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Physicochemical properties and antioxidant capacity of bee pollen collected in Tuzla Canton (B&H)

Fizikalno-kemijske karakteristike i antioksidativni kapacitet pčelinje peludi prikupljene na području tuzlanskog kantona (BiH)

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ABSTRACT

The aim of this research was to evaluate bee pollen load samples collected in Tuzla Canton regarding physicochemical composition and antioxidant properties, with relation to collecting area and time of collection. Bee pollen load samples were collected in two periods: March/April and May/June at 13 locations, dried and analysed for protein, free fat, ash, moisture, total polyphenol and total flavonoid content, and antioxidant properties. The results showed that bee pollen collected in March/April had lower content of proteins, but higher content of total polyphenols and higher antioxidant activity, while total flavonoid content was not influenced by collection period. The location had significant influence on bee pollen properties.

Keywords: bee pollen, physicochemical properties, polyphenol content, flavonoid content, antioxidant activity

SAŽETAK

IZadatak ovog rada bio je odrediti fizikalno-kemijske karakteristike i antioksidativni kapacitet peludi prikupljene na području Tuzlanskog kantona u odnosu na razdoblje prikupljanja i geografsko podrijetlo. Uzorci peludi prikupljene su u razdoblju ožujak-travanj i svibanj-lipanj sa 13 lokacija, uzorci su osušeni i u njima je određen udio proteina, slobodnih masti, pepela i vode zatim udio ukupnih polifenola i flavonoida te antioksidativni kapacitet. Rezultati su pokazali da pelud prikupljena u razdoblju ožujak-travanj ima niži udio proteina, ali viši udio ukupnih polifenola i antioksidativni kapacitet, dok udio flavonoida ne ovisi o vremenu prikupljanja. Geografsko podrijetlo nije imalo utjecaj na karakteristike uzoraka peludi.

Ključne riječi: pelud, fizikalno-kemijske karakteristike, udio polifenola, udio flavonoida, antioksidativna aktivnost

INTRODUCTION

Bee pollen is product formed from floral pollen, nectar or honey, bee secretion and enzymes. Bees use it as food for young bees in the hive, but its nutritional value was recognised by the humans' centuries ago and it has been used as food and medicine.

Its value lies in proteins containing essential amino acids, lipids, minerals and water- and oil-soluble vitamins and polyphenols (Ares et al., 2018). Major bee pollen components are carbohydrates (13 - 55%), proteins (10 - 40%) and lipids (1 - 10%) (Orzáez Villanueva et al., 2002; Bogdanov, 2016). Although present in smaller

amount, vitamins, minerals and bioactive compounds are very important for nutritional and therapeutic properties of bee pollen. Bioactive compounds comprise approximately 70% of bee pollen (Borycka et al., 2015), giving it antioxidative, anti-inflammatory, antimicrobial, anticancerogenic and immunostimulant properties (Harif Fadzilah et al., 2017). Polyphenols, especially phenolic acids and flavonoids, and carotenoids are most important bioactive components of bee pollen (Almeida-Muradian et al, 2005; Komosinska-Vassev et al., 2015; Velásquez et al., 2017).

Floristic composition, geographical region, bee species (stingless or honeybees), preparation conditions, and storage time have impact on chemical composition of bee pollen and consequently its bioactive properties (Mărgăoan et al., 2012; Lilek et al., 2015; Harif Fadzilah et al., 2017, Ares et al., 2018). As a result of many influencing factors, great variability in chemical composition and problem in results comparison exist. To harmonise and standardise bee pollen quality and determination methods, Campos et al. (2008) published international proposal for bee pollen quality with draft basic composition requirements for dried bee pollen. The prescribed limits for physicochemical parameters are used for bee pollen quality evaluation at international level, since only few countries (e.g. Brazil, Bulgaria, Poland and Switzerland) have established pollen quality criteria at national level (Campos et al., 2008; Lilek et al., 2015). Bosnia and Herzegovina has not yet established quality criteria for bee pollen, nor the data of chemical composition and antioxidant capacity of bee pollen from Bosnia and Herzegovina are available in the literature. Therefore, the aim of this research was to determine physicochemical and antioxidant properties of bee pollen collected in Tuzla Canton, with respect to area and period of collection.

MATERIALS AND METHODS

Bee pollen load samples were collected in 13 locations of Tuzla Canton, Bosnia and Herzegovina: Kladanj, Banovići, Živinice, Kalesija, Sapna, Teočak, Čelić, Gradačac, Srebrenik, Tuzla, Lukavac, Gračanica i Doboj-Istok in periods: March/April and May/June 2014.

Samples were collected by professional experienced beekeepers, from healthy and strong bee colonies of *Apis mellifera*. Bee pollen was collected using external and internal pollen traps in 100 g bathes for both periods, every day or in shorter periods, depending on weather conditions and bee colony. Fresh bee pollen was kept in sterile closed cups at -20 °C to prevent bacteria and mould development. After all samples were collected, bee pollen was dried in pollen drier at 40 °C during 48 h. Dried bee pollen was sieved to remove physical impurities, homogenised and stored in closed glass jars, in dry and dark place until analyses.

Chemicals used were: methanol p.a. (99,5%) (T.T.T., Croatia); 2,2-diphenyl-1-picryl-hydrazil (DPPH) (Sigma-Aldrich, Germany), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich, Germany), Folin-Ciocalteu reagent (FC) (Sigma-Aldrich, Germany); gallic acid (Sigma-Aldrich, Germany); 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (98%, Sigma-Aldrich, Germany), ammonium peroxodisulfat (98%, Fluka).

Prior to physicochemical analyses, bee pollen samples were grinded in Grindomix GM 200 (Retsch GmbH; Germany) at 6000 rpm during 20 s. Moisture content was determined by drying at 70 °C (Memmert UFE 400, Memmert GmbH, Germany) until constant weight. Ash content was determined by ashing at 550 °C in muffle furnace (Nabertherm L3/11/P320, Nabertherm GmbH, Germany). Free fat content was determined by extraction with petroleter in Soxtec system (Foss Tecator, Sweden). Protein content by Kjeldahl was determined using factor 6.25 (Almeida-Muradian et al., 2005; de Arruda et al., 2013; Roldán et al., 2011; Human and Nicolson, 2006). Carbohydrate content was calculated from protein, free fat, ash and moisture content and includes raw fibre.

Extracts for further analyses were prepared by extraction with methanol, according to Mărghitaş et al. (2009), Ulusoy and Kolayli (2013) and Morais et al. (2011). Briefly, 20 grams of sample was extracted twice with methanol (first with 100 mL, then with 50 mL)

in ultrasound bath (Bandelin Sonorex, Germany), with agitation (Vibromatic Selecta, Spain). Both extracts and residue were collected and filtered through filter paper (MN 619 eh Nr.6) and diluted to 100 mL for analyses.

Total polyphenol content was determined by Folin-Ciocalteu method, using gallic acid as standard (Moreira et al., 2008, Kroyer and Hegedus, 2001, Mărghitaş et al., 2009). Total flavonoid content was determined by aluminium chloride colorimetric method according to Pascoal et al. (2014), using quercetin as reference standard (Kim et al., 2003). Antioxidant activity by DPPH was determined as described by Morais et al. (2011), FRAP according to Benzie and Strain (1999) and ABTS spectrophotometrically at 734 nm, as % inhibition in 60 s.

To determine botanical origin, bee pollen has to be macerated with ethanol in ultrasound bath during 5 min. The sediment was resuspended two times with 70% ethanol and centrifuged during 3 minutes at 1500 rpm. Pollen sediment is then mixed with water/glycerine (1/1, V/V) mixture, transferred to watch glass and dried during 24 h. After that, one drop of glycerol is added, sample is covered with cover glass and sides are sealed with parafine (Barth et al., 2010). Identification of pollen was done using microscope Olympus BX43F (SN 2H18039 Tokyo, Japan) with maximum magnification 1000x. The microscope is equipped with camera and image processing software Cellsens Standard 1.7.

Statistical analyses were performed using IMB SPSS v.20, Statistica 12 and Excel 2013. The differences were tested using TukeyHSD Post Hoc test ($p>0.05$). The relationship between analysed physicochemical parameters was evaluated using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

In period March/April seven botanical species were determined in bee pollen load samples (Figure 1), most frequent being: dandelion (*Taraxacum officinale*) and *Salix* sp. with 92.3% and *Roasaceae* with 84.6% (results not shown). Monofloral bee pollen contains over 80% pollen of one botanical species according to Feás et al. (2012) and Campos et al. (2008), and ≥ 90% according to Sattler et al. (2015). Dominant pollen species are represented above 45%, accessory with 16-45% and important isolated pollen type with 3-15 % (Sattler et al., 2015; Kostić et al., 2015; Freire et al., 2012). In March/April period only one sample was monofloral (*Salix* ssp., 81% - result not shown) and four were accessory types, three *Salix* and one *Taraxacum officinale*.

In period May/June number of botanical species raised to 13 (Figure 2). Dandelion, pine and *Asteraceae* continue to flower during this period, however, most frequent is blackberry (84.6% - result not shown). All samples in this period were multifloral, with dominant family *Poaceae* spp. and *Trifolium* spp. in two samples, and *Zea* mays and *Plantago* spp. in one sample, respectively.

Botanical origin of bee pollen load samples is a major factor responsible for differences in physicochemical parameters. Several authors have reported that sunflower pollen has low protein and high lipid and carbohydrate content while high protein content is characteristic for willow pollen and pollen of *Brasicaceae* and *Rosaceae* families (Szezęsna, 2006; Nicolson and Human, 2013; Lilek et al., 2015; Velásquez et al., 2017; Spulber et al., 2018). The results of physicochemical parameters obtained in this research confirm with mentioned findings. Namely, bee pollen samples containing high *Salix* pollen and pollen from *Rosaceae* family (*Prunus* and *Rubus* species) had highest proteins while sample from Banovići collected in May-June period that has high share of *Zea mays* and *H. annuus* pollen has low protein content (Figures 1, 2 and Tables 1 and 2). On average, bee pollen load samples collected in Tuzla Canton during March/April period had 4.51±1.01% moisture content (from 2.91±0.04% in Doboj-Istok to 6.07±0.02% in Gradačac), 5.13±1.17% free fat (from 3.47±0.04% in Kalesija to 6.78±0.14% in Kladanj), 2.49±0.39% ash (from 2.10±0.03% in Teočak to 3.08±0.01% in Doboj-Istok) and 20.67±2.31% protein (from 16.69±0.07% in Lukavac to 23.66±0.19% in Gradačac) (Table 1). Calculated value of carbohydrate content was 67.20±2.91%. In May/June period (Table 2), average moisture content increased to 5.52±0.73% and

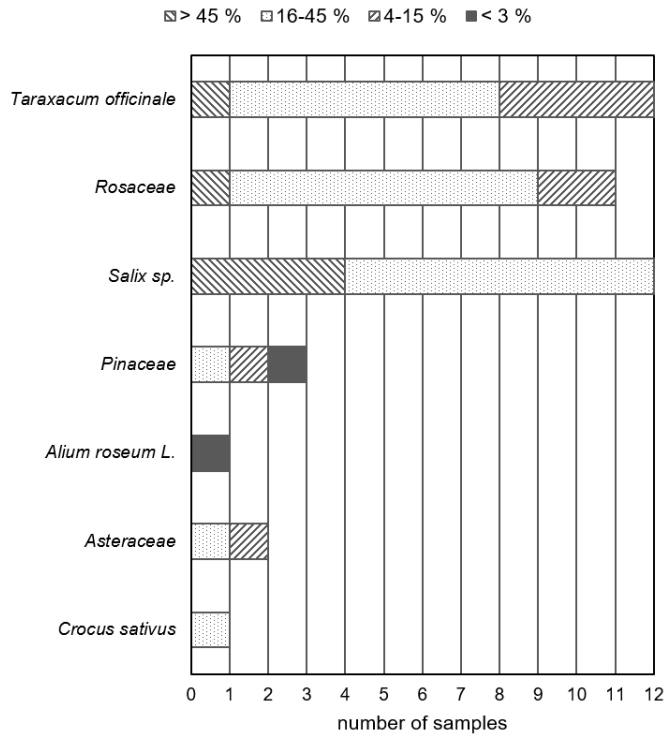

Figure 1. Pollen spectrum of bee pollen load samples collected at the area of Tuzla Canton in period March-April

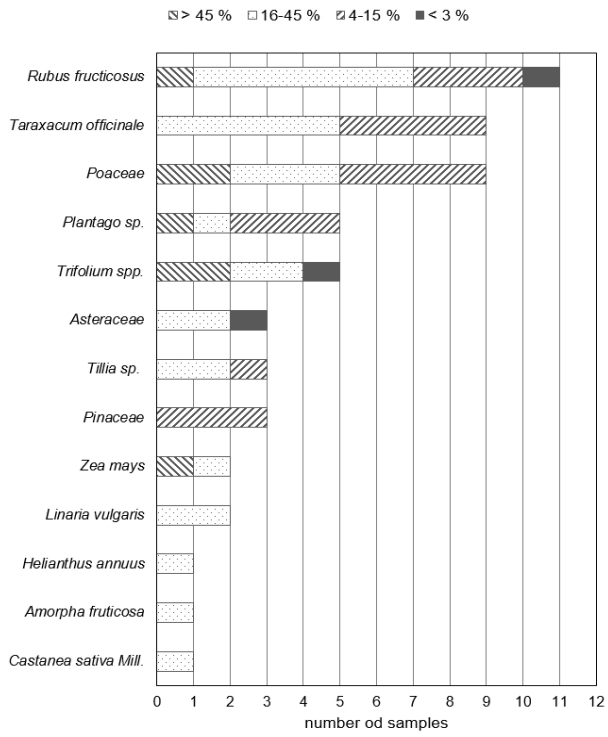

Figure 2. Pollen spectrum of bee pollen load samples collected at the area of Tuzla Canton in period May-June

Table 1. Average values and standard deviation of physico-chemical parametars of bee pollen load samples collected at the area of Tuzla Canton (%) in period March – April

Location	Moisture	Free fat[1]	Ash[1]	Proteins[1]	Carbohydrates[1]
Kladanj	3.61 ± 0.04^{f}	6.78 ± 0.14^{a}	2.21 ± 0.07^{b}	19.26 ± 0.14^{d,f}	68.14 ± 0.03^{b}
Banovići	3.77 ± 0.08^{f}	3.60 ± 0.02^{h}	2.11 ± 0.02^{b}	16.76 ± 0.19^{f}	73.76 ± 0.11^{a}
Živinice	4.47 ± 0.02^{d}	4.24 ± 0.11^{f}	3.07 ± 0.06^{a}	22.20 ± 0.42^{a,b,c}	66.03 ± 0.28^{b,c,d}
Kalesija	5.75 ± 0.04^{b}	3.47 ± 0.04^{h}	2.68 ± 0.38^{a,b}	22.76 ± 1.67^{a,b}	65.33 ± 1.29^{c,d}
Sapna	5.84 ± 0.08^{ab}	5.46 ± 0.04^{c,d}	2.36 ± 0.02^{b}	20.01 ± 1.33^{c,d}	66.34 ± 1.42^{b,c}
Teočak	5.04 ± 0.16^{c}	6.71 ± 0.04^{a}	2.10 ± 0.03^{b}	20.02 ± 0.54^{b,c,d}	66.13 ± 0.70^{b,c,d}
Čelić	5.00 ± 0.03^{c}	5.93 ± 0.06^{b}	2.23 ± 0.03^{b}	21.03± 0.83^{a,b,c,d}	65.81 ± 0.89^{b,c,d}
Gradačac	6.07 ± 0.02^{a}	4.00 ± 0.01^{g}	2.77 ± 0.01^{a,b}	23.66 ± 0.19^{a}	63.51 ± 0.22^{d}
Srebrenik	4.93 ± 0.04^{c}	6.76 ± 0.03^{a}	2.33 ± 0.01^{b}	19.76 ± 0.40^{c,d}	66.22 ± 0.41^{b,c}
Tuzla	4.20 ± 0.02^{d,e}	4.26 ± 0.03^{f}	3.07 ± 0.00^{a}	23.01 ± 0.40^{a}	65.46 ± 0.45^{c,d}
Lukavac	3.07 ± 0.02^{g}	5.31 ± 0.06^{d}	2.13 ± 0.02^{b}	16.69 ± 0.07^{f}	72.81 ± 0.01^{a}
Gračanica	4.06 ± 0.16^{e,f}	5.63 ± 0.05^{c}	2.24 ± 0.00^{b}	20.12 ± 0.15^{b,c,d}	67.96 ± 0.37^{b,c}
Doboj-Istok	2.91 ± 0.04^{g}	4.58 ± 0.02^{e}	3.08 ± 0.01^{a}	23.40 ± 0.11^{a}	66.03 ± 0.18^{b,c,d}

Values in the same column in one period of collecting marked with different superscript are statistically different (P<0.05)
[1] Free fat, ash, protein and carbohydrate values are reported to dry weight

Table 2. Average values and standard deviation of physico-chemical parametars of bee pollen load samples collected at the area of Tuzla Canton (%) in period May – June

Location	Moisture	Free fat[1]	Ash[1]	Proteins[1]	Carbohydrates[1]
Kladanj	5.20 ± 0.08^{f}	4.44 ± 0.01^{b,c}	2.10 ± 0.01^{f,g}	18.99 ± 0.29^{g,h,i}	69.27 ± 0.35^{b,c,d}
Banovići	4.16 ± 0.01^{h}	3.57 ± 0.03^{e}	1.98 ± 0.01^{g}	16.76 ± 0.11^{j}	73.53 ± 0.15^{a}
Živinice	4.78 ± 0.00^{g}	4.60 ± 0.08^{b}	2.44 ± 0.03^{c,d,e}	19.48 ± 0.54^{f,g,h}	68.7 ± 0.42^{d}
Kalesija	5.60 ± 0.04^{e}	2.53 ± 0.08^{h}	2.60 ± 0.06^{b,c}	23.20 ± 0.62^{b}	66.07 ± 0.60^{h}
Sapna	6.32 ± 0.00^{b}	3.20 ± 0.08^{f}	2.25 ± 0.01^{e,f}	19.40 ± 0.18^{f,g,h}	68.84 ± 0.27^{c,d}
Teočak	6.04 ± 0.01^{c}	3.48 ± 0.00^{e}	2.59 ± 0.13^{b,c}	20.10 ± 0.43^{d,e,f}	67.79 ± 0.29^{d,f,g}
Čelić	6.67 ± 0.08^{a}	2.52 ± 0.01^{h}	2.86 ± 0.06^{a}	26.43 ± 0.31^{a}	61.53 ± 0.44^{i}
Gradačac	5.83 ± 0.06^{c,d}	3.06 ± 0.09^{f}	2.72 ± 0.01^{a,b}	22.15 ± 0.53^{b,c}	66.24 ± 0.66$^{g,h.}$
Srebrenik	6.41 ± 0.05^{b}	5.05 ± 0.08^{a}	2.42 ± 0.08^{c,d,e}	19.98 ± 0.45^{d,f,g,h}	66.15 ± 0.40^{g,h}
Tuzla	4.84 ± 0.03^{g}	2.80 ± 0.03^{g}	2.72 ± 0.00^{a,b}	21.52 ± 0.17^{c,d}	68.12 ± 0.11^{d,f}
Lukavac	4.86 ± 0.01^{g}	3.44 ± 0.00^{e}	2.69 ± 0.06^{a,b}	18.35 ± 0.66^{h,i,j}	70.67 ± 0.59^{b}
Gračanica	5.36 ± 0.03^{e,f}	4.27 ± 0.02^{c}	2.46 ± 0.03^{c,d}	20.98 ± 0.37^{c,d,e}	66.93 ± 0.35^{f,g,h}
Doboj-Istok	5.71 ± 0.05^{d}	3.86 ± 0.06^{d}	2.30 ± 0.02^{d,e,f}	17.70 ± 0.13^{i,j}	70.43 ± 0.26^{b,c}

Values in the same column in one period of collecting marked with different superscript are statistically different (P<0.05)
[1] Free fat, ash, protein and carbohydrate values are reported to dry weight

protein content increased to 20.39±2.51%, while free fat content decreased to 3.60±0.79% and ash content remained at 2.47±0.26%. Calculated carbohydrate content was 68.02±2.88%. The obtained values were in accordance with values reported by Anjos et al. (2017) and Feás et al. (2012) for Portuguese bee pollen, Mărgăoan et al. (2012) for Romanian bee pollen, de Arruda et al. (2013) for Brazilian bee pollen and Fuenmayor et al. (2014) for Colombian bee pollen.

Although more botanical species were determined in bee pollen samples collected in May/June period, average total polyphenol content (TPC) as well as flavonoid content were higher in March/April period of collection. Average TPC in March/April period was 9.81±2.31 mg GAE/g d. m. while in May/June period average value was 7.82±1.58 mg GAE/g d. m. Highest TPC values (from 8.72±0.09 mg GAE/g up to 13.37±0.55 mg GAE/g) were measured in samples from March/April period where *Salix* sp. pollen was dominant species (Figure 1, Tables 3 and 4). High total phenolic content of *Salix* sp. pollen was obtained also by Marghitaş et al. (2009). The minimum value was determined for bee pollen collected in Gradačac in April/May (4.95±0.07 mg GAE/g d. m.), and maximum value for bee pollen collected in Živinice in the same period (13.37±0.55 mg GAE/g d. m.) (Table 3). Similar values reported Kroyer and Hegedus (2001), Mărghitaş et al. (2009) for Romanian bee pollen, whereas Ketkar et al. (2014), Freire et al. (2012) and Elamine et al. (2017) reported higher values (18.28 mg GAE/g; 41.5 – 188.6 mg GAE/g and 61.36-123.65 mg GAE/g, respectively). The significant differences in polyphenol content were observed between locations as well, and Čelić and Gradačac were only two locations where polyphenol content increased in May/June period. This can be ascribed to botanical species that were dominant in these samples in that period (Figure 2): blackberry (75% in pollen collected in Čelić) and clover family (54% in Gradačac), both rich in polyphenols (Araujo et al., 2017; Četojević-Simin et al., 2017; Khorasani Esmaeili et al., 2015).

Table 3. Average values and standard deviations of total polyphenol content (TPC), total flavonoid content and antioxidant capacity (determined by ABTS, FRAP and DPPH methods) of bee pollen load samples collected at the area of Tuzla Canton in period March – April

Location	TPC (mg GAE/g)	Flavonoid (mg QE/g)	ABTS (% inhib.)	FRAP (mmol Fe^{+2}/g)	DPPH (IC_{50}, mg/g)
Kladanj	7.91 ± 0.34^{g}	3.27 ± 0.18^{e}	50.78 ± 3.39$^{e.f}$	1.151± 0.063^{g}	4.64 ± 0.37^{b}
Banovići	8.25 ± 0.97$^{f.g}$	4.09 ± 0.05$^{d.e}$	51.33 ± 3.83^{e}	1.358 ± 0.137^{g}	6.00 ± 0.05^{a}
Živinice	13.37 ± 0.55^{a}	10.74 ± 0.40^{a}	83.88 ± 0.96^{a}	3.079 ± 0.116^{c}	1.67 ± 0.02$^{g.h}$
Kalesija	10.64 ± 0.26^{c}	10.24 ± 0.43$^{a.b}$	86.13 ± 2.28^{a}	4.111 ± 0.136^{a}	1.62 ± 0.04$^{g.h}$
Sapna	8.72 ± 0.09$^{e.f.g}$	4.74 ± 0.25^{d}	66.75 ± 2.82$^{c.d}$	1.939 ± 0.096^{f}	3.32 ± 0.30$^{c.d.e}$
Teočak	11.41 ± 0.13^{b}	5.84 ± 0.32^{c}	67.20 ± 3.59$^{c.d}$	2.008 ± 0.151^{f}	3.73 ± 0.07$^{c.d}$
Čelić	7.77 ± 0.24^{g}	4.86 ± 0.31^{d}	42.25 ± 1.86^{f}	1.916 ± 0.055^{f}	3.14 ± 0.21$^{d.e}$
Gradačac	4.95 ± 0.07^{h}	4.85 ± 0.07^{d}	29.08 ± 2.99^{g}	2.526 ± 0.158^{d}	3.34 ± 0.11$^{c.d.e}$
Srebrenik	9.17 ± 0.08$^{d.e.f}$	6.21 ± 0.44^{c}	31.80 ± 2.46^{g}	2.253 ± 0.097^{e}	3.83 ± 0.06^{c}
Tuzla	13.30 ± 0.26^{a}	10.09 ± 0.41$^{a.b}$	79.15 ± 3.22$^{a.b}$	3.663 ± 0.111^{b}	1.43 ± 0.00^{h}
Lukavac	9.79 ± 0.18$^{d.e}$	6.08 ± 0.69^{c}	59.70 ± 4.32$^{d.e}$	1.996 ± 0.118^{f}	2.22 ± 0.05$^{f.g}$
Gračanica	11.16 ± 0.43$^{b.c}$	6.26 ± 0.23^{c}	74.10 ± 4.84$^{b.c}$	1.979 ± 0.052^{f}	2.81 ± 0.04$^{e.f}$
Doboj-Istok	11.16 ± 0.39$^{b.c}$	9.32 ± 0.68^{b}	77.18 ± 6.70$^{a.b}$	3.240 ± 0.124^{c}	1.93 ± 0.02$^{g.h}$

All results were expressed on dry weight;
Values in the same column in one period of collecting marked with different superscript are statistically different (P<0.05); GAE, gallic acid equivalent; QE, quercetin equivalent

Table 4. Average values and standard deviations of total polyphenol content (TPC), total flavonoid content and antioxidant capacity (determined by ABTS, FRAP and DPPH methods) of bee pollen load samples collected at the area of Tuzla Canton in period May - June

Location	TPC (mg GAE/g)	Flavonoid (mg QE/g)	ABTS (% inhib.)	FRAP (mmol Fe^{+2}/g)	DPPH (IC_{50}, mg/g)
Kladanj	6.05 ± 0.80$^{e.f}$	4.93 ± 0.61^{d}	62.55 ± 3.07$^{c.d.e}$	1.432 ± 1.20^{d}	10.06 ± 1.36$^{a.b}$
Banovići	7.15 ± 0.08^{d}	4.69 ± 0.27^{d}	46.80 ± 7.75^{g}	1.329 ± 0.54$^{d.e}$	8.51 ± 0.15$^{a.b.c.d}$
Živinice	6.87 ± 0.56$^{d.e}$	5.91 ± 0.37$^{b.c.d}$	55.75 ± 1.68$^{d.e.f.g.}$	1.555 ± 0.55$^{c.d}$	8.14 ± 0.17$^{a.b.c.d}$
Kalesija	8.34 ± 0.20^{c}	6.80 ± 0.41$^{a.b.c}$	56.63 ± 3.95$^{d.e.f.g}$	2.103 ± 3.14^{b}	4.39 ± 0.21$^{e.f}$
Sapna	7.79 ± 0.17$^{c.d}$	5.45 ± 0.87$^{b.c.d}$	57.23 ± 2.29$^{c.d.e.f}$	1.128 ± 0.41$^{e.f}$	9.86 ± 0.16$^{a.b}$
Teočak	9.83 ± 0.22^{b}	7.75 ± 0.92^{a}	61.13 ± 3.45$^{c.d.e.f}$	1.779 ± 0.76^{c}	5.87 ± 0.01$^{d.e.f}$
Čelić	11.08 ± 0.21^{a}	4.48 ± 0.66^{d}	73.30 ± 4.28$^{a.b}$	1.776 ± 0.86^{c}	7.38 ± 0.11$^{b.c.d}$
Gradačac	6.47 ± 0.77^{e}	5.84 ± 0.67$^{b.c.d}$	67.03 ± 4.15$^{b.c}$	1.476 ± 1.94^{d}	8.81 ± 1.44$^{a.b.c}$
Srebrenik	6.52 ± 0.23^{e}	5.36 ± 0.20$^{c.d}$	55.05 ± 2.03$^{e.f.g}$	0.942 ± 0.68^{f}	10.82 ± 0.42^{a}
Tuzla	7.63 ± 0.12$^{c.d}$	7.58 ± 0.74^{a}	65.73 ± 4.33$^{b.c.d}$	1.755 ± 0.51^{c}	6.55 ± 0.96$^{c.d.e}$
Lukavac	6.95 ± 0.36$^{d.e}$	4.89 ± 0.52^{d}	51.20 ± 6.92$^{f.g}$	1.480 ± 0.99^{d}	7.68 ± 0.09$^{b.c.d}$
Gračanica	10.21 ± 0.35^{b}	7.50 ± 0.49^{a}	78.03 ± 3.21^{a}	3.613 ± 0.96^{a}	3.63 ± 0.01^{f}
Doboj-Istok	6.90 ± 0.33$^{d.e}$	6.89 ± 0.16$^{a.b}$	62.00 ± 1.80$^{c.d.e}$	1.133 ± 0.51$^{e.f}$	8.85 ± 0.91$^{a.b.c}$

All results were expressed on dry weight;
Values in the same column in one period of collecting marked with different superscript are statistically different (P<0.05); GAE, gallic acid equivalent; QE, quercetin equivalent

On average, flavonoid content in March/April period was 6.66±2.50 mg QE/g and in May/June 6.00±1.24 mg QE/g, which corresponds to values reported by Mărghitaş et al. (2009), Carpes et al. (2009) and Araujo et al. (2018). Harif Fadzilah et al. (2017) reported higher values for stingless bee pollen (15.28 – 31.80 mg QE/g), underlining differences in foraging activities, diet and floral species between honey bee and stingless bee. Other authors (Gabriele et al., 2015; Feás et al., 2012) expressed values in rutin or catechin equivalents. Although there is no significant difference in flavonoid content between two examined periods, the difference is significant when considering different municipalities. The highest content of flavonoids was observed in municipalities Tuzla (average in both periods 8.83 mg QE/g), Kalesija (8.51 mg QE/g), Živinice (8.32 mg QE/g) and Doboj-Istok (8.10 mg QE/g).

Antioxidant capacity (AC) of bee pollen was determined by ABTS, FRAP and DPPH methods (Tables 3 and 4). On average, in March/April period, ABTS showed AC of 61.48±18.86%, and 60.95±9.14% in May/June. The lowest value was observed for bee pollen collected in Gradačac in March/April period, and the highest for pollen collected in the same period in Kalesija (Table 3). FRAP method also established higher values for March/April period (av. 2.401±0.858 mmol Fe^{2+}/g compared to 1.653±0.656 mmol Fe^{2+}/g), and differences are remarkable between municipalities as well. The highest value was observed for bee pollen collected in Kalesija in March/April period and the lowest for pollen collected in Srebrenik in May/June. The values determined in this research correspond with the results reported by LeBlanc et al. (2009) and Sardar et al. (2014). Better AC of pollen collected in March/April period showed DPPH method as well (Table 2), with average value of IC_{50} 3.05±1.30 mg/g compared to 7.73 mg/g in May/June. The values determined in this research correspond to values reported by Ulusoy and Kolayli (2014), Feás et al. (2012) and Morais et al. (2011). According to this test, pollen collected in Kalesija had the lowest AC, unlike in two previous tests.

Correlation analysis showed moderate positive correlation between AC determined by FRAP and total polyphenol content, and between AC determined by ABTS and total flavonoid content (Table 5). AC determined by DPPH is in negative, relatively weak negative correlation with AC determined by ABTS and total polyphenol content. Furthermore, relatively weak positive correlation was established between geographical origin and FRAP AC, total polyphenol and total flavonoid content (Table 5).

Table 5. Pearson correlation coefficients (r) between antioxidant capacity and physicochemical parameters, collection period and geographic origin of analysed bee pollen samples ([1]P<0.05; [2]P<0.01)

	DPPH	FRAP	ABTS	Polyphenols	Flavonoids	Proteins	Free fat	Carbohy-rates	Ash	Geographical origin	Collection period
DPPH	1										
FRAP	-0.227	1									
ABTS	-0.309[1]	0.135	1								
Polyphenols	-0.333[1]	0.641[2]	-0.109	1							
Flavonoids	-0.014	0.021	0.643[1]	0.082	1						
Proteins	-0.320[1]	0.000	0.180	0.068	0.198	1					
Free fat	-0.349[1]	0.179	0.227	0.306[1]	0.038	-0.290[1]	1				
Carbohydrates	0.301[1]	-0.010	-0.346[1]	-0.148	-0.370[2]	-0.910[2]	-0.017	1			
Ash	-0.315[1]	0.032	0.136	-0.172	-0.054	0.727[2]	-0.408[2]	-0.574[2]	1		
Geographic origin	-0.183	0.227[2]	0.147	0.162[1]	0.269[2]	0.132	0.024	-0.130	0.295[1]	1	
Collection period	0.8012	-0.442[2]	-0.018	-0.451[2]	-0.165	-0.059	-0.616[2]	0.145	-0.030	0.000	1

Relatively high positive correlation between polyphenols and FRAP was also reported by Velásquez et al. (2017) and Mărghitaş et al. (2009).

CONCLUSION

The presented results showed a great variability in physicochemical and antioxidant properties of bee pollen load samples from Tuzla Canton. Bee pollen collected in March/April had lower content of proteins, but higher content of total polyphenols and higher antioxidant activity, while total flavonoid content was not influenced by collection period. The location had significant influence on bee pollen properties, esp. total polyphenol and total flavonoid content, as well as antioxidant capacity. *Salix* sp. pollen showed high total polyphenol and flavonoid content as well as high AOC and due to the favourable geographical and climatic conditions, Tuzla Canton has a great potential for production of this specific and valuable bee pollen.

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