Bulgarian Journal of Agricultural Science, 28 (No 4) 2022, 764–767

Preliminary study of antioxidant activity of polyfloral and sunflower honey from Bulgaria

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Abstract

Balkanska, R. & Shumkova, R. (2022). Preliminary study of antioxidant activity of polyfloral and sunflower honey from Bulgaria. *Bulg. J. Agric. Sci., 28 (4)*, 764–767

Honey is a good source of natural antioxidants. In the present study we aimed to determine the antioxidant activity of the Bulgarian polyfloral and sunflower honey by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assay and total polyphenols. In selected polyfloral (n=20) and sunflower honey samples (n=10) originated from different regions of Bulgaria antioxidant activity and total polyphenol content were determined. For evaluation of the antioxidant activity two different methods were used: FRAP and DPPH assay. Total polyphenol content was determined by the modified Folin-Ciocalteu method. The average antioxidant activity of polyfloral honey samples measured with FRAP assay was higher than in sunflower honey samples. Sunflower honey had higher average DPPH values than polyfloral honey. The results obtained for total polyphenols for the both honey types are almost identical. The present study confirmed the presence of biologically active compounds in the tested honey samples, which are responsible for the antioxidant activity of honey. The distribution of these compounds is influenced by the floral origin of honey. The polyphenol content correlates with the antioxidant activity of polyfloral and sunflower honey measured with FRAP assay. These results revealed that the Bulgarian honeys studied proved to be a good source of antioxidants that might serve to protect human health.

Key words: antioxidant activity; polyfloral honey; sunflower honey; total polyphenols
Abbreviations: FRAP – Ferric Reducing Antioxidant Power; DPPH – 2,2-diphenyl-1-picrylhydrazyl

Introduction

In recent years, there is a growing demand for natural products with antioxidant activity in human diet. A large number of different plants, fruits and leaves of plants synthesize compounds possessing antioxidant activity. They may be used as a natural source of free radical scavenging compounds (Badakhshan et al., 2012; Pisoschi et al., 2016; Zou et al., 2016; Chaves et al., 2020; Bobis et al., 2020). The majority of these plants are used by the honey bees to collect nectar and pollen. In this respect bioactive components with antioxidant properties can be transferred to honey. Depending on the botanical origin, honey can be classified as monofloral (unifloral) or multifloral (polyfloral) whether it is coming from a single or from several plant species, respectively (Ruoff et al., 2007).

Honey has been used by humans as food and medical product from ancient times (Alvarez-Suarez et al., 2018). It is one of the most important products of beekeeping. The chemical composition of honey is affected by its floral source (Elbanna et al., 2014). The major components of honey are sugars (mainly fructose and glucose), but it also contains amino acids, enzymes, protein, vitamins, minerals elements, organic acids and phenol and flavonoid compounds. All compounds in honey contribute to

its biological activity (Bergamo et al., 2019; Mračević et al., 2020). Phenol and flavonoid compounds, which act as natural antioxidants are becoming increasingly popular because of their potential role in contributing to human health. The antioxidants play an important role in human health. Measuring the total antioxidant activity of honey will provide an understanding of the functional properties of honey. A lot of methods are available to determine the antioxidant activity of honey. In most cases it is necessary to use several assays for determination of antioxidant activity of honey and to obtain good reliability (Fukumoto & Mazza, 2000; Lewoyehu & Amare, 2019). Dzugan et al. (2018) studied antioxidant activity as a biomarker of honey variety. The authors presented different types of honey including multifloral, rape, coniferous honeydew honey. The possibility to classify the botanical origin of honey based on antioxidant activity was proved.

The recognition of antioxidant activity in Bulgarian polyfloral honey can increase its commercial value. This information would have a positive economic impact for the Bulgarian polyfloral honey where it is widely produced. In the present study, we aimed to determine the antioxidant activity of the Bulgarian polyfloral and sunflower honey by DPPH and FRAP assay and total polyphenols.

Materials and Methods

All honey samples were obtained directly from beekeepers operating in Bulgaria. Honey samples were stored in glass jars at room temperature in a dark place before the analysis. Twenty polyfloral and 10 sunflower honey samples were used in this study. The antioxidant activity of honey was estimated by determination of DPPH (2,2-diphenyl-1- picrylhydrazyl), according to the procedure of Brand-Williams et al. (1995) and modified after Liu et al. (2008). FRAP assay (Ferric Reducing Antioxidant Power), (Benzie & Strain, 1996) was performed as described in Mohammadzadeh et al. (2007) with modification of sample preparation. Total polyphenol content was determined by Folin – Ciocalteu colorimetric method (Slinkard & Singleton, 1977) using gallic acid as a calibration standard as described in Liu et al. (2008) with measuring the absorbance at 760 nm. The analyses were carried out in duplicate. Statistical analyses of data were performed using IBM SPSS Statistics version 21 for Windows. Data was expressed as means ± standard deviations (SD). Correlation analyses between the antioxidant activity of polyfloral and sunflower honey samples were done. Level of statistical significance was defined as $p<0.05$.

Results and Discussion

Polyfloral and sunflower honey samples were tested in this study in order to assess their antioxidant activity and possibly to find some relationship between the DPPH, FRAP assays and total polyphenols. The average values and ranges of DPPH and FRAP assays are reported in Table 1.

Table 1. Antioxidant activity of polyfloral and sunflower honey samples

Parameters	Polyfloral honey (n=20)	Sunflower honey (n=10)
DPPH, % inhibition		
Mean±SD	40.05±5.65	48.89±8.43
Ranges (Min – Max)	28.80 – 46.85	39.24 –63.59
FRAP, mmol Fe^{2+}/100 g		
Mean±SD	0.91±0.17	0.59±0.16
Ranges (Min – Max)	0.63 – 1.14	0.42 – 0.95

SD – standard deviation

There is no official method for honey antioxidant activity determination and the different methods allow the measurement of a different group of antioxidants. DPPH assay is one of the frequently used in the evaluation of radical scavengers in natural foods and honey (Dzugan et al., 2018). In the present study obtained results for DPPH in polyfloral honey are comparable to the study of Bobis et al. (2011). The authors received lower average values for DPPH in sunflower honey. Moreover, Dzugan et al. (2018) also determined similar average DPPH values (39.89 ± 15.08% inhibition) for polyfloral honeys of Polish origin compared to our results. The same authors presented large ranges for this parameter (22.45 – 65.78% inhibition). Marghitas et al. (2009) determined very short ranges for DPPH in sunflower honey (40.65 – 49.19% inhibition). In contrast, Predescu et al. (2015) found higher mean values for antioxidant activity with DPPH method in sunflower honey compared to our results. The authors indicate that the higher values mean higher antioxidant activity.

The average antioxidant activity of polyfloral honey measured with FRAP assay was higher than in sunflower honeys (Table 1). However, the analyzed honeys may be considered easily accessible natural sources of antioxidants. Bobis et al. (2011) presented higher average values for this parameter in sunflower honey.

The total antioxidant activity of honey is likely the result of the combined activities and interactions of a wide range of compounds. However, phenolic compounds are well known to be the major contributors of this property (Lianda et al., 2012). In general the total polyphenol content determined by the modified Folin-Ciocalteu method varied greatly among the honey types. In the present study the results for total

polyphenols in the polyfloral and sunflower honey are identical. The average values and standard deviation for the polyfloral honey samples are 46.16±13.02 and 47.45±10.70 mg GAE/100 g for the sunflower honey. The minimal and maximal values for total polyphenol content for the both honey types are presented in Figure 1. Total polyphenol content ranged between 26.48 – 67.45 for polyfloral honey and 32.29 – 68.10 mg GAE/100 g for sunflower honey. The concentrations of polyphenols identified by Bobis et al. (2011) were higher than in the present study for the both honey types. Marghitas et al. (2009) analyzed Romanian honey samples. They determined total polyphenol values for sunflower honey in the ranges 20 – 45 mg GAE/100 g. These results are lower in comparison to the results for this parameter in the present study. Minimal and maximal values obtained by Wilczyńska (2010) regarding total polyphenol content of polyfloral honey originating in Poland demonstrated similarities to Bulgarian polyfloral honey.

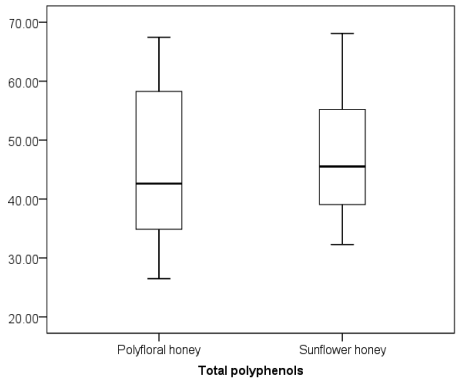

Fig. 1. Box plot diagram of total polyphenols in polyfloral and sunflower honey. Minimal, maximal and median value are shown

Even when the authors use the same method for determination of antioxidant activity very often different modifications are included. In this respect the results of different studies are hard to be compared.

The correlations between FRAP and total polyphenols are presented in Figures 2 and 3. The strongest correlation between these two factors was found for the sunflower honey samples ($r=0.815$, $p<0.05$). The correlation coefficient for the polyfloral honey is $r=0.559$ ($p<0.05$). These results suggest that 31% of the antioxidant activity measured with FRAP method in the polyfloral honey samples and 66% for the sunflower honey samples result from the contribution of total polyphenols. Furthermore, positive linear correlations between the total antioxidant activity, determined by the FRAP method and phenolic content in different honey types including polyfloral honey,

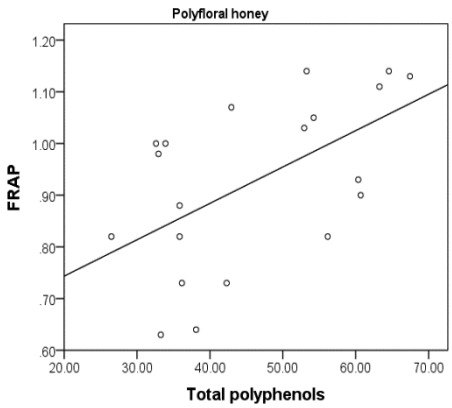

Fig. 2. Correlation between FRAP and total polyphenols in Polyfloral honey ($r=0.559$, $p<0.05$)

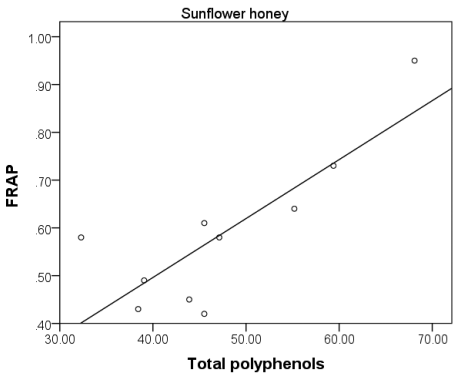

Fig. 3. Correlation between FRAP and total polyphenols in Sunflower honey ($r=0.815$, $p<0.05$)

were observed by Bertoncelj et al. (2007). In the recent study Kulkarni et al. (2020) found a moderate positive correlation between FRAP assay and polyphenols ($r=0.6$, $p<0.05$).

Conclusion

Bulgarian polyfloral and sunflower honeys were characterized by three parameters (DPPH, FRAP and total polyphenols) and compared to products from other countries. The average antioxidant activity of polyfloral honey samples measured with FRAP assay was higher than in sunflower honeys. Sunflower honey had higher average DPPH values than polyfloral honey. The results obtained for total polyphenols for the both honey types are almost identical. The present study confirmed the presence of biologically active compounds in the tested honey samples, which are responsible for the antioxidant activity of honey. The distribution of these compounds is influenced by the floral origin of hon-

ey. The polyphenolic content correlates with the antioxidant activity of polyfloral and sunflower honey measured with FRAP assay. These results revealed that the Bulgarian honeys studied proved to be a good source of antioxidants that might serve to protect human health.

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Received: **December**, **09, 2020**; *Accepted*: **May**, **28, 2021**; *Published*: **August, 2022**