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# Bee breads from Eastern Ukraine: composition, physical properties and biological activities

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**Abstract**: Five samples of polyfloral bee bread originated from two regions of Eastern Ukraine were characterised by microscopic (SEM) and spectroscopic (FT MIR ATR, FT NIR, FT Raman and Vis) methods. Identification of pollens was based on the SEM images. Spectral differences were interpreted in terms of specific contribution of the main chemical constituents (proteins, phenolics, sugars, etc.) and pigments (flavonoids and carotenoids) of bee breads. PCA of FT NIR and Vis data was used for discrimination of bee bread samples according to botanical sources. The morphometric parameters, antioxidant capacity and prebiotic effects of bee breads were also studied. Obtained results expand the knowledge in the composition and properties of this beekeeping product.

**Keywords**: antioxidant capacity; beekeeping products; prebiotics; SEM; spectroscopic methods

The term bee bread refers to the pollen collected by bees from plants and then stored in combs. It can be considered as a food supplement because of the presence of many nutrients (Urcan *et al.* 2012). The pH level of fresh pollen is ~7.2, while in 'mature' honeycomb it decreases to 3.5–4.2, mainly as a result of lactic acid fermentation (Isidorov *et al.* 2009). One benefit of this beekeeping product is its almost unlimited storability in comparison with raw pollen, which rapidly lost its nutritional values after drying or freezing (Bobiş *et al.* 2010). Bee bread is a unique well-balanced natural product, with diverse content (Isidorov *et al.* 2009). The chemical composition of bee bread is determined by the raw flower pollen and varies according to botanic specie, geographic area and collection time. Generally, pollen in honeycomb originated from the honey

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plants; although pollen of wind-pollinated plants can be also present (Wroblewska *et al*. 2010). Knowing bee bread composition, it is possible to determine its botanic source, harvesting region and potential impact on human health. The quality of bee bread depends on the technology of its extraction from combs, further processing and storage conditions.

The aim of this study is evaluation and establishment of distinctions in morphology of pellets, microbial analysis, chemical composition and possible botanic origin of five bee bread samples collected from different habitats in Ukraine by microscopic, spectroscopic and other appropriate methods. Water activity, antioxidant capacity and potential prebiotic properties were also evaluated.

## MATERIALS AND METHODS

***Bee breads***. Five samples of polyfloral bee bread (Table 1) were obtained by beekeepers from localities Pyrjatyn (Poltava region), Pidhorodne, Petrykivka and Solone (Dnipro region) of Eastern Ukraine under the new patented technology developed by the research team of the Department of Beekeeping at the National University of Life and Environmental Sciences of Ukraine in Kyiv (Brovarskyi *et al*. 2017). All these samples were defined as polyfloral, placed in a freezer and conserved at –12°C.

***Morphometry***. Images of bee breads were obtained by automatic macro magnifying glass Zeiss Discovery V12 (Carl Zeiss AG, Germany) using Zeiss AxioVision 4.7.1 software (module Automatic Measurement). The length and width (mm) of 30 randomly chosen pellets of bee bread were measured from the base to the apical part by digital caliper (Proteco, Czech Republic). These pellets were also weighted with analytical scales (Kern ADB-A01S05, Germany). The surface morphology and shape of the pollen was examined by a Scanning Electron Microscope (SEM) Jeol JCM-5700. Samples were sputter-coated with Emitech K550X by a thin 5 nm layer of gold prior to SEM analysis (Synytsya *et al*. 2011).

***Chemical composition***. The composition of bee breads was analysed by physicochemical, chromatographic and spectroscopic methods. Chemical analyses were realized in the Environment laboratory EL spol. s r.o. (Spišská Nová Ves, Slovakia). The contents of dietary fibres, ash, fats and dry matter were determined by gravimetry (IP 5.21, 5.31, 5.5c and 5.7); the former was obtained after enzymatic processing using Megazyme set (Megazyme, Ireland). The protein content was determined by Kjeldahl method (IP 5.9c). All these analyses were made in five parallels.

***Moisture and water activity***. Karl Fischer titration method was done on Volumetric Karl Fischer titrator AF8 (Thermo Orion, USA) for determination of moisture in thawed fresh-frosted bee breads tempered at 25°C (Margreth *et al*. 2010); water activity was measured using an Aqualab 4TEV (MeterFood, USA) and represented as the ratio of partial pressure of water vapour above the surface of the product to pressure of saturated water vapour at 25°C (Mathlouthi 2008). Moisture and water activity of bee breads were measured in five parallels.

***Spectroscopic methods and colour***. FTIR spectra in mid-infrared (4000–400 $cm^{-1}$) and near-infrared (10 000–4000 $cm^{-1}$) regions (FT MIR and FT NIR, respectively) were recorded on FTIR spectrometer Nicolet 6700 (ThermoScientific, USA) using KBr and $CaF_2$ beam-splitters, respectively, and Omnic 7.0 software (Synytsya *et al*. 2011). In the case of FT MIR, the KBr tablets containing bee bread samples made using hand press (Pike, USA) were prepared for the measurements; 64 scans were accumulated with a spectral resolution of 2 $cm^{-1}$. In the case of FT NIR, the measurement was made in glass cuvettes using rotation equipment; 100 scans were accumulated with a spectral resolution of 4 $cm^{-1}$ (Kyselka *et al*. 2018). FT Raman spectra (4000–100 $cm^{-1}$, $\lambda_{ex}$ 1064 nm) of the samples were

Table 1. Specification of polyfloral bee breads

| Sample | Collecting place[a] | Main botanic source[b] | Colour |
|---|---|---|---|
| 1 | Pyrjatin, Poltava region | *Papaver* sp. | dark yellow |
| 2 | Pidhorodne, Dnipro region | *Trifolia* sp. | dark yellow |
| 3 | Petrykivka, Dnipro region | *Helianthus annuus* | golden yellow |
| 4 | Pyrjatin, Poltava region | *Fagopyrum esculentum* | dark yellow |
| 5 | Solone, Dnipro region | *Fagopyrum esculentum* | dark yellow |

[a]locality and region in Ukraine; [b]according to SEM images of this study

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recorded on Nicolet iS50 spectrometer with FT Raman module (Thermo Scientific, USA) using Omnic 9.0 software (ThermoScientific, USA); 100 scans were accumulated with a spectral resolution of 4 $cm^{-1}$. Diffuse reflectance VIS spectra (380–770 nm) were recorded on UV-Vis spectrophotometer UV4 (UNICAM, Great Britain) using Labsphere holder (Labshere, USA) and Vision 3.0 software (UNICAM, Great Britain) (Mot *et al*. 2010). All the spectra were exported in the CSV format for further processing and preparation of graphs in Origin 6.0 (Microcal Origin, USA) software. Second derivatives of normalised NIR spectra (4000–7000 $cm^{-1}$) were calculated and, together with Vis spectra, exported in the CSV format for principal component analysis (PCA) in Statistica 10 (StatSoft, USA) software (Roggo *et al*. 2007). The CIE *L*a*b** colour parameters of bee bread homogenates were obtained based on diffuse reflectance VIS spectra using Chroma 2.0 (Thermo-Spectronic, USA) software (De-Melo *et al*. 2016).

***Antioxidant activity***. Two methods were used for evaluation of bee pollen antioxidant activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2-2´-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) photometric assays. The former one is based on decolouration of stable free radical DPPH$^{\bullet}$ having a strong purple colour (Kedare & Singh 2011); the later one quantifies decolouration of blue-green coloured radical-cation $ABTS^{\bullet+}$ generating from ABTS by metmyoglobin/$H_2O_2$ (Re *et al*. 1999). ABTS assay was applied for the methanolic extracts from bee breads; DPPH assay for both methanolic and aqueous extracts. Bee pollens (0.1 g) were mixed with 25 ml of water or methanol, exposed to ultrasound during 1 hr and then filtrated using syringe filters. Commercial set Randox (Randox Laboratories, USA) was used for ABTS assay. The absorbance was measured at 517 nm (DPPH$^{\bullet}$) and at 600 nm ($ABTS^{\bullet+}$). The resulting antioxidant capacity was expressed as an equivalent of TROLOX concentration (mmol/l) for trapping of the same amount of ABTS or as % of DPPH bleaching.

***Microbial growth stimulation/inhibition assay***. The screening of bee bread samples as potential prebiotics was made on nine strains of *Lactobacillus* sp. with expected probiotic features by disc diffusion method (Ivanišová *et al*. 1999; Reller *et al*. 2009). These strains were isolated from barley leaven and placed in the collection of Food Research Institute Prague. The 5 × 1 mm long hexagonal pieces with a mean weight of 30 mg were cut of bee bread pellets and placed on MRS substrate with an uniformly spread test strain. The test plates were then incubated for 24 h at 25°C. Each test was performed in three replicates. The growth supporting (light) and growth inhibition (dark) zones were examined.

***Statistical evaluation***. For the morphological parameters, chemical composition, water activity and antioxidant capacity, the results were compared among the samples using the ANOVA technique with Statistica 10 (StatSoft, USA). ANOVA indicated differences among the means, and Tukey (HSD) analysis of the differences was used for the comparisons between individual bee pollens. All data are reported as mean values with confidence intervals ($P < 0.05$).

## RESULTS AND DISCUSSION

***Morphology of bee bread pellets***. Bee bread pellets of samples 1–5 are represented in Figure 1. Results of morphometric analysis of the pellets are shown in Table 2. All the average morphometric parameters were the lowest for sample 3; the highest average weight was detected for pellets of sample 1, the average values of length and width were maximal for samples 1 and 5, respectively. The shape and size of honeycomb load are

Table 2. Morphological parameters of bee bread pellets

| Sample | $n$ | $m$ (mg) | $w$ (mm) | $l$ (mm) |
|---|---|---|---|---|
| 1 | 30 | 317.03 ± 13.80$^{b}$ | 5.43 ± 0.11$^{b}$ | 11.18 ± 0.32$^{c}$ |
| 2 | 30 | 312.31 ± 8.17$^{b}$ | 5.50 ± 0.10$^{bc}$ | 10.78 ± 0.23$^{bc}$ |
| 3 | 30 | 232.40 ± 14.02$^{a}$ | 4.65 ± 0.12$^{a}$ | 9.41 ± 0.28$^{a}$ |
| 4 | 30 | 313.53 ± 7.60$^{b}$ | 5.66 ± 0.10$^{bc}$ | 10.50 ± 0.27$^{b}$ |
| 5 | 30 | 307.26 ± 6.09$^{b}$ | 5.73 ± 0.10$^{c}$ | 10.67 ± 0.26$^{bc}$ |
| Pearson correlation$^{**}$ | $m$ (mg) | 1.00 | 0.42 | 0.87 |
| | $w$ (mm) | 0.42 | 1.00 | 0.35 |
| | $l$ (mm) | 0.87 | 0.35 | 1.00 |

$m$–mass; $b$–width; $l$–length; $^{**}P < 0.0001$; different letters indicate significant differences between bee pollens ($P < 0.05$)

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Figure 1. Bee bread. Sample 1 (**A**), 2 (**B**), 3 (**C**), 4 (**D**) and 5 (**E**)

restricted by the combs in honeycomb. Comb diameter is typically about 5.37 mm and the depth of it is 12 mm. The obtained width and length values for bee breads varied in the range 4.08–6.22 mm and 7.38–12.75 mm, respectively, that are roughly corresponded to the comb space. Density and consistence of pollen material should be important for complete packaging in combs, and these features depend on bee pollen composition and botanical origin. On the other hand, the shape of bee bread pellets depends on applied technology of pollen extraction from the honeycombs (Brovarskyi *et al.* 2017). The weight of bee bread pellets varied from 163 to 370.5 mg and, according to Pearson correlation coefficients, it depended on the length (0.87) of the comb in honeycomb construction much more than on its width (0.42). Possibly the bee is able to cram more pollen to longer combs, so the density of resulting bee bread become higher.

***SEM images***. Scanning electron microscopy (SEM) is a powerful tool which allowing obtaining detailed information about the surface of bee bread pellets, morphology and integrity of pollen grains (Dong *et al.* 1994). SEM images of bee bread species are shown in Figure 2. All these samples were defined by beekeepers as poly-floral, and multi-coloured colouring of pellets (Figure 1) confirmed this definition. The mixing of pollen material occurs inside the combs when bees pack them. It is evident, however, that pollen grains of specific morphology predominated in the individual samples. Indeed, the samples 1 and 2 consisted of roundish or slightly elongated pollen grains with smooth surface that is typical for those of genera *Papaver* (popper) or *Trifolia* (clover), respectively. Compression of pollen

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grains along the main axes, wrinkling and appearing of longitudinal folds and furrows is the result of dehydration that is evident for dried pollens. By contrast, the grains in sample 3 have thorny surface and thus can be identified as sunflower (*Helianthus annuus*) pollen. Finally, samples 4 and 5 consisted of rod-like grains that are possibly originated from buckwheat (*Fagopyrum esculentum*). The samples possibly have admixtures of pollens originated from other botanical species than that mentioned above. It was difficult to identify them because pollen grains are immersed into viscous substance, i.e. nectar containing hydrolysate of pollen material. Some grains are evidently destroyed and stuck together forming amorphous conglomerates. Similar destruction of pollen grains has been described for bee collected and stored pollen that could be an evidence of rehydration and following digestion (Human & Nicolson 2006).

***Chemical composition***. Results of chemical composition of bee bread samples are shown in Table 3. It is evident that the contents of proteins (~15–28% m/m in dry matter) were higher to compare with fats

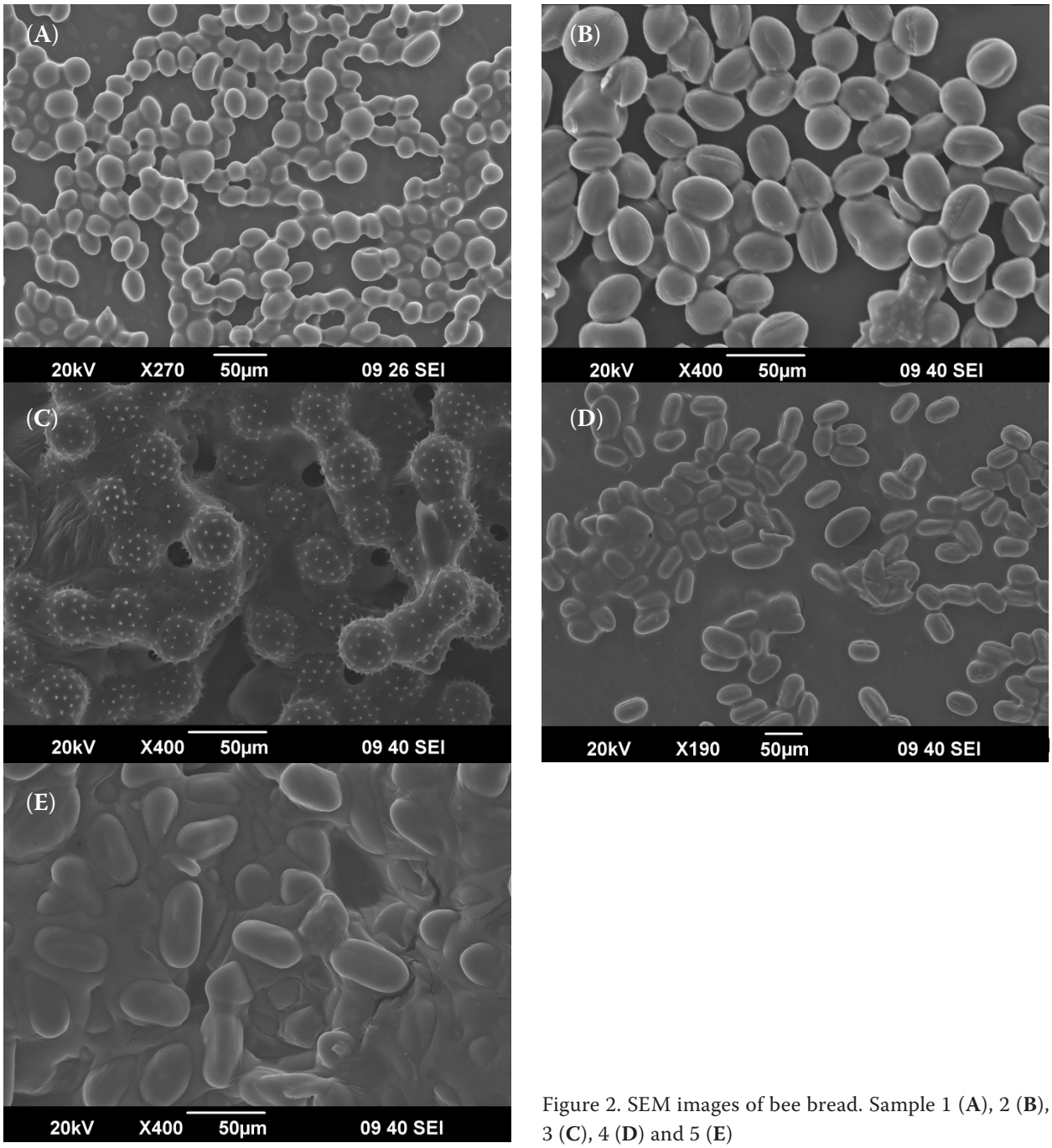


Figure 2. SEM images of bee bread. Sample 1 (**A**), 2 (**B**), 3 (**C**), 4 (**D**) and 5 (**E**)

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(~9–10% m/m in dry matter). Bee bread differs from bee pollen by lower pH (3.8–4.3) due to the presence of lactic acid, a product of fermentation; the former also contains less proteins and fats, but more carbohydrates (Ivanišová *et al.* 1999; Human & Nicolson 2006). Indeed, the protein contents in bee breads of our study (~14–20% in wet matter) are lower than the average value of 22.7% for bee pollen proteins, but fat contents (~6.5–8.3% in wet matter) are higher than the reported content of 5.1% for bee pollen lipids (Komosinska-Vassev *et al.* 2015). Proteins and fats are necessary for the nutrition of bees. Bee bread also contained high amounts of dietary fibres (~37–60% m/m in dry matter) originated from pollen cell walls.

***Moisture and water activity***. Bee pollen as well as derived bee bread is known as highly hygroscopic material and thus susceptible for microbial or fungal growth (Carpes *et al.* 2009). To avoid this, pollen materials are commonly stored in frozen or dried state. The moisture of fresh-frozen bee bread samples 1–5 tempered at 25°C was ~8.8–11.7%; water activity of these samples recorded at the same conditions was in the range of ~0.63–0.73 (Table 3). Moisture and water activity are tightly connected with quality of pollen products; the latter value indicates resistance to microbial contamination (Morgano *et al.* 2011). Water activites obtained for bee breads except sample 5 were below 0.7 and thus low enough to avoid bacterial growth, but yeast growth could still be possible (Sagona *et al.* 2017). Small sugars, phenolics and other polar compounds, which constitute bee breads, contribute to water uptake. During storage of pollens in combs, the rupture of exine made up with chemically inert sporopollenin (Domínguez *et al.* 1999) leads to releasing of many intracellular compounds that may affect bee bread affinity to water. Revealing intine, which is made up mainly with polysaccharides (Heslop-Harrison & Heslop-Harrison 1991), may additionally retain water by its hydrocolloids. Wet conditions, in turn, support grows of microorganisms that decrease safety and sensory properties. On the other hand, phenolics and other bioactive compounds may inhibit microbial growth in bee bread (Abouda *et al.* 2011).

***Vibrational spectra***. FT MIR absorption spectra of bee breads are represented in Figure 3. These spectra are very similar to those of bee pollens that have been published previously (Synytsya *et al.* 2011). Broad band around 3365 $cm^{-1}$ (OH stretching) arose from water, carbohydrates and phenolics. Two narrow bands near 2925 and 2854 $cm^{-1}$ (antisymmetric and symmetric stretching of $CH_2$), a band or shoulder near 3016 $cm^{-1}$ (=CH stretching), weak band at 1740 $cm^{-1}$ (C=O stretching in esters) and shoulders near 1710 $cm^{-1}$ (C=O stretching in carboxylic groups) and 1452 $cm^{-1}$ ($CH_2$ scissoring) and broad band near 1242 $cm^{-1}$ (CO stretching) are originated mainly from lipids including fats and fatty acids (Vlachos *et al.* 2006). Two intense bands near 1655 $cm^{-1}$ (amide I) and 1547 $cm^{-1}$ (amide II) and a shoulder near

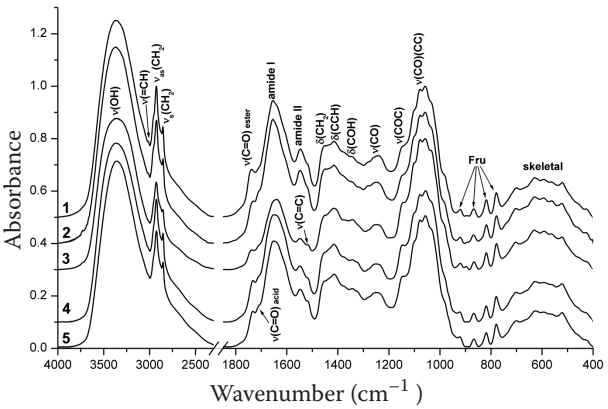


Figure 3. FT MIR absorption spectra of bee breads

Table 3. Chemical composition and water activity of bee breads (% m/m)

| Component | Bee breads | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Proteins | 27.01 ± 2.68$^{c}$ | 28.06 ± 2.78$^{c}$ | 19.50 ± 1.94$^{b}$ | 20.20 ± 2.01$^{b}$ | 15.30 ± 2.09$^{a}$ |
| Fats | 9.08 ± 0.56$^{a}$ | 9.83 ± 0.61$^{a}$ | 9.55 ± 0.63$^{a}$ | 9.95 ± 0.60$^{a}$ | 9.50 ± 0.53$^{a}$ |
| Ash | 3.17 ± 0.24$^{a}$ | 3.66 ± 0.27$^{ab}$ | 4.29 ± 0.32$^{b}$ | 5.15 ± 0.41$^{c}$ | 3.95 ± 0.30$^{b}$ |
| Dietary fibres | 57.81 ± 3.59$^{c}$ | 37.03 ± 2.33$^{a}$ | 57.12 ± 3.58$^{c}$ | 44.70 ± 2.77$^{b}$ | 60.15 ± 3.91$^{c}$ |
| Dry matter | 71.35 ± 1.96$^{a}$ | 71.38 ± 1.81$^{a}$ | 71.73 ± 1.76$^{a}$ | 83.65 ± 2.07$^{b}$ | 72.74 ± 1.73$^{a}$ |
| Moisture | 8.81 ± 0.30$^{a}$ | 10.46 ± 0.52$^{b}$ | 11.65 ± 2.16$^{b}$ | 10.60 ± 0.97$^{b}$ | 10.22 ± 0.60$^{b}$ |
| Water activity | 0.620 ± 0.056$^{a}$ | 0.647 ± 0.070$^{a}$ | 0.623 ± 0.047$^{a}$ | 0.683 ± 0.061$^{a}$ | 0.718 ± 0.066$^{a}$ |

Different letters indicate significant differences between bee pollens ($P < 0.05$); $n = 5$

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1280 $cm^{-1}$ (amide III) arose from proteins (Barth 2007). These spectral features were more pronounced for samples 1 and 2. A shoulder near 1520 $cm^{-1}$ (C=C stretching) indicated phenolic acids (Kačuráková *et al.* 1999). Highly overlapped bands in the region of 900–1200 $cm^{-1}$ (COC, CO and CC stretching) arose mainly from carbohydrates. The shape of the envelope in this region is very similar for all samples. The band at 899 $cm^{-1}$ ($C1_{\beta}H$ bending) was assigned to cell wall cellulose and heteroxylans (Kačuráková *et al.* 1999). This band was pronounced for sample 3 in comparison to the other samples. In addition, several weak to medium bands at 779, 825, 868 and 922 $cm^{-1}$ are typical for amorphous fructose (Ibrahim *et al.* 2006).

Diffuse reflectance FT NIR spectra of bee breads are represented in Figure 4. Like in the case of FT MIR, spectral differences clarify variability in chemical composition of bee breads. Highly overlapping bands corresponding to combinations and overtones of OH, CH and NH vibrations predominate in the NIR region, and band assignment is very complicated because of overlapping. Nevertheless, some publications were devoted to interpretation of Near-infrared spectra (Weyer 1985; Workman & Jerome 1996). The bands near 4258, 4331, 5685, 5785, 7080 and 8292 $cm^{-1}$ (combinations and overtones of $CH_2$ vibrations) arose mainly from lipids (Hourant *et al.* 2000). By contrast, bands near 5157 and 6807 $cm^{-1}$ (combination and overtone of OH vibrations) have a contribution from water, carbohydrates and phenolics (Robert 1998; Soukupová *et al.* 2002). The shoulders near 4864 and 6352 $cm^{-1}$ (combination and overtone of NH vibrations) corresponded to proteins (Miyazawa & Sonoyama 1998). The shoulder near 4405 $cm^{-1}$ (combination of OH and CO stretching) was assigned to carbohydrates. Finally, shoulders near 4667 $cm^{-1}$ (combination of =CH and C=C stretching) and 5835–6012 $cm^{-1}$ (1st overtone of =CH stretching) have a contribution from aromatics and other unsaturated compounds.

Unfortunately, for the bee breads of this study the differences in the mentioned NIR bands are not so pronounced to make evident conclusions about similarity of the samples, and PCA was used to evaluate these differences. Second derivatives were used for PCA instead of the raw NIR spectra to eliminate the background influence. Resulting graph of component score PC1 versus PC2 is shown in Figure 5. This combination of principal components is sufficient for discrimination of bee breads in accordance with their botanical origin evaluated

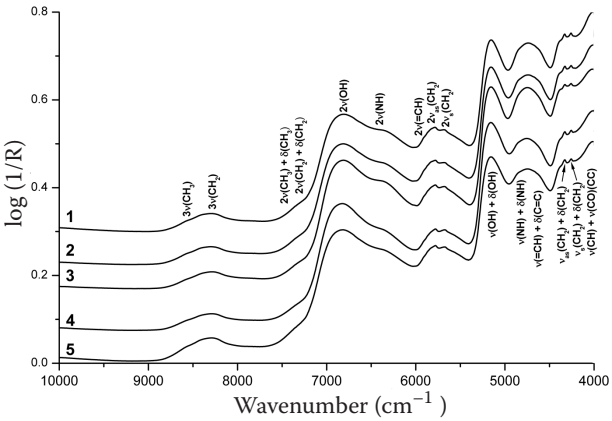


Figure 4. Diffuse reflectance FT NIR spectra of bee breads

by SEM. Indeed, sunflower bee bread 3 with negative PC1 was separated from the other samples showing positive PC1, and the latter ones split based on the sign of PC2, i.e. clover and popper bee breads 1 and 2, respectively, with positive PC2 from buckwheat bee breads 4 and 5 with negative PC2.

FT Raman spectra of bee breads are shown in Figure 6. Spectral differences between samples represent specific composition and ratio between the main constituents and pigments, i.e. proteins, carbohydrates, lipids, phenolics and carotenoids (Synytsya *et al.* 2010). The spectra of sample 3 and, to a lesser extent, sample 1 demonstrated three characteristic bands of carotene at 1529, 1157 and 1004 $cm^{-1}$ (Schulz *et al.* 2005). Carotenoids thus could be chemical markers of botanical origin because only specific pollens like that of sunflower contain these compounds (Sy-

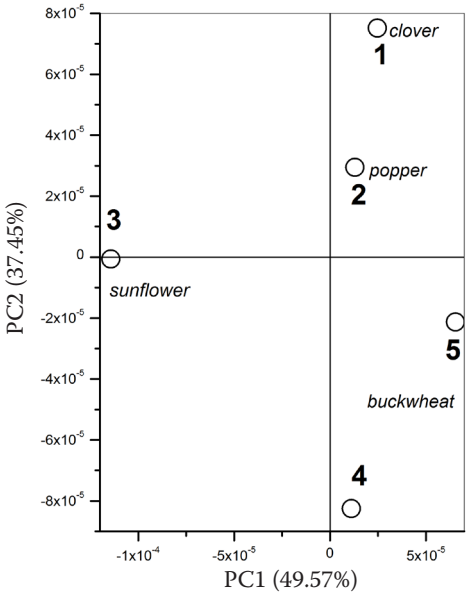


Figure 5. Component score graphs for PCA of diffuse reflectance 2nd derivative FT NIR (4000–7000 $cm^{-1}$)

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NYTSYA *et al.* 2011). The last band is overlapped by the protein band (ring breezing of Phe) at the same wavenumber. Next intense bands near 1650 $cm^{-1}$ (amide I), 1602 $cm^{-1}$ (C=C stretching) and 1444 $cm^{-1}$ ($CH_2$ and $CH_3$ bending) were assigned to proteins, aromatic and aliphatic moieties, respectively (SCHULZ & BARANSKA 2007). Several bands at 1454, 1265, 1074, 917, 865, 821, 713, 628, 518 and 420 $cm^{-1}$ are characteristic for amorphous fructose (SÖDERHOLM *et al.* 1999; IBRAHIM *et al.* 2006).

***Visible reflectance spectra and colour***. Vis spectra of bee breads are represented in Figure 8. Evident spectral difference between the samples indicated specific composition of pigments, i.e. flavonoids and carotenoids. The broad band of sample 3 (less pronounced for sample 1) coincided with golden yellow colour and presence of carotenoids in sunflower pollens (FAMBRINI *et al.* 2010; SYNYTSYA *et al.* 2011). PCA of the spectral region of 450–650 nm, where maximal differences in the spectra were observed, was carried out, and resulting graph of component score PC1 versus PC2 is shown in Figure 6. According to PC1 the samples 1 and 2 (negative PC1) were separated from the other samples (positive PC1), then bee breads 1 and 3 containing carotene (negative PC2) were separated from bee breads 2, 4 and 5 without carotene (positive PC2). It is evident that, like in the case of 2nd derivatives of FT NIR spectra mentioned above, this discrimination of bee breads is consistent with SEM identification of botanical origin.

Colour of plant products depends on the content and ratio of pigments, mainly carotenoids and flavonoids. Therefore, differences in the CIE $L^*a^*b^*$ parameters can be used for distinguishing of bee pollens according to botanical origin. According to the colour diagram (Figure 9), the bee breads are dark yellow with some reddish hue. Among all

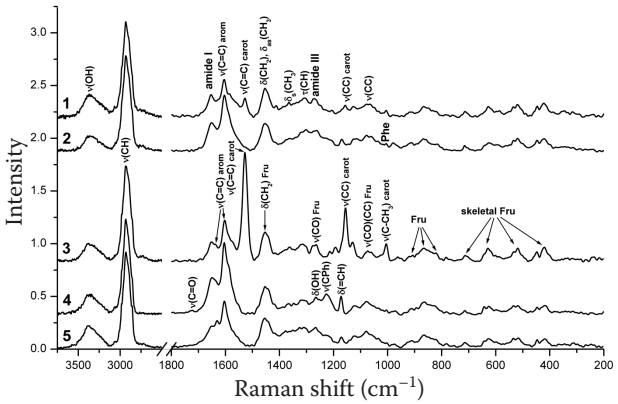


Figure 6. FT Raman spectra of bee breads

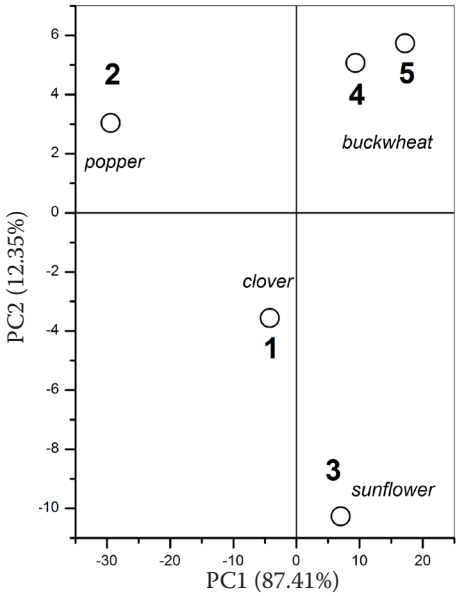


Figure 7. Component score graphs for PCA of raw Vis (450–650 nm) spectra

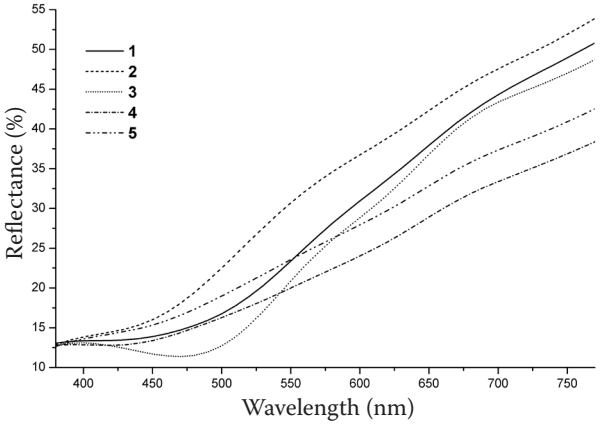


Figure 8. Diffuse reflectance Vis spectra of bee breads

bee breads, samples 2 was the lightest ($L^*$ ~64) and 4 was the darkest ($L^*$ ~53–54). It can be supposed that such bee bread consists of pollen with high content of flavonoids (TAVDIDISHVILI *et al.* 2014; IVANIŠOVÁ *et al.* 2015; RZEPECKA-STOJKO *et al.* 2015). However, sample 3 and, at lesser extent, sample 1 has a significant contribution of the red hue ($a^*$ ~13–16) that could be explained by the presence of carotenoids. The sunflower pollen is known to be rich of carotenoids (FAMBRINI *et al.* 2010; SYNYTSYA *et al.* 2011), so both the colour and SEM images may supports the conclusions about the botanical origin of these bee breads.

***Antioxidant capacity***. Antioxidant activities of bee bread samples are shown in Table 4. Both ABTS and DPPH methods confirmed that bee bread samples had valuable antioxidant activity that is comparable

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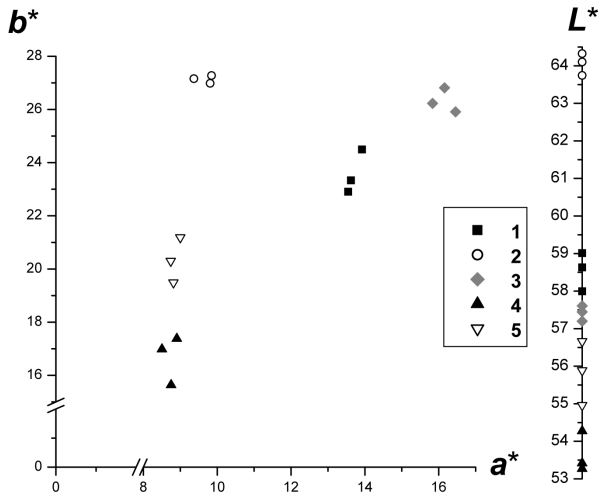


Figure 9. The CIE *L*a*b** colour diagram for bee bread

with results reported for bee pollens (Leja *et al*. 2007; Morais *et al*. 2011; Feás *et al*. 2012). It is known that bee pollens, and thus derived bee breads, contain a lot of hydrophilic and hydrophobic antioxidants including carotenoids, phenolics and polyunsaturated fatty acids (Rice-Evans *et al*. 1996; Krinsky 2001; Richard *et al*. 2008; Rzepecka-Stojko *et al*. 2015). Aqueous and methanolic extracts of bee breads were analysed by DPPH method. For all samples the methanolic extracts showed significantly higher antioxidant capacity (~60–92%) than corresponding aqueous extracts (~14–52%) because the mentioned antioxidants are more soluble in methanol. According to Hudz *et al*. (2017), the total antioxidant activity of extracts obtained from Ukrainian bee breads using DPPH assay depended on the year of pollen collection, botanical origin and the storage time before the preparation of extracts. Ivanišová *et al*. (1999) earlier reported that, among Ukrainian bee bread samples, bee bread from Poltava region having the largest amount of phenolics also demonstrated the highest antioxidant activity estimated by DPPH and phosphomolybdenum methods, so the locality of pollen harvesting could be also important. In our investigation, methanolic and water extracts from bee bread 1 (one of two originated from Poltava region) showed maximal amounts of DPPH bleaching among the samples; this sample also showed maximal value of ABTS assay (Table 5). By contrast, bee bread from sample 3 (sunflower pollen) demonstrated lower antioxidant capacity among methanolic extracts than those from the other samples according to both ABTS and DPPH assays. According to our unpublished results, the sunflower bee pollen also showed lower antioxidant capacity of methanolic and water extracts in comparison with bee pollens of other botanical origin. These results could be explained by the fact that carotenoids, which are plentiful in sunflower pollen (Fambrini *et al*. 2010; Synytsya *et al*. 2011), are less soluble in water and methanol than flavonoids found in pollen of other species.

***Effect on the microbial growth***. The results of the microbial growth stimulation/inhibition assay are summarised in Table 5. The bee breads of this study demonstrated different impacts on the growth of nine *Lactobacillus* sp. isolates in dependence on used sample and strain specificity. All the bee bread samples supported microbial growth of isolates No. 150, 152 and 159, inhibited microbial growth of isolates No. 151 and 153 and showed no effect on the growth of isolate No. 158. In the case of isolates No. 154, 155 and 158 bee breads showed marked differences in their effects on the microbial growth. The strain No. 154 was stimulated only by bee bread samples 1–3, and the strain No. 157 was inhibited only by bee bread samples 3–5; the last two samples showed no effect in both cases. The diversity of bee bread effects was marked for the strain No. 155. Two samples supported its growth (2, 3), another two inhibited (4, 5) and the last one was ineffective. It is known that beekeeping products have selective antibacterial properties, but also may sup-

Table 4. Antioxidant capacity of bee breads

| Sample | ABTS | DPPH (%) | |
|---|---|---|---|
| | Trolox (mm/l) | methanol | water |
| 1 | 2.28 ± 0.45$^{c}$ | 92.07 ± 5.65$^{b}$ | 52.02 ± 2.79$^{d}$ |
| 2 | 1.50 ± 0.25$^{b}$ | 91.94 ± 5.56$^{b}$ | 22.80 ± 1.44$^{c}$ |
| 3 | 0.52 ± 0.10$^{a}$ | 59.70 ± 4.05$^{a}$ | 24.43 ± 1.97$^{c}$ |
| 4 | 1.39 ± 0.22$^{b}$ | 89.55 ± 5.15$^{b}$ | 14.36 ± 0.67$^{a}$ |
| 5 | 1.48 ± 0.27$^{b}$ | 89.17 ± 5.08$^{b}$ | 17.51 ± 1.11$^{b}$ |

Different letters indicate significant differences between bee pollens ($P < 0.05$)

Table 5. Effect of bee breads on the growth of *Lactobacillus* sp.

| Sample | Number of isolate | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 151 | 152 | 153 | 154 | 155 | 157 | 158 | 159 | 150 |
| 1 | – | + | – | + | 0 | 0 | 0 | + | + |
| 2 | – | + | – | + | + | 0 | 0 | + | + |
| 3 | – | + | – | + | + | – | 0 | + | + |
| 4 | – | + | – | 0 | – | – | 0 | + | + |
| 5 | – | + | – | 0 | – | – | 0 | + | + |

$^{0}$no interaction; $^{+}$growth stimulation; $^{-}$growth inhibition

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port growth of specific microorganisms. For example, nutrition with propolis and bee pollen supplement affected both probiotic and pathogen microorganisms in gastrointestinal tract of broiler chickens (Kročko *et al.* 2012). Ivanišová *et al*. (1999) reported that Ukrainian bee bread samples demonstrated antibacterial activity against *Bacillus thuringiensis*, *Escherichia coli* and *Salmonella enteric*; especially active was bee bread from Poltava region having the largest amount of phenolics. Abouda *et al.* (2011) reported that bee breads from different regions in Morocco showed strong antimicrobial activities on the bacterial strains resistant to antibiotics. As a rule, gram positive bacteria were more sensitive to bee bread and bee pollen than gram negative bacteria. According to our results, bee bread is able to support or inhibit the growth of specific *Lactobacillus* dependently on botanical source and chemical composition of this material.

## CONCLUSIONS

In this study bee breads were described by stable shape of pellets as well as acceptable physical and valuable antioxidant properties. Microscopic and spectroscopic methods confirmed diversity in botanical sources, colour and chemical composition of bee breads. SEM images confirmed that, despite expected heterofloral origin, each of bee breads has a prevalence of evident botanical specie. Unlike other samples, bee bread supposedly originated from sunflower pollen was well discriminated from the others by PCA of NIR and Vis spectroscopic data. This bee bread was characterised by the smallest pellets, significant amount of carotene confirmed by golden yellow colour and Raman features and low antioxidant capacity because carotene, the main antioxidant in this case, is less soluble in polar media than phenolics contributed to antioxidant properties of the other samples.

According to results of antioxidant assays, bee bread could be interesting as the source of anti-oxidative compounds for food, pharmacy or cosmetics. Obtained results of microbial growth assay support the conclusion, that bee bread can be used for synbiotic construction with the appropriate microbial strains as probiotics. Such synbiotics may be successful with selected *Lactobacillus* strains for both types of the extracts. All combinations of probiotics and prebiotics in such preparations should be verified *in vivo* before the final confirmation of synbiotic character. Other potential probiotic strains will be tested in future. This new exploitation of bee bread extends the understanding of its potential value for human health.

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