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ANTIOXIDANT CAPACITY OF FLOWER HONEY FROM THE MIDDLE PODRINJE AREA

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ABSTRACT

The aim of this study was to examine the concentration of total phenols and antioxidant capacity of flower honey from the Middle Podrinje area (Bosnia and Herzegovina). The research included a total of 12 samples of flower honey from two municipalities, Srebrenica and Milići. The concentration of total phenols was determined by the Folin-Cicolteu method and the antioxidant capacity by DPPH and FRAP methods. The results of the research showed that the samples of flower honey from the Srebrenica municipality have a higher concentration of total phenols compared to the honeys from the Milići municipality. The antioxidant capacity determined by the DPPH method was from 4.69 to 72.22 mg/ml, and by the FRAP method from 268.7 to 831.7 μM Fe (II). The T test showed a statistically significant difference for the antioxidant capacity of DPPH between the mean values for flower honey samples between the municipalities of Srebrenica and Milići. Perason's coefficient showed a high positive correlation between the antioxidant capacity determined by the FRAP method and the concentration of total phenols.

KEYWORDS: *flower honey, total phenols, antioxidant capacity, Middle Podrinje region*.

1. INTRODUCTION

Honey is an important source of macro- and micronutrients, which bees collect from flower nectars or from secretions made by some insects that live on plants and store in the honeycomb exposing them to enzymatic change. Honey has a strong antioxidant effect because it is a rich source of phytochemicals such as flavonoids and phenols. The total composition of honey as well as the phenolic composition of honey mainly depends on its floral origin, geographical area and processing [1,2,3,4,5]. The composition of phenols can be used as a tool for classification and authentication, especially in the case of single-flowered varieties [4]. Antioxidant capacity of honey depends on floral and geographical origin, climatic conditions and processing, storage and handling of honey [6]. Antioxidant capacity is a measure that demonstrates the ability to reduce and stop harmful oxidative reactions in both food and body. Phenolic compounds are mainly responsible for the antioxidant properties of honey [7,8].

Flower honey is one of the most common types of honey which is produced and consumed in Bosnia and Herzegovina. It can originate from different environments, mountain meadows or lowland areas that are rich in local honey plants.

Flower honey samples from the Middle Podrinje area were examined in this paper, from the two municipalities of Srebrenica and Milići.

2. MATERIALS AND METHODS

2.1. Material

The research included 12 samples of meadow/flower honey collected from beekeepers in 2018, from the middle Podrinje area, Srebrenica and Milići municipalities.

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2.2. Methods

2.2.1. Determining phenol content

The concentration of total phenols was read at 750 nm on a spectrophotometer by the Folin-Ciocalteau method, and the total amount of phenol was calculated as the equivalent of a mg of gallic acid in the extract of 100 grams.

2.2.2. Antioxidant capacity

DPPH method

Determination of the total antioxidant effect in the samples was made according to the free radical scavenging effects of the prepared extracts on DPPH (1,1-diphenyl-2-picrylhydrazyl) in honey by the indirect method. A determined volume of the sample (approximately 10-100 µl) is supplemented with methanol to 2 ml, and 0.5 ml of 0.5 Mm DPPH solution is added to this solution. The mixture should be shaken vigorously and incubated for 30 minutes in the dark at room temperature. Absorbance was measured at 517 nm with methanol as a blank test. The control is 1 ml of 0.5 mM DPPH solution diluted with 4 ml of methanol. Inhibition of DPPH radicals is calculated according to the following equation:

$$I(\%) = \frac{Ak - Aa}{Ak} \cdot 100$$

where
Ak is the absorbance control
Aa is the analysis absorbance

The results are expressed as inhibition of DPPH radicals (%) and as IC50 value (mg/ml), i.e., the concentration of extract that leads to 50% neutralization of DPPH radicals.

FRAP method

Weigh 5 g of honey sample into a glass beaker and dissolve in 20 ml of distilled water and transfer quantitatively to a 50 ml flask and fill up to the mark. A 200 µl sample + 1.8 mL of FRAP reagent was taken for the analysis and incubated for 10 min at 37 ° C. The absorbance was measured at 593 nm with methanol as a blank test. The concentration i.e., µmol/l Fe2+ in the sample, is determined from the equation of the calibration curve.

2.2.3. Statistical analysis

Statistical analysis was performed using SPSS software (version 22). The T-test was used to examine the significance of differences between the arithmetic means of honey samples for phenol content and antioxidant capacity compared to the range, and the Pearson correlation coefficient was used to determine the correlation between physicochemical parameters and antioxidant activity.

3. RESULTS AND DISCUSSION

Table 1 shows the results of the concentration of total phenols and antioxidant capacity of honey samples determined by FRAP and DPPH methods.

Tables:

Table 9. Comparison table for motoring mode

Area	Sample	Total phenols (mg GA /100 g of honey)	FRAP (µM (Fe)(II)) in the 10% honey solution	DPPH, IC_{50} (mg/ml)
Srebrenica	1	33,03 (±4,57)	340,70 (±3,53)	4.90 (±2.30)
	2	48,82 (±2,61)	561,20 (±12,72	6,30 (±2,72)
	3	33,42 (±2,48)	523,20 (±9,89)	4,96 (±2,28)

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	4	31,68 (±2,61)	349,70 (±4,94)	20,97 (±7,98)
	5	35,57 (±3,77)	477,70 (±3,53)	27,47 (±8,33)
	6	61,04 (±2,48)	831,70 (±13,43)	39,43 (±10,17)
Milići	7	41,99 (±2,88)	518,70 (±6,36)	44,89 (±13,74)
	8	20,57 (±0,95)	385,20 (±43,84)	47,12 (±7,64)
	9	31,84 (±0,13)	345,70 (±10,60)	53,51 (±14,33)
	10	38,82 (±5,09)	398,70 (±14,84)	62 (±10,12)
	11	30,58 (±1,58)	268,70 (±2,12)	64,08 (±15,18)
	12	29,14 (±2,48)	304,70 (±6,36)	72,22(±3,67)

The highest concentration of total phenols was in the sample 6 meadow honey from the Srebrenica municipality (61.047 mg/l of honey). Concentrations of total phenols in honey samples are found in the range of 20,571 to 61,047 mg/l of honey. Studies show that darker types of honey contain higher concentrations of total phenols, and thus have a higher antioxidant capacity [9,10,6]Samples from the Srebrenica municipality had a higher concentration of total phenols compared to samples from the Milići municipality. The T-test for the concentration of total phenols did not show a statistically significant difference between the mean values of the samples from Srebrenica and Milići. Analyzing the Pearson coefficient, a moderate negative correlation was observed between the concentration of total phenols and the water content ($r = -0.695$; $p < 0.05$). The results of physico-chemical analysis of flower honey samples from the municipalities of Srebrenica and Milići were published in the work of Brčina et al. [11]. Two methods, FRAP and DPPH, were used to determine antioxidant capacity in the analyzed samples.

Using two methods, FRAP and DPPH methods, it was confirmed that flower honey samples from the Middle Podrinje area have a high antioxidant capacity.

The values obtained by the FRAP method are in the range of 268.7 to 831.7 μM Fe (II). Samples from the Srebrenica municipality had higher values of antioxidant capacity determined by the FRAP method, compared to samples from the municipality of Milići. Botanical origin has the greatest impact on antioxidant activity, while processing, handling and storage have minimal impact. Numerous studies have confirmed that dark honey has a higher antioxidant activity [12]. The T-test did not show a statistically significant difference between the mean values of the samples from Srebrenica and Milići. The results obtained by the DPPH method are expressed as IC_{50} (mg/ml), i.e., as the concentration of honey (mg/ml) required to inhibit 50% of the initial amount of DPPH radicals. This means that the lower the IC_{50} value of the analyzed sample, the higher the antioxidant capacity. The values of the obtained results range from 4.90 to 72.22 mg/ml. Therefore, the highest antioxidant capacity has the sample 1 of meadow honey from the Srebrenica area of 4.90 IC_{50}, and a total phenol content of 33.03 mg of gallic acid/100 g of honey, which is not the highest value of polyphenols. The samples from the Srebrenica area had higher values for the antioxidant capacity of DPPH compared to Milići. The T-test for antioxidant capacity of DPPH showed a statistically significant difference between the mean values of the samples from Srebrenica and Milići. Analyzing the Pearson coefficient, a high negative correlation was observed between the free acid and antioxidant capacity of DPPH ($r = -0.783$; $p<0.01$), and a high positive correlation between the antioxidant capacity determined by the FRAP method and the concentration of total phenols ($r=0.863$; $p<0.01$). These results are consistent with research by other authors [7, 13,14, 15]. The obtained results show that the total phenol content can be part of at least an indicator of the antioxidant activity of honeys from the Middle Podrinje area. The negative coefficient of the Person coefficient of the selected parameters and the DPPH method is due to the fact that the results are expressed via IC_{50}, i.e., the antioxidant capacity is higher the lower the IC_{50} values [15]. Sample 6 from the Srebrenica area had the highest value of antioxidant capacity determined by the FRAP method of 831.7μM Fe (II).

4. CONCLUSION

Based on the conducted analyzes, flower honey samples from the Srebrenica municipality have a higher content of total phenols compared to samples from the Milići municipality. The highest share of phenols was in sample 6

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of meadow honey from the Srebrenica area of. Antioxidant capacity values, determined by DPPH and FRAP methods, samples from Srebrenica municipality are higher than the values for samples from Milići municipality and there is a statistically significant difference for the values obtained by the DPPH method

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