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Physicochemical properties, total phenolic content, and antioxidant activity of the honey samples from the Herzegovina region

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Abstract

Honey is a natural product that is an excellent source of energy containing mainly carbohydrates and water, as well as small amounts of organic acids, vitamins, minerals, flavonoids, and enzymes. Due to the presence of bioactive compounds, it has been shown that honey is beneficial for many diseases, e.g. gastrointestinal diseases, skin diseases, cancer, heart diseases, and neurological degeneration. The study of the physical and chemical properties of honey and the content of bioactive compounds has been increasingly applied in order to determine the quality of honey samples. The aim of this study is to investigate physicochemical properties as well as the total phenol content and antioxidant activity of seven multifloral honey samples from the Herzegovina region. Physicochemical parameters determined in the honey samples (moisture, acidity, electrical conductivity, reducing sugars, sucrose, and insoluble matter) were within the quality standard limits of the Regulation on methods for control of honey and other bee products. Total phenolic content was determined using the Folin-Ciocalteu method and it ranged from 46.98 ± 6.36 to 152.94 ± 4.95 mg GAE/100 g of honey. To determine the antioxidant activity of the honey samples, two methods, FRAP and ABTS, were used. The total phenolic content of honey correlated positively with its antioxidant activity.

Key words: bioactive compounds, total phenol content, FRAP, ABTS, physicochemical parameters

Introduction

Honey is a natural, sweet substance that has been used by people as a sweetener for ages. Even though the health benefits of honey were known from early ancient times, extensive research of biological and chemical properties of honey started in the 1970s. Honey is a natural product that is composed of more than 180 substances whose composition and its properties change with regard to botanical origin, geographical origin, season, environment, treatment of bees (by beekeepers) and with regard to the storage method (de Rodriguez at al., 2004). Carbohydrates account for 73-83% of the total mass of honey, of which 85% of the total carbohydrates include the most represented sugars in honey: glucose and fructose. In addition to simple sugars, honey also contains disaccharides such as sucrose, maltose, isomaltose, turanose, and nigerose and some trisaccharides and oligosaccharides (Ahmed et al., 2018; Rahman et al., 2013). Vitamins and minerals are minor components of honey (0.04% -0.2%). Honey also contains amino acids, proteins, and enzymes such as glucose oxidase, sucrose diastase, and invertase. The colour, taste, and functional properties of honey are determined by the composition of polyphenols rather than variations of carbohydrates and proteins. (Bertoncelj et al., 2007; Yordi et al., 2012). Polyphenols are bioactive substances that are widely spread in plants containing flavonoids, phenolic acids, lignans, and stilbenes. Flavonoids and phenolic acids, which act as antioxidants, are present in honey. The group of flavonoids within honey encompasses flavonols, flavons, and flavanones. Among the phenolic acids detected in honey, one finds hydroxybenzoic acids, hydroxycinnamic acids, and hydroxyphenylacetic acids (Ahmed et al., 2018). The presence of flavonoids and phenolic acids in honey significantly contributes to human health owing to high antioxidant and anti-inflammatory properties they possess.

The quality of honey in Bosnia and Herzegovina is regulated by the Regulation on methods for control of honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09). The regulation prescribes sampling, storage, as well as methods of physical, chemical, and biological analysis for determining the quality of honey and bee products. The minimum and maximum values of certain parameters are also defined. With regard to ripeness, common physicochemical parameters are determined such as the content of reduced sugars, sucrose and moisture, and acidity, while to determine purity, electrical conductivity and water-insoluble matter are determined.

The chemical composition and the content of bioactive compounds differentiate honeys and define their health benefits. Therefore, it is important to

investigate bioactive compounds in honey. The aim of this study is to investigate the quality of honey samples from different locations in the Herzegovina region and to determine the total phenolic content as well as antioxidant activity of these samples.

Material and Methods

Sample collection

Seven samples of honey were collected from beekeepers in 2022 at different sampling sites in the Herzegovina region (Table 1). The major plant species at selected sampling sites were dandelion, mountain germander, breck and thyme, sage, heather, lavender, nettle, clover, saffron, and hawthorn. The honey samples were kept in glass jars in dark places at room temperature until the analysis.

Table 1. Locations of honey sampling sites

Sample	Location	Latitude and longitude of sampling sites
1	Borač, Gacko	43°18′31.9″N 18°34′55.8″E
2	Bošnjaci Bijelo polje, Mostar	43° 25' 52.5288" N 17° 53' 32.064" E
3	Gornja Duboka, Stolac	43° 0' 49.6476" N 17° 57' 35.0172" E
4	Podveležˇ	43° 18' 21.7764" N 17° 54' 44.352" E
5	Orahovica, Stolac	43° 3' 42.9048" N 17° 53' 39.2928" E
6	Bokševica, Jablanica	43° 43' 39.882" N 17° 46' 48.3492" E
7	Gornje Hrasno, Neum	42° 57' 52.128" N 17° 54' 1.26" E

Physicochemical parameters

The physicochemical parameters were determined (moisture, acidity, electrical conductivity, reducing sugars, sucrose, and insolubile matter) according to the Regulation on methods for control of honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09).

Total phenolic content (TPC)

10 g of honey was dissolved in 100 ml of distilled water. The Folin-Ciocalteu method was used to determine the total phenolic content as described by Singletton et al. (1999) with some modifications. 100 µl of the honey solution was mixed with 900 µl of distilled water. 5 ml of diluted a Folin-Ciocalteu reagent (1:10) was added to this mixture and left for 5 minutes at room temperature. Thereafter, 4 ml of sodium carbonate (15%) was added. The mixture was shaken and left undisturbed for two hours in the dark at room

temperature. The absorbance was measured at 765 nm using a Shimadzu UV-1800 spectrophotometer. Gallic acid was used to prepare a calibration curve, and the results were expressed as milligrams of gallic acid equivalents per 100 g of honey (mg GAE/100 g honey).

Ferric reducing antioxidant power (FRAP) assay

10 g of honey was dissolved in 50 ml of distilled water. A modified FRAP method was used to determine the antioxidant activity as described by Re et al. (1999). FRAP reagent (3.8 mL) was added to 200 µl of honey sample. The mixture was incubated for 4 minutes, and the absorbance was measured at 593 nm using the Shimadzu UV-1800 spectrophotometer. The results were expressed as millimoles of Fe2+ equivalents per 100 mg of honey (mmol Fe2+/100 mg honey).

Antioxidant activity using ABTS•+ assay

15 g of honey was dissolved in 50 ml of distilled water. Determination of the antioxidant activity with the ABTS•+ reagent (2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) was carried out following the method by Re et al. (1999) with some modifications. The honey sample (40 µL) was mixed with the ABTS•+ radical solution (4 mL) and left to stand for 6 minutes at room temperature. The absorbance was measured at 734 nm using the Shimadzu UV-1800 spectrophotometer and results were expressed as millimoles Trolox equivalents per 100 mg of honey (mmol TE/100 mg honey).

Statistical analysis

All measurements are expressed as mean ± standard deviations. The statistical differences are considered significant at $p < 0.05$. All the analyses were done in triplicates.

Results and Discussion

Physicochemical parameters of honey

The physicochemical parameters of the honey samples under study are shown in Table 2. Monitoring honey quality is an important tool for the protection and regulation of the honey market, but also classification of honey quality through physicochemical analysis can be of the greatest importance. The physicochemical parameters can help to determine whether the product is defined within the prescribed quality limits and to categorize the honey itself depending on its geographical and botanical origin.

Table 2. Physicochemical parameters of honey samples

Sample	Moisture %t	Acidity (mEq/kg)	Electrical conductivity (mS/cm)	Reducing sugars (%)	Sucrose (%)	Insoluble matter (%)
1	13.40±0.03	22.13±1.44	0.70±0.07	16.32±0.31	1.27±0.17	0.01±0.001
2	13.06±0.05	25.01±1.42	0.35±0.04	11.52±0.52	1.10±0.09	0.09±0.001
3	13.69±0.73	26.77±0.94	0.48±0.22	17.13±0.78	2.65±0.48	0.03±0.02
4	14.21±1.68	26.44±0.52	0.38±0.09	17.12±0.02	1.62±0.16	0.02±0.001
5	14.06±0.77	34.22±0.18	0.99±0.14	18.72±0.23	3.99±0.18	0.03±0.03
6	13.75±0.49	33.75±0.51	1.22±0.32	25.11±1.18	2.28±0.14	0.005±0.007
7	13.51±0.43	32.47±0.85	0.75±0.05	20.32±0.80	4.78±0.55	0.01±0.002

The moisture content is an important parameter since it influences thickness, specific weight, crystallization, and honey taste. It can affect fermentation since high values of moisture contribute to faster honey fermentation (Singh & Singh, 2018). The moisture content in the honey samples analyzed ranged from 13.06 ± 0.05 to 14.21 ± 1.68%, which is in accordance with values defined by the Regulation on honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09) (max 20%). Lower moisture values of the honey samples investigated were due to the fact that samples were harvested in the summer when honey dehydrates much faster.

a maximum permitted value by the Regulation of honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09). This parameter is also considered as a quality parameter since it corresponds to the content of organic acids and influences the fermentation of honey.

Electrical conductivity is considered as a good parameter for the assessment of the botanical origin of honey. It assesses all insoluble organic and inorganic substances and is connected to the concentration of organic acids, proteins, and mineral salts (da Silva et al., 2016). In the honey samples investigated electrical conductivity was in the range from 0.35 ± 0.04 to 1.22±0.32 mS/cm. Two investigated honey samples (5 and 6) had higher values than 0.8 mS/cm, which indicated different origin of these honey samples compared to the others (0.99 ± 0.14 and 1.22 ± 0.32 mS/cm). It was previously reported that higher values of electrical conductivity and higher acidity can be detected in darker honey (Yadata, 2014; Prica et al., 2014). Other studies also reported that electrical conductivity depended on ash and acid content (Rysha et al., 2022; Sakač et al. 2022). Honey samples 5 and 6 had higher acidity than other samples probably due to their content of organic acids or bee secretions (Yadata, 2014).

The proportion of sucrose and reducing sugars in all honey samples analyzed are in accordance with the Regulation of honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09).

Insoluble matter is a measure of the presence of impurities in honey. The lowest insoluble matter 0.005% was found in sample 5 collected in Orahovica, Stolac. The highest value was obtained in sample 2 collected in Bošnjaci Bijelo polje, Mostar (0.09 ± 0.001). All values are under the maximum acceptable content of insoluble matters with a value not higher than 0.1%, which suggests that all honey samples tested were clean.

Total phenolic content and antioxidant activity of honey

Honey serves as a natural source of polyphenol compounds, making them valuable substances for potential use in pharmaceuticals for treating cardiovascular diseases. (Olas, 2020). Total phenolic content ranged from 46.98 ± 6.36 to 152.94 ± 4.95 mg GAE/100 g of honey in the honey samples investigated (Table 3). The results indicated that the total phenolic contents in the honey samples were significantly different.

Table 3. Total phenolic content and antioxidant activity of honey samples

Sample	TPC (mg GAE/100 g honey)	FRAP (mmol Fe^{2+}/100 mg honey)	ABTS (mmol TE/100 mg honey)
1	69.44 ± 0.00^{b}	29.49 ± 1.05^{e}	426.77 ± 105.23^{c}
2	46.98 ± 6.36^{a}	16.36 ± 0.00^{a}	104.01 ± 1.88^{a}
3	74.97 ± 4.95^{c}	22.94 ± 0.80^{c}	357.53 ± 63.86^{b}
4	114.41 ± 7.77^{e}	20.24 ± 2.05^{b}	704.84 ± 58.28^{f}
5	131.88 ± 9.18^{f}	38.83 ± 0.92^{f}	661.50 ± 58.21^{e}
6	152.94 ± 4.95^{g}	41.79 ± 0.38^{g}	7264.54 ± 67.50^{g}
7	82.43 ± 12.72^{d}	24.57 ± 0.92^{d}	593.09 ± 163.54^{d}

*Values with different letters in the same column are significantly different at $p < 0.05$.

The highest phenolic content and antioxidant activity determined with the FRAP and ABTS methods were found in the sample collected in Bokševica, Jablanica, while the lowest was detected in the honey collected in Bošnjaci, Bijelo Polje. Out of all investigated samples, the honey sample collected in Bokševica, Jablanica had the darkest colour. Alves et al. (2013) reported that light honeys have a lower concentration of total phenols. Phenolic compounds contribute to the formation of honey browning by interacting with and attaching themselves to the existing high molecular weight polymers (Brudzynski & Miotto, 2011).

Sakač et al. (2022) determined the total phenolic content in honey ranged from 16.8 ± 0.50 to 26.5 ± 0.76 mg GAE/100 g of honey, which is lower than the results of our research. Atanacković Krstonošić et al. (2019) analyzed phenolic content in mono and polyfloral honeys from Serbia and reported total phenolic content in the range from 23.94 ± 1.26 to 50.90 ± 0.85 mg GAE/100 g of honey. These findings are also lower than those reported in our research. This can be explained by noting that the phenolic content in honey depends on its floral origin. Phenolic content can be used as a tool for classification and authentication of the type of honey especially for monofloral types of honey (Cianciosi et al., 2018).

Honey can prevent and reduce incidence of various diseases caused by oxidative stress due to its antioxidative potential (Larsen & Ahmed, 2022). The antioxidant activity of the honey samples determined by the FRAP assay ranged from 16.36 ± 0.00 to 41.79 ± 0.38 (mmol Fe2+/100 mg of honey). Maksimović & Nedić (2013) investigated antioxidant activity in multifloral honey samples from Serbia by the FRAP method and reported lower values than those obtained in this work. Dżugan et al. (2018) reported that dark honeys show better antioxidant activity as compared with light honeys.

Antioxidant activity of honey samples was evaluated by the ABTS method and was in the range from 104.01 ± 1.88 to 7264.54 ± 67.50 mmol TE/100 mg of honey. Lower values of antioxidant activity in the multifloral Polish honey were reported by Kedzierska-Matysek et al. (2021) (2.9 ± 1.17 mM TE/100 g). The antioxidant activity of honey depends on several factors, such as geographic origin, collection season, mode of storage, bee species, and even interactions between chemical compounds and enzymes in honey (Starowicz et al., 2021). Antioxidant properties of honey are mainly attributed to the presence of phenolic compounds (Beretta et al. 2005; Moniruzzaman et al., 2012). The correlation between total phenolic content and antioxidant activity was statistically significant ($r = 0{,}8753$ and $r = 0{,}7393$, $p < 0.01$).

Conclusion

The majority of the physicochemical parameters assessed in the honey samples, principally moisture, acidity, electrical conductivity, reducing sugars, sucrose, and insoluble matter complied with the quality standard limits set by the Regulation of honey and other bee products (Official Gazette of Bosnia and Herzegovina, No. 37/09). However, the values of the electrical conductivity of 2 investigated samples were higher than 0.8 mS/cm, thus indicating that these two samples belong to the different type of honey than other investigated samples.

Our results indicate that out of the seven honey samples, the honey collected at Bokševica, Jablanica location (Sample no.6) exhibited the highest

total phenol content and the highest antioxidant activity. The total phenolic contents of the investigated honey samples from the Herzegovina region were found to be higher compared to similar studies done elsewhere. The antioxidant activity of the honey samples correlated positively with the total phenolic content. After comparing these results with other studies, it can be concluded that the phenolic content and antioxidant activity of honey samples depends on many factors such as geographic origin, type of honey, seasonality, bee species, etc.

As a natural product rich in polyphenols and antioxidants, honey can be suggested for regular consumption and use in food industries.

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Физичко-хемијска својства, укупни феноли и антиоксидативна активност узорака меда са подручја Херцеговине

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Сажетак

Мед је природни производ који је одличан извор енергије који садржи твари углавном угљикохидрате и воду, као и мале количине органских киселина, витамина, минерала, флавоноида и ензима. Због присуства и биоактивних спојева, мед се учинковито користи у лијечењу многих болести, нпр. гастроинтестиналне болести, кожне болести, карцином, срчане болести и неуролошка дегенерација. Проучавање физичко-хемијских својстава меда и садржаја биоактивних спојева све се више примјењује у сврху одређивања квалитете узорака меда. Циљ овог рада је истражити физичко-хемијска својства, те укупни садржај фенола и антиоксидативно дјеловање седам узорака мултифлорног меда с подручја Херцеговине. Физичко-хемијски параметри утврђени у узорцима меда (влага, киселост, електрична проводљивост, редукујући шећери, сахароза и нерастворљиве материје) били су у границама стандарда квалитета Правилника о методама контроле меда и других пчелињих производа. Укупни садржај фенола одређен је Folin-Ciocalteu методом и кретао се од 46.98 ± 6.36 до 152.94 ± 4.95 mg GAE/100 g меда. За одређивање антиоксидативне активности узорака меда кориштене су двије методе, FRAP и ABTS. Укупни садржај фенола у меду позитивно је корелирао с његовом антиоксидативном активношћу.

Кључне ријечи: биоактивне супстанце, садржај укупних фенола, FRAP, ABTS, физичко-хемијски параметри

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