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Melissopalynological Analysis and Biochemical Assessment of the Nectariferous Plants in Lithuania

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Abstract

One of the most important non-wood forest products are products of the animal origin including bee products. Bee pollen and beebread are vitally important to enhance ecological, economic and social well-being. Valuable constituents of bee products result in healing properties including fatty acids and glucose oxidase.

The present study was aimed to compare fatty acids contents and glucose oxidase activity in pollen and beebread from different plants taxa. Fatty acids ω-3 and ω-6, which are essential for a healthy human diet, were identified in all pollen and beebread samples. Fatty acid patterns were determined in monofloral bee pollen of maple (*Acer* L.), horse chestnut (*Aesculus hippocastanum* L.), dandelion (*Taraxacum officinale* L.) and oilseed rape (*Brassica napus* L.). The pollen from willow (*Salix* spp.), oilseed rape (*Brassica napus* L.) and white clover (*Trifolium repens* L.) were dominated in the beebread used for fatty acid analysis. Fatty acids were determined from C10 to C24 in both pollen and beebread. High levels of the α-linolenic acid, an essential n-3 fatty acid, were found in the samples. The highest content of α-linolenic acid, ranging from 33.5 ± 2.9% to 49.6 ± 2.6%, was identified in all pollen samples, and in beebread from 29.6 ± 1.2% to 42.2 ± 1.4%. Of the saturated fatty acids, a palmitic acid (C16:0) was present in the highest concentration from 14.8 ± 1.9% to 24.8 ± 0.03% in all monofloral pollen, and in beebread, it ranged from 23.3 ± 1.7% to 24.2 ± 1.8%. Lauric acid (C12:0) and stearic acid (C18:0) were found in the highest concentrations only in dandelion (*Taraxacum officinale*) pollen, 11.1 ± 0.8% and 11.6 ± 0.9%, respectively. The enzymatic activity of glucose oxidase (GOX) in beebread and monofloral rape (*Brassica napus*) pollen was statistically significantly higher (at $p < 0.05$) compared to that of willow (*Salix* spp.) and plum (*Prunus domestica* L.) pollen. In beebread, the oilseed rape monofloral pollen, which is characterised by the highest GOX activity, accounted for 81.65%. Both the composition of the pollen in the beebread and its fermentation process in a bee hive can influence GOX activity. It should be emphasized that one of antibacterial constituents released by the enzyme glucose oxidase is hydrogen peroxide. It protects bee colonies against diseases and influences food preservation in a hive.

Key words: non-wood forest products, pollen, beebread, fatty acids, glucose oxidase.

Introduction

Non-wood forest products (NWFPs) are products of "biological origin other than wood derived from forests, other wooded land and trees outside forests" (FAO 1999) including a wide range products from trees, understory plants, fungi or animals. These products are collected from forests and agroforestry. Usually forest honey, pollen and propolis are of high quality because no agricultural activities are carried out in forests. The main two kinds of honey harvested in forests are honeydew and heather. Honeydew honey is generally gathered from coniferous tree species *Abies alba* Mill. and *Picea excelsa* (L.) H. Karst. in Europe (Persano Oddo et al. 2004, Rybak-Chmielewska et al. 2013). In Lithuania, honeydew honey is not found often and abundantly (Čeksterytė et al. 2013). Monofloral heather honey was collected in Jurbarkas

– Tauragė forests in Lithuania (Čeksterytė 2002). Heather honey was collected by *Apis mellifera* L. in forests of the reserve established to protect bee populations indigenous to Lithuania (Balžekas 1995). The study and reproduction of indigenous bee population was discontinued due to its high hybridization level. Bees and forest trees are closely interrelated in the forest ecosystems and adjacent agricultural lands. Bees sustain forests and agricultural crops by pollinating flowering plants and crops (Bradbear 2009). They not only help to increase yield of seeds/fruits but also provide human with bee products. Land transformation including previous deforestation and intensive cultivation has affected insect populations and their adaptation to the new environment conditions (Kremen et al. 2004). There are different causes of losses in honey bee stocks in Europe and America. The honey bee is the main pollinator which uses different floral resources for the colony nutrition. It is maintained that the lack of diversity of floral resources resulting from intensive agricultural activities is also a likely factor contributing to the decline of honey bee population (Decourtye et al. 2010). Recovering of plants diversity required more than 20 years and intensive labour inputs, including biological pest control (Isbell et al. 2013, Bommarco et al. 2013). Providing new measures in nature conservation improves conditions for well-functioning whole ecosystem (Cornell 2010). The nectar and pollen productivity in honey plants varies across different geographical areas depending on the type of vegetation, length of blooming period, and climate conditions. Nectar-producing trees such as *Aesculus hippocastanum* L., *Acer negundo* L. and *Acer platanoides* L., are planted in parks, green areas of Lithuanian cities, homesteads as well as near roadsides (Snieškienė and Juronis 1999, Snieškienė et al. 2011). In Lithuania, *Tilia cordata* Mill. grows on an area of 4,600 ha comprising as little as 0.24% of the total forest area (Semaškienė 2006). Parkways were lined with about 100 small-leaved limes, *Tilia cordata* in Lithuanian estates. Nectar productivity of limes, which were planted in those manor parks about century ago, is not determined yet. In Lithuania, pollen of the above mentioned species is not found in honey every year; whereas *Salix* spp. actually is the main species produced quantities of nectar and pollen in early spring (Baltrušaitytė et al. 2007, Kaškonienė et al. 2010). The Obelynė Manor Park planted by Professor T. Ivanauskas near Kaunas has exceptional dendrological and cultural value for future generations (Straigytė and Vaidelys 2012). The data of melissopalynology analysis of honey show that limes *Tilia cordata* are widely distributed in the Dzūkija National Park and in the Armona Geological Reserve, and *Frangula alnus* Mill. in Lazdijai district. In monofloral honey from the mentioned locations, pollen of *Tilia cordata* comprises 79.0% and 53.9%, respectively, and pollen of *Frangula alnus* 46.1% – 52.1%. Honey with a significant content of pollen from *Rubus idaeus* L. (16.7%), *Robinia pseudoacacia* L. (8.5%) was found in the Gomerta Landscape Reserve in Radviliškis district. However, pollen of *Acer platanoides* L. varied in the narrow range in honey collected in the Varduva Scenic Landscape Reserve in Mažeikiai district, in Lazdijai district and in the Armona Geological Reserve in Ukmergė district (7.0 – 4.7%, respectively), and *Aesculus hippocastanum* (3.1 – 4.9%) in honey from the Salantai Regional Park in Kretinga district and Lazdijai district (Čeksterytė et al. 2013). *Aesculus hippocastanum* is an important medicinal and bee plant (Ćalić-Dragosavac et al. 2009). Sweet chestnut (*Castanea sativa* Mill.) variety 'Judia' is found on two protected areas in Portugal (Dinis et al. 2011). Environmental conditions affect genetic and morphological characteristics of *Castanea sativa* (Lauteri et al. 2004). The concentration of phytopharmaceuticals depends on the species of plants and varies within species and between plant parts and geographical areas of growing (Butkute et al. 2013, Maruška et al. 2014, Carrillo et al., 2014). Fruit of *Castanea sativa* variety 'Judia' in North Portugal contains the highest concentrations of crude protein, soluble sugars, starch and the highest content of unsaturated fatty acids, especially linoleic acid, and the lowest concentrations of saturated fatty acids (Dinis et al. 2012). Black locust *(Robinia pseudoacacia*) was introduced into Jonava forests in Lithuania to replace pines that had become extinct because of the air pollution (Navasaitis 2004). Nectar-producing tree species as maple (*Acer*) genus and *Robinia pseudoacacia* were introduced for increase in the biodiversity and soil improvement in twelve forest districts of the south-western and western Lithuania (Žiogas et al. 2007). Oak (*Quercus* L.) and aspen (*Populus tremula* L.*)* are wind-pollinated and do not produce nectar, but they are useful for honeydew production (Navys 1996). Pollen from the wind-pollinated species as ash (*Fraxinus* sp.), poplar (*Populus* sp.), and oak (*Quercus* sp.) is also found in pollen loads (Keller et al. 2005).

Brassica napus, a worldwide grown agricultural crop, is the main plant producing large amount of nectar and pollen (Kołtowski 2005). However, a more diverse foraging area for bees is better for the development of their colonies and health since early spring. Floral diversity directly affects bee health. Polyfloral pollen is better food for bees compared with monofloral pollen. Polyfloral pollen can influence the diversity and proportion of specific proteins and amino acids re-

quired for bee and tissue development (fat body and hypopharyngeal glands (HPGs). Protein-balanced diet has a positive effect on the build-up of proteins and fats in a bee body (Roulston and Cane 2002, Konopacka et al. 1975). During the biological experiment on the Cander nucleus, it was found that fresh pollen stored at the temperature from -5 to -8 °C for one year was more suitable for bee feeding than dried pollen or conserved with powdered sugar 1:1 (Čeksterytė and Balžekas 1995). Tasei and Aupinel (2008) have reported that bumble-bee larvae fed on a polyfloral blend were heavier than larvae fed on monofloral diets with higher protein content. Lipids of the fat bodies-tissue of bees consist free and bound fatty acids, triacylglycerols, steroids, phospholipids and other compounds. Deficiency of polyunsaturated fatty acids, such as linoleic and linolenic, in the diet of insects is a cause of their slow growth, deformed wings and lower productivity. In the physiology of insects, the other important function of fatty acids is to release energy in flight muscles during the flight (Nation 2002). Sterols (special forms of steroids) are not synthesized by insects; however, they are required ecdysteroid molting hormone during their molting process (De Loof 2006).

Pollen of different plant species varies greatly in fatty acids composition. Investigation of fatty acids composition of the pollen of fifteen Scandinavian plant species revealed that linoleic, linolenic and arachidonic acids were dominant (Solberg and Remedios 1980). Pollen of Australian eucalypts has higher content of linoleic than linolenic acid. However, the percentage of linoleic acid is higher in the pollen of Italian eucalypts compared with Australian ones. A distinctive feature of sunflower pollen is a high content of myristic acid, which can be detrimental to honey bees (Manning 2006). Long chain fatty acids as myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3), arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) were found in the pollen collected in Poland, Korea and China. α-linolenic, palmitic and linoleic acids accounted for the highest content in pollen samples originated in different countries. α-linolenic acid was dominant in Brassicaceae pollen in comparison with polyfloral acid collected in Poland (Szczęsna 2006).

One of the proteins added to nectar by bees is enzyme glucose oxidase, which gives antioxidative and antiseptic properties to honey. Glucose oxidase (GOX) is very rare in the animal kingdom, and is expressed mainly in the hypopharyngeal glands (HPGs) of bees (Ohashi et al. 1999, Bradbear 2009). During honey dilution, the enzyme is activated and oxidises glucose to generate gluconic acid and hydrogen peroxide. GOX catalyses the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide, the latter having antiseptic properties. The antiseptic products are secreted into brood food and sterilize it (White et al. 1963). *Fagopyrum esculentum* Moench (buckwheat) honey possesses higher GOX compared with rape honey (Kretavičius et al. 2010). Hydrogen peroxide provides prevention of diseases and contamination for whole bee colony. Alaux et al. (2010) showed that polyfloral pollen diet induced higher GOX activity in a bee organism compared with monofloral diets and conferred bees with better in-hive antiseptic protection. Polyfloral pollen was found to induce higher GOX activity and immune parameters in a bee body in comparison with monofloral pollen (Alaux et al. 2010). The authors used spring-collected polyfloral pollen of two blends in the bee diet. The pollen of *Erica*, *Cistus*, *Salix*, *Acer* predominated in the first blend, and the pollen of *Quercus*, *Salix*, *Acer*, *Cistus* were dominant in the second one. The monofloral pollen was used from the same blend for the bee feeding (Alaux et al. 2010). Pollen is a primary source of proteins, fatty substances, minerals, and vitamins for the growth of bee queens and worker larvae (Herbert 1992). Worker bees pack the pollen into comb cells located near the brood in the centre of the hive (Dietz 1992). Specific micro flora isolated from pollen and ferment sugars, produces lactic acid, fatty acids, and enzymes. Therefore, these compounds introduced to pollen by the metabolic process conserve them and are important in preservation of beebread in the hive (Gilliam 1979, Gilliam et al. 1989). Symbiotic associations exist among flora, bees and microorganisms, which survive on plants. Bacteria occurring in beebread are also found in floral nectar and are associated with environmental conditions. Beneficial bacteria *Lactobacillus kunkeei*, Acetobacteraceae were identified in beebread samples. These bacteria influenced beebread preservation, bee colony hygiene and health (Anderson et al. 2013). A strain of *Bifidobacterium asteroides* PRL2011 has been recently isolated from the hindgut of *Apis mellifera* var. *ligustica*. *B. asteroides* PRL2011 genome encodes various enzymes that react with toxic compounds (Bottacini et al. 2012). On a world scale, bee products are vitally important for human health and well-being. While the rationale for the sustainable use of tree resources is widely appreciated, by contrast the sustainable use of bee resources is poorly promoted and appreciated. This work will help to deepen the knowledge of the nutritional and bacteriostatic value of non-wood forest products of animal origin.

The aim of the study was to compare fatty acids contents and composition essential for a healthy diet and glucose oxidase activity in beebread and pollen; to evaluate the influence of plant sources on the con-

tent of fatty acids and glucose oxidase activity in the tested products and to identify plant pollen species in bee products using melissopalynological analysis.

Materials and Methods

Preparation of pollen samples for analysis

Pollen loads were collected from early spring to mid July at the divisions of apiary of the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry located at different sites of Kėdainiai district. For collecting bee pollen, a standard pollen trap was mounted on the hive entrance and maintained throughout the collecting period. Every day, pollen was taken from the traps, cleaned and kept in a refrigerator at 5 – 8°C in air-tight plastic bags. At the end of spring, pollen loads of willow, maple, plums, horse-chestnut, dandelion and rape were separated manually by colour from the pollen mixture collected in spring. Fresh pollen samples (100 g of fresh material per sample) were put into small plastic bags, hermetically sealed and kept at -5°C until analysis. Fatty acids were determined in the monofloral pollen loads of *Aesculus hippocastanum*, dandelion *Taraxacum officinale*, *Acer*, *Brassica napus* collected in 2011 and GOX activity in the pollen loads (P1, P2, P3) collected in 2013. The botanical origin of pollen was identified in all samples.

Preparation of beebread samples for analysis

Beebread was collected in the apiary of the Institute of Agriculture LRCAF. Fatty acids were determined in the samples of beebread I, II, III and IV in 2011 (marked as BI, BII, BIII and BIV in Figures). Samples of beebread (I) were collected in spring from all apiary divisions; samples II, III were collected in summer from the locations of Špitolpievis, Gudžiūnai in Kėdainiai district. Sample IV was delivered from Plungė district. After removing from the combs (about 0.5 kg per hive, the beebread was threshed and cleaned. The weight of a composite sample intended for analysis was about 300 g of fresh material. Only pieces of a desirable length 0.3 – 1.0 cm were analysed. Glucose oxidase (GOX) activity was assayed in samples B1, B2, B3 and B4 collected from all apiary divisions in spring 2013. Samples of fresh beebread for GOX activity analysis were manually selected by colour. Fresh beebread was placed in small plastic bags, hermetically sealed and kept at -5 °C until analysis.

Four species of monofloral pollen loads and four types of beebread were prepared for fatty acids analysis and GOX activity; three species of monofloral pollen loads were prepared for GOX activity determination. The composition of the selected pollen loads, and beebread (BI, BII, BIII, BIV and B1, B2, B3, B4) was identified by the melissopalynology method (Louveaux 1978). Identification of beebread botanical and fatty acids composition was done on the same sample collected in 2011. GOX activity analysis was performed on the samples from 2013. GOX activity and botanical analysis was performed on samples of beebread (B1, B2, B3, B4) from 2013.

Preparation of pollen and beebread slides for the determination of botanical origin

A pollen or beebread sample of 10 g was weighed and dissolved in distilled water (20 – 40°C) to the volume of 20 mL. This solution was centrifuged for 10 min at 1,000 g. After that the supernatant liquid was poured off. The sediment was dispersed with 20 mL of distilled water to completely dissolve the remaining sugar crystals and again centrifuged for 5 min at 1,000 *g*. The supernatant was decanted leaving only the sediment. The remaining excess liquid was taken up on absorbent paper. The sediment was collected with plastic Pasteur pipettes (by volume of 1 mL) and spread on a slide over an area of about 20 mm. The slide with the sediment of pollen was dried at 40 °C on a heating plate only for the time strictly necessary to dry. The glycerine jelly (Kaiser's Glycerol Gelatine TM Merck) was liquefied by warming it to 40 °C (either on a heating plate or in a water bath). The cover slips (22 mm × 22 mm) were warmed on the heating plate. One drop of glycerine jelly was deposited onto the cover slip and placed on the slide very slowly to avoid air bubbles. The pollen grain exine and shape were visualized under light microscope Nicon Eclipse E600. Pollen images were taken in two positions: polar and equatorial view, at 400 magnification, focusing on pollen wall and surface sculpture.

Slide preparation of hand-collected pollen

Pollen collected by hand from 85 melliferous plant taxa was prepared also for microscopy. Air-dried plant pollen was shaken from flowers; some part of it was applied on the slide and covered with a cover slip overlaid with liquefied glycerine jelly. These pollen samples are stored as reference. A catalogue of coloured Digital images of Lithuanian melliferous plant pollen grains was created for comparison of images of pollen found in honey to those of known pollen collected manually (Čeksterytė 2012).

Expression of results

About 400–500 pollen grains were counted in each sample. The frequency of pollen of each melliferous taxon is expressed as percentage of the total pollen sum. Honey considered as monofloral is mainly pro-

duced from one plant species or pollen content from one plant species is predominant (constituting more than 45.0%). The pollen content of other plant species is designated as follows: secondary pollen 16–45%; important minor pollen 3–15%; minor pollen < 3.0% (Louveaux et al. 1978).

Determination of fatty acids

Fats were extracted from a 200 mg sample of beebread or pollen using a mixture of 3 ml of chloroform/methanol (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 hydrolysed and methylated simultaneously with a mixture of 100 µL toluene and 0.5 ml boron trifluoride in MeOH for 60 min at 100 °C in a heating block. After cooling, 800 µl distilled water and 800 µL hexane were added. After shaking and settling, the hexane layer (upper layer) containing the fatty acid methylated esters (FAME) was transferred to gas-chromatography (GC) vials and stored at -20°C until analysis. The prepared mixture of methyl esters of fatty acids was analysed by a gas chromatograph GC-3900 equipped with a CP 8400 auto injector and a flame ionization detector (FID) as detector (Varian Assoc., Middelburg, the Netherlands). The FAME were 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 quantitation and identification of peaks. Baseline separation of over 50 FAME peaks was accomplished by means of mixed FAME standards (Sigma). The analytical conditions for GC-FID 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 stepped temperature program: within total run time 57 min (Mamalakis et al. 2006).

Determination of glucose oxidase (GOX) activity

Modified method was used based on measurement of oxygen concentration in the glucose oxidation reaction (Kretavičius et al. 2010). The enzymatic oxygen consumption was detected amperometrically using Clark-type membrane oxygen electrode (Clark et al. 1953). The reaction was performed in the 1.4 ml PMMA cell, thermostated at 37 °C. Analytical grade, previously desiccated D-glucose (Lach-Ner, Czech) solutions were prepared one day before the experiment, for complete mutarotation. The reaction mixture consisted of 3 M glucose in the 0.1 M sodium acetate buffer (pH = 6.0), 0.05 mM NaN_3 was added to suppress a catalase activity. Seven samples were prepared by extraction of 2 g of beebread or pollen specimen in 10 ml of distilled water at 37 °C during three hours; settlings were separated by centrifugation, and GOX activity in the supernatant was tested. A rate of oxygen uptake was recorded after addition of 0.02 ml of the assay solution to the reaction mixture. The controller was used to apply a voltage to the Pt electrode that is – 0.6V versus Ag/AgCl reference electrode. The resultant current proportional to the oxygen concentration in the air-saturated solution was displayed and saved with a data logger. The GOD activity is expressed as IU/g (International Units): micromoles of oxygen consumed in 1 min by the enzyme contained in 1 g of raw material. Measurements are repeated three times.

Statistical analysis

The fatty acids were expressed as % of the total fatty acids present in the chromatogram. The results were analysed using ANOVA software. Results of fatty acids and GOX activity analysis were expressed as a mean ± standard deviation (*SD*) and statistical significance was estimated. The value $p < 0.05$ was taken as statistically significant.

Results

Botanical composition of pollen loads and beebread

Pollen of willow (*Salix* spp.) dominated in the beebread (I) constituting 61.3%, and *Brassica napus*, was secondary – 25.3%. Important minor pollen collected from *Malus domestica* Borkh., accounted for 3.7%. Pollen of *Rubus idaeus*, *Aesculus hippocastanum, Acer platanoides* and *Trifolium repens* constituting a minor part, accounted for 1.8%, 2.3%, 1.4%, 1.2%, respectively, and other below 1.0% (Figure 1). Beebread (II), (III) and (IV) was collected in summer. Pollen of *Brassica napus* constituted the highest proportion 48.1% in beebread (II). The greatest species variety of important minor pollen was found in beebread (II): *Fagopyrum esculentum* M. – 14.5%, *Trifolium pratense* – 11.5%, *Trifolium repens* – 9.8%, *Filipendula* Mill. – 7.3%, *Acer platanoides* – 3.4%.

The beebread (III) was composed mostly of pollen of *Brassica napus* – 42.1%, and beebread (IV) mostly of *Trifolium repens* – 70.8%. Pollen of *Trifolium pratense* and *Trifolium repens* accounted for 22.4% and 16.0% in beebread (III) and in beebread (IV) - *Brassica napus* as low as 4.2%. Important minor pollen of *Filipendula* and *Centaurea cyanus* L. was found in beebread (III), which accounted for 12.1% and 4.3%, accordingly. Important minor pollen of *Salix* spp. and *Brassica napus* in beebread IV constituted 7.6% and 4.2%, respectively. *Filipendula* genus produces small content of sugar in nectar (4.5 – 6.84 mg sugar per 100

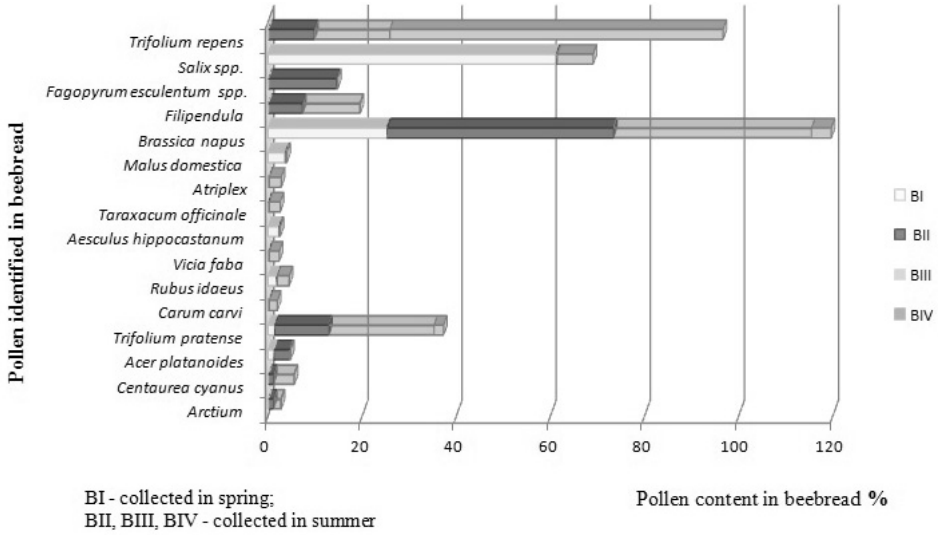

Figure 1. The pollen content in beebread (I, II, III, IV) expressed as a percentage of the total pollen sum

flowers). Bees mainly collect pollen from the plants of this genus; however; some species serve as a nectar source (Pelmenev 1985). Plant species *Filipendula vulgaris* attracts butterflies and is vital for the survival of their populations (Eilers et al. 2013).

Composition of fatty acids in pollen and beebread

Thirty two fatty acids were identified in monofloral pollen. α-linolenic acid (C18:3n3) dominated in all samples of monofloral pollen (Figure 2 a, b).

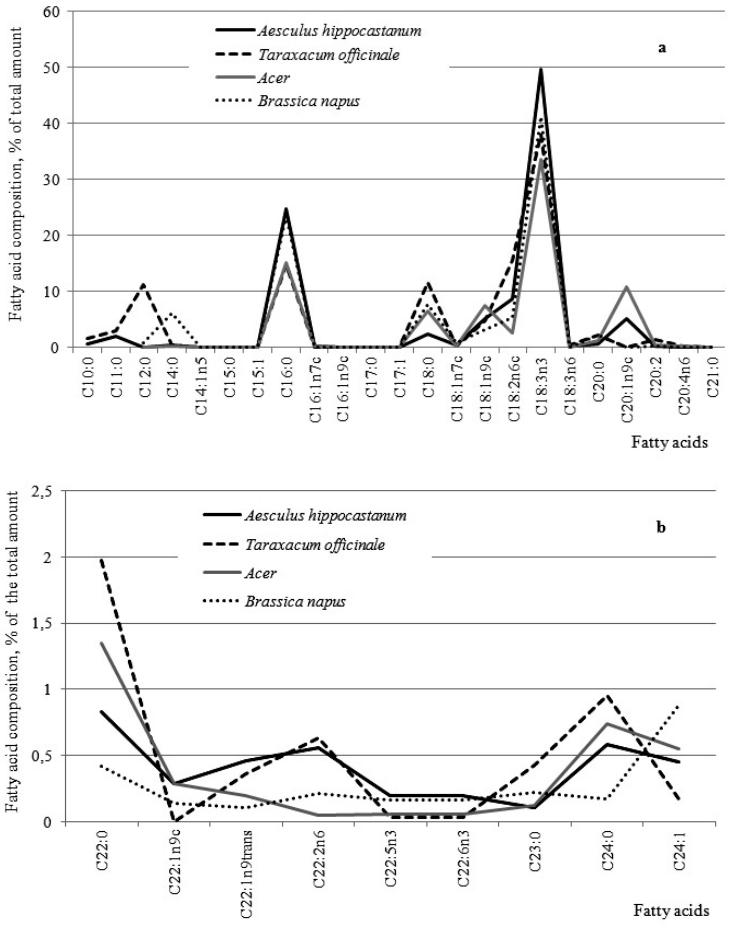

Figure 2. The composition of short and medium chain (a) and long chain (b) fatty acids in the pollen (*Aesculus hippocastanum, Taraxacum officinale, Acer* and *Brassica napus*) of species expressed as a percentage of the total amount of fatty acids present in the sample

Pollen of *Aesculus hippocastanum* had the highest content of α-linolenic acid (49.6%) and significantly differed from its content in maple (*Acer*) pollen, which accounted for 33.5%, the difference was significant at $p < 0.05$. No significant differences in α-linolenic acid content were found between *Taraxacum officinale* and *Brassica napus* pollen ($p < 0.05$). Linolenic acid C18:2n6c was found in the highest concentration of 15.5% in *Taraxacum officinale* pollen, the difference was significant compared to other pollen tested. Palmitic acid (C16:0) was present in the highest concentration from 14.8% to 24.8% compared to other saturated fatty acids in all monofloral pollen. The concentration of palmitic acid (24.8%) in *Aesculus hippocastanum* pollen did not statistically differ from that (23.6%) in *Brassica napus*. However, the content of palmitic acid was lower in *Taraxacum officinale* and maple *Acer* spp. pollen at $p < 0.05$. *Taraxacum officinale* pollen was distinguished by the highest content of saturated fatty acids – lauric (C12:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), tricosanoic (C23:0), lignocern (C24:0), accordingly 11.1%, 11.6%, 2.3%, 2.0%, 0.4%, 1.0%. The content of oleic (C18:1n9c) and eicosenoic (C20:1n9c) acids was the highest in *Acer* spp. pollen compared to other monofloral pollen. The content of eicosenoic acid (10.9%) in *Acer* spp. pollen significantly differed from that in *Taraxacum officinale* pollen by 78 times and in rape pollen by 72 times. *Brassica napus* pollen stood out only by the highest content 6.2% of myristic acid (C14:0). The content of myristic acid in *Aesculus hippocastanum*, *Taraxacum officinale* and *Acer* spp. pollen was significantly lower compared to *Brassica napus* pollen and accounted for 0.46%; 0.4% and 0.32%, respectively.

Beebread and pollen share the same fatty acids composition (Figure 3 a, b), except for brassidic acid (C22:1n9 trans), which is present only in the pollen samples. Among total 29 fatty acids, identified in beebread, 13 of them were saturated and 16 unsaturated.

The content of brassidic acid in *Aesculus hippocastanum* pollen was the highest (0.46 %) and in *Brassica napus* pollen it was the lowest (0.11%), and the difference was statistically significant at $p < 0.05$. A very low content of elaidic acid (C18:1n9trans) was identified in all beebread samples, while in pollen it was not present at all.

The findings show that there were no statistically significant differences among all fatty acids content in beebread except for eicosenoic acid (C20:1n9c). The content of eicosenoic acid in beebread collected in spring (I) was 0.23% and higher compared to summer beebread (II, III, IV). The difference was statistically significant at $p < 0.05$. Content and composition of identified fatty acids show domination of unsaturated acids and there variety in tested beebread (Table 1).

Of the total 29 fatty acids, identified in beebread, 13 were saturated and 16 were unsaturated. The con-

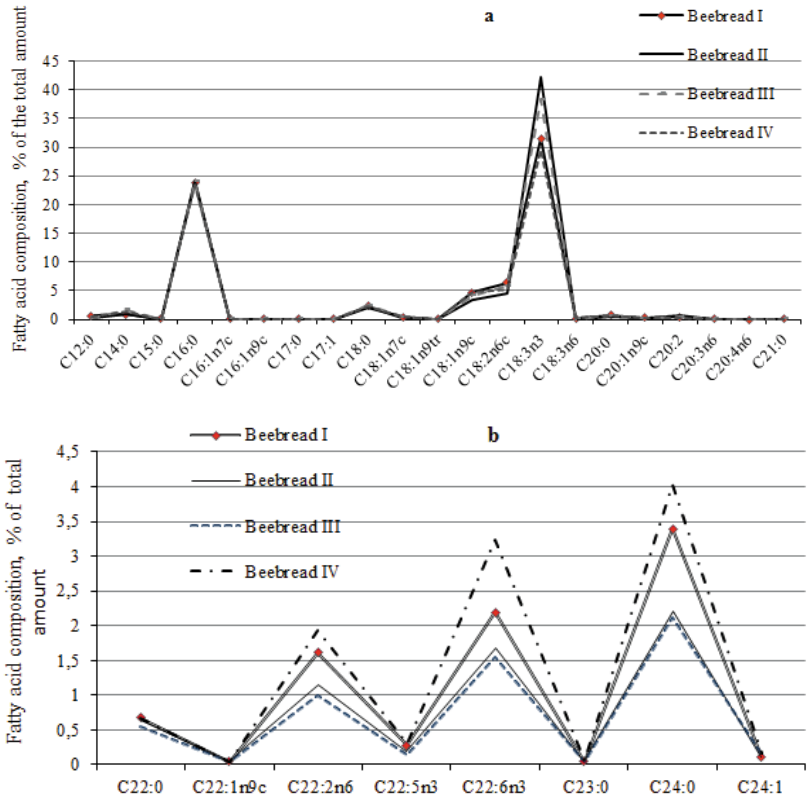

Figure 3. The composition of short and medium chain (**a**) and long chain (**b**) fatty acids in the beebread of four kinds expressed as a percentage of the total amount of fatty acids present in the sample

Table 1. The content of different groups of fatty acids in samples of beebread, %

Σ of fatty acids (FA)	Beebread samples I	II	III	IV	LSD_{05}
Saturated (FA)	33.20 ± 2.02	31.74 ± 1,57	32.52 ± 2,0	33.37 ± 4,0	16.781
n-3 unsaturated (UFA)	33.97 ± 13.6	44.02 ± 1.8	40.03±2.0	33.16 ± 0.5	20.495
n-6 unsaturated	8.55 ± 2.66	5.98 ± 0,84	7.33 ±1.19	7.95±2.15	6.822
LSD_{05}	36.40	6.29	9.08	13.18	
n-3/n-6	0.98	0.72	0.81	1.01	
SFA/UFA	0.78	0.63	0.69	0.81	
	Groups of fatty acids				
total Σ of fatty acids by samples	saturated 32.71	unsaturated n-3 37.79	unsaturated n-6 7.45		LSD_{05} 5.14

$p < 0.05$

tent and composition of identified fatty acids show the domination of unsaturated acids and their variety in the tested beebread. Significant differences were established between the total sum of saturated fatty acids and n-3, n-6 unsaturated acids in the samples of beebread (II). The total sum of fatty acids having n-6 double bonds significantly differed from that of saturated and n-3 acids in samples III and IV. The total content of saturated, n-3 and n-6 fatty acids present in the beebread samples (I) did not show significant differences among their groups. Mean content of fatty acids of all samples shows significant differences between saturated fatty acids and n-6 acids unsaturated (at $p < 0.05$). However, no significant differences achieved between samples in different groups saturated and n-3 unsaturated acids. The ratio of n-3/n-6 varied in the range of 0.98 – 1.01 and showed beneficial composition of unsaturated fatty acids for human nutrition (Simopoulos 2004). The concentration of į-Linolenic acid, which is the most valuable for human nutrition medium chain n-3 fatty acid, was the highest and ranged from 29.61 ± 1.2% to 42.15 ± 1.42%. The content of long chain – docosahexaenoic acid (DHA) ranged from 1.54 ± 0.47% to 2.19 ± 0.86% and was higher comparing to the other long chain unsaturated fatty acids identified in the beebread samples. The amount of the other long chain fatty acids: docosadienoic one, which has n-6 structure, varied in the range from 1.0 ± 0.49% to 1.94 ± 0.37% and that of saturated lignoceric from 2.12% to 4.03%. The ratio of SFA/UFA amounting values from 0.63 to 0.81 indicated higher content of unsaturated fatty acids in beebread.

The content of cis isomers of C16 fatty acid range hexadecenoic and methyl palmitoleate was very low and varied from 0.09±0.01% to 0.11±0.01%. The same tendency was noticed with the cis isomer of C20 fatty acid range. The content of trans isomer of C18 is lower compare to the content of cis isomers above-mentioned fatty acids. Of all fatty acids identified in beebread, such unsaturated fatty acid like eicosatrienoic (C20:3n6) was present at the lowest concentration ranging from 0.02 ± 0.01% to 0.10 ± 0.05%.

Activity of glucose oxidase (GOX) in pollen and beebread

The highest GOX activity (21.6 ± 3.4 U/g) was determined in beebread (B1) containing 81.7% of *Brassica napus* pollen (Table 2).

Table 2. Glucose oxidase (GOX) activity in beebread and pollen, U/g

No	Botanical origin of samples, %	GOX activity, U/g
B1	*Brassica napus* L. – 81.65; *Salix* spp. – 12.95; *Acer* spp. – 2.52; *Trifolium pratense* L. – 0.72; *Frangula alnus* Mill. – 0.72; *Centaurea cyanus* L. – 0.72; *Carum carvi*. L.– 0.72; honeydew – 1.42	21.6 ± 3.4
B2	*Brassica napus* L. – 71.69; *Frangula alnus* Mill. – 20.48; *Salix* spp. – 6.63; *Acer* spp. – 1.2	14.7 ± 0.63
B3	Salix spp. – 72.4; *Brassica napus* L – 21.8; *Acer* spp. – 4.0; *Aesculus hippocastanum* L. – 0.5; orchard – 1.3	10.1 ± 0.3
B4	*Acer* spp. – 32.64; *Salix* spp. – 49.59; *Brassica napus* L. – 9.91; *Aesculus hippocastanum* L.– 1.24; *Frangula alnus* Mill. – 3.31; *Malus* spp. – 3.31	5.99 ± 0.84
P1	*Brassica napus* L. – 100.0	7.44 ± 0.54
P2	*Prunus cerasifera* L.– 86.83; *Salix* spp. – 13.17	0.78 ± 0.01
P3	*Salix alba* L., *Salix caprea* L. – 100.0	0.87 ± 0.01

B1, B2, B3, B4 – samples of beebread
P1, P2, P3 – samples of pollen

Monofloral *Brassica napus* pollen exhibited also the highest GOX activity – 7.44 ± 0.54 U/g, which was as many as 9.5 – 8.5 times higher compared to that of the *Salix* spp. and plum (*Prunus cerasifera* L.) pollen. The lowest GOX activity, 5.99 ± 0.84 U/g, was recorded for beebread composed of *Salix* spp. – 49.59%, *Acer* spp. – 32.64%, *Brassica napus* – 9.91 % pollen and the share of other pollen tested varied in the range of 1.24 – 3.31%. The lowest GOX activity was determined in the monofloral pollen of *Prunus cerasifera* and *Salix* spp. pollen, respectively 0.78 ± 0.01 U/g and 0.87 ± 0.01 U/g. The activity of GOX was the high-

est in beebread pollen (B1) containing *Brassica napus* – 81.65% and the differences were statistically significant at $p < 0.05$ compared with all other samples tested. However, there was only negligible difference in the activity of GOX between spring pollen (P2) composed of *Prunus cerasifera* – 86.83% and monofloral pollen (P3) composed of *Salix* spp.

Discussion

The beebread of various floral origins, collected in Lithuania, contained fatty acids from C10:0 to C24:1 carbon atoms. Among those fatty acids there were saturated fatty acids of carbon length C18, C20, C22, and C23–C24. Fatty acids of carbon length C18 possess 4 unsaturated bonds; 3–4 in C20 and 2–3 in C22. Those findings show a variety of fatty acids in pollen and beebread and current technical possibilities to identify unsaturated long chain fatty acids (Čeksterytė et al. 2008, Čeksterytė and Jansen 2012). Our study showed marked differences in fatty acids composition among different monofloral pollen; however, beebread collected from different locations had similar contents of fatty acids.

Carotenoid pigments, neutral lipids, terpenoids are concentrated on the pollen coat surface (Dobson 1988). Neutral lipids have higher diversity of free fatty acids compared to internal lipid fractions. Neutral lipids and free fatty acids underlying on in the pollen coat have anti-microbial activity within bee hive (Morris et al. 1979). Lipids from the coat surface of pollen, leak to the brood cell walls and inject antiseptic protection for colony. Lipids of dandelion pollen are useful for hive antiseptic protection. Linoleic, linolenic and lauric acids dominate in lipids of dandelion pollen coat and possess antimicrobial properties. Pollen of dandelion (*Taraxacum officinale*) is short in dietary essential amino acids tryptophan and phenylalanine, arginine for bees; however, it can be attractive for its high content of fats displayed on the coat surface (Herbert et al. 1970, Manning 2006). Rape pollen is attractive to honey bees because it has higher nutritional value and contains a greater proportion of essential amino acids compared to other pollen (Keller et al. 2005). External lipids of rape pollen account for 9.8% and internal 21.9% of the total lipid content. Palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids are predominantly present in the lipids of rape pollen coat and in the internal layer it is palmitic and stearic acids (Rothnie et al. 1987). Our data also confirm high content of linoleic, linolenic and lauric acids in pollen of dandelion (*Taraxacum officinale*) and palmitic (C16:0), stearic (C18:0) and myristic (C14:0) acids in rape (*Brassica napus*) pollen as well as high content of α-linolenic acid (C18:3n3).

Lauric acid (C12:0) prevails in *Salix* spp., *Calendula* L. and some pine species pollen (Manning 2006). Honey bee foragers gathered higher quantities of some pollen types than of others. Preferences in pollen collection are generally associated with pollen quality or other factors such as aroma or visual signals of plant pollen (Somerville 2005, Farré-Armengol et al. 2013).

Different GOX activity was determined in beebread and honeybee-collected pollen. It is obvious that GOX activity was higher in beebread samples B1, B2 and B3 and in monofloral pollen of *Brassica napus* (P1) compared to monofloral pollen of P2 and P3. GOX is a carbohydrate-metabolizing enzyme and is secreted to the nectar from hypopharyngeal gland of bees while processing nectar into honey (Ohashi et al. 1999). After pollen is deposited in a comb, the process of fermentation starts, which causes an increase in GOX activity in fermented beebread. The bees add salivary secretions to the pollen while processing beebread. The process of secretion to pollen in a bee hive is longer than to pollen loads. We can state that the repeatable secretion and conditions of bee hive result in higher GOX activity in beebread compared to pollen.

The low GOX activity identified in pollen in our experiment can evidently show that not all kinds of monofloral pollen are beneficial in bee diet. Beebread is composed of different pollen and its fermentation in a bee hive causes an increase in GOX activity. Beebread, which has higher GOX activity than pollen, can positively influence in-hive antiseptic conditions, bee diet and immune system.

Conclusions

The study showed pollen and beebread to be similar in composition of fatty acids and to markedly differ in their contents. α-linolenic acid (C18:3 n3) was the most abundant fatty acid and found in very high concentrations in all kinds of pollen and beebread. The content of α-linolenic acid in pollen varied in a wider range compared to beebread, accordingly 33.5 ± 2.1% to 49.6 ± 1.0% and 29.6 ± 0.8% to 42.2 ± 1.0%.

A characteristic feature of the horse-chestnut pollen fatty acids composition is the presence of the highest content of α-linolenic and palmitic acids, 49.59% and 24.79%, respectively. Rape pollen also possesses high content of α-linolenic (41.12%) and palmitic (23.56%) acids; however, it is lower compared to the *Aesculus hippocastanum* but higher compared to *Acer* spp. and *Taraxacum officinale* pollen.

A distinctive feature of *Taraxacum officinale* pollen is significantly higher content of saturated fatty acids compared to other pollen tested: linoleic 15.47% and stearic 11.59% acids.

Maple pollen was distinguished by the highest content of eicosenoic acid (10.90%), which content in other tested pollen varied in the range from 0.14% to 5.23% and in beebread from 0.04% to 0.05%.

Beebread and pollen contain a higher amount of α-linolenic and a lower content of linoleic acids. The composition of unsaturated fatty acids in beebread is more various and there content is higher compare to saturated fatty acids. This composition of fatty acids is favourable for human nutrition. Isomers of fatty acids occurred at low concentration.

Monofloral pollen of *Brassica napus* and beebread composed of the dominant pollen of *Brassica napus* possesses high GOX activity. Further research should be done to investigate the influence of different kinds of pollen on the activity of GOX in beebread.

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ОПРЕДЕЛЕНИЕ ИСТОЧНИКОВ НЕКТАРА ЛИТОВСКОЙ ФЛОРЫ МЕТОДОМ МЕЛИССОПАЛИНОЛОГИИ И НЕКОТОРЫЕ ИХ БИОХИМИЧЕСКИЕ ПАРАМЕТРЫ В ПЫЛЬЦЕ

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Резюме

Одним из наиболее важных недревесных лесных продуктов являются продукты животного происхождения, включая продукты пчеловодства. Пчелиная пыльца и перга имеют жизненно важное значение для повышения экологического, экономического и социального благополучия. Ценные компоненты продуктов пчеловодства обладают целебными свойствами, включая жирные кислоты и ферменты. Пыльца и перга были собраны на пасеке Института сельского хозяйства, филиале Литовского исследовательского центра сельского и лесного хозяйства. Ботанический состав собранных образцов определён методом мелиссопалинологии. Жирные кислоты определялись в монофлорной пыльце рапса (*Brassica napus* L.), конского каштана (*Aesculus hippocastanum* L.), клёна (*Acer* L.) и одуванчика (*Taraxacum officinale* L.). Пыльца ивы (*Salix* spp.), рапса (*Brassica napus* L.) и белого клевера (*Trifolium repens* L.) доминировали в перге, использованной для анализа жирных кислот. Длина цепи всех установленных жирных кислот колебалась от C10 до C24. Высокий уровень омега-3 б-линоленовой кислоты обнаружен во всех образцах. Самое высокое содержание б-линоленовой кислоты во всех образцах пыльцы колебалось в диапазоне от 33,5 ± 2,9 % до 49,6 ± 2,6 %, а в перге от 29,6 ± 1,2% до 42,2 ± 1,4%. Из насыщенных жирных кислот, пальмитиновая кислота (C16:0) присутствует в высоких концентрациях, от 14,8 ± 1,9 % и 24,8 ± 0,03 % во всех монофлорных образцах пыльцы, а в перге она колебалась от 23,3 ± 1,7% до 24,2 ± 1,8%. Лауриновая (C12:0) и стеариновая (C18:0) кислоты были обнаружены в самой высокой концентрации только в пыльце одуванчика (*Taraxacum officinale* L.) – 11,1 ± 0,8% и 11,6 ± 0,%, соответственно. Ферментативная активность глюкозооксидазы (ГО) в перге и монофлорной пыльце рапса (*Brassica napus* L.) оказалась статистически значимо выше (при *p* В 0,05) по сравнению с образцами пыльцы ивы (*Salix* spp.) и сливы (*Prunus domestica* L.). В перге, отличащейся высоким показателем ГО, содержание монофлорной пыльцы рапса составило 81,65 %. На активность ГО в перге может влиять не только состав пыльцы, но и процесс ферментации в сотах.

Ключевые слова: недревесные лесные продукты, пыльца, перга, жирные кислоты, глюкозооксидаза