it is the most abundant amino acid in honey. Although the concentration of proline is not a parameter of honey stipulated by law, a concentration lower than 180 mg/kg may indicate an immaturity of honey or an adulteration of honey with sugar syrups (Bogdanov, 2009). The measured proline contents of honeys from Czech and Slovak beekeepers are summarized in Table 1. The determined proline concentrations showed statistically most significant difference ($p < 0.01$) between nectar and honeydew honeys. In comparison with our results, Persano Oddo and Piro (2004) determined very similar proline content in European black locust honeys (222 ± 58 mg/kg), rape honeys (235 ± 49 mg/kg), lime honeys (352 ± 102 mg/kg) and honeydew honeys (468 ± 127 mg/kg). Czipa et al. (2019) also measured similar proline content (245 ± 26 mg/kg) in Hungarian black locust honeys. Flanjak et al. (2016) recorded lower proline content (157.0 ± 21.5 mg/kg) in Croatian samples of black locust honey. Among the analyzed market honeys, a proline concentration less than 180 mg/kg was detected in 16.1 % of analyzed samples, specifically in four samples of nectar honeys from Czech Republic with the proline concentrations (mg/kg) ranging from 109 ± 12 to 161 ± 8 and one blend of the non-EU nectar honeys (173 ± 8). In contrast, except one sample of black locust honey with a proline concentration (155 ± 22 mg/kg), all analyzed honeys directly from Czech and Slovak beekeepers had a proline concentration higher than 180 mg/kg. Persano Oddo and Piro (2004) also recorded a proline content lower than 180 mg/kg in black locust honeys (112-337 mg/kg). Non-significant difference ($p > 0.05$) between the proline concentrations of beekeeper and market honeys was observed. On the other hand, we observed significant difference ($p < 0.05$) between the proline concentrations of Czech beekeeper and market Czech nectar honeys.

**Color intensity of Czech and Slovak beekeeper honeys**

The color intensities of the Czech and Slovak beekeeper honeys ranged from 18 ± 1 mAU to 1122 ± 13 mAU. The average values of color intensity (mAU) for the analyzed unifloral honeys were the following: black locust (26 ± 5) < rape (48 ± 11) < lime nectar honeys (78 ± 7) < lime honeydew honey (154 ± 6). The color intensities (mAU) of Czech multifloral honeys ranged from 73 ± 1 to 225 ± 3 and honeydew honeys from 154 ± 6 to 307 ± 0. The color intensities (mAU) of Slovak multifloral honeys ranged from 57 ± 3 to 153 ± 3 and honeydew honeys from 271 ± 4 to 1122 ± 13. The average color intensities of honeys from Czech and Slovak beekeepers are summarized in Table 1.

Beretta et al. (2005) reported the average values of color intensity for black locust honeys (25 mAU) and honeydew honeys (466 mAU) from different regions in Italy. These findings were in great agreement with our results for black locust and honeydew honeys. Similarly, Flanjak et al. (2016) determined very similar color intensities (mAU) for Croatian black locust honeys (34 ± 11) and honeydew honeys (430 ± 244). Flanjak et al. (2016) and Bertoncelj et al. (2007) detected higher average value of color intensity (mAU) in lime honeys (128 ± 35 and 123 ± 25). Within the analyzed Czech and Slovak multifloral honeys was determined lower average color intensity (132 ± 60 mAU) in comparison with reported value (415 mAU) by Beretta et al. (2005). In contrast, Bertoncelj et al. (2007) reported higher color intensity in Slovenian multifloral (344 ± 57 mAU) and black locust honeys (70 ± 15 mAU) in comparison with our results. Tomczyk et al. (2019) determined several times higher color intensities (mAU) for black locust (221 ± 220), rape (197 ± 37), and lime honeys (306 ± 74) directly from Slovak beekeepers. Karabagias et al. (2016) observed significant variations in color intensity (mAU) of Greek honeys, according to their botanical origin: pine (405 ± 135) > fir (289 ± 89) > thyme (209 ± 71) > orange blossom (164 ± 49). Differences in the determined values of color intensity indicate the influence of several pre-extraction factors such as presence of various natural plant pigments, pollen grains, minerals, flavonoids, and also post-extraction factors such as Maillard products, on the honey color intensity.

**Color intensity of market honeys**

The color intensities of all analyzed market honeys are presented in Table 2. The color intensities (mAU) of market Czech nectar honeys ranged from 75 ± 1 to 557 ± 17. The color intensity (mAU) of blends of the EU and the non-EU nectar honeys ranged from 127 ± 8 to 841 ± 1 and blends of the EU and the non-EU honeydew honeys from 1049 ± 12 to 1498 ± 13. The determined average color intensity (mAU) for market Czech nectar honeys (285 ± 157) was 2.8-times higher, in comparison with

the average value of color intensity (101 ± 73) for nectar honeys from Czech beekeepers and the most significant difference ($p < 0.01$) was observed. Ahmed et al. (2016) analyzed color intensity of Algerian honeys before and after heat treatment at various temperature levels. The color intensity of the unheated Sahara honey samples ranged from 1260 to 1440 mAU, with detected effect of heating to color intensity. The effect of different honey processing to color intensity of Tualang honey samples investigated Khalil et al. (2015). The average values of color intensity (mAU) of the Tualang honey samples ranged from 266.6 ± 37.6 to 474.0 ± 322.2. Khalil et al. (2015) also detected effect of different processing conditions on color intensity of Tualang honeys. In addition, the most significant difference ($p < 0.01$) between the color intensity of beekeeper and market honeys and between the color intensity of nectar and honeydew honeys were observed.

### Color of Czech and Slovak beekeeper honeys

The color of honeys (mm Pfund) from Czech and Slovak beekeepers ranged from 8.5 ± 0.5 to 238.7 ± 0.0; with the average value for nectar honeys 47.1 ± 25.6 and honeydew honeys 138.6 ± 81.5. The average (mm Pfund) color values of the analyzed unifloral honeys were the following: black locust (15.4 ± 5.2), rape (25.2 ± 2.6), lime nectar (55.9 ± 5.3) and lime honeydew honey (95.4 ± 0.5). The color (mm Pfund) of Czech multifloral honeys ranged from 64.2 ± 0.5 to 92.0 ± 1.1 and honeydew honeys ranged from 92.8 ± 0.0 to 113.2 ± 0.5. The color (mm Pfund) of Slovak multifloral honeys ranged from 33.0 ± 0.5 to 70.9 ± 0.5 and honeydew honeys ranged from 108.0 ± 1.6 to 283.7 ± 0.0. Fig. 1 shows the color of honey samples directly from Czech and Slovak beekeepers.

The USDA classified honey color, according to Pfund scale, into seven color grades: < 8 mm (water white), 9–16 mm (extra white), 17–34 mm (white), 35–50 mm (extra light amber), 51–85 (light amber), 86–114 (amber) and >114 mm (dark). According to this color classification, a color of the black locust honey samples varied from extra white to white, a color of the lime honeys varied from extra light amber to amber and a color of the rape honeys was white. The color of all the analyzed honeydew samples was classified as amber, except one sample with the highest obtained color value 238.7 ± 0.0 classified as dark.

Czipa et al. (2019) and Persano Oddo and Piro (2004) reported similar average color values (mm Pfund) for black locust Hungarian honeys 12 ± 5 and European black locust honeys 12.9 ± 5.6. The color values (mm Pfund) for black locust honeys presented by Juan-Borrás et al. (2014) were lower (4.3 ± 1.3) in comparison with our results. Flanjak et al. (2016) also presented the lower measured colors (mm Pfund) for Croatian black locust honeys (3 ± 2). For rape honeys, Persano Oddo and Piro (2004) determined very similar color value (26.2 ± 4.1 mm Pfund). Our determined average color value (mm Pfund) for lime nectar and lime honeydew honeys (63.8 ± 18.2) were higher than the values reported by Persano Oddo and Piro (2004) for European lime honeys (33.3 ± 13.1) and by JuanBorrás et al. (2014) for lime honeys (42.2 ± 17), respectively. Flanjak et al. (2016) also determined lower color values for Croatian lime honeys (17 ± 6 mm Pfund). Persano Oddo and Piro (2004) and Flanjak et al. (2016) determined lower average color values for European (86.0 ± 16.4 mm Pfund) and Croatian honeydew honeys (88 ± 24 mm Pfund) in comparison with our results for Czech and Slovak honeydew honeys (138.6 ± 81.5 mm Pfund).

### Color of market honeys

The measured color values of all market honeys are summarized in Table 2. The color of market Czech nectar honeys (mm Pfund) ranged from 45.2 ± 1.0 to 122.1 ± 0.5. Fig. 2 shows the color of honey samples from markets. Czech and Slovak beekeepers. The colors of blends of the EU and the non-EU nectar honeys (mm Pfund) were similar and ranged from 44.9 ± 2.6 to 201.2 ± 0.5 and honeydew honeys from 241.0 ± 0.5 to 288.5 ± 2.6. The determined average value of color (mm Pfund) for nectar honeys from Czech beekeepers (54.8 ±

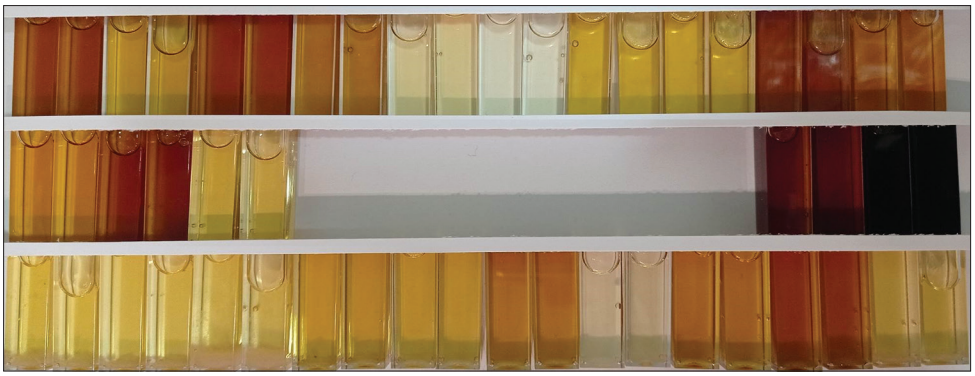

**Fig 1.** The color of honeys from Czech beekeepers (the first line and the left part of the second line and Slovak beekeepers (the right part of the second line and the third line).

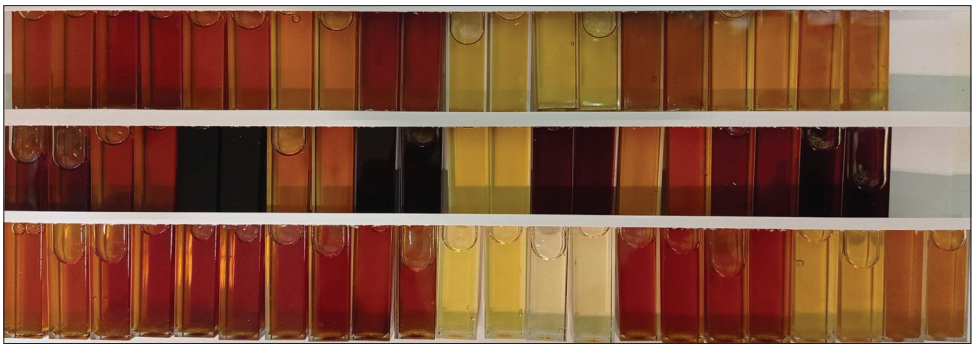

**Fig 2.** The color of honeys from markets: the first line - multifloral honeys from the Czech Republic, the second line and the third line: blend of the (European Union) EU and the non-EU honeys.

28.3) was 1.6-times lower in comparison with the average value (86.7 ± 25.8) for market Czech nectar honeys and a significant difference ($p < 0.05$) was observed. The most significant difference ($p < 0.01$) between the colors of beekeeper and market honeys, and between the color of the analyzed nectar and honeydew honeys were determined. Detected higher color and color intensity values in market honeys reflected influence of honey origin, also processing and storage conditions on honey color and color intensity.

### Total phenolic content of Czech and Slovak beekeeper honeys

The results showed that the determined total phenolic contents varied greatly among the honey types, as presented in Table 1. The total phenolic content (mg GAE/100 g) of honeys from Slovak beekeepers varied from 17.8 ± 0.0 to 103.0 ± 4.1; with a mean value 40.6 ± 22.40. In contrast, the total phenolic contents (mg GAE/100 g) of Czech honeys were similar and varied from 19.1 ± 0.6 to 66.7 ± 1.5; with a mean value 46.1 ± 15.5. The lowest concentrations (mg GAE/100 g) were determined in black locust honeys (22.0 ± 6.4), followed by rape honeys (31.3 ± 1.93), lime nectar honeys (40.8 ± 2.7) and lime honeydew honey (57.2 ± 3.9). The highest phenolic contents (mg GAE/100 g) were measured for honeydew honeys (67.6 ± 21.8). The average total phenolic contents (mg GAE/100 g) for Czech and Slovak multifloral honeys are presented in Table 1.

Tomczyk et al. (2019) detected very similar total phenolic contents (mg GAE/100 g) in black locust (20 ± 5), rape (21 ± 4), and lime honeys (35 ± 1) honeys directly from Slovak beekeepers. In comparison with our results, Bertoncelj et al. (2007) reported higher average values of total phenolic content (mg GA/kg) for black locust (44.8 ± 14.8), lime (83.7 ± 14.3) and for multifloral honeys (157.3 ± 20.9), too. Higher average values of total phenolic content (mg GA/kg) for black locust honeys (39.1 ± 6.8), lime honeys (85.8 ± 17.4) and honeydew honeys (318.6 ± 132.6) were also reported by Flanjak et al. (2016). Comparable results of the total phenolic content for black locust honeys were investigated by Czipa et al. (2019); they determined the range of total phenolic content (mg GAE/100 g) of 10.5 to 22.1, with a mean value of 16.5 ± 3.0. The reported data about the total phenolic content in honey may be very variable among the authors. This is probably caused by different modification of Folin–Ciocalteu method used, and the obtained results are not comparable in some cases.

### Total phenolic content of market honeys

The average total phenolic content (mg GAE/100 g) of market Czech honeys (49.4 ± 10.2) was in close agreement with the results obtained for Czech multifloral honeys gained directly from beekeepers (51.2 ± 7.0). In comparison the average total phenolic content (mg GAE/100 g) in blends of the EU and the non-EU nectar honeys was higher (63.8 ± 17.8) and ranged from 40.2 ± 0.4 to 96.3 ± 6.7. The total phenolic content (mg GAE/100 g) in blends of the EU and the non-EU honeydew honeys ranged from 112.8 ± 0.4 to 142.6 ± 4.6. The most significant difference ($p < 0.01$) between the total phenolic content of beekeeper and market honeys and between the nectar and honeydew honeys were determined. The difference between the total phenolic content of beekeeper Czech nectar honeys and market Czech nectar honeys was insignificant ($p > 0.05$). The reported data about the total phenolic content in beekeeper and market honeys were very variable, with the greatest influence of honey´s botanical and geographical origin.

### Correlation between the parameters

All determined correlation coefficients are summarized in Table 4. Significantly very high positive Pearson correlation coefficient ($r$) between the total phenolic content and color ($r = 0.9422$) and color intensity ($r = 0.9527$) was detected. Determined very high correlation coefficients indicated that phenolics are one of the main components responsible for the honey color and color intensity. This result also confirmed the findings of Anand et al. (2018). Our correlations were

**Table 4: Correlation matrix of the analyzed parameters (Pearson correlation coefficients)**

| Variables($n$ = 56) | TPC | Color intensity | Color | Proline | Diastase | HMF | EC | Moisture |
|---|---|---|---|---|---|---|---|---|
| TPC | 1 | | | | | | | |
| Color int. | 0.9527* | 1 | | | | | | |
| Color | 0.9422* | 0.9307* | 1 | | | | | |
| Proline | 0.8428* | 0.8205* | 0.7878* | 1 | | | | |
| Diastase | 0.4090* | 0.3204* | 0.3680* | 0.6175* | 1 | | | |
| HMF | 0.3355* | 0.4294* | 0.3931* | 0.1916 | -0.3114* | 1 | | |
| EC | 0.7353* | 0.6530* | 0.7249* | 0.6395* | 0.4058* | 0.0183 | 1 | |
| Moisture | -0.1205 | -0.0253 | -0.0428 | -0.0797 | -0.1440 | 0.2617* | -0.3311* | 1 |

(*) significant at P < 0.05, TPC – total phenolic content, HMF – 5-hydroxymethylfurfural, EC – electrical conductivity

in a great agreement with the finding of Pontis et al. (2014) who observed very high positive correlation ($r$ = 0.967) between the total phenolic content and color, and Bertoncelj et al. (2007) who observed very high positive correlation ($r$ = 0.908) between the total phenolic content and color intensity. Compared to our results, Tomczyk et al. (2019) calculated significantly high positive correlation between the color intensity and the total phenolic content ($r$ = 0.703). Significantly high positive correlation was also observed between the honey color and the color intensity ($r$ = 0.9307). Significantly high positive correlation was also observed between the proline content and the total phenolic content ($r$ = 0.8428), the color intensity ($r$ = 0.8205) and the color ($r$ = 0.7878).

Determined significantly very high and high positive correlations between color and total phenolic content, electrical conductivity (mineral content) and proline, confirms that the color of honey depends mainly on the content of these substances. Normally, there is a positive correlation between color and electrical conductivity (ash content), however we have also confirmed that the color of honey also depends on the proline content. Proline and diastase are components of bee origin, and their correlation was moderate positive ($r$ = 0.6175). Correlations between moisture content and other parameters were negligible and mostly non-significant. These findings were in great agreement with Flanjak et al. (2016). Honey color also depends on the presence of Maillard reaction products, mainly on HMF content, that confirmed determined positive correlations between color/color intensity and HMF content, which were significantly low.

## CONCLUSIONS

This paper brought a complex evaluation of quality parameters in honeys directly from beekeepers and honeys from markets. The determined physicochemical parameters and bioactive compounds varied depending on the botanical and geographical origin of honeys. The identified most significant differences ($p$ < 0.01) between the analyzed beekeepers and market honeys in color, color intensity, total phenolic content, moisture content and 5-hydroxymethylfurfural point to the different properties and impact of honey processing/handling on honey composition. The diastase activities, proline concentrations, colors, color intensities and total phenolic contents showed statistically most significant difference ($p$ < 0.01) between nectar and honeydew honeys. In several parameters such as color, color intensity, total phenolic content, proline content and electrical conductivity a very good correlations with botanical origin of honey were observed and beside the melissopalynological analysis these parameters participated on characterization and authentication of unifloral honeys. The color intensity strongly correlated with phenolic content and statistically significant ($p$ < 0.05) very high positive Pearson correlation ($r$ = 0.9527) was obtained. Normally, there is a positive correlation between color and electrical conductivity, however we have also confirmed that the color of honey also depends on the proline content.

The results of this study confirmed that the origin (beekeeper/market) and botanical source of honeys (unifloral/nectar/honeydew) had a significant effect on honey quality, biological value and finally health benefits of honey.

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### Author contributions

Research concept and design: Tkáč, M., Vorlová, L., Borkovcová, I. and Golian, J.; Collection of data: Tkáč, M. and Golian, J.; Data analysis and interpretation: Tkáč, M. and Borkovcová, I.; Writing the article: Tkáč, M.; Critical revision of the article: Tkáč, M., Vorlová, L., Borkovcová, I. and Golian, J.; Final approval of the article: Tkáč, M., Vorlová, L., Borkovcová, I. and Golian, J.

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