Original Article

DETERMINATION OF ANTIOXIDANT ACTIVITY, PHENOLIC COMPOUND, MINERAL CONTENTS AND FATTY ACID COMPOSITIONS OF BEE POLLEN GRAINS COLLECTED FROM DIFFERENT LOCATIONS

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Abstract

The objective of the present work was to investigate the influence of locations on bioactive propertiest, phenolic compounds and mineral contents of bee pollens. The oil content of pollen grains changed between 3.50% (Alanya) and 6.85% (Russia-Perm Region). The highest total phenolic content (720 mg/100g) and antioxidant activity values (81.4%) were observed in pollens obtained from the Russia-Perm Region and Alanya districts, respectively. Additionally, the highest carotenoid was found in a pollen sample collected from Karaman (Sarıveliler) (98.6 mg/g). The major phenolic compounds were (+)-catechin (66.75-337.39 mg/100g) and quercetin (61.2-1221.7 mg/100g) in all pollen samples. The pollen samples were observed to be a significant source of potassium (3846-6287 mg/kg), phosphorus (2947-5010 mg/kg), calcium (1022-2424 mg/kg) and sulfur (1744-2397 mg/kg). All of the analysis results were significantly affected by supplying locations. The antioxidant activity values of pollens were found partly similar and varied depending on locations. The content of saturated fatty acid (palmitic) was high (20-30%) in the tested pollen samples but did not exceed the content of linoleic acid.

Keywords: bee pollen, bioactive properties, carotenoid, fatty acids, minerals, oil, phenolic compounds

INTRODUCTION

Pollen has nutritional and physiological properties (Kroyer & Hegedus, 2001). The antiatherosclerotic action of pollen extract is probably due to the metabolic conversion of α-linolenic acid into eicosapentaenoic acid (Wójcicki et al., 1986; Seppanen et al., 1989) as well as its polyphenolic substances, flavonoids (Kroyer & Hegedus, 2001; Campos et al., 2003) and an-

timicrobial activity (Garcia et al., 2001; Basim, Basim, & Özcan, 2006; Carpes et al., 2009). Pollen grains are rich in phosphorus (6923.63 mg/kg), calcium (1031.98 mg/kg), potassium (5116.76 mg/kg), magnesium (754.64 mg/kg), manganese (5116.8 mg/kg) and zinc (50.6 mg/kg pollen) (Carpes et al., 2009). Flavonol, glycossides and aglycones, and hydroxycinnamic acids present in free forms or combined with other pollen components are the major phenolic profile of bee pollen (Chantarudee et al., 2012; Fanali, Dugo, & Rocco, 2013). Antioxidant activity values of pollen grains decrease after one year (Campos et al., 2003). Bee pollen constitutes one of nature's most complete and nutritious foods because it gathers almost all nutrients necessary for humans. From the information found on the literature it was possible to depict some of the most investigated effects of bee pollen. The constituents of pollen depends on botanical and geographical origin, plant status and climatic conditions (Carpes et al., 2009; Morais et al., 2011). The objective of the present work was to evaluate the effect of locations on the oil content, total phenolic content, antioxidant activity, carotenoid content, phenolic compounds and mineral content and fatty acids of bee pollen grains.

MATERIAL AND METHODS

Samples

Samples of bee pollen were collected during the 2016 beekeeping season (May - July) from the Turkish provinces of Antalya (Alanya), Hatay, Karaman (Sarıveliler), Konya (Hadim), Konya (Taşkent), Konya (Karapınar), Niğde (Bor). Two bee pollen samples were also provided from the Russia-Altay Mountain and Russia-Perm region in the same year. About 50 g of bee pollen sample was harvested from beehives found from the seven locations. The polen samples were transferred to a laboratory in cooler bag. The pollen wase collected from the tray, placed in a clean paper bag and left for 24 hours to dry at room temperature. The samples were homogenised and kept in 500 mL glass flasks. After drying, the samples were frozen at -18°C until being used. For the analyses, the mixed bee pollen was used. Prior to the chemical analyses, the pollen pellets were ground. All analyses were carried out in triplicate, and all results were expressed as mean±standard deviation.

Methods

Sample extraction

Pollen was extracted according to Ares et al. (2016) with some modifications. About 12 mL of methanol was added to 0.5 g ground pollen samples in an erlenmeyer flask. The mixture was shaken by vortex for 1 min and sonicated for 10 min, followed by centrifugation at 6000 rpm for 10 min. These steps were repeated twice. After the supernatants were collected, extract was concentrated at 37°C in a rotary evaporator. The volume of the extracts were completed to 5 mL methanol and then filtered with a 0.45 µm nylon filter.

Total phenolic content

Total phenol contents of the pollen samples were determined with the Folin-Ciocalteu (FC) reagent according to Yoo et al., (2004). Total phenolic content of the pollen extract was measured as 750 nm wave length in a spectrophotometer (Shimadzu, Japan). The results were given as mg gallic acid equivalent (GAE)/100 gram of fresh weight.

Antioxidant activity

The free-radicalscavenging activity of the pollen samples was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (1998). After the extract was mixed with 2 mL methanolic solution of DPPH, the absorbance was recorded at 517 nm with the use of a spectrophotometer.

Carotenoid content

Carotenoids were extracted according to Silva da Rocha et al. (2015). Acetone (25 mL) was added to 2 g ground bee pollen sample in a 100 mL flask. The mixture was shaken by vortex for 10 min and filtrated with filter paper (Whatman No. 1), followed by a seperation funnel. The filtrate was fractionated with 20 mL

of petroleum ether and washed with 100 mL of distilled water in order to remove the acetone. These steps were performed twice. Whatman No. 1 covered with anhydrous sodium sulfate (5 g) for removing residual water was used to filtrate the petroleum ether layer. The volume of the extracts was completed to 25 mL by petroleum ether. After these procedures, the absorbance was measured at 450 nm.

Determination of phenolic compounds

Phenolic compounds of bee pollen extracts were determined using Shimadzu-HPLC equipped with PDA detector and Inertsil ODS-3 (5 µm; 4.6 x 250 mm) column. 0.05% Acetic acid in water (A) and acetonitrile (B) mixture of the mobile phase were used for phenolic constituents analyses by HPLC. The flow rate of the mobile phase was 1 mL /min at 30°C and the injection volume was 20 µL. The peaks were recorded at 280 and 330 nm with PDA detector. The gradient program was as follows: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The total running time per sample was 60 min. Phenolic compounds were determined according to the retention time and absorption spectra of peaks of standard compounds. The total area under peak was used to quantify each phenolic.

Mineral content

About 0.5 g of ground pollen was put into a heated cup contained 15 mL of pure NHO_3 and 2 mL H_2O_2 (30% v/v). The sample was incinerated in a MARS 5 microwave oven at 210°C. The filtrates were analysed through Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) against standard solutions of known concentrations (Skujins, 1998).

Oil extraction

Pollen grains were extracted in a Soxhlet apparatus for 5 h, and the solvent (petroleum ether) was removed with a rotary evaporator at 50°C (AOAC,1990). The oil obtained was kept at +4°C till analysis.

Fatty acid composition

Pollen oil was esterificated according to the ISO-5509 (1978) method, and fatty acid methyl esters were analyzed through the use of gas chromatography (Shimadzu GC-2010) equipped with flame-ionization detector (FID) and capillary column (Tecnocroma TR-CN100, 60 m x 0.25 mm, film thickness: 0.20 µm). A standard fatty acid methyl ester mixture (Sigma Chemical Co.) was used to determine sample peaks (AOAC, 1990).

Statistical Analyses

Results were subjected to analysis of variance with mean separation by Duncan's multiple range test. Differences were considered statistically significant at the $p<0.05$ levels. All analyses were carried out in triplicate, and all results were expressed as mean±standard deviation (MSTAT C) of independent pollen samples (Püskülcü & İkiz, 1989).

RESULTS

The oil, total phenolic content, antioxidant activity values and carotenoid contents of bee pollen samples are reported in Tab. 1. The oil contents of bee pollens changed between 3.37% (Alanya) and 6.85% (Russia-Perm Region). The total phenolic content ranged from 434.17 mg GAE/100g (Hatay) to 719.58 mg GAE/100g (Russia-Perm region). Carotenoid contents of pollen samples changed between 12.78 mg/g and 98.62 mg/g. In addition, the bee pollen collected from Karaman (Sarıveliler) had the maximum carotenoid content, with a value of 98.62 mg/g. The polyphenol content of bee pollen samples showed a change to location supplied. A relationship was observed among total phenol antioxidant activity values of bee pollen extracts depending on locations ($p<0.05$) (Tab. 1). A linear relationship has been observed between total phenol and antioxidant activity values in Russian bee pollen samples ($p<0.05$) (Tab. 1).

The phenolic compounds of bee pollen samples are presented in Tab. 2. (+)-Catechin and quercetin were determined as the main phenolic

Table 1.
Oil content, total phenolic contents, antioxidant activity, carotenoid contents of pollen grains

Locations	Oil content (%)	Total phenolic content (mg/100g)	Antioxidant activity (%)	Carotenoid content (mg/g)
Konya (Karapınar)	4.83±0.01*^{c}	599.44±0.01^{c}	73.07±0.01^{d}	74.03±0.01^{c}
Konya (Taşkent)	3.59±0.02^{d}	677.92±0.01^{b}	63.88±0.01^{g}	67.03±0.03^{e}
Konya (Hadim)	3.37±0.02^{d}	652.22±0.01^{bc}	74.41±0.01^{cd}	48.47±0.03^{f}
Russia-Altay Mountains	4.53±0.02^{c}	668.19±0.01^{b}	75.41±0.01^{c}	24.11±0.09^{g}
Russia-Perm Region	6.85±0.02^{a}	719.58±0.01^{a}	66.35±0.03^{f}	12.78±0.01^{h}
Karaman (Sarıveliler)	6.73±0.01^{a}	509.86±0.02^{c}	60.35±0.03^{fg}	98.62±0.02^{a}
Hatay	5.21±0.06^{b}	434.17±0.01^{de}	78.78±0.02^{b}	72.34±0.05^{d}
Antalya (Alanya)	3.50±0.09^{d}	656.04±0.01^{bc}	81.41±0.02^{a}	40.03±0.07^{f}
Niğde(Bor)	4.60±0.03^{c}	499.44±0.04^{d}	70.83±0.02^{e}	81.74±0.09^{b}

* mean±standard deviation (n:3);
** Values within each column followed by different letters are significantly different ($p<0.05$)

compounds in all bee pollen samples. The maximum (+)-catechin and quercetin contents were found in pollens of Hadim (337.40 mg/100g) and Karapınar (1221.73 mg/100g), respectively. Based on the location of pollens, the major gallic acid, naringenin, 1,2-dihydroxybenzene, rutin trihydrate and 3,4-dihydroxybenzoic acid were determined in bee pollens harvested from Russia-Altay Moutains (101.77 mg/100g), Sarı Veliler (501.13 mg/100g), Hadim (114.97 mg/100g), Alanya (80.47 mg/100g) and Niğde-Bor (94.74 mg/100g), respectively (Tab. 2). In addition to these phenolic compounds, all pollens had minor amounts of syringic acid, caffeic acid, *trans*-ferulic acid, apigenin 7 glucoside, *p*-coumaric acid, resveratrol, *trans*-cinnamic acid, kaempferol and isorhamnetin. Statistically significant differences were observed between phenolic compounds and locations. Statistically significant differences were found among gallic acid, 3,4-dihydroxybenzoic acid, (+)-Catechin, 1,2-dihydroxybenzene, rutin trihydrate, resveratrol, quercetin ($p<0.05$) (Tab. 2).

Table 3 shows micro- and macro-elements of pollens. Pollen grains are a significant source of potassium (K), phosphorus (P), calcium (Ca), sulfur (S), magnesium (Mg). Other such minerals as Al, Fe, Na, Zn, Mn, B and Cu were found at lower levels. In addition, the highest potassium content was observed in pollens collected from Russia-Altay Mountains (6287.39 mg/kg), followed by Konya (Taşkent) (5851.94 mg/kg) and Konya (Hadim) (5834.58 mg/kg), Antalya (Alanya) (5723.87 mg/kg), Russian-Perm Region (5208.03 mg/kg) and Niğde (Bor) (4728.45 mg/kg). The highest P contents (5009.95 mg/kg) was determined in pollen samples obtained from Russia-Altay Mountains, while pollens collected from Niğde-Bor and Russia-Perm Region had the highest Ca (2424.45 mg/kg) and S (2397.00 mg/kg) contents, respectively (Tab. 3). Sulfur content of pollen changed between 1743.58 mg/kg (Karaman-Sarıveliler) and 2397.00 mg/kg (Russia-Perm Region).

The fatty acid composition of bee pollen oils is shown in Tab. 4. The key fatty acids of pollen samples were palmitic, oleic, linoleic and linolenic. In general, the identified saturated fatty acids included lauric, myristic, palmitic, arachidic and stearic. Of all saturated acids, the palmitic acid contents of pollen samples changed between 10.08% (Russia-Perm Region) and 29.20% (Taşkent). The highest palmitic acid content was found in bee pollen sample collected from Taşkent (29.20%), followed by Konya (Hadim) (22.86%), Hatay (14.20%), Antalya (Alanya)

Table 2.

Phenolic compounds of pollen grains (mg/100g)

Phenolics	Locations								
	Konya (Karapınar)	**Konya (Taşkent)**	**Konya (Hadim)**	**Russia-Altay mountains**	**Russia-Perm region**	**Karaman (Sarıveliler)**	**Hatay**	**Antalya (Alanya)**	**Niğde (Bor)**
Gallic acid	32.89±0.62^{*b}	22.0±0.03^{e}	18.09±0.45^{f}	101.77±0.16^{a}	29.26±0.70^{c}	30.58±0.68^{b}	25.27±0.39^{d}	11.71±0.67^{g}	6.17±0.08h
3,4-dihydroxy-benzoic acid	39.37±0.86^{e**}	23.53±0.05^{f}	46.57±0.99^{c}	53.26±0.52^{b}	28.19±0.62^{e}	23.00±0.64^{f}	44.46±0.48^{d}	17.09±0.56^{g}	94.74±2.99^{a}
(+)-catechin	88.40±1.51^{e}	93.93±1.14^{d}	337.40±0.87^{a}	125.48±1.71^{b}	73.88±5.35^{f}	96.72±1.19^{c}	87.00±0.53^{e}	92.91±0.14^{d}	66.75±0.84^{g}
1,2-dihydroxy-benzene	34.72±0.91^{g}	8.34±0.48ı	114.97±0.03^{a}	39.40±1.06^{f}	49.51±0.74^{e}	28.83±0.02h	77.16±0.61^{b}	65.73±1.61^{c}	54.52±0.39^{d}
Syringic acid	12.16±0.27^{e}	23.77±0.01^{a}	16.35±0.22^{c}	11.63±0.41^{f}	19.73±0.45^{b}	10.55±0.70^{f}	14.75±0.05^{d}	12.55±0.14^{e}	5.56±0.01^{g}
Caffeic acid	12.08±0.44^{d}	6.63±0.45^{g}	14.03±0.17^{c}	7.93±0.48^{f}	5.84±0.35h	8.70±0.59^{ef}	16.24±0.41^{b}	9.18±0.37^{e}	23.86±0.63^{a}
Rutin trihydrate	54.50±2.73^{b}	14.46±0.28^{f}	11.28±0.04^{g}	20.46±0.09^{d}	41.84±0.56^{c}	9.82±0.62h	17.58±0.27^{e}	80.47±0.46^{a}	16.49±0.49^{ef}
p-coumaric acid	1.90±0.08^{d}	2.09±0.15^{c}	10.46±0.18^{a}	4.33±0.32^{b}	2.73±0.19^{c}	1.39±0.10^{de}	2.63±0.24^{c}	4.08±0.19^{b}	2.68±0.27^{c}
Trans-ferulic acid	3.25±0.13^{g}	18.27±0.06^{c}	23.01±0.93^{b}	23.68±0.37^{b}	42.24±0.25^{a}	4.75±0.45^{f}	12.48±0.81^{d}	4.89±0.49^{f}	5.19±0.34^{e}
Apigenin 7 glucoside	4.24±0.17^{g}	8.23±0.55^{e}	24.31±0.41^{a}	20.13±0.08^{c}	22.23±0.72^{b}	4.91±0.16^{g}	5.77±0.39^{f}	10.76±0.25^{d}	5.95±0.49^{f}
Resveratrol	17.59±0.30^{e}	14.20±0.28^{g}	82.02±0.04^{a}	75.11±0.19^{b}	15.71±0.09^{f}	4.24±0.23h	3.83±0.09ı	36.19±0.68^{c}	23.56±0.81^{d}
Quercetin	1221.73±1.11^{a}	159.93±1.03^{f}	685.36±0.60^{b}	141.63±0.30^{g}	195.40±0.08^{e}	61.23±0.76ı	362.03±0.01^{c}	120.35±4.89h	265.18±2.93^{d}
Trans-cinnamic acid	1.57±0.18h	7.75±0.58^{e}	5.84±0.23^{g}	26.73±0.30^{c}	31.13±0.36^{b}	181.33±0.25^{a}	13.46±0.40^{d}	6.07±0.30^{f}	30.77±0.01^{bc}
Naringenin	5.90±0.67h	8.96±1.06^{g}	44.84±0.01^{d}	58.84±1.20^{b}	11.86±0.01^{f}	501.13±2.38^{a}	41.78±0.64^{c}	4.43±0.21ı	18.48±0.52^{e}
Kaempferol	1.91±0.10^{e}	19.24±2.01^{b}	2.86±0.01^{d}	39.37±0.14^{a}	39.15±0.88^{a}	-***	19.90±0.17^{b}	4.98±0.34^{c}	-
Isorhamnetin	2.21±0.08h	13.11±0.62^{e}	3.26±0.02^{g}	71.23±0.40^{a}	59.74±0.32^{b}	23.01±0.87^{c}	16.12±0.31^{d}	17.83±0.01^{d}	11.97±0.15^{f}

*mean±standard deviation (n:3); **Values within each column followed by different letters are significantly different ($p<0.05$);***nonidentified

(13.74%) and Niğde (Bor) (13.15%). The highest lauric acid (7.37%) was found in Sarıveliler pollen sample. Oleic acid contents of pollens ranged from 12.66% to 54.35%, and the highest oleic acid amount was found in the pollen from Karapınar (54.35%). The linoleic and linolenic acid contents of pollen oils changed between 6.27% (Russia-Perm Region) and 25.66% (Alanya) to 2.02% (Alanya) and 24.80% (Hadim), respectively ($p<0.05$) (Tab. 4). In addition to these

Table 3.

Mineral contents of pollen grains (mg/kg)

Locations	Al	Mo	Ca	B	Cd	Cr	Cu	Fe
Konya(Karapinar)	63.13±0.12^{*c}	0.24±0.02^{g}	2197.52±5.08^{b}	34.57±0.08^{b}	0.04±0.01^{e}	0.80±0.54^{a}	11.57±0.12^{a}	90.15±0.14^{d}
Konya (Taşkent)	37.83±0.33^{i**}	0.47±0.02^{c}	1626.78±2.80^{e}	8.83±0.06^{i}	0.09±0.01^{d}	0.17±0.02^{d}	5.89±0.07^{e}	73.60±0.12^{f}
Konya (Hadim)	55.73±1.10^{d}	0.48±0.01^{b}	2114.68±4.17^{c}	13.29±0.08^{f}	0.07±0.01^{d}	0.38±0.04^{c}	7.11±0.06^{c}	129.07±0.09^{a}
Russia-Altay mountains	47.51±0.53^{e}	0.16±0.02^{h}	1655.13±6.19^{e}	11.54±0.04^{g}	0.19±0.04^{a}	-***	7.02±0.07^{c}	68.15±0.14^{g}
Russia-Perm Region	12.06±0.07^{h}	0.45±0.01^{d}	1974.46±4.80^{d}	39.97±0.08^{a}	0.11±0.03^{c}	0.13±0.05^{e}	6.54±0.07^{d}	38.26±0.09^{i}
Karaman (Sarıveliler)	89.28±0.66^{a}	0.04±0.01^{i}	1694.73±9.34^{e}	26.14±0.03^{d}	0.06±0.01^{e}	0.45±0.06^{b}	7.18±0.08^{c}	92.03±0.08^{c}
Hatay	85.58±0.67^{b}	0.37±0.02^{e}	2164.56±4.11^{b}	27.15±0.05^{c}	0.04±0.01^{e}	0.87±0.07^{a}	8.50±0.09^{b}	103.63±0.09^{b}
Antalya (Alanya)	27.26±0.65^{g}	0.33±0.03^{f}	1021.94±6.00^{f}	9.68±0.04^{h}	0.14±0.04^{b}	0.39±0.05^{c}	6.69±0.07^{d}	62.37±0.08^{h}
Niğde (Bor)	48.54±0.55^{e}	0.54±0.03^{a}	2424.45±4.08^{a}	23.14±0.04^{e}	0.04±0.01^{e}	0.13±0.03^{e}	7.56±0.09^{c}	84.53±0.08^{e}
Locations	**K**	**Mg**	**Mn**	**Na**	**Ni**	**P**	**Pb**	**Zn**
Konya(Karapinar)	4854.43±10.1^{d}	754.45±9.03^{e}	22.51±0.08^{e}	141.59±6.04	0.84±0.06^{e}	4123.04±5.00^{c}	-	28.91±0.07^{f}
Konya (Taşkent)	5851.94±7.0^{b}	1188.79±6.52^{a}	30.43±0.08^{c}	61.90±0.12^{g}	0.58±0.07^{f}	5261.63±7.03^{a}	0.55±0.09^{c}	36.44±0.10^{d}
Konya (Hadim)	5834.58±8.7^{b}	1187.44±5.50^{a}	58.66±0.11^{a}	112.50±4.50^{d}	2.42±0.07^{a}	4396.41±7.50^{c}	0.31±0.05^{d}	38.52±0.07^{c}
Russia-Altay mountains	6287.39±8.5^{a}	1025.37±7.03^{c}	38.23±0.09^{b}	133.02±4.00^{c}	1.63±0.09^{b}	5009.95±7.00^{b}	0.64±0.07^{a}	42.45±0.11^{b}
Russia-Perm Region	5208.03±5.58^{c}	1213.46±5.50^{b}	23.87±0.07^{d}	67.49±5.50^{f}	0.39±0.07^{g}	4494.14±9.21^{c}	0.18±0.04^{f}	28.00±0.06^{f}
Karaman (Sarıveliler)	3845.96±9.83^{e}	712.83±5.62^{f}	11.06±0.07^{g}	273.21±5.52^{a}	0.31±0.06^{h}	2947.20±9.58^{e}	0.25±0.05^{e}	24.07±0.06^{h}
Hatay	4264.57±6.1^{d}	725.23±6.01^{e}	19.51±0.08^{f}	133.83±5.53^{c}	0.85±0.04^{e}	3744.29±8.61^{d}	0.34±0.06^{d}	161.99±5.57^{a}
Antalya (Alanya)	5723.87±6.0^{b}	683.03±6.00^{g}	24.88±0.07^{d}	88.91±3.00^{e}	1.48±0.15^{c}	3511.29±6.51^{d}	0.60±0.08^{b}	29.91±0.11^{g}
Niğde (Bor)	4728.45±7.04^{d}	886.54±5.50^{d}	21.25±0.09^{ef}	133.96±5.56^{c}	0.98±0.01^{d}	4060.67±7.02^{c}	0.25±0.06^{e}	33.17±0.09^{e}

*mean±standard deviation (n:3); **Values within each column followed by different letters are significantly different ($p<0.05$);***nondetermined

Table 3. (continued) (mg/kg)

Locations	S
Konya(Karapınar)	2161.09±7.53[c]
Konya (Taşkent)	2061.17±6.01[d]
Konya (Hadim)	2015.51±4.50[d]
Russia-Altay mountains	2162.76±8.51[c]
Russia-Perm Region	2397.00±7.55[a]
Karaman (Sarıveliler)	1743.58±5.50[f]
Hatay	1871.50±7.05[e]
Antalya (Alanya)	1839.72±6.02[e]
Niğde (Bor)	2204.28±6.51[b]

* mean±standard deviation (n:3);
** Values within each column followed by different letters are significantly different (p<0.05)

Table 4.

Fatty acid compositions of pollen grain oils (%)

Fatty acids	Locations								
	Konya (Karapınar)	Konya (Taşkent)	Konya (Hadim)	Russia-Altay mountains	Russia-Perm region	Karaman (Sarıveliler)	Hatay	Antalya (Alanya)	Niğde (Bor)
Lauric	0.67±0.05*[f]	0.85±0.01[d]	0.75±0.02[e]	1.20±0.03[b]	0.96±0.01[c]	7.37±0.06[a]	0.87±0.02[d]	0.58±0.04[g]	1.14±0.05[b]
Myristic	0.28±0.07[g]**	0.72±0.02[f]	1.63±0.05[c]	0.62±0.02[f]	3.29±0.13[a]	0.87±0.13[e]	0.38±0.01[g]	1.05±0.06[d]	2.79±0.16[b]
Palmitic	10.40±0.54[f]	29.20±0.01[a]	22.86±1.05[b]	12.53±0.12[e]	10.08±0.04[f]	12.83±0.99[e]	14.20±0.02[c]	13.74±0.62[d]	13.15±0.88[d]
Stearic	3.11±0.16[c]	3.17±0.02[c]	3.98±0.18[c]	7.07±0.16[a]	3.44±0.03[c]	6.23±0.01[b]	2.92±0.02[d]	3.27±0.14[c]	2.49±0.17[d]
Elaidic	0.83±0.76[a]	-***	-	0.34±0.01[b]	-	0.11±0.01[c]	-	-	-
Oleic	54.35±0.91[a]	12.66±0.01[g]	18.77±0.86[f]	36.27±0.46[c]	23.30±0.07[e]	19.38±0.01[f]	47.23±0.06[b]	35.94±0.59[c]	27.05±0.89[d]
Linole-laidic	0.16±0.06[c]	-	-	-	0.91±0.00[b]	-	-	-	1.31±0.03[a]
Linoleic	10.22±0.50[g]	13.74±0.01[e]	10.27±0.52[g]	17.05±0.22[c]	6.27±0.13[h]	19.59±0.03[b]	11.83±0.04[f]	25.66±0.13[a]	16.78±0.61[d]
Arachidic	0.95±0.02[c]	0.67±0.02[d]	1.55±0.06[b]	0.44±0.01[e]	0.48±0.00[e]	0.40±0.01[e]	1.54±0.02[b]	0.63±0.05[d]	2.25±0.16[a]
Linolenic	9.40±0.09[f]	22.90±0.01[b]	24.80±0.28[a]	11.29±0.23[e]	11.09±0.02[e]	18.37±0.76[d]	11.42±0.03[e]	2.02±0.08[g]	20.35±0.43[c]
Behenic	0.45±0.05[e]	0.74±0.01[b]	0.59±0.01[d]	0.48±0.02[e]	2.76±0.01[a]	0.56±0.09[d]	0.61±0.01[c]	0.52±0.11[d]	0.71±0.04[b]
Arachi-donic	0.38±0.14[g]	5.09±0.01[a]	2.06±0.04[c]	0.40±0.03[f]	0.82±0.03[e]	1.08±0.19[d]	1.11±0.11[d]	0.47±0.02[f]	4.27±0.01[b]

* mean±standard deviation (n:3);
** Values within each column followed by different letters are significantly different (p<0.05);
*** nonidentified

fatty acids, pollen samples had minor amounts of lauric, myristic, stearic, arachidic, behenic and arachidonic acids. Statisticaly significiant differences were not observed among the stearic acid contents of Russian-perm region, Konya (Hadim), Antalya (Alanya), Konya (Karapınar) and Konya (Taşkent) locations (Tab. 4).

DISCUSSION

According to the study by Feas et al. (2012), the phenolic contents of organic bee pollen varied between 12.9 and 19.8 mg GAE/g of extract. In a previous study, the highest polyphenol content had been found in monofloral bee pollen from

Brassica napus subsp*. napus* L. (1389.67 mg GAE/kg), followed by *Papaver somniferum* L. bee pollen (817.33 mg GAE/kg) (Fatrcova-Sramkova et al., 2013). Pascoal et al. (2014) determined that the highest and the lowest values of phenolics in eight commercial bee pollens were 32.15 mg/g and 18.55 mg/g, respectively. In another study, the phenolic contents of bee pollens were found to be between 10.5 and 16.8 mg GAE/g (Morais et al., 2011). The total phenolic contents of pollen grains ranged from 19.28 to 48.90 mg GAE/ g (Carpes et al., 2009). The average polyphenol concentration in fresh samples of ethanol exracts was 21.3 mg GAE/g of extract (Rzepecka-Stojko et al., 2012). In previous studies, this value for this extract was 24.6 mg GAE/g (Kroyer & Hegedus, 2001), 12.4 mg GAE/g (Bonvehi, Torrento, & Lorente, 2001) and 32.4 mg GAE/g (Rzepecka-Stojko et al., 2010), 30.46 mg GAE/g (Carpes et al., 2009).

Pollen collected from Alanya demonstrated the highest antioxidant activity in the range of 81.41%. The lowest quantity of antioxidants (60.35%) was determined in the pollen collected from Sarıveliler. In an experiment by Kim et al. (2015), the antioxidant activity of pollen provided from Korea ranged from 12.7% to 50.1%. The antioxidant activity of monofloral bee pollens ranged from 0.135 and 2.814 mmol Trolox/g (Marghitas et al., 2009). Another study showed that the antioxidant activity of 25 µg/mL methanol extracts of pollen samples varied between 86.3 and 90.2% (Negri et al., 2011). Ethanolic bee pollen extracts exhibited high free radical scavenging activity with DPPH inhibition values ranged from 37.95% (EC50: 641.3 µg/mL) of *Rubus* extracts and 94.45 (EC50:215.2 µg/mL) of Castanea extracts (Gabriele et al., 2015). Carotenoid contents of pollens were found to be between 12.78 mg/g and 98.62 mg/g. The highest carotenoid content was determined in pollen collected from Sarıveliler (98.62 mg/g), followed by Niğde-Bor (81.74 mg/g) and Karapınar (74.03 mg/g). The content of pollen obtained from the Russia-Altay mountains was minimum with 12.78 mg/g. Statistically significant differences were observed between the flavonoid content of the pollen depending on the locations ($p<0.05$). Bee pollen constitutes a natural source of antioxidants such as phenolic acids and flavonoids, which are responsible for its biological activity (Rzepecka-Stojko et al., 2010). In previous studies, pollen grains contained snaringenin, isorhamnetin 3-o-rutinoside, rhamnetin 3-o-neohesperidoride, isorhamnetin, quercetin 3-o-rutinoside, quercetin 3-o-neohesperidoside, vanillic acid, protocatechuic acid, gallic acid, p-coumaric acid, hesperidin, rutin, apigenin, luteolin, isorhamnetin, kaempherol and quercetin (Bonvehi et al., 2001; Han et al., 2012). Fanali et al. (2013) used nano-liquid chromatography for the determination of polyphenols in bee pollen, and identified nine polyphenols, namely o-cumaric acid, p-coumaric acid, ferulic acid, myricetin, cinnamic acid, quercetin, naringenin, hesperetin and kaempferol. Different flavonoid such as quercetin, apigenin, isorhamnetin were identified in honey and pollen (Baltrušaitytė et al., 2007; Isidorov et al., 2015).

Pollen grains are a significant source of potassium (K), phosphorus (P), calcium (Ca), sulfur (S), magnesium (Mg), but mineral contents of pollen grains change depending on location. Pollen grains are rich in phosphorus (6923.63 mg/kg), calcium (1031.98 mg/kg), potassium (5116.76 mg/kg), magnesium (754.64 mg/kg), manganese (5116.8 mg/kg) and zinc (50.6 mg/kg pollen), and and our results showed slight differences according to those of Carpes et al. (2009). Several other such factors as plant varieties, climatic conditions, moisture and pollen collection time affected bioactive properties of pollen grains.

The kind of bee pollen plays an important role in fatty acid composition and the shelf-life of the product due to polyunsaturated fatty acids. This is because high levels of linoleic and linolenic acid of pollen oils were found. The composition and oil properties of pollen grains depend upon the locations, flora type and such plant growing conditions as climatic factor and soil structure. Loublier et al. (1991) extracted the pollen grains and identified high levels of palmitic, α-linolenic and eicosenoic acids. Saa-Otero et al. (2000) reported that several pollen types contained

14.7 to 31.9% palmitic, 1.1 to 3.8% stearic, 5.3 to 22.7% oleic, 4.9 to 38.0% linoleic and 10.3 to 38.4% linolenic acids. Nicolson & Human (2013) determined 28.70% lauric, 5.20% myristic, 25.95% palmitic, 2.12% stearic, 5.91% oleic, 4.80% linoleic, 19.94% α-linoleinic and 7.38% eicosenoic acids in stored pollen grains. The list of fatty acids found in the pollen studied here is similar to those identified by Serra-Bonvehi et al. (1987), Loublier et al. (1991), Saa-Otero et al. (2000), Hassan (2011) who reported that palm pollen oil contained 4.82% lauric, 13.33% myristic, 34.45% palmitic, 2.04% stearic, 7.32% arachidic, 7.07% palmitoleic, 7.19% oleic, 14.24% linoleic and 1.27% γ-linolenic, and 4.57% arachidonic acids. Bastos et al., (2004) found oleic, linoleic, and palmitic acids in pollen oil. Pollen samples changed widely in the quantity of individual and total fatty acids with some samples having several times the quantity found in other samples. Generally, the fatty acid compositions of pollens were significantly affected by location.

As a result, oil contents of pollen grains are low, but total phenol contents are very high. Pollen samples contain different saturated and unsaturated long-chain fatty acids. Antioxidant activity values of pollen were found to be partly similar and varied depending on locations. The content of saturated fatty acid (palmitic) was high (20-30%) in the tested pollen samples but did not exceed the linoleic acid content. Bee pollen grains can be applied as a suitable dietary supplement due to high saturated and unsaturated fatty acids, minerals and phenolic compounds. Very few differences in fatty acid composition were found among pollen types.

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