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Abstract. The element concentration (Cu, Fe, Zn, Mn, Cr, Co, Ni, Se, K, Na, Ca and Mg), heavy metal concentration (Cd, Hg, Pb and As) and fatty acid composition of 12 Serbian bee bread samples from different geographical origins were examined. The element concentration was examined using ICP-MS, and total lipids for fatty acid determination were extracted from homogenized bee bread samples with hexane/isopropanol mixture by accelerated solvent extraction. Potassium was the major element, ranging between 5515±361.20 mg/kg and 7487±381.50 mg/kg. The highest As and Pb concentrations were found in bee breads from Lazarevac. This bee bread also contained the highest level of PUFA and SFA. Also, the n-6/n-3 ratio ranged between 0.86±0.28 and 1.40±0.05, indicating bee bread can be a good source of unsaturated fatty acids. Bee bread could be useful in monitoring environmental contamination by heavy metals (Cd, Hg, Pb and As), although complex studies of all bee products give sufficient information on this topic.

1. Introduction

Honey bee products, due to their nutritional and medical properties, are widely used in human diet and medicine. Therefore, many studies have presented the chemical composition of these products. A majority of publications refer to honey, wax, or propolis composition [1-7]. However, the composition of bee bread, including fatty acid composition and element concentration, has not been equally studied. Fermented bee pollen is called bee bread (Figure 1) and mainly includes pollen, honey, and secretions of bees' salivary glands [8]. This work presents, for the first time, the fatty acid composition and element concentration of 12 samples of beebread, obtained from different geographical locations in Serbia, where bee bread, due to its nutritional and physiological properties, is used in human diets.

Figure 1. Bee bread (in beekeeping frame) (J. Ciric)

2. Material and Methods

A total of 12 bee bread samples were obtained from apiaries located in different Serbian regions (I – Gornji Milanovac, II – Lazarevac and III – Ležimir) between May and August of 2018. The bee bread samples were hand collected from four healthy beehives in apiaries with 30-70 colonies and kept at -20 °C.

Approximately 0.5 g of homogenized bee bread was transferred into a Teflon vessel with 5 ml nitric acid (67% Trace Metal Grade, Fisher Scientific, Loughborough, UK) and 1.5 ml hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MO, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) program consisted of three steps: 5 min from RT to 180 °C, 10 min hold at 180 °C, and 20 min cooling. Analysis of the following 16 elements: Fe, Zn, Cu, Mn, Se, Cr, Co, Ni, Na, K, Mg, Ca, Cd, Pb, Hg and As, was performed by inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany). Multielemental internal standard was introduced into the ICP-MS during the measurements.

Total lipids for fatty acid determination were extracted from homogenized bee bread with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, GmbH, Idstein, Germany). After evaporation of solvent until dryness under a stream of nitrogen, total lipids were converted to fatty acid methyl esters (FAME) by trimethylsulfonium hydroxide. FAMEs were determined using a Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cyanopropyl HP-88 capillary column (100 m x 0.25 mm x 0.20 mm) [9]. Temperature of the injector and detector were 250 °C and 280 °C, respectively. FAMEs were identified on the basis of relative retention time, compared with the relative retention times of the individual compounds in a standard mixture of fatty acid methyl esters, Supelco Component 37 FAME mix (Supelco, Bellefonte, USA). Quantification of fatty acids was determined relative to an internal standard, heneicosanoic acid, C21:0. The level of fatty acids is expressed as a percentage (%) of the total identified fatty acids.

The statistical analysis was performed using the GraphPad Prism version 7.00 software. The fatty acid composition and concentrations of elements in bee bread samples were expressed as the mean ± standard deviation and were subjected to analysis of variance (One-way ANOVA). The parameters were analyzed using the Student's t-test at the probability of 0.01.

3. Results and Discussion

The element concentrations (Cu, Fe, Zn, Mn, Cr, Co, Ni, Se, K, Na, Ca and Mg) and heavy metal concentrations (Cd, Hg, Pb and As) of the bee bread are shown in Table 1. The Na content differed widely among the geographical locations. Bee bread from Ležimir was characterized by the significantly highest mean Na concentration ($p<0.01$). The highest Mg and K concentrations were

found in the bee bread from geographical location I (Gornji Milanovac). Potassium was the major element determined in the bee breads. The mean Ca content in the bee bread ranged between 1190±76.38 mg/kg and 1806±44.98 mg/kg. The lowest Mn, Fe and Ni concentrations were measured in bee bread from Gornji Milanovac (19.51±3.74 mg/kg, 43.07±3.84 mg/kg and 1.30±0.26 µg/kg, respectively). The mean Cr concentration in all tested bee bread was between 106.90±12.92 µg/kg (Ležimir) and 183.80±29.77 µg/kg (Lazarevac). Se was not detected in any of the examined bee bread (<0.2 mg/kg). The concentrations of elements in bee bread have been reported in several studies [10-13], but for Serbian bee bread, data is very limited. However, similar results to ours were reported previously by Stanciu et al. [10], Villaneuva et al. [11], Somerville et al. [12] and Salamanca et al. [13].

The Fe concentration of the bee bread was significantly different ($p<0.01$) in different locations and ranged between 43.07±3.84 mg/kg (Gornji Milanovac) and 57.52±4.28 mg/kg (Ležimir). Similar results were obtained by Villaneuva et al. [11] and Stanciu et al. [10]. The mean Co concentrations in all tested bee breads was significantly different ($p<0.01$). The concentrations of Cu and Zn were relatively higher in bee bread collected from Ležimir (10.72±1.67 µg/kg**,** 46.94±8.37 mg/kg, respectively).

The concentrations of heavy metals (As, Cd, Pb and Hg) in bee bread collected from apiaries in different locations were presented in Table 1. Data obtained revealed the highest levels of As (43.37±4.39 µg/kg) and Pb (183.20±8.95 µg/kg) occurred in bee bread from Lazarevac. On the other hand, the lowest concentrations of Cd (32.18±0.73 µg/kg) were recorded in bee bread from Gornji Milanovac. Hg was not detected in any of the examined bee breads (<1.0 µg/kg). The differences in concentrations of heavy metals (As, Cd, Pb) in bee bread between the three study locations could be attributable to different local contamination/pollution sources. The main contaminants of bee products are heavy metals [1, 2, 14], and pesticides originating from the environment [15, 16] and from agricultural and apiculture practices [17]. The present study revealed the highest As and Pb concentrations occurred in bee bread from Lazarevac. These results, as previously mentioned, emphasize the likely elevated level of environmental pollution with these heavy metals in this location.

Table 1. Element concentrations ($\overline{X}$ ± Sd) in Serbian bee breads from different geographical locations

	Geographical location		
Element (units)	I*	II	III
Na (mg/kg)	32.90 ± 1.69^{A}	34.91 ± 1.50^{A}	49.07 ± 3.03^{B}
Mg (mg/kg)	845.80 ± 83.20^{A}	692.20 ± 39.93^{B}	714.70 ± 40.64^{C}
K (mg/kg)	7487 ± 381.50^{A}	5944 ± 730.40^{B}	5515 ± 361.20^{C}
Ca (mg/kg)	1190 ± 76.38^{A}	1266 ± 248.80^{B}	1806 ± 44.98^{C}
Cr (µg/kg)	131.30 ± 15.68^{A}	183.80 ± 29.77^{B}	106.90 ± 12.92^{C}
Mn (mg/kg)	19.51 ± 3.74^{A}	38.15 ± 4.99^{B}	204.80 ± 1.17^{C}
Se (mg/kg)	<0.2	<0.2	<0.2
Fe (mg/kg)	43.07 ± 3.84^{A}	54.97 ± 6.79^{B}	57.52 ± 4.28^{B}
Co (µg/kg)	65.50 ± 9.70^{A}	37.52 ± 4.65^{B}	55.43 ± 3.88^{C}
Ni (µg/kg)	1.30 ± 0.26^{A}	1.33 ± 0.12^{B}	3.67 ± 0.30^{C}
Cu (µg/kg)	5.29 ± 0.25^{A}	4.59 ± 0.11^{A}	10.72 ± 1.67^{B}
Zn (mg/kg)	41.59 ± 3.63^{A}	27.31 ± 2.25^{B}	46.94 ± 8.37^{A}
As (µg/kg)	33.27 ± 9.63^{A}	43.37 ± 4.39^{B}	20.50 ± 3.96^{C}
Cd (µg/kg)	32.18 ± 0.73^{A}	43.50 ± 4.15^{B}	136.50 ± 8.48^{C}
Pb (µg/kg)	100.90 ± 6.06^{A}	183.20 ± 8.95^{B}	55.85 ± 6.42^{C}
Hg (µg/kg)	<1.0	<1.0	<1.0

* I – Gornji Milanovac, II – Lazarevac and III – Ležimir; Different letters ($^{A, B, C}$) show statistically significant different average concentrations between bee breads from different locations, $p<0.01$

Table 2 contains data on the fatty acid composition of the investigated bee bread. The average fatty acid compositions of the bee breads from different geographical locations differed significantly ($p<0.01$). The most abundant fatty acid in bee bread from Gornji Milanovac, Lazarevac and Ležimir was C16:0 (39.23±3.57%) followed by C18:3n-3. The bee breads contained high levels of saturated (SFA) and polyunsaturated fatty acids (PUFA). The total n-3 fatty acid content was the highest in bee breads from Lazarevac (25.64±2.90 %) and the lowest in bee breads from Ležimir (7.30±0.99%). The content of n-6 fatty acids varied between 10.21±1.97% (Ležimir) and 22.00±1.16% (Lazarevac). The most favourable n-6/n-3 fatty acid ratio was found in bee bread from Gornji Milanovac. The proportion of total n-6/n-3, fulfilling the demands of health-conscious consumers (reducing the risk of many diseases), should be from 1 to 5 [18]. Similar results were presented in the studies by Kaplan et al. [19] and Isidorov et al [1].

Table 2. Fatty acid composition (% of total fatty acids or fatty acid ratio; $\overline{X}$ ± Sd) of Serbian bee breads from different geographical locations

Fatty acid	Geographical location		
	I*	II	III
C14:0	0.63±0.13^{A}	0.41±0.15^{A}	8.15±1.09^{B}
C15:0	5.28±0.04^{A}	5.34±0.10^{A}	2.98±0.16^{B}
C16:0	23.00±2.80^{A}	18.46±2.60^{A}	39.23±3.57^{B}
C16:1	0.30±2.80^{A}	0.32±1.80^{A}	ND[1]
C18:0	5.54±0.25^{A}	5.05±0.66^{A}	4.12±0.29^{A}
C18:1cis	17.74±0.03^{A}	17.72±0.06^{A}	15.82±0.07^{B}
C18:2cis	20.17±0.73^{A}	21.00±1.03^{A}	7.54±1.02^{B}
C20:0	1.99±0.04^{A}	1.90±0.08^{A}	1.33±0.05^{B}
C18:3n-6	ND	ND	1.06±0.02
C18:3n-3	21.20±2.79^{A}	24.66±3.01^{A}	5.37±0.36^{B}
C20:1	0.92±0.01	ND	ND
C20:2n-6	0.70±0.04^{A}	0.62±0.06^{A}	0.70±0.03^{A}
C20:3n-6	0.39±0.01^{A}	0.37±0.02^{A}	1.98±0.01^{B}
C20:3n-3	0.89±0.12^{A}	0.97±0.10^{A}	1.93±0.01^{B}
C24:0	3.97±0.48^{A}	3.01±0.30^{A}	8.51±0.60^{B}
C24:1	0.15±0.01^{A}	0.14±0.01^{A}	1.31±0.01^{B}
SFA	39.64±3.75^{A}	34.17±4.20^{A}	64.30±5.69^{B}
MUFA	17.86±0.31^{A}	18.19±0.29^{A}	17.12±2.30^{A}
PUFA	42.50±4.85^{A}	47.64±3.99^{A}	18.57±1.58^{B}
n-6	20.97±1.11^{A}	22.00±1.16^{A}	10.21±1.97^{B}
n-3	22.27±3.69^{A}	25.64±2.90^{A}	7.30±0.99^{B}
n-3/n-6 ratio	1.03±0.10^{A}	1.17±0.13^{A}	0.71±0.03^{B}
n-6/n-3 ratio	0.87±0.37^{A}	0.86±0.28^{A}	1.40±0.05^{B}

* I – Gornji Milanovac, II – Lazarevac and III – Ležimir; Different letters ($^{A, B, C}$) show statistically significant differences between bee breads from different locations, $p<0.01$; [1] ND – Not detected

4. Conclusion

To our knowledge, this study is the first to compare the chemical composition and fatty acid composition of bee breads from different geographical locations in Serbia. The results of the analyses show that beebread contains large quantities of unsaturated fatty acids and sometimes has very favourable n-6/n-3 fatty acid ratios. This composition indicates the high nutritive value of bee bread.

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