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Composition and content of fatty acids in beebread of various floral origin, collected in Lithuania and prepared for storage in different ways

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Important fatty acid counterparts in the diet are α-linolenic acid (ALA) which is an ω-3 fatty acid, and linoleic acid (LA) which is an ω-6 fatty acid. The optimal ratio of unsaturated fatty acids ω-6 : ω-3 in the diet is thought to be 1 : 2, but in modern diet, however, this ratio is in unbalance (1 : 15–20). Pollen and beebread is a source which partly could restore this unbalance.

The aim of the present study is to determine the content and composition of fatty acids in beebread after using different preparation methods for storing. Beebread was collected in 2009 from Institute of Agriculture, Lithuanian Research Centre and Forestry's apiaries situated in different locations. Combs with beebread were distracted in spring from hives situated in four different locations. Beebread samples were dried at 35 °C or 40 °C to the moisture level of 8.0–10.0%. Parts of samples were wetted for 2 hours and dried at 40 °C. The other samples with fresh beebread were kept in a refrigerator at 5–8 °C. The fatty acids were extracted, hydrolyzed and methylated in one step and analyzed by gas chromatography with FID detection (Varian Ass).

Oilseed rape (*Brassica napus* var. *oleifera* DC) pollen ranged from 54.5% to 80.0% and willow (*Salix alba* L., *Salix caprea* L.) from 8.8% to 34.6%. The highest content of α-linolenic acid (27.04% to 43.83%) was found in all samples of beebread. The content of ω-6 linoleic acid in them varied from 5.44% to 9.11%. Of all saturated acids, palmitic acid was present at the lowest content of 20.5% and arachidic acid at the highest content of 2.82% in samples of beebread that contained the lowest amount of rape (*Brassica napus* var. *oleifera* DC) pollen 54.5% and highest willow (*Salix alba* L., *Salix caprea* L.) 34.6%. The highest content of palmitic acid (25.02–26.21%) was observed in samples where rape (*Brassica napus* var. *oleifera* DC) pollen accounted for 67.2% to 80.0%. The highest reduction in the content of ω-6, ω-9 and saturated fatty acids was observed in wetted and dryed beebread. Investigations of long-chain fatty acids of different botanical origin of beebread show that beebread has a higher ω-3 / ω-6 ratio which is more suitable for human diet than many plant oils.

Introduction

Omega-3 fatty acids ω-3 are polyunsaturated fats that the body cannot synthesize, but they are essential for multiple physiological functions and for the prevention and modulation of certain diseases common in Western civilization [1]. Omega-3 fatty acids are essential for the growth and development of infants and have an impact on health in different populations throughout the life cycle; consequently, they should be included in the diets of all humans [2–4]. Studies indicate that humans evolved on a diet with a ratio of ω-6 : ω-3 fatty acids of approximately 1 or lower. Dietary habits were changing over the centuries. Today, the ratio ω-6 : ω-3 in food is approximately 10 : 1 to 20–25 : 1, indicating that the intake of omega-3 fatty acids is much lower than needed. Studies indicate that in human organism α-linolenic (ALA) is converted into other omega-3 acids – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Omega-6 fatty acid interferes with the conversion of ALA to its long-chain metabolites EPA and DHA. In both omega-3 and omega-6 routes, the same enzymes – elongases and saturases are involved, – but in humans the conversion to very long-chain fatty acids is not very efficient. The consumption of food with fish and fish oils or algae provides the organism with EPA and DHA and can spare enzymes from competing for the conversion of ALA to EPA and DHA [5].

Bee pollen is a plant product which has a high content of ALA as compare with other fatty acids present in pollen. Pollen may lower serum cholesterol level, inhibit platelet aggregation and promote vessel wall production for prostacyclin. Prostacyclin is both a potent inhibitor of platelet aggregation and a powerful vasodilator. Polish and Finnish researchers have noted that the anti-atherosclerotic action of pollen extract may be in part due to metabolic conversion of α-ALA into EPA [6, 7].

Beebread is a mixture of pollen. During its collection and packing to hive frames, bees mix it with nectar, glandular secretion, and lactic acid bacteria. Beebread is fermented by the honey lactic acid bacteria flora added to the pollen. In the process of fermentation, microorganisms conserve the pollen and convert it into beebread. This process changes the composition of pollen during its presence in the hive [8]. Lactic acid bacteria existing in beebread, called probiotics, which contain beneficial bacteria that give strength to the body, support the digestive tract and effectively increase nutrient absorption. Studies have shown that lacto-fermentation can inhibit the growth of pathogenic bacteria. Fermented foods are also rich in enzymes [9–11]. Beebread contains usually the same fatty acids as fresh pollen. The total content of fatty acids in bee-collected pollen and beebread, however, is lower as compared with fresh pollen [12].

Fatty acids ω-6 and ω-3 were identified in Lithuanian beebread [13]. In this study, it was determined that the ratio of ω-6 to ω-3 mostly close to 1 : 1 or 1 : 2 and depends on the botanical composition of beebread. Findings suggest that beebread has not only a balanced fatty acid composition, but also acts on human health by moderating immunoglobulin level [14].

The aim of the present study was to determine the content and composition of different fatty acids in beebread.

Materials and methods

PREPARING SAMPLES FOR ANALYSIS. Preparing beebread for analysis was accomplished by the beekeeper Aloyzas Barkauskas (Šiauliai). Upon removing from the combs it was cleaned, and only the desirable length of beebread pieces (0.3–1.0 cm) were used for the analysis. Beebread samples were dried at 35 °C or 40 °C to the moisture level of 8.0–10.0. Parts of samples were wetted for 2 hours and dried at 40 °C. The samples with fresh beebread were kept in a refrigerator at 5–8 °C in hermetically sealed dishes. In total, 16 samples were prepared for analysis.

DETERMINATION OF FATTY ACIDS. The extraction of fats from beebread was accomplished by using a chloroform / methanol mixture (1 / 1) and 750 µl of water. The chloroform layer was transferred into another tube and the solvent was removed by evaporation. Then the fatty acid esters were hydrolyzed and methylated simultaneously with a mixture of 100 µl toluene and 0.5 ml BF3/MeOH for 60 min at 100 °C in a heating block. After cooling, 800 µl of distilled water and 800 µl hexane were added. After shaking and settling, the hexane layer (upper layer) containing fatty acid methylated esters (FAME) was transferred to GC vials and stored at -20 °C until analysis. The prepared mixture of methyl esters of fatty acids was analyzed with a GC-3900 gas chromatograph equipped with a CP 8400 auto injector (Varian Assoc.). The FAME was separated on a 100 × 0.25 mm ID WCOT fused silica capillary column coated with a 0.25 µm of CP-Select CB provided by Varian Ass. The Galaxie software was used for the quantisation and identification of peaks. The baseline separation of over 50 FAME peaks was accomplished with the help of mixed FAME standards (Sigma). The analytical conditions employed were as follows: volume injected 1 µl, carrier gas nitrogen (1.1 ml/min), injector temperature 250 °C, FID 275 °C, split ratio 1 : 20, and oven temperature from 185 °C to 245 °C with a stepped temperature program within the total run time of 57 min [15].

The botanical composition of beebread was identified by the melissopalinology method. The estimation of the botanical composition of pollen from beebread was based on the frequency of pollen from specific nectar-producing plants [16].

STATISTICAL ANALYSIS. The fatty acids have been expressed as % of the total fatty acids present in the chromatogram. The results were analyzed using ANOVA programme; estimated were the mean of measurement ($\bar{x}$), sample standard deviation (Sx), standard deviation of the mean (SD), the coefficient of variation (CV), and the statistical significance of results (p). The value $p < 0.05$ was taken as statistically significant.

Results and discussion

Twenty-two fatty acids were identified in spring beebread. Our findings indicate that beebread contains medium-chain (C10–C18) and long-chain (C20–C24) saturated fatty acids (Table 1). Medium-chain saturated fatty acids are present in higher amounts as compared with long-chain fatty acids, from which palmitic acid (C16:0) is present in the highest amount (from 20.50 ±1.52% to 26.21 ±1.26%) and arachidic (C20:0) from 1.90 ±0.23% to 2.82 ±0.37%. Beebread collected in spring had the highest content of ω-3 α-linolenic fatty acid (from 27.04 ±3.09% to 43.83 ±1.59%), while the content of ω-6 fatty linoleic acid was lower (from 5.47 ±0.63% to 8.39 ±1.34%).

The highest reduction in the content with a significant difference ($p < 0.05$) was found for myristic, stearic and arachidic fatty acids after drying beebread at 40 °C and wetting for 2 h, followed by drying at 40 °C. The reduction of the content of unsaturated fatty acids was significant ($p < 0.05$) for linoleic and oleic acids after wetting beebread and drying at 40 °C. The results showed that the reduction in the average content of ω-9 fatty acids was greater as compared with ω-6 ($p < 0.05$) (Table 2).

Table 1. Fatty acid composition in beebread collected in spring

Fatty acids	Chemical structure	*Relative percentage of fatty acids, %				
		Fresh, stored at 5–8 °C	Dried at 35 °C	Dried at 40 °C	Wetted for 2 h and dried at 40 °C	LSD_{05}
Capric	C10:0	0.64 ±0.12	0.66 ±0.09	0.57 ±0.14	0.47 ±0,09	0.36
Undecanoic	C11:0	2.33 ±0,40	2.62 ±0.04	2.01 ±0.13	1.85 ±0.13	1.07
Lauric	C12:0	0.33 ±0.07	0.38 ±0.05	0.60 ±0.19	0.62 ±0.09	0.34
Myristic	C14:0	5.02 ±0.82	6.41 ±0.35	2.18 ±0.51	1.77 ±0.22	1.60
Pentadecanoic	C15:0	0.09 ±0.01	0.07 ±0.00	0.09 ±0.02	0.11 ±0.00	0.04
Palmitic	C16:0	20.50 ±1.52	25.02 ±1.43	26.21 ±1.26	25.28 ±0.51	3.12
Heptadecanoic (Margaric)	C17:0	0.18 ±0.03	0.11 ±0.02	0.11 ±0.02	0.13 ±0.02	0.08
Stearic	C18:0	6.61 ±0.94	5.82 ±0.22	3.10 ±0.67	3.68 ±0.30	1.79
Oleic	C18:1n9c	4.54 ±0.58	5.25 ±0.35	4.52 ±0.65	3.21 ±0.22	1.31
Linoleic	C18:2ω-6c	8.39 ±1.34	5.44 ±0.27	8.24 ±1.14	5.47 ±0.63	2.59
γ-Linolenic	C18:3ω-6	0.13 ±0.04	0.14 ±0.03	0.14 ±0.03	0.08 ±0.06	0.10
α-Linolenic	C18:3 ω-3	34.59 ±3.68	27.04 ±3.09	33.98 ±5.23	43.83 ±1.59	11.73
Arachidic	C20:0	2.82 ±0.37	2.35 ±0.19	2.38 ±0.20	1.90 ±0.23	0.62
Eicosenoic	C20:1n9c	0.25 ±0.11	0.30 ±0.06	0.19 ±0.03	0.14 ±0.01	0.24
Eicosadienoic	C20:2(ω-6)	0.19 ±0.02	0.16 ±0.01	0.26 ±0.06	0.17 ±0.05	0.14
Eicosatrienoic	C20:3ω-3	0.09 ±0.02	0.14 ±0.01	0.42 ±0.18	0.42 ±0.07	0.13
Behenic	C22:0	0.22 ±0.05	0.23 ±0.07	0.41 ±0.04	0.35 ±0.03	0.03
Brassidic	C22:1 ω-9 trans	0.31 ±0.07	0.25 ±0.05	0.77 ±0.21	0.40 ±0.03	0.39
Erucic	C22:1 ω-9cis	0.12 ±0.02	0.15 ±0.02	0.09 ±0.01	0.09 ±0.01	0.06
Tricosanoic	C23:0	0.06 ±0.01	0.06 ±0.00	0.08 ±0.01	0.03 ±0.01	0.03
Lignocerin	C24:0	1.60 ±0.30	2.18 ±0.80	1.46 ±0.45	0.93 ±0.34	1.80
Nervonic acid	C24:1	0.56 ±0.21	0.29 ±0.03	0.30 ±0.13	0.52 ±0.17	0.54

*Results are given in mean ± standard deviation Sx.

Table 2. Effect of beebread preparation method on fatty acid content

Fatty acids (FA)	$\bar{x}$	Sx	Min.	Max.	SD	V%
ω-3	35.49	3.08	29.20	43.19	6.17	25.68
ω-6	6.67	0.72	5.55	8.52	1.41	4.43
ω-9	5.02	0.70	3.84	5.57	0,25	3.75
Saturated (FA)	41.18	1.65	37.77	45.53	3.31	8.03
Unsaturated (FA)	47.18	2.71	40.26	53.36	5.41	11.47
ω-3 / ω-6	5.20	–	5.20	5.00	–	–
SFA / UFA	0.87	–	0.94	0.85	–	–

$p < 0.05$.

The ratio of ω-3 / ω-6 was high and constant (5.0–5.2), independently of the content, showing an exceptionnally beneficial composition of fatty acids in the beebread. The ratio of saturated to unsaturated fatty acids (SFA / UFA) was 0.87 and had a low variation in the range 0.85–0.94. The content of ω-3 fatty acids varied in the range from 29.20% to 43.19%; as a result, the coefficient of variation (CV) was the highest for these fatty acids (25.68%). Similar results as in previous investigations were found in the SFA / UFA ratio in beebread mixed with honey. The ω-6 / ω-3 ratio in the samples was close to unity [14].

Rape (*Brassica napus* var. *oleifera* DC) pollen prevailed in beebread collected in spring (Table 3). Willow (*Salix alba* L., *Salix caprea* L.) pollen was present in sample I of beebread with the highest content (34.6%), while in samples III and IV it comprised 8.8%. A significant content of horse-chestnut (*Aesculus hippo-*

castanum L.) was found in beebread samples III and IV (12.8%). The presence of Raspberry (*Rubus idaeus L.*), white clover (*Trifolium repens* L.) and apple-tree (*Malus domestica* Borkh.) pollen was minor (1.6–2.0%).

Table 3. Botanical composition of beebread collected in spring (on 6 April 2009)

Samples	Location of apiary	Pollen composition in beebread, %
I	Akademija	Rape (*Brassica napus* var. *oleifera* DC) – 54.5; willow (*Salix alba* L., *Salix caprea* L.) – 34.6; raspberry (*Rubus idaeus L.*) – 2.0; charlock (*Sinapis arvensis* L.) – 6.9; white clover (*Trifolium repens* L.) – 2.0
II	Alksnėnai	Rape (*Brassica napus* var. *oleifera* DC) – 80.0; willow (*Salix alba* L., *Salix caprea* L.) – 20.0
III	Gudžiūnai	Rape (*Brassica napus* var. *oleifera* DC) – 76.8; willow (*Salix alba* L., *Salix caprea* L.) – 8.8; horse-chestnut (*Aesculus hippocastanum* L.) – 12.8; apple-tree (*Malus domestica* Borkh.) – 1.6
IV	Krakės	Rape (*Brassica napus* var. *oleifera* DC) – 67.2; willow (*Salix alba* L., *Salix caprea* L.) – 8.8; horse-chestnut (*Aesculus hippocastanum* L.) – 12.8; lilac (*Syringa*) 9.6; apple-tree (*Malus domestica* Borkh.) – 1.6

The initial moisture content in beebread samples ranged from 14.0% to 17.0%, indicating that it they has been collected for analysis in the optimal condition. Moisture level after drying at 35 °C and 40 °C was 8.0–10.0%, which is considered suitable for products of plant origin. The damage level of fatty acids in beebread during the drying process can be similar to that of rapeseed which have a high content of fatty acids [18]. The losses of fats can be associated with the greatest moisture content and leakage of fats caused by the damage of pollen grains in beebread in the process of drying. Wet pollen grains swell very fast. We can state that the decreased content of most of the fatty acids in samples wetted for 2 hours is associated with an increased moisture content in pollen grains. The test showed that the variation of concentrations was statistically insignificant for most fatty acids in the samples dried at 35 °C and 40 °C.

Nutrition experts recommend replacing some ω-6 fatty acids in the diet with those of the ω-3 family [4]. Plant oils, such as canola or others, have a high content of vitamin E which is a natural antioxidant which protects unsaturated fatty acids from oxidation and is beneficial to human health [17–19]. Plant sources of omega-3 fatty acids are important in the food. Wild plants, such as purslane, walnuts, figs and others, accumulate higher amounts of ALA, vitamin E, vitamin C, and other antioxidants as compared with cultivated plants [1]. Honey and beebread contain flavonoids, have antioxidant properties and exhibit a free radical scavenging activity [20, 21]. Therefore, the total composition of the biologically active beebread components may be important for human health hen beebread is used as a supplement.

Conclusions

1. Beebread is a mixture of pollen collected from cultivated and wild plants, which contains different saturated and unsaturated long-chain fatty acids, including the nutritionally important omega-3 linolenic acid (ALA) whose content was the highest (27.04% to 43.83%) compared with the other unsaturated and saturated acids present in the beebread.
2. The content of saturated fatty acid (palmitic) was high (20.50–26.21%) in the tested samples, but it did not exceed the content of omega-3 linolenic acid.
3. Beebread can be applied as a suitable dietary supplement due to a high content of omega-3 linolenic acid and a beneficial ratio of ω-3 / ω-6 fatty acids and unsaturated-to-saturated fatty acids.
4. Beebread should not be stored in wet conditions. Reduction of the content of 10 fatty acids from the 22 fatty acids present in the beebread samples was noted after storing for 2 h in wet conditions and a subsequent drying at 40 °C.

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RIEBALŲ RŪGŠČIŲ SUDĖTIS IR KIEKIS ĮVAIRIOS BOTANINĖS SUDĖTIES BIČIŲ DUONELĖJE SURINKTOJE LIETUVOJE, PARUOŠTOJE LAIKYMUI SKIRTINGAIS BŪDAIS

S a n t r a u k a

Dietologijoje yra svarbios nesočios riebalų rūgštys – α-linoleno rūgštis 18:3 ω-3 (ALA) ir ω-6 linolo (LA). Optimalus bendras nesočiųjų riebalų rūgščių ω-6 : ω-3 santykis subalansuotoje žmonių mityboje yra 1 : 2, tačiau šiuolaikinėje mityboje šis santykis yra išbalansuotas ir tapo žymiai didesniu (1 : 15–20). Žiedadulkės ir bičių duonelė yra produktai, kurie dalinai gali papildyti šį išbalansuotą santykį.

Šio darbo tikslas yra nustatyti riebalų rūgščių sudėtį ir kiekius skirtingais būdais paruoštoje bičių duonelėje. Bičių duonelė surinkta Žemdirbystės instituto Lietuvos agrarinių ir miškų mokslų centro bityne 2009 m. Koriai su bičių duonele buvo išimti pavasarį iš avilių, išdėstytų keturiose skirtingose vietovėse**.** Bičių duonelės mėginiai buvo išdžiovinti 35–40 °C temperatūroje iki 8,0–10,0 % drėgnio. Dalis mėginių buvo drėkinti 2 h, po to išdžiovinti 40 °C. Riebalų rūgštys buvo ekstrahuojamos, hidrolizuojamos ir metilinamos, po to analizuojamos dujų chromatografu su integruotu detektoriumi FID (Varian Ass).

Rapsų (*Brassica napus* var. *oleifera* DC) žiedadulkių kiekis bičių duonelėje yra nuo 54,5 % iki 80,0 %, karklų (*Salix alba* L., *Salix caprea* L.) – nuo 8,8 % iki 34,6 %. α-Linoleno rūgšties (27,04–43,83 %) rasta daugiausia visuose bičių duonelės mėginiuose; ω-6 linolo rūgšties kiekis kito nuo 5,44 iki 9,11 %. Palmitino rūgšties buvo mažiausia – 20,5 %, lyginant su kitomis sočiosiomis riebalų rūgštimis, o arachido daugiausia 2,82 % bičių duonelėje, kurioje rapsų (*Brassica napus* var. *Oleifera* DC) žiedadulkių buvo mažiau, negu kituose mėginiuose – 54,5 %, tačiau daugiausia karklų (*Salix alba* L., *Salix caprea* L.) žiedadulkių – 34,6 %. Didžiausias palmitino rūgšties kiekis 25,02–26,21 % nustatytas mėginiuose, kuriuose dominavo rapsų (*Brassica napus* var. *Oleifera* DC) žiedadulkės nuo 67,2 iki 80,0 %. Daugiausiai ω-6, ω-9 ir sočiųjų riebalų rūgščių sumažėjo sudrėkintuose ir po to džiovintuose bičių duonelės mėginiuose. Nesočiųjų riebalų rūgščių, turinčių ilgą grandinę, tyrimai parodė, jog riebalų rūgščių ω-3 / ω-6 santykis bičių duonelėje yra aukštesnis ir palankesnis dietologijoje, negu daugumoje augalų aliejų.