

Correlation between antioxidant and physicochemical parameters of honey samples from Bosnia and Herzegovina and Turkey

Samardžić, M., Ibragić, S.*

Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina

Article info

Received: 04/01/2022
Accepted: 22/09/2022

Keywords:

Honey
Antioxidant activity
Colour
Proline
Ascorbic acid

*Corresponding author:

Saida Ibragić

E-mail: saidetun19@gmail.com
Phone: 00-387-33-279-914

Abstract: The aim of this study was to determine the association value between the following parameters and the antioxidant properties of honey: colour, phenolics content (TPC), flavonoid content (TFC), proline and ascorbic acid content. The samples were collected in Bosnia and Herzegovina and Turkey and included honeydew, monofloral and polyfloral honey. The antioxidant activity of honey samples was determined using the ABTS and DPPH assays. Based on the correlation matrix, the main findings revealed a strong correlation between antioxidant activity and TPC, TFC, proline content and colour. Honey colour was in best correlation with the TFC ($r = 0.910$, $p < 0.001$) where dark coloured honeys showed a higher TFC, however antioxidant activity showed a highly significant dependence on the TPC (DPPH-TPC: $r = -0.872$; ABTS-TPC: $r = -0.783$, $p < 0.001$). Ascorbic acid was not established as a predictive parameter that can be used to estimate the antioxidant properties of honey and did not significantly correlate with any of the remaining variables.

INTRODUCTION

Honey is a natural dietary antioxidant that has been in use since ancient times. The biological activities of honey are derived from its chemical composition and that mainly depends on the botanical origin, climatic and environmental conditions. The honey composition is also influenced by production methods, handling, and storage, however only to a lesser extent (Kıvrak and Kıvrak, 2017; Manyi-Loh, Ndip and Clarke, 2011; Al-Mamary, Al-Meerri and Al-Habori, 2009; Beretta *et al.*, 2005). A typical honey composition includes a mixture of sugars, various phenolic compounds, minerals, proteins, enzymes, vitamins and volatile compounds (Manyi-Loh *et al.*, 2011). Special reference is given to phenolic constituents as they have been shown to possess antioxidant properties by acting through various mechanisms (Alvarez-Suarez *et al.*, 2009).

Several authors have reported the honey colour to be correlated with the polyphenol content of honey. Dark honeys, being richer in polyphenols are consequently better antioxidants (Beretta *et al.*, 2005; Ferreira *et al.*, 2009; Alvarez-Suarez *et al.*, 2010; Perna *et al.*, 2013). The antioxidant properties have been reported to be

effective in reducing the risk of heart disease, cancer and immune-system decline (The National Honey Board, 2002). In addition to that, honey is known for its antimicrobial, antiviral, anti-inflammatory activities (Bogdanov *et al.*, 2008). This study evaluated the correlation between the antioxidant activity and various biochemical and physicochemical parameters in monofloral and polyfloral honey samples from two geographical regions, obtained commercially or directly from beekeepers. The selected parameters that were correlated to the results obtained by antioxidant assays included colour, the total phenolic (TPC) and total flavonoid content (TFC).

In addition, proline, which is used as a determinant of the honey quality, was reported to contribute to the reducing ability and radical scavenging potential of honey (Khalil *et al.*, 2012) and was therefore included in our study. Finally, ascorbic acid, previously used as a simple marker to discriminate the botanical origin of honeys (León-Ruiz, Vera, González-Porto, & Andrés, 2011), has been assessed as a predictive parameter useful in the evaluation of antioxidant activity.

To the best of our knowledge, although numerous studies worldwide evaluated the antioxidant properties of honey, no study reported a characterization of Bosnian honey by the selected parameters, nor their interrelation evaluated by their correlation coefficients.

EXPERIMENTAL

Chemicals

All chemicals and solvents used were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, quercetin ($\geq 95\%$), *o*-phenylenediamine (99.5%), 2-propanol (99.9%) and DL-proline (99%) were obtained from Sigma Aldrich, Co. (St. Louis, MO, USA). L(+)-Ascorbic acid and ninhydrin were obtained from Merck (Germany). Folin-Ciocalteu reagent, aluminium chloride hexahydrate, ammonium chloride, ammonium hydroxide, potassium persulfate, sodium carbonate anhydrous, acetone, ethanol (96%), methanol (99.5%) and formic acid were sourced from Semikem (B&H).

Honey samples

Honey samples were collected from different regions in Bosnia and Herzegovina (B&H), either as commercially available products or directly from beekeepers. Honey samples from Turkey were all commercially available products purchased in Istanbul. The honey samples were prepared as aqueous solutions in various concentrations as required by the utilised methods.

Colour

Samples were diluted in water (1:1; w/v) and the absorbance was measured at 635 nm. Results were calculated using the following formula: $P_{\text{fund}} = -38.70 + 371.39 \times \text{ABS}$ (Ferreira *et al.*, 2009) and expressed in millimetres on a Pfund scale (Fell, 1978).

pH

The pH was determined using the method described by Bogdanov, Martin and Lullmann (2002). Honey samples were weighed (10 g), dissolved and homogenised in 75 mL of distilled water. Direct readings were taken for each honey sample using a calibrated pH meter (pH meter PH-20W).

Total phenolic content

The Folin-Ciocalteu method was used to determine the TPC as reported by Singleton and Rossi (1965), with some modifications. The volume of 200 μL of honey solution (100-300 mg/mL) was mixed with 1 mL of Folin-Ciocalteu reagent. After 5 minutes of incubation at room temperature, 800 μL of sodium carbonate solution (7.5%, w/v) was added. The mixture was incubated for 30 minutes at room temperature, and the absorbance was measured at 734 nm by using a UV-Visible spectrophotometer. Calibration curve was prepared using known concentrations of gallic acid (10-110 mg/L) and results expressed in milligrams of gallic acid equivalents (mg GAE/100 g of honey).

Total flavonoid content

The TFC was determined by using the Dowd method as adapted by Arvouet-Grand *et al.* (1994). 500 μL of honey solution (300-500 mg/mL) was mixed with 500 μL of 2% aluminium trichloride solution in methanol. The mixture was incubated for 30 minutes at room temperature, and the absorbance was measured at 415 nm by using a UV-Visible spectrophotometer. Calibration curve was prepared using known concentrations of quercetin (2.5-20 mg/L) and results expressed in milligrams of quercetin equivalents (mg QE/100 g of honey).

DPPH and ABTS

The DPPH assay was performed as described by Blois (1958). The volume of 100 μL of honey solutions at various concentrations were mixed with 1.9 mL of a DPPH solution (0.05 mmol/L in methanol) and kept in dark. After 30 min of reaction at room temperature, the absorbance of the solution was measured at 517 nm.

ABTS radical-scavenging activity of honey samples was determined according to Re *et al.* (1999). The volume of 100 μL of honey solutions at various concentrations were mixed with 900 μL of ABTS solution (7 mmol/L in ethanol) and the absorbance was recorded at 734 nm. The ABTS and DPPH scavenging ability were expressed as IC_{50} (mg/ml). The scavenging activity in both assays was calculated using the following formula:

$$\text{scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample/standard solution.

Ascorbic acid content

Ascorbic acid was determined using the method described by Wu *et al.* (2003), with some modifications. The volume of 500 μL of honey solution (20-30 mg/mL) was mixed with 500 μL of *o*-phenylenediamine solution in 0.1 mol/L hydrochloric acid (0.5%, w/v) and 750 μL of $\text{NH}_3\text{-NH}_4\text{Cl}$ buffer solution (pH 9.4). The mixture was then diluted to 5 mL with water and thoroughly mixed by shaking. After 5 minutes, the fluorescence intensity was measured using a 1 cm quartz cell at excitation and emission wavelengths of 330 and 430 nm, respectively. Calibration curve was prepared using known concentrations of ascorbic acid (1 – 70 $\mu\text{g/mL}$) and results expressed in milligrams of ascorbic acid equivalents (mg AAE/100 g of honey).

Proline content

The proline content was determined using the method described by Bogdanov, Martin and Lullmann (2002), based on the original method of Ough (1969). The volume of 375 μL of honey solution (50-130 mg/mL) was mixed with 750 μL of formic acid and 750 μL of ninhydrin solution (3% in acetone). The tube containing the mixture was shaken vigorously for 15 minutes and placed in a boiling water bath. After 15 minutes, the tube was transferred to a 70 °C water bath for an additional 10 minutes. 3.75 mL of the 50% 2-propanol water solution was added to the mixture and left to cool. The absorbance was measured at 510 nm, 45 minutes after removal from the 70 °C water bath. Water was used as the blank. Calibration curve was prepared using the standard proline solution (200 mg/L), and the result expressed as mg of proline/kg of honey.

Statistical analysis

Except for the determination of proline content, all measurements were performed in triplicates and results are reported as mean \pm SD. The Pearson's correlation coefficient (r) was calculated to find the interdependence between the variables and draw conclusions whether there is a significant association between the investigated parameters describing the colour, TPC, TFC, proline content, ascorbic acid content and antioxidant activity. Statistical significance was set at two levels: $p < 0.05$ (significant) and $p < 0.001$ (highly significant).

RESULTS AND DISCUSSION

The current study investigated 15 honey samples of different botanical and geographical origin, as presented in Table 1. The samples were classified as monofloral honey, polyfloral honey and honeydew. The pH value is a useful parameter that aids the determination of origin. According to Gomes *et al.* (2011) forest honey has higher pH values than flower honey. This study included only one sample of forest honey, which is insufficient for further interpretation. In general, depending on the botanical source, the pH of the nectar, soil and the presence of acids and minerals, the pH of honey ranges between 3.5 and 5.5 (Bogdanov, Martin and Lüllmann, 1997). The honey samples presented pH values ranging from 3.7 - 4.6. The average pH value of monofloral samples (4.15) was very similar to the pH value of

polyfloral samples (4.00), however honeydew samples showed slightly lower acidity (4.43).

Botanical and geographical origin are also determinants of another parameter - colour. The colour of honey is due to pigments but also other factors such as beekeeper's interventions, aging and storage conditions may affect it (Khalil *et al.*, 2012). The international markets demand specific honey colour since American consumers prefer light honey with delicate, light taste while the population in some European areas asks for dark honey that has a stronger taste (Delmoro *et al.*, 2010). The consumer's colour preference is not necessarily a reflection of the honey quality. The colour of the honey samples in this study varied from extra white to dark amber. The majority of samples was in the range of 119.73 - 239.06 mm, which corresponds to dark amber.

Table 1. Origin, pH and colour of honey samples

Sample ID	Honey description	Country	pH	mmPfund	Colour
<i>Monofloral</i>					
1 #	Sage honey	B&H	4.1 ± 0.01	164.1	dark amber
2 #	Chestnut honey	B&H	4.3 ± 0.00	119.73	dark amber
3 ##	Extracted chestnut honey	B&H	4.5 ± 0.00	54.7	light amber
4 ##	Raspberry and goji honey	B&H	3.7 ± 0.01	123.04	dark amber
<i>Polyfloral</i>					
5 ##	Forest honey	B&H	4.1 ± 0.02	124.28	dark amber
6 ##	Meadow honey	B&H	3.8 ± 0.00	136.69	dark amber
7 #	Mountain honey	Turkey	3.9 ± 0.00	10.55	extra white
8 #	Mountain honey	Turkey	3.8 ± 0.01	17.86	white
9 #	Floral honey	Turkey	4.4 ± 0.00	80.04	light amber
10 #	Polyfloral honey	Turkey	3.8 ± 0.01	68.63	light amber
11 #	Foral honey	Turkey	4.0 ± 0.02	90.23	amber
12 #	Floral honey	Turkey	4.2 ± 0.02	140.72	dark amber
<i>Honeydew</i>					
13 ##	Honeydew	B&H	4.6 ± 0.00	239.06	dark amber
14 #	Cedar	Turkey	4.2 ± 0.01	89.94	amber
15 #	Pine	Turkey	4.5 ± 0.01	195.03	dark amber

commercially obtained, ## obtained from beekeepers

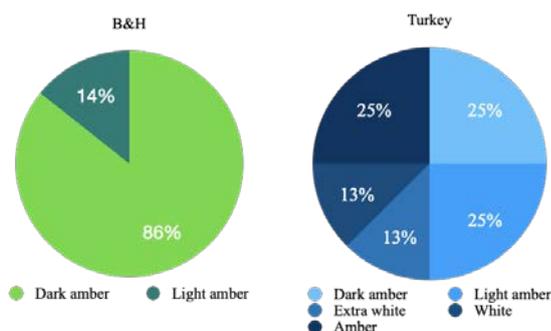


Figure 1. Colour of the investigated honey samples classified according to geographical origin.

The content of single phenolic or other compounds present in honey is too low to have a major antioxidant significance (Gheldof, Wang and Engeseth, 2002), it is rather the combination and interaction of enzymes, vitamins, amino acids, organic acids and other components that contribute to the overall antioxidant capacity (Nayik *et al.* 2016; Ferreira *et al.*, 2009). Therefore, to evaluate the antioxidant activity of the

selected honey samples, a series of parameters have been determined, as shown in Table 2.

It has been demonstrated that some amino acids have antioxidant properties (Wu *et al.*, 2003; Udenigwe and Aluko, 2011). A study on Burkina Fasan honey confirmed the radical scavenging potential of proline (Meda *et al.*, 2005). Proline is the prevalent among the described free amino acids in honey (Belitz, Grosch and Schieberle, 2009) and its content is commonly used to estimate the quality and maturity of honey, as well as in detection of sugar adulteration. Genuine honey will have a minimum value of 180 mg proline per kg, however it should be kept in mind that the proline content varies in different types of honey (Bogdanov *et al.*, 1999). To illustrate that, 6719 honey samples of European origin were analysed and it was found that the proline content ranged from 222 to 956 mg/kg depending on the plant species (Oddo and Piro, 2004; Piazza and Oddo, 2004). Except for three flower honey samples from Turkey (sample 10-12), all other samples of this study had a proline content above the minimum prescribed value and can therefore be considered genuine. The raspberry honey had the highest proline content of 1305.29 mg/kg.

Table 2. Proline, ascorbic acid, phenolic and flavonoid content in honey samples and their antioxidant activity

Sample ID	Sample description	Proline mg/kg	Ascorbic acid mg AAE/100g	TPC mg GAE/100g	TFC mg QE/100g	DPPH IC ₅₀ mg/mL	ABTS IC ₅₀ mg/mL
<i>Monofloral</i>							
1B	Sage honey	768.08	22.01 ± 0.14	83.68 ± 1.69	4.65 ± 0.02	480.13 ± 11.89	51.87 ± 2.52
2B	Chestnut honey	461.98	96.46 ± 2.09	57.98 ± 0.54	4.1 ± 0.04	745.36 ± 12.14	69.70 ± 1.79
3B	Extracted chestnut honey	330.92	110.78 ± 1.88	48.8 ± 0.90	2.82 ± 0.08	1132.33 ± 7.75	93.09 ± 2.82
4B	Raspberry and goji honey	1305.29	41.81 ± 1.77	87.85 ± 1.21	4.69 ± 0.02	670.31 ± 20.49	78.99 ± 2.84
<i>Polyfloral</i>							
5B	Forest honey	721.35	22.18 ± 1.28	73.17 ± 1.29	4.58 ± 0.03	464.94 ± 15.67	63.94 ± 3.37
6B	Meadow honey	984.30	38.19 ± 2.52	58.32 ± 1.81	4.69 ± 0.09	567.52 ± 30.34	84.93 ± 1.83
7T	Mountain honey	324.15	4.57 ± 0.40	12.72 ± 0.26	1.28 ± 0.01	2891.78 ± 1.76	174.60 ± 1.02
8T	Mountain honey	282.28	3.75 ± 1.01	28.06 ± 0.07	0.96 ± 0.12	2645.90 ± 28.14	169.75 ± 8.19
9T	Floral honey	377.49	12.46 ± 0.36	70.57 ± 3.07	3.34 ± 0.02	443.57 ± 10.64	49.67 ± 0.58
10T	Polyfloral honey	145.44	7.97 ± 0.44	26.11 ± 0.30	1.76 ± 0.04	**	335.06 ± 13.13
11T	Foral honey	76.88	6.06 ± 0.41	21.07 ± 0.47	1.46 ± 0.03	**	173.88 ± 8.47
12T	Floral honey	85.54	9.56 ± 0.89	29.36 ± 0.74	3.31 ± 0.05	**	232.75 ± 4.96
<i>Honeydew</i>							
13B	Honeydew	677.17	30.46 ± 0.63	89.8 ± 2.35	6.87 ± 0.08	264.04 ± 5.36	37.22 ± 2.31
14T	Cedar	232.26	21.18 ± 1.36	56.06 ± 0.60	3.21 ± 0.02	564.61 ± 15.02	64.59 ± 1.83
15T	Pine	1233.58	22.18 ± 1.85	54.86 ± 0.64	4.27 ± 0.04	599.58 ± 6.40	69.73 ± 0.43

** Honey solution in the concentration of 1 mg/mL was insufficient to obtain the IC₅₀ values. Higher concentrations lead to inhomogeneous turbid solutions inappropriate for the assay.

B – samples from B&H; T- samples from Turkey

Ascorbic acid is a naturally occurring antioxidant that has been analysed to describe both the nutritional value and antioxidant properties of honey. The number of studies is rather scarce, as ascorbic acid is unstable and prone to chemical and enzymatic oxidation (León-Ruiz *et al.*, 2013). The use of different analytical methods makes the comparison of results difficult. In the present study, the ascorbic acid content was determined spectrophotometrically, based on its condensation reaction with *o*-phenylenediamine. The obtained results were in the range between 22.01 - 110.78 mg AAE/100 g and 3.75 - 22.18 AAE mg/100 g in Bosnian and Turkish honey, respectively. Two studies (Kesic *et al.*, 2009; León-Ruiz *et al.*, 2013), based on a volumetric method using 2,6-dichlorophenolindophenol, reported a different concentration range in honey from B&H (37.22 - 378.3 mg/100g) and honey from Spain (0.77 - 57.15 mg/100 g). Phenolics are one of the most important classes of compounds found in honey (Khalil *et al.*, 2012) and flavonoids as a subclass represent the main functional components of scientific and therapeutic significance (Yao *et al.*, 2004; Alvarez-Suarez *et al.*, 2012). The mechanisms by which phenolic compounds exhibit their antioxidant activity include free radical-scavenging, metal ion chelation and hydrogen donation (Havsteen, 2002). The TPC values of the analysed honey samples were in the range between 12.72 - 89.8 mg GAE/100 g, with a mean value of 71.37 mg GAE/100 g for honey from B&H and 37.35 mg GAE/100 g for Turkish honey. Similar values (12.64 - 90.57 mg GAE/100 g) were obtained for Croatian honey (Piljac-Žegarac, Stipčević and Belščak, 2009) but also for African honey sorts (32.59 - 114.75 mg GAE/100 g), Brazilian honey (28.9 - 69.0 mg GAE/100 g), Greek honey (55.0 - 92.0 mg GAE/100 g) and Portuguese honey (22.62 - 72.77 mg GAE/100 g) (Meda *et al.*, 2005; Cruz *et al.*, 2014; Stagos *et al.*, 2018; Ferreira *et al.*, 2009). Honeydew followed by raspberry and goji honey had the highest TPC, while mountain honey (sample 7) had the lowest TPC. The TFC values varied between 0.96 - 6.87 mg QE/100 g, with the following mean values for Bosnian and Turkish honey respectively: 4.63 mg QE/100 g and 2.45 mg QE/100 g. Compared to other studies, the obtained values were similar to the TFC in African honey (0.17 - 8.35 mg QE/100 g) and Brazilian honey (0.90 - 4.86 mg QE/100 g) (Meda *et al.*, 2005; Pontis *et al.*, 2014).

Until today, there is no universal method that can be used to determine the *in vitro* antioxidant activity. The two most commonly employed methods are the DPPH and ABTS assays and there is no equivocal view as for which of the two assays is more appropriate for honey samples (Lachman *et al.*, 2010; Piljac-Zegarec *et al.*, 2009; Meda *et al.*, 2005; Turkmen *et al.*, 2006). The results describing the antioxidant activity were expressed as IC₅₀, where a lower IC₅₀ value implies a stronger antioxidant activity. The antioxidant activity determined by the DPPH assay was stronger in polyfloral than in monofloral honey collected in both countries, however the Bosnian honey samples appeared to have a superior radical scavenging capacity than Turkish honey. This is also true when observing the AA obtained by the ABTS assay. The IC₅₀ ranged between 37.22 - 93.09 mg/mL and 49.68 - 335.06 mg/mL for Bosnian and Turkish honey, respectively. The IC₅₀ values of Bosnian honey are similar to those of Greek honey reported by Stagos *et al.* (2018), however direct comparison of AA from literature data is difficult given the various reaction conditions used by different authors. The main differences from honey samples collected in Turkey and B&H are presented in Fig. 2.

According to the obtained results, among the Bosnian honey samples, honeydew had the best antioxidant properties, whereas the same is true for the polyfloral honey (sample 9) among the Turkish honeys. The correlation matrix (Table 3) was calculated taking into account all samples of this study, regardless of botanical and geographical origin. Clearly, there is a highly significant correlation between the TPC and TFC ($r = 0.8709$, $p < 0.001$). Findings from previous studies regarding the TPC and AA have been contradictory. Some authors found no significant correlation between these parameters (Bueno Costa *et al.*, 2016; Stagos *et al.*, 2018), while others found a significant correlation (Al *et al.*, 2009; Alvarez-Suarez *et al.*, 2010; Pontis *et al.*, 2014). The reasoning behind is that for some plants, the AA depends not only on the quantity of phenolics, but mainly on the chemical composition of phenolic compounds (Stagos *et al.*, 2012), which is then reflected onto the chemical profile of honey. In this study, the TPC and TFC showed a highly significant or significant correlation to the results obtained by both antioxidant assays.

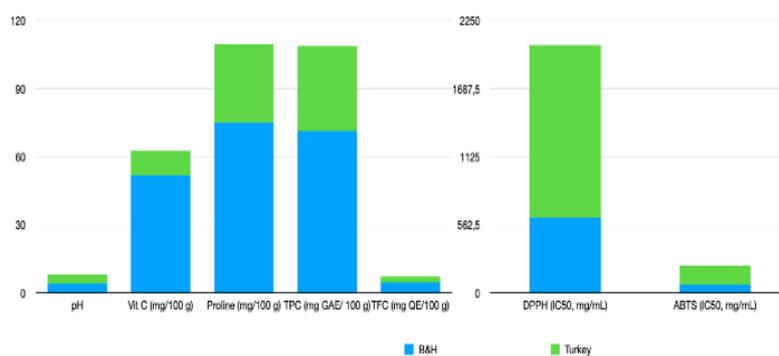


Figure 2. Comparison of physicochemical parameters in honey samples from B&H and Turkey.

Table 3. Pearson's correlation coefficients among antioxidant activity and other investigated parameters

	TPC	TFC	DPPH	ABTS	Colour	Proline	Ascorbic acid
TPC	1						
TFC	0.879*	1					
DPPH	-0.872*	-0.847*	1				
ABTS	-0.783*	-0.676**	0.979*	1			
Colour	0.678**	0.910*	-0.772**	-0.449	1		
Proline	0.628**	0.616**	-0.447	-0.547**	0.557**	1	
Ascorbic acid	0.297	0.295	-0.262	-0.381	0.065	0.109	1

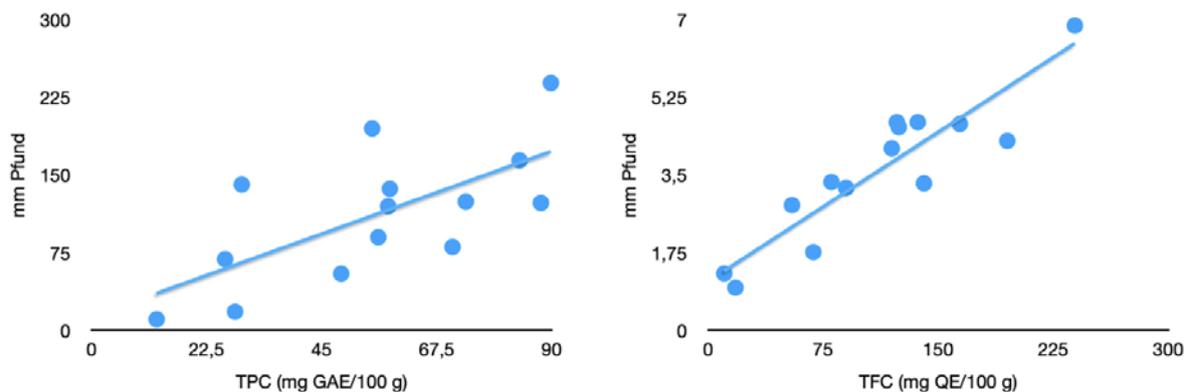
* - highly significant ($p < 0.001$), ** - significant ($p < 0.05$)

Judging by the correlation coefficient between the ABTS and DPPH ($r = 0.979$, $p < 0.001$), both assays are equally suitable for measurements of the radical scavenging capacity in honey. The possible relationship between phenolics, flavonoids, AA and colour has also been commonly investigated and demonstrated (Pontis *et al.*, 2014; Ferreira *et al.*, 2009; Khalil *et al.*, 2012). Numerous studies have shown that in comparison to light coloured honeys, dark honeys demonstrate a higher TPC and are therefore characterised by a stronger AA (Pontis *et al.*, 2014; Perna *et al.*, 2013; Cabrera *et al.*, 2017). At the significance level of $p < 0.05$, the TPC and the colour of the analysed samples had a correlation of $r = 0.678$, however there was a stronger association ($r = 0.910$, $p < 0.001$) between the colour and the TFC content, as shown in Fig. 3.

The colour of honeys is due to various pigments that are chemically classified as flavonoids (Havsteen, 2002), which explains the obtained results. Interestingly, the AA was significantly correlated to the honey colour only when determined by the DPPH assay. When interpreting

honey samples from Turkey it is important to note that they were predominantly light-coloured honeys which, as can be seen from the correlation matrix, implies a lower phenolics content and limited antioxidant defence.

Several authors suggested the total proline content to be a critical factor responsible for the AA of honey (Khalil *et al.*, 2012; Saxena, Gautam and Sharma, 2010). Our study confirmed these findings showing lower but significant correlation between proline content and TPC, TFC, colour and the AA determined by the DPPH assay. Ascorbic acid content in Algerian honey has been found to be associated with flavonoids, colour and DPPH scavenging activity (Khalil *et al.*, 2012), however our results differ. There was no correlation between ascorbic acid and any of the variables presented in Table 3. Among the investigated samples, the best example that follows the correlation matrix is honeydew, which is dark amber in colour, and characterised by the highest TPC and TFC values and the strongest AA. Concluding from the same parameters, polyfloral honeys were of better quality than monofloral honeys.

**Figure 3.** Correlation between colour and A) TPC and B) TFC.

CONCLUSION

Based on the results obtained from the analysis of 15 honey samples of different botanical and geographical origin it can be concluded that the following parameters are strongly associated with the antioxidant activity: colour, TPC, TFC and proline. The colour of honey is influenced by the pigments which are in chemical terms classified as flavonoids. The obtained results confirmed that dark honeys are rich in phenolics and particularly in flavonoids. There was an excellent correlation between the DPPH and ABTS antioxidant assays ($r = 0.979$, $p < 0.001$), however DPPH showed a better correlation with other parameters and can therefore be used preferentially. Our study also confirmed the total proline content is a parameter directly associated with the AA of honey, however ascorbic acid was not found to be in correlation with any of the remaining variables related to AA. The honey samples from Bosnia were richer in phenolics and of stronger AA compared to the samples from Turkey, which may be a consequence of the variety of the collected samples. Dark amber was the predominant colour of Bosnian honeys, whereas the Turkish honey had a higher share in light coloured samples. The novelty of the study is also in the description of the antioxidant properties of Bosnian honey evaluated by the selected parameters. Bosnian honey, containing significant amounts of compounds related to the AA, can consequently be recommended for use for its beneficial health effects. Polyfloral samples were found to have better antioxidant properties compared to monofloral honeys, whereas such a difference was not evident between the commercially purchased honeys and honeys obtained from beekeepers.

REFERENCES

- Al, M. L., Daniel, D., Moise, A., Bobis, O., Laslo, L., Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry*, 112(4), 863–867. <https://doi.org/10.1016/j.foodchem.2008.06.055>
- Al-Mamary, M., Al-Meer, A., Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research*, 22(9), 1041–1047. [https://doi.org/10.1016/S0271-5317\(02\)00406-2](https://doi.org/10.1016/S0271-5317(02)00406-2)
- Alvarez-Suarez, J. M., Giampieri, F., González-Paramás, A. M., Damiani, E., Astolfi, P., Martínez-Sánchez, G., Bompadre, S., Quiles, J. L., Santos-Buelga, C., Battino, M. (2012). Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food and Chemical Toxicology*, 50(5), 1508–1516. <https://doi.org/10.1016/j.fct.2012.01.042>
- Alvarez-Suarez, J. M., Tulipani, S., Díaz, D., Estevez, Y., Romandini, S., Giampieri, F., Damiani, E., Astolfi, P., Bompadre, S., Battino, M. (2010). Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with colour, polyphenol content and other chemical compounds. *Food and Chemical Toxicology*, 48(8–9), 2490–2499. <https://doi.org/10.1016/j.fct.2010.06.021>
- Alvarez-Suarez, J., Tulipani, S., Romandini, S., Vidal, A., Battino, M. (2009). Methodological Aspects about Determination of Phenolic Compounds and In Vitro Evaluation of Antioxidant Capacity in the Honey: A Review. *Current Analytical Chemistry*, 5(4), 293–302. <https://doi.org/10.2174/157341109789077768>
- Arvouet-Grand, A., Vennat, B., Pourrat, A., Legret, P. (1994). Standardisation d'un extrait de propolis et identification des principaux constituants [Standardization of propolis extract and identification of principal constituents]. *Journal de pharmacie de Belgique*, 49(6), 462–468.
- Beretta, G., Granata, P., Ferrero, M., Orioli, M., Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*, 533(2), 185–191. <https://doi.org/10.1016/j.aca.2004.11.010>
- Belitz, H.-D., Grosch, W., Schieberle, P. (2004). Sugars, Sugar Alcohols and Honey. In *Food Chemistry* (pp. 862–891). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-07279-0_20
- Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, 181(4617), 1199–1200. <https://doi.org/10.1038/1811199a0>
- Bogdanov, S., Jurendic, T., Sieber, R., Gallmann, P. (2008). Honey for nutrition and health: A review. *Journal of the American College of Nutrition*, 27(6), 677–689. <https://doi.org/10.1080/07315724.2008.10719745>
- Bogdanov, S., Lüllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., Oddo, L. P., Sabatini, A. G., Marcazzan, G. L., Piro, R., Flamini, C., Morlot, M., Lhéritier, J., Borneck, R., Marioleas, P., Tsigouri, A., Kerkvliet, J., Ortiz, A., Ivanov, T., ... Vit, P. (1999). Honey quality and international regulatory standards: Review by the international honey commission. *Bee World*, 80(2), 61–69. <https://doi.org/10.1080/0005772x.1999.11099428>
- Bogdanov, S., Martin, P., Lüllmann, C. (1997). Harmonised methods of the European Honey Commission. *Apidologie*, 28, 1–59.
- Bogdanov, S., Martin, P., Lullmann, C. (2002). Harmonised methods of the international honey commission. *Swiss Bee Research Centre, FAM, Liebefeld*.
- Bueno-Costa, F. M., Zambiazzi, R. C., Bohmer, B. W., Chaves, F. C., Silva, W. P. da, Zanusso, J. T., Dutra, I. (2016). Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT - Food Science and Technology*, 65, 333–340. <https://doi.org/10.1016/j.lwt.2015.08.018>
- Cabrera, M., Perez, M., Gallez, L., Andrada, A., Balbarrey, G. (2017). Colour, antioxidant capacity, phenolic and flavonoid content of honey from the Humid Chaco Region, Argentina. *Phyton*, 86(1), 124–130. <https://doi.org/10.32604/phyton.2017.86.124>

- Cruz, L. C., Batista, J. E. S., Zemolin, A. P. P., Nunes, M. E. M., Lippert, D. B., Royes, L. F. F., Soares, F. A., Pereira, A. B., Posser, T., Franco, J. L. (2014). A study on the quality and identity of Brazilian Pampa biome honey: Evidences for its beneficial effects against oxidative stress and hyperglycemia. *International Journal of Food Science*, 2014. <https://doi.org/10.1155/2014/470214>
- Delmoro, J., Muñoz, D., Nadal, V., Pranzetti, V. (2010). El Colour En Los Alimentos: Determinación De Colour En Miel. *Invenio*, 13(25), 145–152.
- Fell, R.D. (1978). Colour grading of honey. *American Bee Journal*, 118(12), 782-789.
- Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M., Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, 114(4), 1438–1443. <https://doi.org/10.1016/j.foodchem.2008.11.028>
- Gheldof, N., Wang, X. H., Engeseth, N. J. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry*, 50(21), 5870–5877. <https://doi.org/10.1021/jf0256135>
- Gomes, T., Feás, X., Iglesias, A., Estevinho, L. M. (2011). Study of organic honey from the northeast of Portugal. *Molecules*, 16(7), 5374–5386. <https://doi.org/10.3390/molecules16075374>
- Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics*, 96(2), 67–202. [https://doi.org/https://doi.org/10.1016/S0163-7258\(02\)00298-X](https://doi.org/https://doi.org/10.1016/S0163-7258(02)00298-X)
- Kesić, A., Mazalović, M., Crnkić, A., Čatović, B., Hadžidedić, Š., Dragošević, G. (2009). The influence of L-ascorbic acid content on total antioxidant activity of bee-honey. *European Journal of Scientific Research*, 32(1), 96–102.
- Khalil, M. I., Moniruzzaman, M., Boukraâ, L., Benhanifia, M., Islam, M. A., Islam, M. N., Sulaiman, S. A., Gan, S. H. (2012). Physicochemical and antioxidant properties of algerian honey. *Molecules*, 17(9), 11199–11215. <https://doi.org/10.3390/molecules170911199>
- Kıvrak, Ş., Kıvrak, İ. (2017). Assessment of phenolic profile of Turkish honeys. *International Journal of Food Properties*, 20(4), 864–876. <https://doi.org/10.1080/10942912.2016.1188307>
- Lachman, J., Orsák, M., Hejtmánková, A., Kovářová, E. (2010). Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT - Food Science and Technology*, 43(1), 52–58. <https://doi.org/10.1016/j.lwt.2009.06.008>
- León-Ruiz, V., Vera, S., González-Porto, A. V., Andrés, M. P. S. (2011). Vitamin C and sugar levels as simple markers for discriminating spanish honey sources. *Journal of Food Science*, 76(3). <https://doi.org/10.1111/j.1750-3841.2011.02041.x>
- León-Ruiz, V., Vera, S., González-Porto, A. V., Andrés, M. P. S. (2013). Analysis of Water-Soluble Vitamins in Honey by Isocratic RP-HPLC. *Food Analytical Methods*, 6(2), 488–496. <https://doi.org/10.1007/s12161-012-9477-4>
- Manyi-Loh, C. E., Ndip, R. N., Clarke, A. M. (2011). Volatile compounds in honey: A review on their involvement in aroma, botanical origin determination and potential biomedical activities. *International Journal of Molecular Sciences*, 12(12), 9514–9532. <https://doi.org/10.3390/ijms12129514>
- Meda, A., Lamien, C. E., Romito, M., Millogo, J., Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, 91(3), 571–577. <https://doi.org/10.1016/j.foodchem.2004.10.006>
- Nayik, G. A., Suhag, Y., Majid, I., Nanda, V. (2018). Discrimination of high altitude Indian honey by chemometric approach according to their antioxidant properties and macro minerals. *Journal of the Saudi Society of Agricultural Sciences*, 17(2), 200–207. <https://doi.org/10.1016/j.jssas.2016.04.004>
- Oddo, L. P., Piro, R. (2004) Main European unifloral honeys: descriptive sheets. *Apidologie*, 35(Suppl. 1), S38-S81. <https://doi.org/10.1051/apido:2004049>
- Ough, C. S. (1969). Rapid Determination of Proline in Grapes and Wines. *Journal of Food Science*, 34(3), 228–230. <https://doi.org/10.1111/j.1365-2621.1969.tb10327.x>
- Perna, A., Simonetti, A., Intaglietta, I., Gambacorta, E. (2013). Antioxidant properties, polyphenol content and colourimetric characteristics of different floral origin honeys from different areas of Southern Italy. *Journal of Life Sciences*, 7(4), 428-436.
- Piazza, M. G., Oddo, L. P. (2004). Bibliographical review of the main European unifloral honeys. *Apidologie*, 35(Suppl. 1), S94-S111. <https://doi.org/10.1051/apido:2004046>
- Piljac-Žegarac, J., Stipčević, T., Belščak, A. (2009). Antioxidant properties and phenolic content of different floral origin honeys. *Journal of ApiProduct and ApiMedical Science*, 1(2), 43-50. <https://doi.org/10.3896/ibra.4.01.2.04>
- Pontis, J. A., da Costa, L. A. M. A., da Silva, S. J. R., Flach, A. (2014). Colour, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology*, 34(1), 69–73. <https://doi.org/10.1590/S0101-20612014005000015>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biology and Medicine*, 26(9), 1231–1237. [https://doi.org/https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/https://doi.org/10.1016/S0891-5849(98)00315-3)
- Saxena, S., Gautam, S., Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, 118(2), 391–397. <https://doi.org/10.1016/j.foodchem.2009.05.001>

- Singleton, V. L., Rossi, J. A. (1965). Colourimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144 LP – 158
- Stagos, D., Soultisiotis, N., Tsadila, C., Papaconomou, S., Arvanitis, C., Ntontos, A., Karkanta, F., Adamou-Androulaki, S., Petrotos, K., Spandidos, D. A., Kouretas, D., Mossialos, D. (2018). Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *International Journal of Molecular Medicine*, 42(2), 726–734. <https://doi.org/10.3892/ijmm.2018.3656>
- The National Honey Board. (2002). Honey – health and therapeutic qualities. Available from: <https://www.biologiq.nl/UserFiles/Compendium%20Honey%202002.pdf>.
- Turkmen, N., Sari, F., Poyrazoglu, E. S., Velioglu, Y. S. (2006). Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry*, 95(4), 653–657. <https://doi.org/10.1016/j.foodchem.2005.02.004>
- Udenigwe, C. C., Aluko, R. E. (2011). Chemometric analysis of the amino acid requirements of antioxidant food protein Hydrolysates. *International Journal of Molecular Sciences*, 12(5), 3148–3161. <https://doi.org/10.3390/ijms12053148>
- Wu, X., Diao, Y., Sun, C., Yang, J., Wang, Y., Sun, S. (2003). Fluorimetric determination of ascorbic acid with o-phenylenediamine. *Talanta*, 59(1), 95–99. [https://doi.org/10.1016/S0039-9140\(02\)00475-7](https://doi.org/10.1016/S0039-9140(02)00475-7)
- Wu, H. C., Shiau, C. Y., Chen, H. M., Chiou, T. K. (2003). Antioxidant activities of carnosine, anserine, some free amino acids and their combination. *Journal of Food and Drug Analysis*, 11(2), 148–153. <https://doi.org/10.38212/2224-6614.2720>
- Yao, L. H., Jiang, Y. M., Shi, J., Tomás-Barberán, F. A., Datta, N., Singanusong, R., Chen, S. S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*, 59(3), 113–122. <https://doi.org/10.1007/s11130-004-0049-7>

Summary/Sažetak

Cilj ovog rada je bilo odrediti korelaciju između antioksidativne aktivnosti uzoraka meda i sljedećih parametara: boja, ukupan sadržaj fenola (TPC), ukupan sadržaj flavonoida (TFC), sadržaj prolina i askorbinske kiseline. Uzorci su sakupljeni u Bosni i Hercegovini i Turskoj, a obuhvatali su mednu rosu, monofloralne i polifloralne medne vrste. Antioksidativna aktivnost je određena ABTS i DPPH metodom. Na osnovu korelacionog matriksa utvrđena je dobra korelacija antioksidativne aktivnosti sa TPC, TFC, sadržajem prolina i bojom meda. Boja meda je bila u najboljoj korelaciji sa TFC ($r = 0.910$, $p < 0.001$), pri čemu su se tamniji medovi odlikovali višim TFC vrijednostima. Antioksidativna aktivnost je bila značajno ovisna o TPC (DPPH-TPC: $r = -0.872$; ABTS-TPC: $r = -0.783$, $p < 0.001$). Sadržaj askorbinske kiseline se nije pokazao kao prediktivan parametar koji bi se koristio za procjenu antioksidativnih svojstava meda, te nije značajno korelirao ni sa drugim varijablama.