ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Technol. Aliment. 21(2) 2022, 213–222

www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://dx.doi.org/10.17306/J.AFS.2022.1024

ORIGINAL PAPER

Received: 20.01.2022
Accepted: 2.06.2022

INFLUENCE OF BOTANICAL ORIGIN ON PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF MONOFLORAL BEE POLLEN

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ABSTRACT

Background. Bee pollen, a source of nutrients for adult honey bees and larvae, is produced from plant flower pollen which bees collect and mix with nectar or secretions from their salivary glands. Bee pollen contains nutritionally essential substances like proteins, lipids, amino acids, mineral substances, and vitamins but also carotenoids, flavonoids, and polyphenols. The amount of each substance contained in bee pollen depends on the botanical origin and the source region. Recently, many investigations have been concerned with the antioxidant properties of different food products. The aim of this research was to examine the antioxidant capacity of bee pollen and investigate its relationship with total phenolic and flavonoid contents.

Materials and methods. Samples were collected from three locations in Croatia from April to June 2019. Sixteen bee pollen pooled samples were classified according to color and, after melissopalynological analysis, total phenolic (Folin-Ciocalteu method) and total flavonoid contents and antioxidant capacity (FRAP assay) were determined in fourteen monofloral bee pollen samples.

Results. The monofloral bee pollen samples had 82–100% of their pollen originating from one botanical species. The highest total phenolic content (TPC) was measured in *Prunus* spp. and *Salix* spp. monofloral bee pollens (15.80 and 13.75 mg GAE/g, respectively), which also had the highest ferric reducing-antioxidant power (FRAP) values (124.44 and 147.61 μmol Fe^{2+}/g, respectively). The samples with the lowest TPC (*Crepis biennis* L. and *Taraxacum officinale* F.H. Wigg.; 4.00 and 5.34 mg GAE/g, respectively) also had the lowest FRAP values (25.24 and 34.74 μmol Fe^{2+}/g, respectively). The values for total flavonoid content (TFC) did not vary a lot between the analyzed samples (5.05–9.71 mg QE/g).

Conclusion. In comparison to some other food products, bee pollen, like most bee products, appears to be a good source of antioxidants. The botanical family or botanical species of bee pollen affects the antioxidant properties of the bee pollen. Due to a lack of research on monofloral bee pollen in comparison to pooled samples, knowledge about specific parameters of different monofloral bee pollen samples should be broadened.

Keywords: monofloral bee pollen, botanical origin, antioxidant capacity, total flavonoids, total phenolic content

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INTRODUCTION

Bee pollen is used by honey bees to produce bee bread. Honey bees collect pollen from plant anthers, mix it with secretions from their salivary glands and nectar and place it in baskets situated on their hind legs. Pollen loads are packed into honeycomb cells and covered with a thin layer of honey and wax (Komosinska-Vassev et al., 2015). Beekeepers collect the bee pollen by using a pollen trap, and collected loads must be dried before human consumption (Coe, 2007). Depending on the plant species, the pollen grains differ in shape (round, cylindrical, bell-shaped, triangular, or thorny), color (bright yellow to black), size, and weight (a dozen or several dozens of micrograms). Mostly, pollen baskets consist of pollen from one dominant plant species, but often, they also consist of many different plant species (multicolored pollen loads; Komosinska-Vassev et al., 2015; Shubharani et al., 2013). For confirmation of the monoflorality of bee pollen samples, pollen loads need to be separated according to color and then each subsample needs to undergo melissopalynological analysis.

Due to its valuable chemical composition, characterized by its high protein content (22.7% on average), which includes essential amino acids (10.4% on average; Komosinska-Vassev et al., 2015), lipids, carbohydrates, minerals, water and oil soluble vitamins, and polyphenols (Ares et al., 2018), bee pollen is an effective food supplement which can supply the human body with a wide range of different health promoting nutrients (Kostić et al., 2020). The chemical composition, and therefore the bioactive properties, of bee pollen depends on its botanical and geographical origin, as well as the bee species and storage conditions (Ares et al., 2018; Lilek et al., 2015; Mărgăoan et al., 2012). The most important bioactive components of bee pollen are polyphenols, especially phenolic acids and flavonoids but also carotenoids (Almeida-Muradian et al., 2005; Velásquez et al., 2017), which are responsible for the anti-inflammatory, antimicrobial, antioxidative, anticancerogenic, and immunostimulant properties of bee pollen (Mărgăoan et al., 2019).

The antioxidant properties of the bee pollen have been examined in many studies, using different methods. Kostić et al. (2019) examined methanolic and ethanolic extracts of monofloral *Helianthus annuus* L. bee pollen and concluded that it has high antioxidant activity and flavonoid content. Nanda and Thakur (2019) found Brassicaceae bee pollen (*Brassica napus* L.) to be a good polyphenol source and used it in the production of polyphenol-rich milk powder. The composition of bee pollen affects its antioxidant properties, but numerous studies that look at the composition and properties of various bee pollen samples present a diversity of results (Kocot et al., 2018). Some studies confirmed the positive correlation between TPC and antioxidant capacity (Kalaycıoğlu et al., 2017; Freire et al., 2012), while others did not find this relationship (Leja et al., 2007). Preparation of pollen extracts and type of solvent seem to be important for the properties of pollen extracts (Kim et al., 2015). This diversity of the factors affecting the properties of bee pollen leads to problems with comparing the results obtained from different research (Aličić et al., 2014). In Croatia, research on bee pollen is scarce in comparison to research on other bee products, e.g., honey. The antioxidant activity of bee pollen collected in eastern Croatia was published in 2018 (Bilić Rajs et al., 2018), and mainly referred to bifloral and polifloral bee pollen samples, while Prđun et al. (2021) published characterizations of bee pollen according to physico-chemical properties, headspace composition, and FTIR spectral profiles. The aim of this work was to evaluate the antioxidant capacity of monofloral bee pollen samples collected in Croatia and verify possible connections within examined parameters. Given the fact that bee pollen is reputed to be a source of antioxidants, the results will be compared with other food products known to be frequently consumed and to have health promoting properties.

MATERIALS AND METHODS

Sixteen samples of bee pollen were collected from three locations in Croatia (Otočac – Mountain region, Senj – Mediterranean region and Krapina – Continental region) during the beekeeping season of 2019, from April to June. After collection, the pollen loads were classified according to color with the aim of getting as many monofloral subsamples as possible. The samples were frozen at –18°C before further analysis in order to preserve their biological and chemical properties. All analyses were conducted in duplicate.

Botanical origin of bee pollen

Microscopic slides were prepared using the bee pollen samples, which were classified according to color using modified methods of Barth et al. (2010). Two grams of bee pollen consisting of the same color loads extracted from one group sample was weighed into a 12 mL centrifuge tube and mixed with 70% ethanol up to 10 mL. After vortexing, the mixture was placed for five minutes in an ultrasonic bath (Bandelin, Sonorex, Super RK 100 H, Berlin, Germany) and then left to stand for 25 minutes. The tube was then put in a centrifuge (Sigma 2–16, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) for three minutes at 1500 rpm. The obtained sediment was resuspended with ethanol and the procedure was repeated. A water (7 mL) and glycerol solution (1:1) was added to the sediment and left for 30 minutes. The mixed solution was dissolved two times with the water and glycerol solution, and the obtained sediment was stirred with a Pasteur pipette and spread on a microscopic slide. Melissopalynological analysis was conducted to confirm if the sample could be labeled as monofloral bee pollen (>80% of pollen grains from one botanical species according to the Campos et al., 2008). At least 500 pollen grains were counted in each microscopic slide and identified using 400× magnification (B-800 Series; Optika Microscopes, Ponteranica, Italy). For the botanical origin identification, CMS Celle's Melissopalynological Collection (von der Ohe, 2003) and Ponet Pollen databank were used.

Bee pollen extracts

Samples of ten grams of monofloral bee pollen loads were added to a 100 mL volumetric flask filled to the label with methanol (Merck KGaA, Darmstadt, Germany) and ultra-sonicated two times for 30 minutes. The suspension was filtered and stored at –18°C before analysis. Each sample was made in duplicate.

Total phenolic content

The Folin-Ciocalteu method (FC) was used to determine the total phenolic content (Singleton et al., 1999), expressed as mg of gallic acid per gram of bee pollen. Water (6 mL) and FC reagent (0.5 mL; Reagecon, Shannon, Ireland) were added to 100 µL of diluted bee pollen extract (0.1 mL extract + 0.1 mL distilled water). A solution of 20% Na_2CO_3 (1.5 mL; Panreac, Barcelona, Spain) was added in the sixth minute, and a 10 mL volumetric flask was filled to the mark with distilled water and left to stand in the dark for two hours. A calibration curve was made with testing solutions of gallic acid (98% purity, Sigma-Aldrich, Switzerland) in concentrations from 0.1 to 1.0 mg/mL, and the absorbance was measured at 760 nm (UV-Vis spectrophotometer UV-1800; Shimadzu Corp., Kyoto, Japan). The analyses were conducted in duplicate.

Total flavonoid content

The method described by Pascoal et al. (2014) was used to determine the total flavonoid content (TFC), expressed as mg quercetin per gram of bee pollen sample (Kim et al., 2003), where quercetin (Sigma-Aldrich, Switzerland) was used as a reference standard. 5% $NaNO_2$ (Gram-mol, Zagreb, Croatia) and 10% $AlCl_3$ (Kemika, Zagreb, Croatia) in the amount of 60 µL were added to 50 µL of bee pollen extract and 950 µL of distilled water. After six minutes, 400 µL NaOH (1 M; Gram-mol, Zagreb, Croatia) was added. The absorbance was measured at 510 nm (UV-Vis spectrophotometer UV-1800; Shimadzu Corp., Kyoto, Japan) and the results were calculated from the calibration curve made with the quercetin solutions (0.001–0.5 mg/mL). The analyses were conducted in duplicate.

FRAP assay

The antioxidant activity was determined according to the method described by Benzie and Strain (1999), expressed as µmol Fe^{2+} per gram of bee pollen. The FRAP reagent was made fresh every day consisting of acetate buffer (pH = 3.6; 300 mmol/L; J.T. Baker, Fisher Scientific, USA), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) reagent (98%; Sigma-Aldrich, Switzerland) and $FeCl_3 \times 6H_2O$ (Riedel-de Haën, Germany) in 10:1:1 ratio. The FRAP reagent (3 mL) was added to 0.1 mL of diluted bee pollen extract (extract/water 1:10) and incubated at 37°C in a thermostatic bath (TKS, Zagreb). The absorbance was measured at 593 nm (UV-Vis spectrophotometer UV-1800; Shimadzu Corp., Kyoto, Japan) after 5 minutes of incubation. A calibration curve was made with water solutions of $FeSO_4$ (Kemika, Zagreb, Croatia) (0.1–1 mM/L). The analyses were conducted in four repetitions.

Data analysis

Mean values, ranges and SDs were calculated for each parameter. In order to evaluate any statistical differences between the analyzed parameters, factorial analysis of variance (ANOVA) and the post-hoc Tukey HSD test were performed using software STATISTICA® 14.0.0.15, and p-values <0.05 were considered significant.

RESULTS

From the sixteen collected pooled samples, after being sorted according to color (Fig. 1) and melissopalynological analysis, fourteen bee pollen samples were confirmed as monofloral and used for further analysis (A1, A2, A3, C1, C3, I1 – Continental region; B1, E2, G1, M1, N1 – Mountain region; D2, D3, H1 – Mediterranean region). The pollen spectrum of the obtained monofloral samples are given in Table 1. From all the obtained samples, half were composed from pollen of which 100% came from one botanical species, while the percentage of dominant pollen in the other samples was from 82–99%.

The results of the TPC, TFC, and FRAP analyses are given in Table 2. All analyses were conducted in two (TPC and TFC) or four repetitions (FRAP assay), and in Table 2 the mean values, standard deviations, and minimal and maximal values are given to show results of statistical difference between the analyzed parameters (Tukey HSD test). Total phenolic

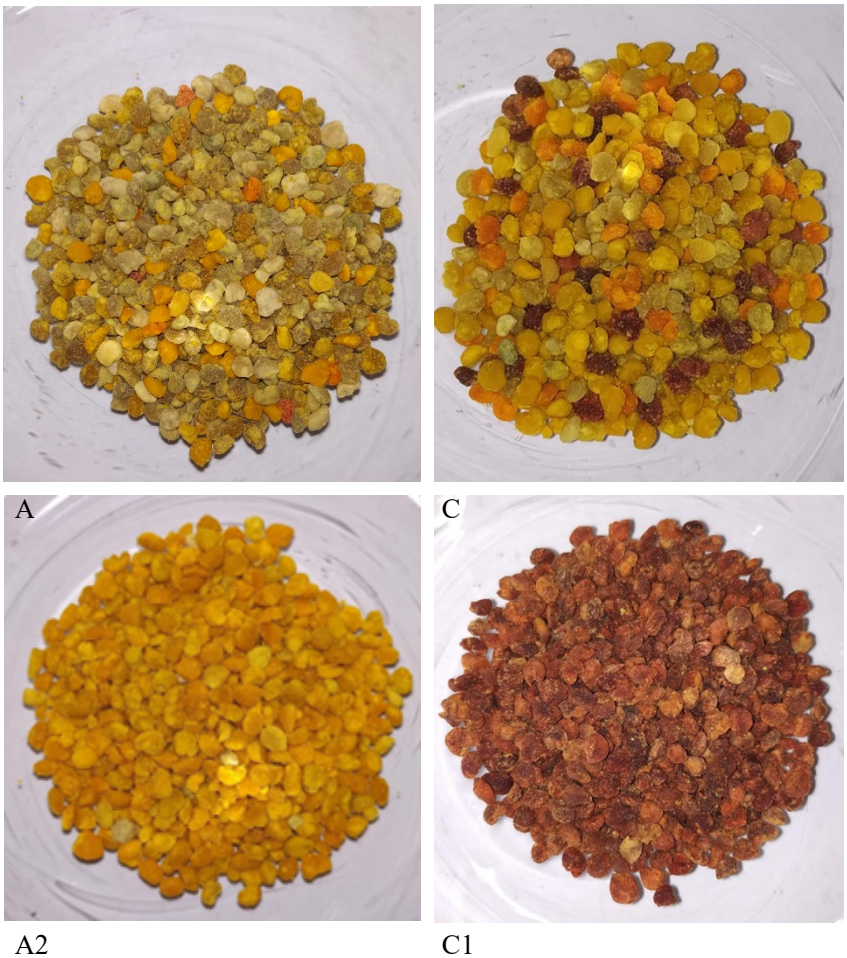

Fig. 1. Agregate (A and C) and unifloral (A2 – *Salix* spp. and C1 – *Aesculus hippocastanum* L.) samples of bee pollen

Table 1. Pollen spectrum of collected monofloral bee pollen samples

Sample	Main pollen type (≥80%)		Important minor pollen (3–15%)		Minor pollen (<3%)		Undetermined pollen
	species	%	species	%	species	%	%
A1	*Taraxacum officinale* F. H. Wigg.	86	*Acer* spp.	7	–	–	2
			Salix spp.	5			
A2	*Salix* spp.	100	–	–	–	–	–
A3	*Prunus spinosa* L.	90	*Salix* spp.	8	–	–	2
B1	*Prunus spinosa* L.	100	–	–	–	–	–
C1	*Aesculus hipposcastanum* L.	100	–	–	–	–	–
C3	*Salix* spp.	99	–	–	*Carex* spp.	1	–
D2	*Crepis biennis* L.	94	*Helianthemum* spp.	3	*Fabaceae*	2	1
D3	*Prunus mahaleb* L.	100	–	–	–	–	–
E2	*Prunus* spp.	100	–	–	–	–	–
G1	*Quercus pubescens* Willd.	84	*Rhamnus* spp.	5	*Juglans regia* L.	2	2
					Fabaceae	2	
			Liliaceae	3	*Fraxinus* spp.	1	
					Carex spp.	1	
H1	*Quercus pubescens* Willd.	82	*Fraxinus* spp.	13	*Rhamnus* spp.	2	1
					Liliaceae	1	
					Dactylis glomerata L.	1	
I1	*Papaver rhoeas* L.	100	–	–	–	–	–
M1	*Filipendula vulgaris* Moench.	97	–	–	*Dactylis glomerata* L.	1	1
					Plantago spp.	1	
N1	*Phacelia tanacetifolia* Benth.	100	–	–	–	–	–

content – TPC was expressed as mg GAE per g of pollen sample, and the difference between the minimal and maximal values was about 11 mg GAE g^{-1}. The values for TFC varied less with the difference between the extreme values, which was about 4 mg QE per g of pollen sample. The FRAP values, expressed as µmol Fe^{2+} per gram of pollen sample, had the largest range between the pollen samples, depending on the botanical source. The obtained results will be compared with literature values for some foodstuffs often consumed or known to be functional foods or food supplements.

DISCUSSION

The melissopalynological analysis conducted on the collected bee pollen subsamples confirmed that fourteen of them had a share of pollen originating from one botanical species >80%, which classifies them as monofloral bee pollen samples according to Campos et al. (2008) and Feás et al. (2012). Seven of the samples were 100% monofloral (*Salix* spp., *Prunus spinosa* L., *Aesculus hipposcastanum* L., *Prunus mahaleb* L., *Prunus* spp., *Papaver rhoeas* L. and *Phacelia*

Table 2. Total phenolic content, total flavonoid content, and antioxidant capacity (FRAP assay) determined in monofloral bee pollen samples, mean ±SD

Sample	Total phenolic content mg GAE/g	Total flavonoid content mg QE/g	FRAP µmol Fe^{2+}/g
A1	5.34 ±0.23^{a}	6.68 ±0.07^{a}	34.74 ±0.59^{a}
A2	13.73 ±0.07^{b}	9.19 ±0.10^{b}	123.00 ±2.96^{b}
A3	11.50 ±0.49^{c}	5.05 ±0.01^{c}	60.16 ±1.73^{c}
B1	13.43 ±0.06^{b}	5.55 ±0.21^{c,d}	89.95 ±2.23^{d}
C1	13.31 ±0.04^{b}	9.71 ±0.12^{b}	97.86 ±3.63^{e}
C3	13.75 ±0.28^{b}	9.14 ±0.32^{b}	147.61 ±1.56^{f}
D2	4.00 ±0.09^{d}	5.94 ±0.09^{d}	25.24 ±0.11^{g}
D3	13.59 ±0.23^{b}	5.64 ±0.07^{c,d}	63.17 ±1.75^{e,h}
E2	15.80 ±0.30^{e}	7.11 ±0.22^{a}	124.44 ±2.55^{b}
G1	11.58 ±0.54^{c}	7.01 ±0.08^{a}	87.81 ±2.09^{d}
H1	13.27 ±0.45^{b}	8.06 ±0.18^{e}	105.77 ±0.54^{i}
I1	9.44 ±0.06^{f}	5.51 ±0.11^{c,d}	80.89 ±1.86^{j}
M1	8.79 ±0.05^{f}	5.99 ±0.22^{d}	68.86 ±1.86^{h,k}
N1	12.03 ±0.21^{c}	5.30 ±0.03^{c}	65.96 ±3.82^{c,k}
Minimum	4.00 ±0.09	5.05 ±0.01	25.24 ±0.11
Maximum	15.80 ±0.30	9.71 ±0.12	147.61 ±1.56

Values represented with the same letters are not statistically different according to Tukey HSD test ($p > 0.05$).

tanacetifolia Benth.; Table 1), while the share of the main pollen in the other seven samples was from 82–99%. Five of the samples had important isolated pollen (3–15% of bee pollen from one botanical species) in a notable share. The highest value was in the *Q. pubescens* Willd. (H1) sample, where *Fraxinus* spp. was present with a 13% share of the pollen. Minor amounts of pollen (<3% of bee pollen from one botanical species) were present in five of the samples. The samples collected at the beginning of May (G1 and H1) had the highest diversity of botanical species. The share of undetermined pollen, which was present in six of the samples, was very low (1 or 2%), so it should not have an impact on the results of any further analyses.

The main factor responsible for the differences in physicochemical properties, such as antioxidant properties, is the botanical origin of the bee pollen samples. The TPC of the analyzed samples ranged from 4.00 mg GAE/g in the *C. biennis* L. (D2) sample to 15.80 mg GAE/g in the *Prunus* spp. (E2) sample (Table 2). The samples that belonged to the *Prunus* genus (A3, B1, D3 and E3) all had high TPC values (11.50–15.80 mg GAE/g), while the samples belonging to the Asteraceae family (A1 and D2) had the lowest TPC values (5.34 and 4.00 mg GAE/g, respectively). Statistical analysis (Tukey HSD test) showed that the *Taraxacum officinale* F.H. Wigg sample (A1) differed from all the other samples according to its TPC values (Table 2). Kostić et al. (2019) reported in their work on the Asteraceae family (*Helianthus annuus* L.) average TPC values of pollen in the amount of 3.82 mg GAE/g, which is quite similar to the values in this research. For the same pollen type, Fatrcová-Šramková et al. (2016) obtained higher results (6.92 mg GAE/g), but that was using

ethanolic pollen extracts. Kostić et al. (2019) indicated the influence of the used solvent on the end results, explaining this with the difference in protein content in the final extracts. Two samples belonging to *Salix* spp. (A2 and C3) had similar values for TPC (13.73 and 13.75 mg GAE/g, respectively). The *Q. pubescens* Willd. samples (G1 and H1) varied in their TPC values (11.58 and 13.27 mg GAE/g), which could be a consequence of higher shares of pollen from other botanical origins, because those two samples had pollen shares of *Q. pubescens* Willd. of 82 and 84% (Table 1). The TPC value for the *T. officinale* pollen sample (5.34 mg GAE/g) is comparable with values obtained in the research of Mărgăoan et al. (2012; 5.63 mg GAE/g), while Mărghitaş et al. (2009) and Stanciu et al. (2008) reported higher values (16.2 and 19.2 mg GAE/g, respectively), but the results were expressed based on the dry matter of pollen. Available literature values for the TPC of *Salix* spp. pollen ranged from 7.69 (Mărgăoan et al., 2012) to 16.4 mg GAE/g (Mărghitaş et al., 2009) of the dry matter of pollen. The TPC of the *Salix* spp. bee pollen in this study expressed on the dry matter of pollen (Prđun et al., 2021) was 16.20 mg GAE/g and 16.52 mg GAE/g, which is comparable with the results of Mărghitaş et al. (2009). Velásquez et al. (2017) reported 33.34 mg GAE/g in *Prunus* spp. pollen (52.2% of *Prunus* spp. pollen), which is much higher than the results obtained for all *Prunus* genus pollen samples in this research. Generally, the values for TPC obtained by Velásquez et al. (2017) are higher than in other research, e.g., by Feás et al. (2012) and Pascoal et al. (2014). Blando et al. (2015) conducted research on *P. mahaleb* L. fruit, with results for TPC of 19.80–31.04 mg GAE/g of fruit, which indicate that the fruit of this botanical species is richer in phenolic content than the pollen from the same botanical species. The TPC values for some other monofloral pollen types could be found in the work of Thakur and Nanda (2021), where coriander pollen had a high value of 25.63 mg GAE/g, while the values for rapeseed and coconut bee pollen were lower (16.84 and 15.50 mg GAE/g, respectively). The TFC did not vary a lot among the samples, and the minimal and maximal values were 5.05 and 9.71 mg QE/g, respectively. Although the range of the TFC values between the samples was not large, statistical analysis of the data showed that differences exist, and the H1 sample was statistically different from all the other samples (Table 2). The highest values were observed in the samples belonging to the Sapindaceae (C1 – 9.71 mg QE/g) and Salicaceae families (A2 – 9.19 mg QE/g and C3 – 9.14 mg QE/g). Most of the samples had values between 5 and 6 mg QE/g (Table 2), and the range of the obtained results is similar to those reported by Aličić et al. (2020) and Araújo et al. (2017). Kostić et al. (2019) obtained lower average TFC values for the Asteraceae family (*Helianthus annuus* L.; 0.84 mg QE/g).

The antioxidant capacity of the analyzed monofloral bee pollen samples was determined by FRAP assay (Table 2). The biggest differences between the tested samples, according to the Tukey HSD statistical test, were found for the FRAP parameter. Samples with higher TPC values had higher FRAP values (C3 – 147.61 μmol Fe^{2+}/g, A2 – 123.00 μmol Fe^{2+}/g, E2 – 124.44 μmol Fe^{2+}/g). The same situation was found in the research of Thakur and Nanda (2021), where coriander pollen had the highest TPC and FRAP values. The lowest FRAP values were in pollen samples coming from the Asteraceae family, which also had the lowest TPC values (D2 – 25.24 μmol Fe^{2+}/g and A1 – 34.74 μmol Fe^{2+}/g). A positive correlation between FRAP values and TPC was also reported by Aličić et al. (2020), Mărghitaş et al. (2009), and Velásquez et al. (2017). It can also be concluded that being part of the same plant family doesn't lead to similar FRAP values. Samples D3 (*P. mahaleb* L.) and E2 (*Prunus* spp., wild cherry) are both of the *Rosaceae* family, with 100% share of pollen from one plant species. The samples are from different subfamilies, which could be a partial reason for the differences in FRAP values (63.17 and 124.44 μmol Fe^{2+}/g, respectively). Another factor which has an influence on the results is the type and share of other pollen species present if the sample does not consist of 100% pollen from the same botanical origin (e.g. samples G1 and H1).

Comparing the obtained results and those from the literature for other foodstuffs often consumed or used as food supplements, it can be concluded that bee pollen is a good source of antioxidants. In comparison to the literature, the values for TPC are higher than those for blueberry (0.77–8.12 mg GAE/g) and blackberry fruit (1.73–3.05 mg GAE/g; Koca and Karadeniz, 2009), and in the range of or lower than the values for aronia fruit juice (30.02 mg/GAE/g) and tea (14.94–34.36

mg/GAE/g; Tolić et al., 2013). Vegetable, fruit, fruit juice, and tea FRAP values (Fu et al., 2011; Koca and Karadeniz, 2009; Pellegrini et al., 2003; Ryan and Prescott, 2010; Tolić et al., 2013; Zujko and Witkowska, 2013) are also lower than those obtained in this research. Only dark chocolate (146.7 μmol Fe^{2+}/g; Zujko and Witkowska, 2013) has a similar value to the *Salix* spp. pollen sample (C3 – 147.61 μmol Fe^{2+}/g). The amount of product that is consumed daily or monthly should also be considered. 1–2 teaspoons of bee pollen is usually consumed per day, which means not more than approximately 10 g of product (Bogdanov, 2017). According to Flanjak et al. (2016), the phenolic contents for chestnut and honeydew honey were 1.62 and 3.19 mg GAE/10 g of honey, respectively, while black locust, lime, and sage honey had 0.39, 0.86, and 0.97 mg GAE/10 g of honey, respectively. Taking this into account, some pollen types have several times higher TPC contents than honey types known for their good antioxidant sources (chestnut and honeydew). The same is found if the TPC pollen content is compared to the content in plum (sanhua) or black grape, which have 49.5 and 7.70 mg GAE/10 g (Chen et al. 2014), which is again several times lower than the content in some pollen. Because of its good techno-functional properties (Kostić et al., 2015), bee pollen is often utilized in other food products, which also opens doors for further investigation in this direction.

Monofloral bee pollen is a good source of antioxidants, particularly some specific types, like *Salix* spp. or *P. mahaleb* L. This research confirms that botanical origin greatly affects the antioxidant properties of bee pollen, which can be seen beyond the botanical families or specific botanical species. The correlation between some specific parameters is much clearer to confirm if the research is conducted on monofloral samples, but that type of research has been carried out less than that done on pooled samples. Broadening the amount of research done on bee pollen, as well as the parameters studied, is necessary in Croatia and further afield because there is an obvious lack of pollen quality criteria and data about the bioactive properties of bee pollen.

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