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The specificity of monofloral bee pollen fatty acid composition from Croatia and its nutritional value

Specifičnost sastava masnih kiselina monoflorne pčelinje peludi iz Hrvatske i njezina nutritivna vrijednost

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ABSTRACT

The aim of this study was to evaluate the influence of botanical origin on the fatty acids composition of bee pollen and its nutritional value. The seven monofloral bee pollen samples from different locations of Croatia were analysed. A total of 26 fatty acids (from C12 to C24) were identified with a significant difference in their shares in the samples, and nine of them were common to all samples. Assessing bee pollen fatty acids composition through the nutritional quality indicators (high unsaturated fatty acids/saturated fatty acids ratios, high n-3/n-6 and α-linolenic acid/linoleic acid ratios, very low atherogenic and thrombogenic indexes) it is evident that all analysed bee pollen samples are valuable products, which included in the diet can improve the fatty acids balance and could have a positive impact on human health.

Keywords: bee products, health benefits, saturated fatty acids, polyunsaturated fatty acids, n-6/n-3 ratio

SAŽETAK

Cilj ovog rada bio je ispitati utjecaj botaničkog podrijetla na sastav masnih kiselina pčelinje peludi i njezinu nutritivnu vrijednost. Analizirano je sedam monoflornih uzoraka pčelinje peludi prikupljenih na različitim lokacijama u Hrvatskoj. U uzorcima je identificirano ukupno 26 masnih kiselina (od C12 do C24) sa značajnom razlikom njihovih udjela u uzorcima, a devet ih je bilo zajedničko svim uzorcima. Procjenom sastava masnih kiselina pčelinje peludi kroz pokazatelje nutritivne kvalitete (visoki omjeri nezasićenih masnih kiselina/zasićenih masnih kiselina, visoki omjeri n-3/n-6 i α-linolenske kiseline/linolne kiseline, vrlo niski aterogeni i trombogeni indeksi) vidljivo je da su svi analizirani uzorci pčelinje peludi vrijedni proizvodi koji uvrštavanjem u prehranu mogu poboljšati ravnotežu masnih kiselina i pozitivno utjecati na ljudsko zdravlje.

Ključne riječi: pčelinji proizvodi, dobrobit za zdravlje, zasićene masne kiseline, polinezasićene masne kiseline, n-6/n-3 odnos

INTRODUCTION

Bee pollen consists of plant flower pollen mixed with nectar or secretion from salivary glands and contains a large number of components - proteins, carbohydrates, lipids, crude fiber, vitamins (β-carotene, tocopherols, thiamine, riboflavin, folic acid, biotin, pantoteic acid, pyridoxine, niacin, ascorbic acid) and minerals (K, Mg, Ca, Na, P, Fe, Zn, Mn, Cu), polyphenol compounds and enzymes (Kieliszek et al., 2018; Qiang-Qiang et al., 2018; Thakur et al., 2020). Bee pollen, as well as honey, has been used in human nutrition since ancient times, and today bee pollen is the best-selling bee product after honey (Bogdanov, 2016). The nutritional value of bee pollen is well known, but the bioavailability of nutrients is affected by the presence of the pollen shell (intine and exine), which can reduce bioavailability by 50% or more (Kostić et al., 2020). Today, the focus of bee pollen research is the increase of nutrients bioavailability using innovative methods of processing that will break down the pollen cell walls and at the same time will not affect the degradation of the bioactive components (Aylanc et al., 2021).

Many therapeutic properties are associated with bee pollen and its composition like antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, antiallergic, hepatoprotective, cardioprotective etc (Qiang-Qiang et al., 2018; Mărgăoan et al., 2018; Mărgăoan et al., 2014). One of the most important bee pollen components are fatty acids, building components of lipids. The composition and amount of fatty acids in diet are important for both bees colonies and humans. In humans, the fatty acids are involved in the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases (Simopoulos, 2008; Saini at al., 2018; Jenkins et al., 2015). In bees, the fatty acids mostly provide energy, but they also have antibiotic properties against several pathogens (e.g. American foulbrood) and could enhance bees cognitive performance (Ruedenauer, 2021). The nutritional and/or therapeutic value of fatty acids of food for years have been expressed and evaluated through different nutritional indicators (Polyunsaturated fatty acids (PUFA)/Saturated fatty acids (SFA), α-linolenic acid (ALA)/ linoleic acid (LA), n-3/n-6 ratio etc.) (Ulbricht and Southgate, 1991; Chen and Liu, 2020). Although there are plant species of bee pollen in which saturated fatty acids are dominant, different studies (Mărgăoan et al., 2014; Manning, 2001; Féas et al., 2012; Yang et al., 2013) show that bee pollen of many plant species is a good source of unsaturated fatty acids, especially n-3 and n-6 polyunsaturated fatty acids in favorable proportions, which makes bee pollen important for human nutrition.

Bee pollen composition varies widely depending on plant source, bee taxon, climatic conditions, beekeeping practice and geographical origin (Bogdanov, 2016; Mărgăoan et al., 2014; Manning, 2001). Regarding the plant source, in addition to the expected differences in the fatty acid composition of multifloral samples, which are a unique combination of pollen of different plant origin, studies also show large differences in the fatty acid composition of monofloral samples from different geographical areas (Thakur and Nanada, 2018). Fatty acid profile of multifloral or/and monofloral bee pollen of many countries has been well described (Kostić et al., 2017; Markowicz et al., 2004; Dong et al., 2016; Čeksteryte et al., 2014) but according to our knowledge, so far, in Croatia only three papers deal with the composition of bee pollen (Prđun et al., 2021; Bilić Rajs et al., 2018; Bilić Rajs et al., 2022) but they do not deal with fatty acids composition.

Considering that the chemical composition of bee pollen is strongly related to botanical origin, and that the vegetation is specific to a particular geographical area, the aim of this study was to evaluate the influence of botanical origin on the fatty acids composition of bee pollen collected from different location in Croatia, and its nutritional value.

MATERIALS AND METHODS

Samples

Pooled bee pollen samples were collected in three locations in Croatia (Otočac – Mountain region;

44°48'46.78'', 15°21'35.58'', Senj – Mediterranean region; 44°59'6.20'', 14°54'24.29'' and Krapina – Continental region; 46°9'4.20'', 15°52'4.76''). Bee pollen samples were collected using front-mounted pollen traps placed at the entrance of the Langstroth-Rooth hives. Five honey bee experimental colonies (*Apis mellifera carnica* Polmann 1879) were selected at each location. The collection of bee pollen was performed every 15 days, starting from 1 April to 15 June in beekeeping season of 2019. In total, 16 pooled (multifloral) bee pollen samples were collected. Pooled samples were classified after the collection according to color in order to obtain subsamples (in total seven monofloral samples) and frozen at -18 °C before further analysis in order to preserve their biological and chemical properties.

Botanical origin of bee pollen

Microscopic slides of classified bee pollen samples were prepared using the modified methods of Barth et al. (2010). Bee pollen consisting of the same color loads extracted from the one pooled sample was weighed into a 12 mL centrifuge tube and mixed with 70% ethanol up to 10 mL. Mixture was vortexed and placed for five minutes in ultrasonic bath (Bandelin, Sonorex, Super RK 100 H, Berlin, Germany) and then left to stand 25 minutes. Tube was than centrifuged (Sigma 2–16, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) for three minutes at 1500 rpm. After resuspension of sediment with ethanol and repeated procedure, sediment was left to stand 30 minutes with added water and glycerol solution (1:1) two times. Obtained sediment was stirred with Pasteur pipette and spread on microscopic slide. Confirmation of sample monoflorality was done by melissopalynological analysis (>80% of pollen grains from one botanical species according to the Campos et al. (2018). Analysis were conducted using 400× magnification (B-800 Series; Optika Microscopes, Ponteranica, Italy) where at least 500 pollen grains were counted in each microscopic slide. For botanical origin identification CMS Celle's Melissopalynological Collection (Von der Ohe, 2003) was used. This analysis confirmed seven monofloral bee pollen samples: *Taraxacum officinale* (L.) Webber, *Salix* spp., *Prunus spinosa* L., *Aesculus hippocastanum* L., *Prunus mahaleb* L., *Quercus pubescens* Willd. and *Filipendula vulgaris* Moench.

Total lipid extraction and preparation of fatty acids methyl esters (FAMEs)

Lipids were extracted from monofloral bee pollen samples with Folch method (Folch et al., 1957). Samples were weighed and homogenized with the mixture of chloroform (Carlo Erba, France): methanol (J. T. Baker, Poland) (2:1) up to final volume twenty times higher than sample weight. After 20 minutes of shaking in orbital flat shaker (IKA KS 260, China) at room temperature samples were centrifuged (Sigma, Germany) two times at 2000 rpm for twenty minutes. Solution was filtered and 0.9% NaCl (Merck, Germany) solution was added after which lower chloroform phase with lipids was evaporated at vacuum rotary evaporator. Flasks with lipids were dried until constant weight. For fatty acids methyl esters (FAMEs) preparation 0.1 g of sample lipid was weighed with addition of heptane (Carlo Erba, France) and 2 mol/L KOH (Gram Mol, Croatia) methanolic solution. Upper layer containing FAMEs was injected to the gas chromatograph (GC).

Fatty acids determination using gas chromatography with flame ionization detector

FAMEs separation was performed on a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corp., Kyoto, Japan) equipped with a flame ionization detector (FID) (Shimadzu Corp., Kyoto, Japan) and fitted with an SH-FAMEWAX™ capillary column (30 m × 0.32 mm, 0.25 µm film thickness) (Shimadzu Corp., Kyoto, Japan). Temperatures of injector and detector were set at 240 ˚C and 250 ˚C, respectively. Split ratio was 1:100 and injection volume 2 µL. Nitrogen was used as the carrier gas with the constant flow rate of 1.26 mL/min. The GC oven program was as follows: 120 ˚C hold for 5 min, then increased to 220 ˚C at 5 ˚C/min, and hold for 20 min. FAMEs were identified in the samples based on the comparison with retention times of certified reference standard (Supelco F.A.M.E. Mix, C4-C24, St. Louis,

USA) under the same conditions. The quantification was performed using area normalization method and the results were expressed as an average percentage of identified fatty acid on total fatty acids (%) and the analyses were performed in triplicate.

Calculation of lipid quality indices

Lipid quality indices that are most commonly used for characterization of fatty acid composition (SFA, MUFA, PUFA n-6, PUFA n-3, n-3/n-6, n-6/n-3, ALA/LA, LA/ALA, PUFA/SFA, UFA/SFA, IA, IT, HH) were calculated in this paper. Total saturated fatty acids (SFA), total monosaturated fatty acids (MUFA), total n-6 polyunsaturated fatty acids (PUFA n-6) and total n-3 polyunsaturated fatty acids (PUFA n-3) are expressed as sum of particular fatty acid type.

Polyunsaturated Fatty Acid/Saturated Fatty Acid ratio (PUFA/SFA) is calculated as ΣPUFA/ΣSFA. Ulbricht and Southgate (1991) proposed method for calculation index of atherogenicity (IA) and index of thrombogenicity (IT) using the following equations:

$$IA = [C12:0 + (4 \cdot C14:0) + C16:0] / \sum MUFA + \sum n - 6\ PUFA + \sum n - 3\ PUFA,$$

$$IT = C14:0 + C16:0 + C18:0 / [(0.5 \cdot \sum MUFA) + (0.5 \cdot \sum n - 6\ PUFA) + (3 \cdot \sum n - 3\ PUFA) + (n\text{-}3/n\text{-}6)].$$

Ratio between hypocholesterolemic fatty acids (C18:1n9 + ΣPUFA) and hypercholesterolemic fatty acids (C12:0 + C14:0 + C16:0) is expressed as hypocholesterolemic/hypercholesterolemic ratio (HH) (Mierlită, 2018). Long-chain fatty acids n-3 and n-6 were expressed and calculated also as n-3/n-6 and n-6/n-3 ratio. Linoleic acid (LA, C18:2n-6) and α-linolenic acid (ALA, C18:3n-3) were calculated as LA/ALA and ALA/LA ratio. Health lipid indices UFA/SFA was calculated as unsaturated fatty acids (UFA) and saturated fatty acid (SFA) ratio.

RESULTS AND DISCUSION

Fatty acid composition

A total of 26 fatty acids (from C12 to C24) were identified with a significant difference in their proportions in the samples, and nine of them: pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0), oleic and elaidic (C18:1n-9c+t), linoleic (C18:2), ɤ-linolenic (C18:3n-6), α-linolenic (C18:3), and arachidic acid (C20:0) were common to all samples (Table 1).

Many studies of the bee pollen fatty acids composition reported different results regarding fatty acids that are common to all samples. Manning et al. (2006) reported five fatty acids (palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3), Kostić et al. (2017) six (caprylic (C8:0), palmitic (C16:0), stearic acid (C18:0), oleic (C18:1), linoleic (C18:2) and α-linolenic (C:18:3) acid), Mayda et al. (2020) six (palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3) and eicosenoic (C20:1) acid) and Markowicz et al. (2004) only tree (oleic (C18:1), linoleic (C18:2) and arachidic (C20:0) acid). In this study, in addition to mentioned above fatty acids, in all samples were found and pentadecanoic acid (C15:0), hepadecanoic acid (C17:0); ɤ-linolenic acid (C18:3n-6). Mannig et al. (2006) along with five basic fatty acids reported arachidic acid in 99.5% samples, C17:0 in 77.5%; C15:0 in 54.4% of samples. Fatty acids have an essential role in the reproduction, development and growth of honey bees. Fatty acid such as myristic, lauric acid, linoleic acid and linolenic acid, have bactericidal activity and they are important for colony hygiene (Manning, 2001).

Considering the average share of all samples, the most abundant fatty acids in this study were C18:3n-3, C18:2n-6, C16:0 and C18:1. Considering however, the proportion of fatty acids by individual samples, ALA was also the most represented fatty acid, it was the dominant fatty acid of four samples (*Taraxacum officinale* L.; *Salix* spp., *Aesculus hippocastanum* L., *Filipendula vulgaris*) and linoleic acid in two samples (*Prunus spinosa* L., *Prunus mahaleb* L.) with shares of 48.86% to 12.86% and 32.48% to 5.45%, respectively. Palmitic acid (C16:0) was dominant fatty acid only in *Quercus pubescens* bee pollen sample (32.70%), but practically in the same proportion as linoleic acid (32.48%). Manning et al. (2001) indicate that the majority of pollens dominant in palmitic acid were also dominant in linoleic acid.

Table 1. Fatty acids composition (% of total fatty acids) of monofloral bee pollen samples

Samples and their dominate botanical source (%)	*Taraxacum officinale* L. 86%	*Salix* spp. 100%	*Prunus spinosa* L. 100%	*Aesculus hippocastanum* L. 100%	*Prunus mahaleb* L. 100%	*Quercus pubescens* 84%	*Filipendula vulgaris* 97%
Fatty acids*							
C12:0	0.98±0.27	0.70±0.20	0.38±0.13	0.12±0.03	nd	nd	nd
C13:0	1.42±0.13	nd	0.89±0.22	nd	nd	nd	nd
C14:0	1.31±0.08	0.68±0.12	0.48±0.03	0.21±0.01	0.28±0.08	nd	nd
C14:1	nd	nd	nd	nd	nd	nd	nd
C15:0	19.75±1.77	0.41±0.02	2.75±0.41	0.12±0.01	4.82±0.39	0.17±0.05	0.94±0.09
C15:1	nd	nd	nd	nd	nd	nd	nd
C16:0	16.67±0.84	14.78±0.97	17.47±0.48	18.88±0.91	17.48±0.58	32.70±2.03	19.17±0.34
C16:1	nd	0.13±0.02	0.14±0.01	0.12±0.00	0.15±0.03	0.21±0.03	nd
C17:0	1.29±0.13	0.96±0.09	6.27±0.65	0.92±0.16	0.46±0.05	0.50±0.14	1.06±0.09
C17:1	nd	0.21±0.01	nd	0.06±0.01	nd	nd	0.39±0.09
C18:0	2.41±0.67	3.61±0.58	1.58±0.27	2.79±0.27	2.15±0.57	3.39±0.53	5.60±0.38
C18:1n-9c+t	2.04±0.59	5.16±0.21	6.63±1.31	8.09±1.29	20.50±2.24	15.03±1.26	9.77±0.61
C18:2n-6c	14.83±0.53	16.50±1.99	30.11±1.11	5.45±0.35	26.08±2.01	32.48±1.18	15.42±1.17
C18:2n-6t	nd	nd	nd	nd	nd	nd	nd
C18:3n-6	0.33±0.06	2.78±0.28	2.92±0.24	7.84±1.17	0.87±0.09	0.20±0.05	1.27±0.17
C18:3n-3	33.79±0.55	27.87±0.81	22.98±0.34	48.86±2.01	20.46±0.81	12.86±0.49	25.95±1.22
C20:0	0.37±0.06	0.87±0.07	0.45±0.03	0.36±0.05	0.66±0.02	0.59±0.05	0.82±0.04
C20:1	1.68±0.17	3.22±0.27	0.29±0.05	4.36±0.62	0.60±0.03	0.30±0.02	nd
C20:2n-6	nd	13.20±1.34	2.10±0.50	0.27±0.03	1.53±0.21	0.43±0.09	2.68±0.21
C20:3n-6	nd	0.43±0.07	0.62±0.06	0.09±0.02	1.15±0.13	nd	0.64±0.13
C21:0	nd	nd	nd	nd	nd	nd	nd
C20:4n-6	nd	nd	nd	nd	nd	nd	1.14±0.30
C20:3n-3	nd	nd	0.13±0.04	0.25±0.02	0.35±0.11	nd	2.93±0.41
C20:5n-3	nd	0.92±0.30	nd	nd	nd	0.26±0.07	0.47±0.14
C22:0	nd	0.69±0.08	0.92±0.04	0.19±0.01	0.96±0.05	0.36±0.08	1.78±0.19
C22:1n-9	1.79±0.27	0.29±0.04	nd	0.12±0.01	0.39±0.05	nd	1.01±0.12
C22:2	nd	5.37±0.83	1.46±0.50	0.39±0.06	0.81±0.12	0.42±0.08	4.72±0.37
C23:0	nd	nd	0.55±0.04	0.30±0.02	0.48±0.04	nd	5.04±0.68
C24:0	nd	0.87±0.06	0.88±0.06	0.26±0.01	1.02±0.08	0.80±0.11	nd

Continued. Table 1.

Samples and their dominate botanical source (%)	*Taraxacum officinale* L. 86%	*Salix* spp. 100%	*Prunus spinosa* L. 100%	*Aesculus hippocastanum* L. 100%	*Prunus mahaleb* L. 100%	*Quercus pubescens* 84%	*Filipendula vulgaris* 97%
Fatty acids*							
C22:6n-3	nd	nd	nd	nd	nd	nd	nd
C24:1	1.33±0.13	0.33±0.04	nd	0.61±0.03	nd	nd	nd
SFA	44.19±0.68	23.57±1.58	32.62±1.0	24.15±0.66	28.31±1.31	38.51±1.95	34.41±0.95
MUFA	6.84±0.20	9.34±0.52	7.07±1.30	13.36±1.29	21.63±2.23	15.54±1.26	11.17±0.60
PUFA n-6	15.16±0.53	38.28±0.41	37.21±0.11	14.04±1.30	30.43±2.12	33.53±0.91	25.87±1.00
PUFA n-3	33.79±0.55	28.79±1.06	23.11±0.31	49.11±1.99	20.81±0.74	13.12±0.44	29.35±1.12

Values represent mean and standard deviation of the three measurements

*SFA- total saturated fatty acids; MUFA- total monosaturated fatty acids; PUFA n-6 – total n-6 polyunsaturated fatty acids; PUFA n-3 – total n-3 polyunsaturated fatty acids; nd – not detected

The presence of low amounts of lauric (C12:0) and myristic acid (C14:0) (maximal value for C12:0 was 0.98% and 1.31% for C14:0) was detected in 4 and 5 out of 7 analysed samples. Studies reported that number of pollen species have ALA as dominant fatty acid: *Zea mays*, *Cruciferae* spp., etc. (Yang et al., 2013; Kostić et al., 2017; Manning, 2006). The highest share of ALA (from all analysed samples) and the lowest share of LA were found in *A. hippocastanum* sample, and almost identical data are given by Čeksterytė et al. (2014). *A. hippocastanum* bee pollen contained also high proportion of ɤ-linolenic acid (7.84%). ALA is also most abundant fatty acid of *Salix* spp. bee pollen, share of ALA, LA and palmitic acid are in agreements with results of Conte et al. (2017) who analysed *Salix alba*, unlike the study of Manning et al. (2001) where oleic and linoleic acids are most prevalent, or study of Kostić et al. (2017) where palmitic acid was the most prevalent fatty acid of willow bee pollen. The *T. officinale* pollen, in addition to the already mentioned ALA as the dominant fatty acid, also contains significant amounts of linoleic acid and palmitic acid and a very large proportion of odd chain saturated fatty acids, especially C15:0. Odd-chain fatty acids, pentadecanoic (C15:0) i heptadecanoic acid (C17:0), were found in all samples, ranged from 0.12% to 6.27%, with the exception of *T. officinale* bee pollen where pentadecanoic acid share was 19.75%. Presence of odd chain saturated fatty acids (C15:0 and C17:0) in trace or small amounts reported by several authors (Manning, 2006; Qiang-Qiang et al., 2018; Kostić et al., 2017; Conte et al., 2017) in bee pollen, and Čeksterytė et al. (2014) and Kaplan et al. (2016) in bee bread. Odd-chain saturated fatty acids are present in small amounts in dairy fat, some fish and plants and have been associated with lower risks of cardiovascular disease, adiposity, type 2 diabetes and many other diseases (Venn-Watson et al., 2020; Pfeuffer and Jaudszus, 2016). Recently, there has been a growing interest in their role in the organism as well as in data on food source. Estimated daily intake of odd–chain fatty acids is approximately 1.0 g (Dąbrowski et al., 2022) and considering pentadecanoic acid (C15:0) evidence, Ven-Watson et al. (2020) propose it as a potential essential fatty acid. The chemical composition of bee pollen is strongly related to botanical origin, however, significant differences of the results, within a species from different locations and different authors are identified. For example, in rapeseed bee pollen (*Brassica napus* 95.6%) from China the most represented fatty acids are α-linolenic and myristic acid (Yang et al., 2013), while in the same pollen type from India (*B. napus* 100%) the most represented are ɤ-linolenic, eicosatrienoic (C20:3n-3) and palmitic acid (Thakur and Nanda, 2018). Furthermore, the

bee pollen analysis of six different Serbian maize hybrids showed large differences in the fatty acid composition (Kostić et al., 2017) where it should be emphasized that it is only one species. There are, of course, examples where the similarities between the results of one species of bee pollen from different geographical areas are more pronounced than the differences. Bearing in mind the definition of uniformity (Campos et al., 2008) (min 80%) only a small part of differences can be attributed to the difference due to location, i.e. due to differences in accompanying plants. In general, despite the large number of studies to date, due to the great diversity in plant species, there is still not enough studies on monofloral samples to provide insight into the "natural" variability of fatty acid composition, or other compounds.

Nutritional aspect/indices

A lot of different indices (SFA, PUFA, UFA, n-3, n-6 and their ratios, IA, IT etc.) are used to assess the impact of food products fatty acids composition on health. The Table 1 shows total SFA, MUFA, PUFA n-6 and PUFA n-3 content in analysed samples bee pollen, and Table 2 selected indices (ratios) used to assess nutritional quality of bee pollen fatty acids. n-3 fatty acids were the most prevalent in *A. hippocastanum* bee pollen (49.11%) and the least present in *Q. pubescens* (13.12%). ALA was dominant fatty acid in n-3 fatty acids, represented between 88.4% (*F. vulgaris*) and 100% (*T. officinale*) (Table 1). Eicosatrienoic (C20:3n-3) and eicosapentaenoic (C20:5n-3) acid were present in small proportions, while docosahexaenoic acid (C22:6n-3) was not found in any sample.

The proportion of n-6 fatty acids in the samples ranged between 14.04% in *A. hippocastanum* bee pollen up to 38.28% in *Salix* spp. bee pollen, with a dominance of linoleic acid in proportions between 39% (*A. hippocastanum*) and 98% (*T. officinale*) (Table 1). *Salix* spp. pollen contains high proportions of eicosadienoic acid (C20:2n-6) (13.20%) and docosadienoic acid (C22:2n-6) (5.37%). Arachidonic acid was found only in a *F. vulgaris* sample (1.14%).

According to the literature, very long chain fatty acids (VLCFA) shares vary from very low (trace) to even relatively high, depending on bee pollen botanical origin. The share of VLCFAsc in Serbian bee pollen samples varied between 0.0 and 10.91% of total fatty acids (behenic acid (C22:0). The eicosatrienoic acid, γ-linolenic acid, eicosapentaenoic acid, heptadecenoic acid, petroselinic acid and dihomo-γ-linolenic acid are for first time detected in bee pollen from some regions of India, and authors suggest those as bee pollen chemical markers of regions under study (Thakur and Nanda, 2018).

Table 2. Average values of selected nutritional indices of bee pollen fatty acids

Nutritional index*	*Taraxacum officinale* L.	*Salix* spp.	*Prunus spinosa* L.	*Aesculus hippocastanum* L.	*Prunus mahaleb* L.	*Quercus pubescens*	*Filipendula vulgaris*
n-3/n-6	2.23	0.75	0.62	3.50	0.68	0.39	1.13
n-6/n-3	0.45	1.33	1.61	0.29	1.46	2.55	0.88
ALA/LA	2.28	1.69	0.76	8.96	0.78	0.40	1.68
LA/ALA	0.44	0.59	1.31	0.11	1.27	2.53	0.59
PUFA/SFA	1.11	2.85	1.85	2.62	1.81	1.21	1.60
UFA/SFA	1.26	3.24	2.07	3.17	2.57	1.61	1.93
IA	0.41	0.24	0.29	0.26	0.26	0.53	0.29
IT	0.18	0.17	0.21	0.13	0.22	0.56	0.23
HH	2.69	4.47	3.65	3.71	4.04	1.89	3.39

*ALA - α-linolenic acid (C18:3n-3); LA - linoleic acid (C18:2 n-6); PUFA - polyunsaturated fatty acids; SFA – total saturated fatty acids; UFA – total unsaturated fatty acids; IA - index of atherogenicity; IT - index of thrombogenicity; HH - hypercholesterolemic index

The monounsaturated fatty acids were the least presented, between 6.84% (*T. officinale*) and 21.63% of total fatty acids (Table 1). Oleic acid isomers make up between 29.8% (*T. officinale*) and 96.7% (*Q. pubescens*) of monounsaturated fatty acids, and the largest amount was found in *P. mahaleb* pollen (20.50%).

The saturated fatty acids proportion ranged between 23.57% and 44.19%. Palmitic acid (C16:0) was dominant saturated fatty acid in most analysed samples, ranged from 14.78% in *Salix* spp. pollen to 32.70% in *Q. pubescens* pollen. The stearic acid (C18:0) share was low, between 1.58% in *P. spinosa* and 5.60% in *F. vulgaris* bee pollen.

PUFA/SFA ratio is the basic and most commonly used index for evaluating nutritional value of dietary foods, based on the hypothesis that polyunsaturated fatty acids lower LDL-C, while saturated fatty acids increase it. It follows that higher ratios have greater positive effects on human health (Ulbrischt and Southgate, 1991; Chen and Liu, 2020). The PUFA/SFA bee pollen ratio ranged from 1.11 (*T. officinale*) to 2.85 (*Salix* spp.) (Table 2), while the PUFA/SFA ratio 22 samples organic bee pollen from Spain ranged from 1.67 to 4.51 (Féas et al., 2012). For the comparation with other foods, PUFA/SFA ratios are for fish (0.80-1.52) (Rincón-Cervera et al., 2020), muscle baby-beef cattle on corn-based diet (0.12-0.65) (Karolyi et al., 2009), or traditional fermented pork sausage Slavonski kulen 0.20 (Pleadin et al., 2014).

As a result of the observed shortcomings of this index, new indices were created UFA/SFA, IA, IT (Ulbrischt and Southgate, 1991), HH (Mierlită, 2018; Santos-Silva et al., 2002). UFA/SFA ratio takes into account beneficial impact of MUFA on LDL-C, but not the differences between saturated fatty acids. The UFA / SFA bee pollen ratio ranged from 1.26 (*T. officinale*) to 3.24 (*Salix* spp.), on average 2.26. With the exception of *P. mahaleb* bee pollen, which has the highest C18:1 share, the same sample distribution as for the PUFA / SFA ratio was maintained. Very low value UFA/SFA ratio (and PUFA/SFA ratio too) of *T. officinale* pollen is a consequence of palmitic acid (C16:0) and heptadecanoic acid (C15:0) share. The literature data of UFA/SFA bee pollen ratio, as can be expected, vary in wide ranges: (0.39 - 1.33) (Kostić et al., 2017), (1.91- 5.91) (Féas et al., 2012), (2.2-6.7) (Thakur and Nanda, 2018), (0.40-10.98) (Yang et al., 2013) reflecting the large number of plant species represented in either multifloral or monofloral samples.

The atherogenicity index (IA) represents an inverted PUFA/SFA ratio, so that foods with high IA value have a higher atherogenic potential, i.e., contribute more to the increase in LDL-C and total cholesterol. In addition, in relation to the PUFA/SFA ratio, lower chain length SFA (≤ C10) and stearic acid are excluded from saturated fatty acids (not counted) because they do not raise serum cholesterol, the myristic acid coefficient is included due to its atherogenic potential, while polyunsaturated ones expand with monounsaturated fatty acids which are as effective as PUFA in lowering LDL-C. Very low IA values have plant oils, sunflower 0.07, olive 0.14 with the exception of palm and coconut oils (0.88 and 13.63, respectively) and high dairy products (2.03) (Ulbrich and Southgate, 1991). The bee pollen atherogenicity index values (0.24-0.53) are similar or more favorable then the values of chicken meat (0.50) (Ulbrich and Southgate, 1991) or fish, like sardines and mackerel, 0.60 and 0.48, respectively (Fernandes et al., 2014).

Index of thrombogenicity (IT) describes the thrombogenic potential of fatty acids, i.e. their tendency to thrombus formation. IT gives the ratio of prothrombogenic saturated fatty acids myristic (C14:0), palmitic (C16:0) and stearic (C18:0), and antithrombogenic MUFAs and PUFAs - taking into account their efficiency. Antithrombogenic activity is most pronounced in the n-3 series, especially of EPA and DHA. Therefore, in the choice of food, preference should be given to foods with lower IT values. The IT values of bee pollen ranged between 0.13 (*A. hippocastanum*) and 0.56 (*Q. pubescens*) (Table 2) are comparable with the values for different species of fish (0.14-0.87) (Chen and Liu, 2020).

Hypocholesterolemic/hypercholesterolemic ratio (HH) presents impact of FA composition on cholesterol. However, there are differences in the formulas for HH indices, some authors do not include C12:0, and

unsaturated fatty acids are calculated as the sum of oleic acids and all PUFAs, or oleic acid and selected PUFAs. The obtained HH index values for bee pollen samples ranged widely from 1.89 to 4.47, and are comparable to the literature data for fish (1.54 to 4.83) (Chen and Liu, 2020). For dairy and meat products, the HH ranges are 0.32-1.29 and 1.27-2.79, respectively (Chen and Liu, 2020). Oils in general (except coconut and palm), due to their composition, low amounts of saturated fatty acids and high amounts of unsaturated, have high values of HH index, e.g. camelina oil 11.2-15.0 (Ratusz et al., 2018). It is clear that the HH and IA indices deal with the same aim, assessing the impact of fatty acids or food products on cholesterol level, but HH gives the ratio of selected unsaturated and saturated fatty acids, and IA vice versa.

The n-3/n-6 and linoleic acid/α-linolenic acid ratio

PUFAs are generally considered to have a positive effect on human health, but n-6 and n-3 PUFAs have opposing effect on metabolic functions. Therefore, absolute contents, as well as their proportion, especially LA and ALA that are essential fatty acids, plays a significant role in the regulation of homeostatic processes (Simopoulos, 2008; Saini and Keum, 2018). Research shows that humans have evolved on a diet where the ratio is of n-6 to n-3 essential fatty acids was 1 to 2:1, whereas today, in Western diets this ratio is 15-20:1 indicating a deficiency of n-3 fatty acids and an excess of n-6 fatty acids. This disbalance promote the pathogenesis of many diseases, cardiovascular disease, cancer, inflammatory and autoimmune diseases, whereas lower n-6/n-3 ratio has suppressive effects (Simopoulos, 2008). The analyzed samples of monofloral bee pollen are rich in n-3 fatty acids, especially ALA, which contributes to optimal n-6/n-3 and linoleic acid/α-linolenic acid ratios. The n-6/n-3 ratios ranged from 0.29 (*A. hippocastanum*) to 2.55 (*Q. pubescens*) which is the largest of all, but still optimal. It is also applicable to a reciprocal value, n-3/n-6 and α-linolenic acid/linoleic acid ratios ranged between 0.39 and 3.50, and 0.40-8.96, respectively (Table 2). Similar value of n-6/n-3 ratio reported by many authors: Mărgăoan et al. (2014) 0.15-1.64 for 16 samples, Feas et al. (2012) 0.11-0.96 for 22 samples, and Conte et al. (2017) for willow (0.59) and horse chestnut (1.67) bee pollen.

Assessing bee pollen fatty acids composition through the nutritional indices, it is evident that bee pollen is characterized by high PUFA/SFA and UFA/SFA ratios, high n-3/n-6 and α-linolenic acid/ linoleic acid ratios, very low atherogenic and thrombogenic index and high HH index values.

CONCLUSION

The selected types of monofloral bee pollen were analysed and numerous specifics in fatty acids composition have been identified. Assessing bee pollen fatty acids composition through the nutritional indices, it is evident that all bee pollen samples are a valuable product, which as food/diet supplement can have a positive impact on human health. Diversity in plant species which effect on fatty acid composition of bee pollen and finding of odd chain fatty acids in significant proportions in some bee pollen type are a strong motivation for further researches.

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