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Fatty acid composition in beebread

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Pollen and fatty acid composition were studied in the beebread collected in spring and summer. Willow pollen in spring beebread comprised 45.1 ± 3.0%, while rape pollen in summer beebread constituted 78.7 ± 4.5%. Twenty-two fatty acids were identified in beebread, including five ω-3, four ω-6 and three ω-9 polyunsaturated fatty acids. The ratio of ω-6 and ω-3 fatty acids was 1 : 1 in beebread samples where rape pollen constituted 45.1 ± 3.0% and 61.7 ± 4.0%, while this ratio was 2 : 1 in the beebread with a higher content of rape pollen, 78.7 ± 4.5%.

Key words: pollen, beebread, fatty acid composition

INTRODUCTION

Some polyunsaturated fatty acids (PUFAs), such as ω-3 and ω-6, are essential for human nutrition and should be consumed with food because the human body is unable to synthesize these acids in the gastrointestinal tract. The ω-3 fatty acids most important in human diet include α-linolenic (ALA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids [1, 2]. Ethyl esters of EPA and DHA have been reported to reduce the level of serum triglycerides [3]. EPA and DHA also possess cardioprotective properties through reducing blood cholesterol, triglyceride level and exerting an anti-arrhythmic, anti-thrombotic, anti-inflammatory impact. The primary source of DHA and EPA is fish and fish oil [4]. Clinical studies [5] showed that flaxseed oil is beneficial for the human health, particularly due to the presence of the essential ω-3 ALA. PUFAs are required for a number of essential body functions. For instance, ω-3 PUFAs reduce the inflammatory effects associated with ω-6 fatty acids [1]. Dietary specialists recommend food with the optimum ratio of ω-6 and ω-3 acids 1 : 1 or 2 : 1. However, the American diet contains 6–20 times more ω-6 fatty acids than ω-3. Such an imbalanced diet raises the rate of joint and heart diseases. The other plant sources containing ALA are canola, soybean, hemp seed, safflower and wheat germs [6].

Pollen and beebread may also be considered as a source which to some extent could compensate for the imbalance of fatty acids in the diet. For instance, 12 fatty acids were determined in almond (*Prunus dulcis*) pollen and beebread; two of them, oleic and linoleic, are unsaturated [7]. The composition of fatty acids in pollen depends on plant species [8]. Linoleic acid prevailed in dandelion and apple tree pollen, constituting 24.9 and 23.1 mg/g, respectively. However, the content of other fatty acids in clover, charlock pollen, except for dandelion, was found to be less than 2 mg/g [9]. The content of essential PUFAs, linoleic, linolenic and arachidonic in beebread accounted for 48.0% of the total fat content, linoleic acid being the major one – 34.0% [10]. However, the pollen composition of beebread in this study was not reported. Previously performed studies of beebread and pollen fatty acid composition did not reveal the presence of DHA and EPA, which are received by the human body mainly with foods of animal origin [11]. It is likely that some fatty acids may occur in the beebread by transferring them from the bee's organism with royal jelly during the pollen conservation process in a beehive. Therefore, the main aim of our study was to revise the data on fatty acids composition in beebread, particularly attempting to find out whether DHA and EPA are present in the product among the other essential fatty acids.

MATERIALS AND METHODS

Beebread was collected in the apiary of the Lithuanian Institute of Agriculture in 2006. Combs with beebread were removed from the bee colony at the end of June and August. Because combs and beebread after honey extraction remain wet due to the honey residue, they were placed in a bee colony for half a day to dry (the bees are licking up the combs to make them dry). Then the combs were transferred to a thermostat and dried

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additionally for 24 h at 30–40 °C. Finally, the combs with beebread were transferred to a refrigerator set at (–5)–(–10) °C, at which the wax becomes breakable while the beebread solidifies. Frozen beebread was trashed shortly. The small parts of beebread and small pieces of wax were blown up with the air stream after trashing. The length of the finally prepared beebread pieces was 0.3–1.0 cm. Beebread was dried again at 40–45 °C to the moisture level of 8.0–10.0%.

The samples of dried beebread were kept in the refrigerator at 5–8 °C in hermetically sealed dishes. The other portions of beebread were ground and mixed with honey at the ratio 1 : 1; the products obtained in such a way were preserved in the same conditions as dried beebread. It should be noted that the beebread and honey mixture was used for a clinical study at the Kaunas County Hospital for the possible influence on the immune system of patients suffering from rheumatoid arthritis [12]. Pollen content in beebread was studied by the method of melissopalynology, which is based on the evaluation of the relative frequency of pollen from nectar-secreting plants [13, 14].

To extract fats from 0.5 g of beebread, 10 ml of a chloroform / methanol mixture (2 : 1) was used [15]. The extraction was accomplished overnight at room temperature. The sample was filtered, diluted with 20 ml of 0.74% potassium chloride and strongly shaken for a full separation of layers. The lower layer was sampled with a syringe and transferred into a test-tube for evaporation in a vacuum thermostat at 50 °C. The extracted fatty acids were methylated in fresh 2.0% sodium methylate according to Christopherson and Glass [16]; 5 ml of sodium methylate were added to the test tube with fat and left for 1 h at room temperature. After that, 7 ml of 1 M hydrochloric (aqueous) acid was added. The test tube was hermetically sealed and shaken for 1 min. The sample was left still till the layers separated; the upper layer was transferred to the angular tube and evaporated. The prepared mixture of methyl esters of fatty acids was analysed with a GC-2010 SHIMADZU gas chromatograph equipped with a flame ionization detector. Fatty acids were separated using an AT™-FAME capillary column (30 m long, 0.25 mm id, 0.25 μm film thickness). The temperature of the column was programmed from 150 °C (6 min hold) to 240 °C at a rate of 4 °C/min. Nitrogen was used as a carrier gas at a flow rate of 0.33 ml/min. The total GC analysis time was 60 min.

Statistical analysis. The data are expressed as a mean of three measurements ± standard deviation. Statistical significance was estimated at $p < 0.01$. Correlation coefficients to determine the relationship between two different parameters and significant differences were evaluated using the SPSS (version 11.5) statistical software.

RESULTS

Beebread I contained pollen from plants flowering in spring, while pollen in samples II and III was mainly from plants flowering in summer (Table 1). Beebread I, collected in spring, contained almost equal parts of rape and willow pollen – 45.1 ± 3.0% and 41.8 ± 2.0%, respectively (Table 1). Pollen from the other plants flowering in spring was present in minor quantities; for instance, dandelion and apple-tree pollen constituted 2.0 ± 0.4 and 1.7 ± 0.3%, respectively. The content of rape pollen increased in summer beebread II and III from 61.7 ± 4.0% to 78.7 ± 4.5%, while the content of willow pollen decreased from 17.9 ± 3.5% to 5.6 ± 1.2%. Dandelion and apple-tree pollen, which were present in beebread I and II, were not found in beebread III. Data indicate that bees use some pollen for brood rearing mostly in spring and early summer.

Rape pollen prevailing in beebread, when mixed with honey, accounted for 40.9 ± 2.5%. The content of other pollen collected from summer blossom varied from 2.6 ± 0.2% to 9.9 ± 1.0%; for instance, willow pollen accounted for up to 8.3 ± 0.6%.

No significant differences were found between the total sum of ω-6 and ω-3 fatty acids in beebread and beebread mixed with honey ($p > 0.01$) (Table 2). The ratio of these acids in beebread samples where rape pollen constituted 45.1 ± 3.0% and 61.7 ± 4.0% was 1: 1, while in the beebread with a higher content of rape pollen (78.7 ± 4.5%) this ratio was 2 : 1. The preferable ω-6 and ω-3 fatty acid ratio (1 : 1) was found in beebread mixed with honey.

A strong correlation was found between the content of rape pollen and 13 fatty acids from those 22 present in beebread sam-

Table 1. **Botanical composition of beebread and beebread mixed with honey according to pollen content (%)**

Beebread (I)
Rape (*Brassica napus* var. *oleifera* DC) – 45.1 ± 3.0; willow (*Salix alba* L., *Salix caprea* L.) – 41.8 ± 2.0; bluebottle (*Centaurea cyanus* L.) – 4.1 ± 0.5; raspberry (*Rubus idaeus* L.) – 2.1 ± 0.3; dandelion (*Taraxacum officinale* L.) – 2.0 ± 0.4; apple-tree (*Malus domestica* Borkh.) – 1.7 ± 0.3; white clover (*Trifolium repens* L.) – 1.6 ± 0.5; charlock (*Sinapis arvensis* L.) – 1.6 ± 0.4
Beebread (II)
Rape (*Brassica napus* var. *oleifera* DC) – 61.7 ± 4.0; willow (*Salix alba* L., *Salix caprea* L.) – 17.9 ± 3.5; bluebottle (*Centaurea cyanus* L.) – 7.1 ± 2.2; charlock (*Sinapis arvensis* L.) – 4.2 ± 1.0; raspberry (*Rubus idaeus* L.) – 2.5 ± 0.8; white clover (*Trifolium repens* L.) – 2.5 ± 0.5; heather (*Calluna vulgaris* L.) – 1.7 ± 0.4; dandelion (*Taraxacum officnale* L.) – 1.2 ± 0.3; apple-tree (*Malus domestica* Borkh.) – 1.2 ± 0.3
Beebread (III)
Rape (*Brassica napus* var. *oleifera* DC) – 78.7 ± 4.5; willow (*Salix alba* L., *Salix caprea* L.) – 5.6 ± 1.2; bluebottle (*Centaurea cyanus* L.) – 5.3 ± 1.0; lime (*Tilia* L.) – 2.8 ± 0.4; white clover (*Trifolium repens* L.) – 2.3 ± 0.5; charlock (*Sinapis arvensis* L.) – 2.3 ± 0.7; raspberry (*Rubus idaeus* L.) – 1.7 ± 0.3; caraway (*Carum carvi*. L.) – 1.3 ± 0.3
Beebread mixed with honey (IV)
Rape (*Brassica napus* var. *oleifera*) – 40.9 ± 2.5; caraway (*Carum carvi*. L.) – 9.9 ± 1.0; charlock (*Sinapis arvensis* L.) – 9.7 ± 0.7; bluebottle (*Centaurea cyanus* L.) – 9.6 ± 0.5; white clover (*Trifolium repens* L.) – 8.8 ± 0.4; willow (*Salix alba* L., *Salix caprea* L.) – 8.3 ± 0.6; alder (*Frangula* L.) 4.4; lime (*Tilia* L.) – 3.0 ± 0.5; red clover (*Trifolium pratense* L.) – 2.8 ± 0.3; raspberry (*Rubus idaeus* L.) – 2.6 ± 0.2

Table 2. **Fatty acid composition in beebread**

Fatty acid	Abbreviation	Content, %			
		I	II	III	IV
Capric	C10 : 0	nd	0.78 ± 0.03	1.44 ± 0.10	0.75 ± 0.07
Lauric	C12 : 0	0.82 ± 0.08	0.86 ± 0.08	0.66 ± 0.04	0.93 ± 0.03
Myristic	C14 : 0	2.66 ± 0.03	4.18 ± 0.43	1.02 ± 0.04	1.39 ± 0.17
Myristoleic	C14 : 1ω-5	0.91 ± 0.14	0.69 ± 0.01	0.67 ± 0.06	0.73 ± 0.11
Palmitic	C16 : 0	13.08 ± 0.74	8.8 ± 0.06	2.62 ± 0.16	7.61 ± 0.55
Margaric	C17 : 0	4.25 ± 0.31	3.33 ± 0.17	4.88 ± 0.11	2.71 ± 0.30
Stearic	C18 : 0	1.42 ± 0.00	1.44 ± 0.05	0.84 ± 0.11	1.50 ± 0.01
Oleic	C18 : 1ω-9	12.06 ± 0.28	14.39 ± 0.58	19.22 ± 0.21	14.22 ± 0.31
Linoleic	C18 : 2ω-6	1.59 ± 0.33	1.10 ± 0.23	0.63 ± 0.02	1.20 ± 0.22
γ-Linolenic	C18 : 3ω-6	1.19 ± 0.13	0.77 ± 0.21	1.38 ± 0.10	1.51 ± 0.39
α-Linolenic	C18 : 3 ω-3	8.53 ± 0.18	5.83 ± 0.46	1.12 ± 0.02	4.00 ± 0.04
Arachidic	C20 : 0	10.41 ± 0.27	12.11 ± 0.86	13.43 ± 0.09	15.91 ± 0.23
Eicosenoic	C20 : 1ω-9	nd	nd	0.55 ± 0.05	0.73 ± 0.12
Eicosatrienoic	C20 : 3 ω-6	nd	nd	0.58 ± 0.08	nd 0
Arachidonic	C20 : 4 ω-6	10.94 ± 0.87	13.98 ± 0.72	23.36 ± 0.26	13.18 ± 0.40
Eicosapentaenoic	C20 : 5 ω-3	7.74 ± 0.38	8.00 ± 0.06	9.11 ± 0.29	9.63 ± 0.34
Behenic	C22 : 0	0.74 ± 0.02	0.62 ± 0.12	0.84 ± 0.07	nd
Erucic	C22 : 1 ω-9	0.70 ± 0.12	0.67 ± 0.08	nd	nd
Docosapentaenoic	C22 : 5 ω-3	0.62 ± 0.09	1.54 ± 0.07	1.24 ± 0.11	3.19 ± 0.28
Docosahexaenoic	C22 : 6 ω-3	5.60 ± 0.26	4.92 ± 0.33	3.97 ± 0.26	4.58 ± 0.31
Lignoceric	C24 : 0	2.90 ± 0.72	3.80 ± 0.18	3.23 ± 0.06	2.74 ± 0.40
Non identified		13.87 ± 0.57	11.03 ± 0.42	9.27 ± 0.39	12.50 ± 0.08
Total sum of ω-6	ω-6	6.06 ± 2.84	5.28 ± 2.76	8.44 ± 4.72	5.30 ± 2.50
Total sum of ω-3	ω-3	5.62 ± 1.17	5.09 ± 0.90	3.86 ± 1.23	5.35 ± 0.96
Total sum of ω-9	ω-9	6.40 ± 3.27	7.53 ± 3.97	9.89 ± 5.39	7.47 ± 3.90

I – beebread containing 45.5% of pollen from plants flowering in spring,

II, III – beebread containing summer pollen,

III – beebread mixed with honey at the ratio 1 : 1.

nd = not detected.

ples I, II and III. The correlation coefficient for the arachidic, arachidonic, oleic, linoleic and capric acids varied in the range 0.954–0.994 ($p < 0.01$); for the eicosatrienoic, eicosenoic, eicosapentaenoic it was 0.859–0.864 ($p < 0.05$). A negative correlation was found between rape pollen and palimitic and α-linolenic acids (–0.990 and –0.985, $p < 0.01$, respectively), as well as for stearic, erucic and linoleic acids (0.827, –0.882 and –0.861, $p < 0.05$, respectively). There was no correlation between rape pollen and the other nine identified fatty acids.

Beebread present in the market is most often a mixture of products of different origin; therefore, it is reasonable to compare the average concentrations of fatty acids present in various beebread samples. The average content of α-linolenic acid (4.32%) in all samples (I–IV) was not significantly different from that of DHA (4.24%) at $p > 0.01$. However, a significant difference was found between the content of α-linolenic acid and EPA (7.68%) and arachidonic acid (11.52%) at $p < 0.01$. On average, arachidonic and oleic acids constituting 16.09 ± 2.38% and 15.22 ± 1.35%, respectively, were the major ones in beebread. Their content in the analysed samples varied within 10.07–23.62% and 11.77–19.43%, respectively. The average content of arachidic acid was 11.98 ± 0.60% (10.14 to 13.52%), of EPA 8.31 ± 0.29% (7.36 to 9.40%) and of palmitic acid 8.18 ± 1.93% (2.45 to 13.82%). The next quantitatively important acids were α-linolenic (5.16 ± 1.37%), DHA (4.83 ± 0.33%) and margaric (4.15 ± 0.33%). The highest coefficient of variation in the content was found for α-linolenic acid (65.29%). The differences of fatty acid content in beebread samples I, II and III were not significant ($p > 0.01$) as compared with beebread mixed with honey at a ratio 1 : 1. Therefore, we could state that honey in beebread IV had no influence on the fatty acid content. Previously, the composition and content of fatty acids was studied mainly in pollen. For instance, Szczęsna [17] reported α-linolenic, palmitic and linoleic acids which on average constituted 43.0%, 28.0% and 14.0%, respectively, as the prevailing acids in pollen collected in Poland, Korea and China. α-Linolenic was the major acid in pollen from rape. The composition and content of fatty acids in various samples of pollen or beebread is associated with the botanical origin of pollen. Manning [18] found 73 fatty acids in 577 different pollen samples and stated that only five (palmitic, stearic, oleic, linoleic and linolenic) were common to all samples. Linoleic acid in pollen from calendula (*Arctotheca calendula*) comprised 50.4%, eucalyptus (*Eucalyptus* spp.) 44.2%, dandelion (*Taraxacum officinale*) 14.3%, willow (*Salix* spp.) 9.61%, rape (*Brassica napus*) 7.7%, sunflower (*Helianthus annus*) 5.38%, red clover (*Trifolium pratense*) 5.01%. Linolenic acid in pollen from *Trifolium pratense* constituted 41.4%; in pollen from *Salix* spp., *Brassica napus* and *Taraxacum officinale* it accounted for 21.7 to 25.4%, while in pollen from *A. calendula* and *Helianthus annus* its content was remarkably lower – 3.7 and 3.63%, respectively.

The predominant acid in *Helianthus annus* was myristic (47.6%), while in the other above mentioned pollen its content varied within 0.39–13.3%. Manning did not indicate the presence of EPA (C20 : 5 ω-3) and other long-chain (C22 : 1 ω-9, C22 : 5 ω-3, C22 : 6 ω-3, C24 : 0) fatty acids in pollen. However, a decrease in pollen fatty acid content when they become beebread in the hive and are stored in its combs was observed [19]. In fact, pollen fatty acid composition begins to change during their collection when bees add nectar and glandular secretion to make pollen loads [20]. Human & Nicolson [21] reported changes in fatty acids in fresh pollen collected by bees and in beebread from aloe (*Aloe greatheadii* var. *davyana*). They found that the total content of all the 18 fatty acids identified in their study was higher in fresh pollen than in pollen collected by bees or in beebread. The total content of monounsaturated fatty acids in pollen fat was 53.24%, while in beebread it constituted 36.61% (decreased by 16.63%); the content of gadoleic acid decreased from 41.53% to 24.16%. However, the content of oleic acid, which in pollen accounted for 11.71%, in beebread slightly increased to 12.45%. The total content of polyunsaturated fatty acids in pollen (20.08%) was found to be lower than in beebread by 1.3%. The content of linoleic acid in beebread was lower by 4.42% as compared with pollen, while the content of ricinoleic acid was higher by 3.01%. The same authors [21] identified DHA in fresh *Aloe greatheadii* var. *davyana* pollen, however, it was absent in bee-collected pollen and in beebread. Loper & Stanfifer [22] found remarkable differences between hand- and bee- collected almond (*Prunus Dulcis*) pollen. Eicosenoic acid (C 20 : 1) was found in the pollen of coniferous plants, eicosadienoic C20 : 2 in *Aloe greatheadii* var. *davyana* pollen, arachidonic in *Taraxacum officinale* pollen [23]. To the best of our knowledge, the presence of EPA in pollen or beebread was not previously reported in available literature sources. It is known that EPA is produced by microorganisms, fungi and algae [24].

The glandular secretion of each bee species is different; it depends on their physiological stage. Therefore, when injected to beebread, it may impart individual sensory properties [25]. Some fatty acids, such as trans-10-hydroxy-δ-2-decanoic and n-decanoic (capric), were reported to be present in royal jelly and beebread [26]. Nectar and glandular secretion added to the pollen during their collection, as well as bacterial flora present in the pollen and participating in pollen preservation process in the hive, change the nutritive value of pollen and convert them into beebread [27].

DISCUSSION

Pollen found in beebread to a great extent reflect the flora spread throughout the areas visited by the bees. Beebread in Lithuania is collected by bees in the active season during early spring, from March, to the end of summer in August and sometimes in the beginning of autumn in September. In this continuous work, pollen collecting depends on the plant flowering season, plant growing area, weather conditions, bee colony strength. It is difficult to collect pollen with pollen traps early in spring or in bad weather conditions [28].

The microbiological action of *Lactobacillus* species and changes of acidity in the process of pollen conversion into beebread in a beehive has been discribed by Chevtchik [29]. This process has been comprehensively studied. However, little evidence is available on fatty acid production by micro-organisms in the process of pollen conversion to beebread [30]. Five species of bacteria belonging to the genus *Bacillus* were identified in pollen and beebread. *Bacillus subtilis* is a metabolically active bacterium producing antibiotics and methyl-branched fatty acids [31]. The moulds isolated from pollen and beebread produce enzymes involved in lipid metabolism [32]. The ability of microorganisms to produce fatty acids is studied for the synthesis of commercially useful fatty acids, such as arachidonic and docosahexaenoic [33].

The beebread could be regarded as an extra source of essential polyunsaturated fatty acids in human diet. Beebread also exhibits antioxidant properties [34] and does not require any specific preservation with chemicals for a longer storage. Honey mixed with beebread preserves it and contributes aromatic components [35]. Thermally processed (40–45 °C) beebread containing 8.0–10.0% of moisture, or beebread mixed with honey was used together with medicaments for treating diabetes and arthritis [36, 37]. Most people tolerate it very well and use it instead of other relevant supplements.

CONCLUSIONS

1. Arachidonic and oleic acids were the prevailing fatty acids in beebread constituting on average 16.09 ± 2.38% and 15.22 ± 1.35%, respectively.

2. The content of α-linolenic in the beebread varied mostly from 1.10 to 8.71 %**.** The highest content of this acid comprised 65.29%.

3. The average content of α-linolenic acid (4.32%) in all samples (I–IV) was not significantly different from that of DHA (4.24%). A significant difference was found in the content of α-linolenic acid and EPA (7.68%).

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RIEBALŲ RŪGŠČIŲ SUDĖTIS BIČIŲ DUONELĖJE

Santrauka

Buvo tirta žiedadulkių ir riebalų rūgščių sudėtis bičių duonelėje. Pavasarinėje bičių duonelėje rasta 45,1 ± 3,0% karklų, o rinktoje vasarą – 78.7 ± 4.5% rapsų žiedadulkių. Tyrimo duomenimis, bičių duonelėje yra įvairios struktūros riebalų rūgščių. Nustatyta dvidešimt dvi riebalų rūgštys, iš kurių penkios – ω-3 struktūros, keturios – ω-6, trys – ω-9 polinesočiosios riebalų rūgštys. Bičių duonelėje, kurioje rapsų žiedadulkės sudarė 45,1 ± 3,0% ir 61,7 ± 4,0%, polinesočiųjų rūgščių ω-6 ir ω-3 santykis 1 : 1. Bičių duonelėje, kurioje rapsų žiedadulkių buvo 78,7 ± 4.5,%, šių rūgščių santykis 2 : 1.