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BIOACTIVE COMPOUNDS EVALUATION IN DIFFERENT TYPES OF CZECH AND SLOVAK HONEYS

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ABSTRACT

Honey contains important bioactive compounds (enzymes, phenolic compounds, vitamins, and minerals) with several positive health effects for humans. In the study six types of honey (acacia, rape, floral, multi flower, forest, and honeydew honeys), of Czech and Slovak origin, were evaluated for bioactive compounds by means of color, polyphenols and antioxidant capacity analyses. The brightest color of honeys, the lowest values measured spectometrically, had acacia and rape honeys, followed by floral, and darker multi flower and forest honeys, and honeydew honeys. Polyphenols (PP) amount, determined by spectrophotometric method with Folin-Ciocalteu reagent, was highest for the darkest honeydew honeys, followed by multi flower and forest honey, brighter floral honeys, and rape and acacia honey. Honeys polyphenols were in the range from 54.0 to 254.2 mg GAE.100g^{-1}. The total antioxidant capacity (TAC) was analyzed by spectrometric methods with ABTS and DPPH reagents. Antioxidant capacity values are in agreement with the PP contents order. They were highest also for honeydew honeys (59.2 – 89.6 and 73.1 – 118.7 mg TE.100g^{-1}), followed by multi flower (66.0 and 56.7 mg TE.100g^{-1}) and forest honey (56.0 and 49.1 mg TE.100g^{-1}), then floral honeys (33.0 – 49.2 and 27.8 – 38.7 mg TE.100g^{-1}) and the lowest values for rape (19.0 and 28.1 mg TE.100g^{-1}) and acacia (15.5 and 11.3 mg TE.100g^{-1}) honey. A positive correlation between color, PP amount and TAC was evaluated for analyzed honeys. Darker honey samples showed higher values of phenolic compounds and antioxidant potential, therefore they belong to the honey types with higher amount of bioactive compounds such as antioxidants.

Keywords: honey; color; polyphenol content; antioxidant capacity; DPPH; ABTS

INTRODUCTION

Honey could be generally defined as foodstuff containing mainly sugars, monosaccharides fructose and glucose that form about 70% of sugar content, and about 10% of oligosaccharides (disaccharides and trisaccharides; sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, kojibiose and trisaccharides maltotriose and melezitose). It is quite energetic food due to sugar content. Further there are enzymes, with important biological activity, such as catalase and glucoseoxidase; amino acids and proteins; organic acids; vitamins (such as ascorbic acid, vitamin E), carotenoid derivatives (β-carotene), minerals, and polyphenols **(Miguel et al., 2017; Bertoncelj et al., 2007; Gheldof et al., 2002)**.

As **Miguel et al. (2017)** mentioned honey is an effective nutraceutical foodstuff and its biological activity is mainly dependent on honey's floral or geographic origin. Composition of honey depends on several parameters such as honey type (blossom nectar or honeydew honey), geographical origin (locality of the collection), flora, soil, weather and season, and also post-harvest conditions and honey storage **(Bogdanov et al., 2008)**.

Honey could be defined as product of honey bees (*Apis mellifera*) as a nectar of flowers, flowering plants, they are floral honeys; unifloral if they are produced from the nectar of one type of flowers; or multifloral. They could be also non-floral, honeydew honeys, which are from honeydew (secretion), a sugar-rich substance secreted by various animals such as secretions of aphids plant sucking insects **(Pita-Calvo and Vázquez, 2017)**. Honeydew honey is chemically different from common blossom nectar honey because nectar is dissimilar from honeydew that is usually darker and has higher mineral content **(Grembecka and Szefer, 2012)**. Its darker color is produced by sugars, minerals and amino acids **(Sanz et al., 2005)**. Even nectar honeys from the same floral origin can vary in their chemical composition.

Honey is quite expensive natural product that was found to be adulterated sometimes **(Bušová and Kouřimská, 2018)**. The situation about the honey quality in the Czech

Republic is specified in the Situational Outlook Report of the Czech Ministry of Agriculture **(MZe, 2017)**. The honey consumption in the Czech Republic is only about 0.7 kg per person per year, in Slovakia is marked higher consummation **(Guziy et al., 2017)**.

Honey is known as a potential therapeutic product with bioactive compounds for the treatment and prevention of various diseases. There were studies of honey and its antioxidant, antibacterial, antiviral, anti-inflammatory, antihypercholesterolemic, vasodilatative, and hypotensive activities **(Viuda-Martos et al., 2008; Hadagali and Chua, 2014)**.

Honey is supposed to be a good natural antioxidant for foods. Honey addition can help to prevent or delay food spoilage due to oxidative reactions, to enhance the oxidative stability of the meat, such as dry honey applied to turkey products **(Antony et al., 2000; Antony et al., 2006)**. Dark honeys have higher content of phenolic compounds and possess better antioxidant activity, so they could be used as a good complement of these products **(Beretta et al., 2005; Bertoncelj et al., 2007)**. Free radical scavenging activity of honeys was found to be related with the water-soluble vitamins **(Chua et al., 2013)**. Also **Gheldof et al. (2002)** found an association between antioxidant activity and water-soluble honey fraction consisted of phenolics, peptides, proteins, organic acids, gluconic acid, ascorbic acid, the enzymes glucose oxidase, catalase and peroxidase.

Due to the study of **Schramm et al. (2003)** consumption of honey (buckwheat honey) increased plasma total phenolic content and also plasma antioxidant and reducing capacities. So phenolic antioxidants from processed honey are bioavailable, and increase antioxidant activity of plasma. It is speculated that phenolic antioxidants may augment defenses against oxidative stress and might be able to protect humans from oxidative stress.

The goal of this study was to evaluate amount of several bioactive compounds of few honey types produced from the Czech Republic and Slovakia by determination of polyphenols and antioxidant capacity.

Scientific hypothesis

The scientific hypothesis of this study was to examine the differences in various types of honeys from Czech and Slovak beekeepers due to their bioactive compounds evaluation measured by polyphenolic content and antioxidant capacity of two methods (DPPH and ABTS tests), and correlations between them.

MATERIAL AND METHODOLOGY

Honey samples

There were evaluated 11 honey samples (nectars and honeydew honeys) of 6 honey types of Czech (produced in Moravian region, CZ) and Slovak origin (SK). There were 1 sample of acacia honey (A1) sample (SK), 1 rape honey (R1; CZ), 3 samples of floral honey (FL1-FL3; all CZ), 1 multi flower honey (MF1) sample (SK), 1 sample of forest honey (FO1; SK), 4 samples of honeydew honeys (HD1-HD4, all CZ), collected from private beekeepers, seasons 2013 and 2014. The samples were stored in glass jars in the dry dark place at room temperature (approximately 20 °C) and after jar opening they were analyzed up to ten days.

Determination of Honey Color

For the determination of honey color a modified spectrometric method of **Beretta et al. (2005)** was used. The honey samples were diluted to 50% (w/v) solution with distilled water. They were sonicated for 5 min and filtered through a paper filter and used for color analyses. Absorbances of samples were measured at two wavelengths, 450 nm and 720 nm against blank on the spectrometer (Libra S6 Biochrom, GB). The difference in absorbances (ΔA) was expressed. Determinations were made in triplicate.

Determination of Polyphenolic Content

The polyphenolic (PP) content in honeys was evaluated by a modified spectrophotometric method with Folin-Ciocalteau reagent **(Socha et al., 2009)**. Samples for PP evaluation were prepared with 10 g honey and 40 mL of distilled water. After sonication (5 min) the solutions were filtered through a paper filter and quantitatively transferred into a 50 mL volumetric flask. Afterwards the samples were made up to 50 mL with distilled water and used for the analyses of PP. To extracts (0.1 mL) with 1 mL of distilled water, Folin-Ciocalteau reagent (1 mL; 10% (w/v); Penta Chemicals, CZ) was added and after agitation it was left for 5 min in the dark at room temperature, then 1 mL of sodium carbonate (10% (w/v); Penta Chemicals, CZ) solution was added and mixed again. After 15 min of standing in the dark at lab temperature absorbance of samples was measured at $\lambda = 750$ nm against blank using a Libra S6 Biochrom spectrometer (GB). Gallic acid was used as a standard and PP values were expressed as gallic acid equivalents (GAE) in $mg.100g^{-1}$ sample. Determinations were made in triplicate.

Determination of Antioxidant Capacity

For the total antioxidant capacity (TAC) determinations modified spectrometric methods using ABTS and DPPH reagents **(Škrovánková et al., 2018; Beretta et al., 2005)** were used.

The procedures for honey samples extraction were same for both determinations. There were mixed 10 g of honey sample and 40 mL of distilled water. After sonication (5 min) the solutions were filtered through a paper filter and quantitatively transferred into a 50 mL volumetric flask. Afterwards the samples were made up to 50 mL with distilled water and used for the analyses.

ABTS method: To 50 μL of honey extract the reactive radical mixture (4 mL), composed of ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; Sigma Aldrich, CZ) (12 mL; 3.5 mM) with $K_2S_2O_8$ (0.06 M; Lukes, CZ) and acetic buffer (pH 4.3), was added. The reaction mixture was shaken vigorously on a Vortex mixer and for 30 min it was left to react without light exposure at room temperature. Honey samples absorbance (A) and absorbance of control samples (AC) were after time limit measured at $\lambda = 734$ nm against blank by spectrometer Libra S6 Biochrom (GB). Inactivation (I) was calculated from the decrease of absorbance (%) according to relation (1). Results of TAC (ABTS) were calculated from

inactivation using calibration curve with trolox as standard. It was expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

$$I = \frac{AC-A}{AC} . 100 \quad (1)$$

DPPH method: To prepared honey extract (0.2 mL) a DPPH (1,1-diphenyl-2-picrylhydrazyl) solution in ethanol (1.9 mL; 0.02 mM; Sigma Aldrich, CZ) and acetate buffer solution (1 mL; pH 5.5) were added. The mixture was shaken vigorously on a Vortex mixer in capped glass and left without light exposure for 1 h at room temperature. Absorbance of samples (A) and absorbance of control samples (AC) was measured at λ = 517 nm against blank on the spectrometer (Libra S6 Biochrom, GB). Their inactivations (I) were also calculated from the decrease of absorbance according to relation (1) and the values were expressed as trolox equivalents (TE) in mg.100g^{-1} sample. Average results were obtained from three parallel determinations.

Statistic analysis

All analyses were provided in triplicate, and the data were expressed as mean values ± standard deviation (SD). Statistic evaluation of the results was made by Statistica program, StatSoft version 9.0 (Dell, USA) using parametric test comparing mean values of two independent assortments (Student *t*-test). Differences at a 95% confidence level (p <0.05) were considered statistically significant. Correlations between the parameters evaluated were obtained using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Color of honeys

In terms of our measurements and also due to sensorial examination, honey color ranges from white pale, pale yellow to amber and dark brown with the values ΔA from 0.139 to 0.817, the average 0.478 (Table 1). As expected, as the darkest honeys with the highest values were evaluated honeydew honeys (HD samples). Their values (average 0.779) were nearly five times higher than the brightest ones, acacia and rape honeys (average 0.161); and nearly three times to the values of floral honeys (0.268). Multi flower and forest honeys belong to the group of darker honeys. Several compounds, pigments are responsible for honey color. To the most important honey pigments belong water soluble polyphenols, flavonoids, and lipid soluble carotenoids **(Isla et al., 2011)**.

Also mineral composition is important for honey color. As **González-Miret et al. (2005)** evaluated lightness is significantly related with minerals such as S, Ca, Fe, As, Pb, and for the dark honey types (chestnut, and honeydew honeys) also Cd is considerable. Due to these facts, there are therefore expectations of higher content of polyphenols and probably also higher antioxidant values for darker honeys **(Bertoncelj et al., 2007; Kuś et al., 2014)**.

Content of phenolics

In the determination with Folin-Ciocalteau reagent electron-donating antioxidants such as polyphenols, ascorbic acid, and vitamin E are evaluated. The total polyphenols (PP) contents of the honey samples (Table 1) range from 54 to 254.2 mg GAE.100g^{-1} with the average 150 mg GAE.100g^{-1} honey. There were marked differences between honeys (p <0.05, Student *t*-test). Also **Bertoncelj et al. (2007)** mentioned that total phenolic content differs widely among different honey types. As it was expected due to other scientist researches **(Moniruzzaman et al., 2014; Alvarez-Suarez et al., 2010)** brighter, pale honeys had lower polyphenol values. PP results for honeys were in agreement with literature sources; lowest for acacia honey, where also **Beretta et al. (2005)** evaluated the lowest content (5.5 mg GAE.100g^{-1}). It was followed by rape honey and floral ones, forest and multi flower honey, whereas the highest PP content had the darkest honeydew honeys with the nearly five times higher values in comparison to the lowest ones. These findings are in agreement with the values previously reported for other European honeys, as **Kuś et al. (2014)** determined in Polish honeys total phenolic content in the range 12.2 – 117.4 mg GAE.100g^{-1}. The composition of honeys is dependent on the botanical origin, floral source, and also seasonal and environmental factors, as well as processing **(Kıvrak and Kıvrak, 2017; Cavazza et al., 2013; Dimitrova et al., 2007)**.

Honey samples exhibited similar order of samples for color and PP values. To characterize the relationships between color and polyphenolic content the correlation (Figure 1) was evaluated. They are strongly related with a correlation factor r = 0.8294. **Sant'ana et al. (2014)** also discovered that the lowest total phenolic content corresponded to light-colored honey and the highest values to dark honeys. Positive correlation between color and PP determined also other scientists **(Alvarez-Suarez et al., 2010; Kuś et al., 2014).**

Honey color thus seems to be a relatively reliable parameter to indicate high PP content in honey.

Antioxidant capacity

The antioxidant potential that means overall hydrogen/electron-donating activity of present antioxidants, was measured by two methods, with ABTS and DPPH test. The total antioxidant capacity (TAC) results for honey samples are demonstrated in Table 1. The TAC values of ABTS method were in the range from 15.5 to 89.6 mg of trolox equivalents per 100 grams of honey sample with the average 54.5 mg TE.100g^{-1}; and from 11.3 to 118.7 mg TE.100g^{-1} for DPPH method with the average 58.4 mg TE.100g^{-1}, respectively. There were marked differences between honeys (p <0.05, Student *t*-test). Also, **Frankel et al. (1998)** showed in their study that great variations exist in the chemical nature of honey from different floral sources as they found in best honey source 20.3 times higher concentration of antioxidants in comparison with that of the lowest one. The least active honeys in our research were, similarly like in PP evaluation, the brightest honeys **(Beretta et al., 2005; Kuś et al., 2014),** pale acacia **(Bertoncelj et al., 2007)** and rape honeys, for both methods.

Higher potency in scavenging of DPPH free radical and also ABTS test showed floral honeys (average 34.8 and 40.2 mg TE.100g^{-1}, respectively), forest and multi flower honey. Honeys with the best antioxidant potency were evaluated honeydew honeys (average 98.1 mg and 80.6 mg TE.100g^{-1}, respectively). The highest results for TAC (DPPH and ABTS test) were nearly eleven and nearly six times higher, respectively, than the lowest TAC value. Also, **Ferreira et al. (2009)** obtained the highest antioxidant values in the dark honeys.

Table 1 Color evaluation (ΔA), polyphenols content (PP) and total antioxidant capacity (TAC) of honey samples.

Honey sample	ΔA ±SD	PP (mg GAE.100g^{-1} ±SD)	TAC (ABTS) (mg TE.100g^{-1} ±SD)	TAC (DPPH) (mg TE.100g^{-1} ±SD)
A1	0.139 ±0.009^{a}	54.0 ±1.7^{a}	15.5 ±2.4^{a}	11.3 ±1.3^{a}
R1	0.183 ±0.016^{b}	56.8 ±1.2^{a}	19.0 ±0.2^{b}	28.1 ±0.9^{b}
FL1	0.238 ±0.013^{c}	126.5 ±0.6^{b}	33.0 ±1.0^{c}	37.9 ±2.0^{c}
FL2	0.273 ±0.010^{d}	111.3 ±0.5^{c}	49.2 ±6.5^{d}	38.7 ±2.8^{c}
FL3	0.293 ±0.016^{e}	146.2 ±0.9^{d}	38.5 ±1.6^{e}	27.8 ±0.8^{b}
MF1	0.467 ±0.019^{f}	155.3 ±0.4^{e}	66.0 ±3.0^{f}	56.7 ±0.3^{d}
FO1	0.552 ±0.024^{g}	150.1 ±0.5^{d,e}	56.0 ±1.1^{g}	49.1 ±0.9^{e}
HD1	0.745 ±0.027^{h}	164.5 ±0.4^{f}	59.2 ±4.3^{g}	73.1 ±1.3^{f}
HD2	0.753 ±0.025^{h,i}	203.0 ±1.5^{g}	84.5 ±2.0^{h}	99.5 ±1.1^{g}
HD3	0.801 ±0.020^{i,j}	224.6 ±1.3^{h}	89.1 ±9.0^{h}	101.2 ±1.8^{g}
HD4	0.817 ±0.031^{j}	254.2 ±1.4^{i}	89.6 ±2.5^{h}	118.7 ±1.3^{h}

Note: Means within a column with at least one identical superscript are not significantly different by Student's *t*-test (p <0.05).

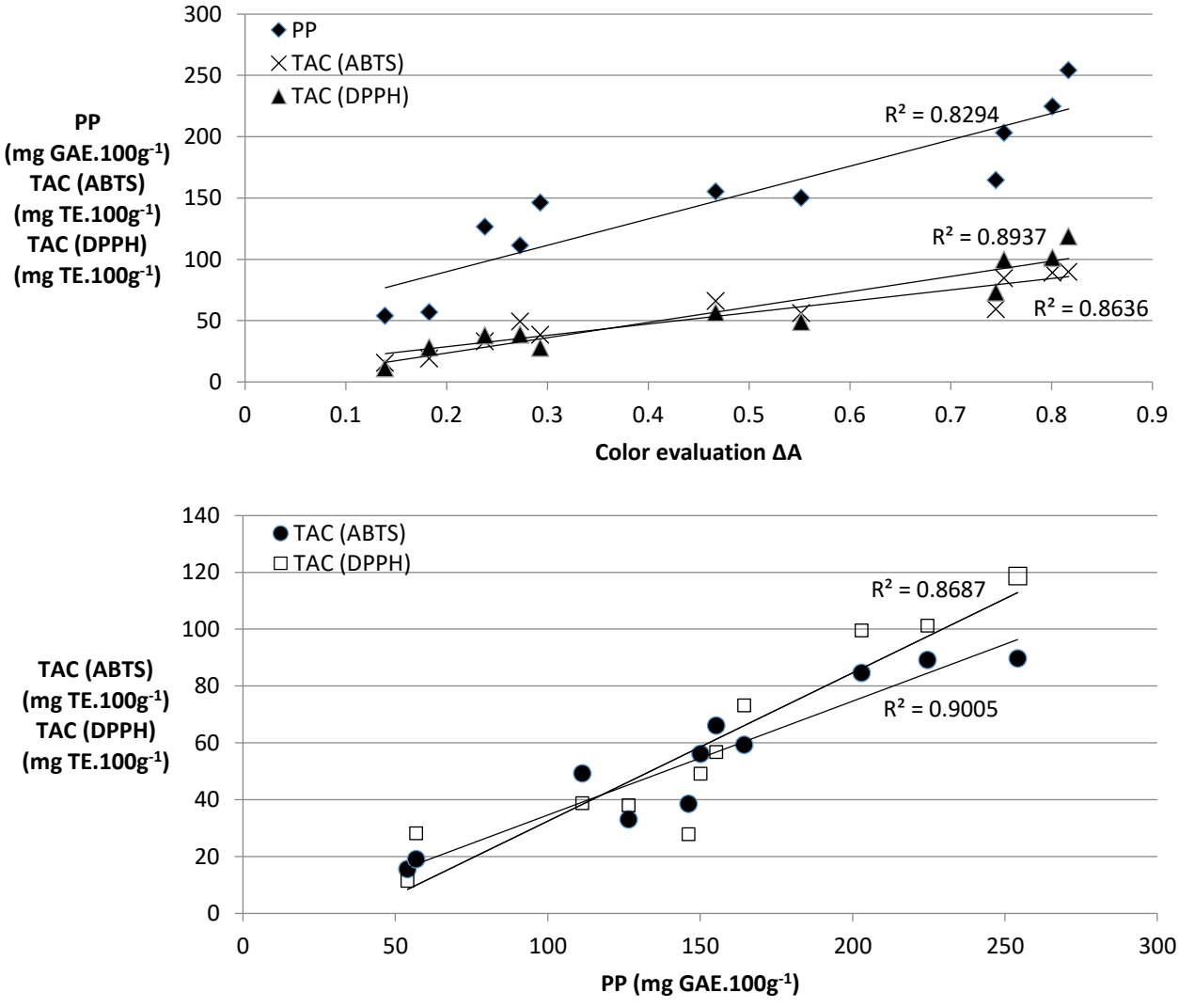

Figure 1 Correlations between color evaluation (ΔA) and PP and TAC values (top), and PP and TAC values (bottom).

Similar descending order of TAC values was observed also for honey color. The correlations to examine the relationships between them (Figure 1) were evaluated. Antioxidant capacity showed a strong relationship comparing both assays and color intensity, for ABTS evaluation $r = 0.8836$, and for DPPH test $r = 0.8937$. High correlation between color and antioxidant capacity was determined also by other researchers **(Sant'ana et al., 2014; Pontis et al., 2014; Beretta et al., 2005; Bertoncelj et al., 2007)**. Honey color thus seems to be a relatively reliable parameter to indicate not only PP content but also antioxidant potential in honey.

Antioxidant capacity determined by both analyses was also strongly positively associated with the polyphenolic content ($r = 0.9005$ for ABTS; $r = 0.8687$ for DPPH), as shown in Figure 1. Therefore high PP contents predicate high TAC values in analyzed honey samples. Also **Wilczyńska (2014), Sant'ana et al. (2014), Pontis et al. (2014), Beretta et al. (2005),** and **Bertoncelj et al. (2007)** found positive correlation between PP and TAC for honey samples. Although **Pontis et al. (2014)** determined high flavonone and dihydroflavonol content in some honeys, no correlations between them and antioxidant potential they observed. Generally, our findings and data in the literature have shown a linear relationship between honey color, phenolic compounds and antioxidant capacity.

CONCLUSION

Honey has several positive health effects for humans. They are related to their bioactive compounds such as enzymes, phenolic compounds, vitamins and some minerals. Honeys with the best polyphenols content and antioxidant capacity values evaluated by both methods are honeydew honeys, the darkest ones. The color of honeys was determined in the progression: pale acacia, then rape honey, floral, multi flower and forest honeys, and dark honeydew honeys. The polyphenol content in honeys was in the range from 54.0 to 254.2 mg GAE.100g^{-1}, in the descending order: honeydew honeys, multi flower and forest honey, followed by floral honeys, then rape and acacia honey. Antioxidant capacity values by two evaluation methods (ABTS, DPPH), are in agreement with polyphenols content order. They were highest also for honeydew honeys (59.2 – 89.6 and 73.1 – 118.7 mg TE.100g^{-1}), followed by multi flower (66.0 and 56.7 mg TE.100g^{-1}) and forest honey (56.0 and 49.1 mg TE.100g^{-1}), then floral honeys (33.0 – 49.2 and 27.8 – 38.7 mg TE.100g^{-1}) and the lowest values for rape (19.0 and 28.1 mg TE.100g^{-1}) and acacia (15.5 and 11.3 mg TE.100g^{-1}) honey. There was established a positive correlation between the color, polyphenolic amount and antioxidant capacity of the evaluated honeys. Darker honey samples showed higher content of phenolic compounds and increased antioxidant capacity.

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