Food Chemistry 115 (2009) 1056–1063

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Gas chromatographic–mass spectrometric investigation of the chemical composition of beebread

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ARTICLE INFO

Article history:
Received 17 May 2008
Received in revised form 31 October 2008
Accepted 7 December 2008

Keywords:
Beebread
Chemical composition
Gas chromatography–mass spectrometry

ABSTRACT

Beebread consumption has a very long tradition; however, its composition and bioactive properties have not been studied thoroughly up to now. This study is expected to expand the knowledge of chemical composition of this bee product as a natural remedy and functional food ingredient. With the help of successive extraction with organic solvents of different polarity, more than 200 compounds were extracted from five samples of beebread and then identified by GC–MS method. The content of some phenol compounds (*p*-coumaric acid, kaempherol, isorhamnetin) with antioxidant properties has been determined quantitatively. Different content of free aminoacids have been detected in the analyzed samples, which is assumed to be caused by Maillard reaction between aminoacids and carbohydrates.

1. Introduction

The apicultural products are widely used from ancient times in human diet and folk medicine due to their nutritional and medical properties. Therefore many publications are devoted to the study of the chemical composition of these products. The majority of publications refer to honey composition and its dependence on the botanic composition of plants, from which bees collect nectar. A great number of works also present studies of the chemical composition of propolis (Bankova, Popova, Bogdanov, & Sabatini, 2002; Maciejewicz, Daniewski, Bal, & Markowski, 2001; Marcucci, 1995; Pietta, Gardana, & Pietta, 2002; Prytzyk et al., 2003; Adelmann et al., 2007), bee-collected pollen (Herbert & Shimanuki, 1978; Human & Nicolson, 2006; Kroyer & Hegedus, 2001; Roulston & Cane, 2000; Silva et al., 2006) and wax (Jiménez, Bernal, Aumente, Toribio, & Bernal, 2003; Jiménez, Bernal, Aumente, Toribio, & Bernal, 2004; see also Hamilton, 1995; Kolattukudy, 1976). Special original publications and reviews deal with methodology of studying the chemical composition of apicultural products including samples preparation and their analysis by modern methods such as high performance liquid chromatography (HPLC) and capillary gas chromatography with mass spectrometric detection (GC–MS) (Alklam, 1998; Bogdanov, Ruoff, & Persano Oddo, 2004; Gómez-Caravaca, Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernándes-Gutiérrez, 2006). However, the composition of apicultural products has not been studied evenly. It is stated in the introduction to the Alklam's (1998) review cited above that such products as beebread and royal jelly are not considered in the review due to the complete lack of publications on the topic. Indeed, we could find only one publication (Baltrušaitytė, Venskutonis, & Čkstarytė, 2007) on the subject of beebread composition. In this work radical scavenging activity of extracts from honey and beebread are discussed.

The pollen collected by bees from plants is the original stock for beebread. In the process of its storage in cells, the chemical composition of pollen changes, apparently mainly because of bees' glandular secretions. Among other factors, there is a change in acidity: pH level of fresh pollen is approximately 7.2, but in 'mature' beebread it decreases to 3.5–4.2, mainly as a result of lactic acid formation. It can be expected that the chemical composition of beebread will be considerably determined by the composition of pollen collected by bees, which, according to literature data, varies widely depending on species composition of plants in a particular region (Stanley & Linskens, 1985; Talpay, 1981).

This work presents for the first time the chemical composition of five samples of beebread, obtained from different parts of the Baltic Region, where beebread has been used in traditional medicine as well as in food diets due to its nutritional and physiological properties.

2. Experimental

2.1. Chemicals

Pyridine and bis(trimethylsilyl)trifluoroacetamide (BSTFA) with addition of 1% of trimethylchlorosilane were purchased from

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doi:10.1016/j.foodchem.2008.12.025

Sigma–Aldrich (Poznań, Poland). Commercial *p*-coumaric acid, kaempherol, isorhamnetin, fructose, glucose and sucrose were purchased from Fluka (Poznań, Poland). Beebread extraction was carried out by *n*-hexane, diethyl ether, and methanol (POCH SA, Gliwice, Poland).

2.2. Beebread samples

Beebread samples were obtained from apiarists from different countries of the Baltic Region (Fig. 1). The sample BB-1 was collected in Poland (Kórnik, Wielkopolska Province, 51°47′ N-17°23′ E). The sample BB-2 was obtained from Russia (St. Petersburg, collected in Leningrad region, 59°37′ N-30°29′ E), and the other three samples were obtained from Latvia. First of them (BB-3) originates from the eastern part of Latvia (Madona region, 56°46′ N-26°11′E); the sample BB-4 originates from the western part (Tukums region, coast of Riga Gulf, 56°56′ N-23°19′ E), and the sample BB-5 originates from the central part (Daugmales, Riga region, 56°49′ N-24°35′ E) of the country. All the samples except the latter one were collected in autumn of 2007 and were dried at 50–70 °C. The sample BB-5 was extracted from honey-combs in January, 2008 and was not dried before analysis.

2.3. Sample preparation and analysis

Cooled at −18 °C beebread samples were powder-ground. 3.2 ± 0.2 g of the powder was placed in a 50 mL flask and extracted, constantly stirred, successively with 3 × 25 mL of *n*-hexane, 3 × 25 mL of diethyl ether, and 3 × 25 mL of methanol. The duration of each extraction cycle was 1 h. The combined extracts were filtered through a paper filter and the solvent was completely removed on a rotor evaporator. After the mass of oil-like residue left on the walls was determined, it was washed out by 10 mL of appropriate solvent (hexane, ether or methanol). 0.5 mL of ether or methanol solution was put into a vial of 2 mL in volume. After evaporation of solvent, 220 μL of pyridine and 80 μL of BSTFA were added into the vial. The reaction mixture was sealed and heated during 0.5 h at 60 °C to obtain trimethylsilyl (TMS) derivatives. All steps of analytical procedure applied by us may be illustrated by an analysis flow chart in Fig. 2.

Hexane extracts were separated on a Perkin-Elmer Turbo Mass apparatus which was fitted with PE-5HT (30 m × 0.25 mm I.D.; 0.10 μm film thickness) fused silica capillary column. TMS derivatives were separated on an Agilent 6890 gas chromatograph with mass selective detector MSD 5973 (Agilent Technologies, USA). Gas chromatograph was fitted with autosampler HP 7683, electronic pressure control and split/splitless injector. Separation was performed on the HP-5ms fused silica column (30 m × 0.25 mm I.D.; 0.25 μm film thickness). Helium flow rates through both columns was 1 mL/min. The injectors worked in split (1:30) mode; injectors temperature 250 °C, ionization voltage 70 eV. The analyses were carried out at temperature increasing from 40 to 310 °C at the rate 3 °C/min where it was held for 15 min. Detection was performed in the full scan mode from m/z 41 to 700.

A hexane solution of C_8–C_{28}, C_{30}, C_{32}, C_{34}, C_{36}, C_{38} and C_{40}, *n*-alkanes were previously separated in the conditions described above, and their retention times were determined. The values of retention times of *n*-alkanes and analytes were used to calculate

Fig. 1. Map giving the approximate position of beebread origin/sampling.

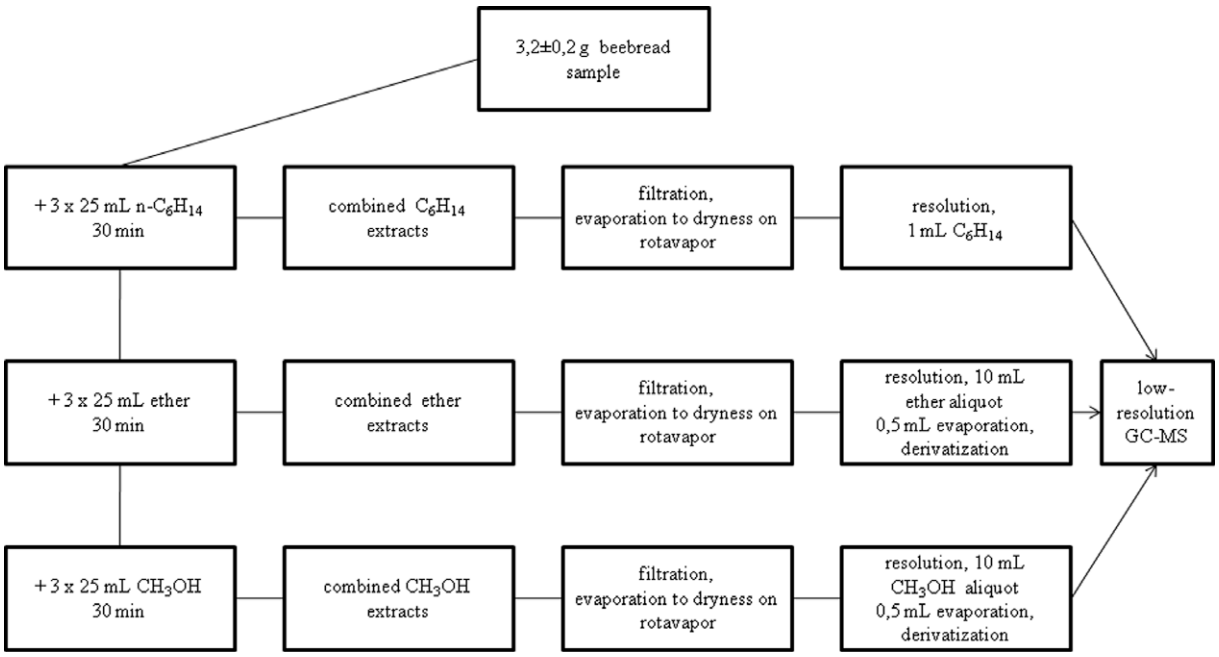

Fig. 2. Analysis flow chart.

linear temperature programmed retention indices (LTPRI) from equation:

$$\mathrm{LTPRI} = 100\left(z + n\frac{t_x - t_z}{t_{z+n} - t_z}\right)$$

where $n \geqslant 1$, t_x is the retention time of the analyte, t_z is the retention time of the n-alkane eluting directly before the analyte, t_{z+n} is the retention time of the n-alkane eluting directly after the analyte, z is the number of carbon atoms for the n-alkane eluting directly before the analyte. After integration, the fraction of each component in the total ion current (TIC) was calculated. The components were identified with the aid of an automatic system of processing data of GC–MS supplied by NIST mass spectra library. The MS library search was performed by using PBM (Probability–Based Matching) algorithm. Each analyte peak was evaluated for peak purity and resolution from the nearest eluting peak.

To enhance the reliability of identification we used both mass spectra library search and LTPRI of registered chromatographic peaks. A computer home-made program was developed for identification. It is supplied with the database of randomized literature and measured in our laboratory LTPRI values for more than 6100 organic compounds. Identification was considered reliable if the results of computer search at MS library were confirmed by the measured LTPRI, the deviation of which from the database values did not exceed ±5 u.i.

2.4. The precision of analytical procedure

The method precision was studied by three replicate extractions and analyses of the hexane, ether and methanol extracts. The precision was expressed by relative standard deviation (R.S.D). The peek areas of the extract components obtained by replicate analyses were used for calculation of their R.S.D. values, which amounted to 17% in average. Fairly high values of relative standard deviations are apparently conditioned by multi-staging procedure of compound extraction.

To calibrate MSD 5973 mass spectral detector, a series of five solutions of *p*-coumaric acid, kaempherol and isorhamnetin in acetone covering the concentration range 20–2000 mg/L was prepared by successive dilutions. For preparation of TMS derivatives, 1 mL of calibration solution was transferred to the vial of 2 mL in volume. Solvent was gently evaporated in a stream of warm air. To the residue, 220 μL of pyridine and 80 μL of BSTFA were added. The vials with the obtained mixtures were closed; the contents were heated at 60 °C for 0.5 h, and next subjected to GC–MS analysis in the conditions described above. On the basis of the analysis results, regression equations were calculated. The procedure revealed linear behavior over the whole concentration range tested with $R^2 > 0.990$ for all three compounds. The limits of detection (LOD) were determined by comparison the signal-to-noise (S/N) ratio of the lowest concentration to S/N = 3 and were found to vary between 0.06 and 0.08 μg/μL. The method enables quantitation of these phenol compounds in ether extracts at concentrations $\leqslant$0.24 μg/μL.

3. Results and discussion

3.1. A choice of analytical procedure

In this investigation, the sample preparation procedure includes successive beebread extraction with nonpolar (*n*-hexane), slightly polar (diethyl ether) and polar (methanol) solvents. The aim of extraction with hexane was to separate the components of honey-comb waxes. More polar carboxylic acids and phenols are well soluble in ether. In turn, methanol dissolves highly polar carbohydrate compounds: mono- and disaccharides, carbohydrate acids and alcohols (cyclitols). The used procedure does not required expensive solvents and special equipment.

This approach is more time consuming and less accurate at the stage of quantitative determination of components in comparison with a "single injection" method (Molnár-Perl, 1999). Nevertheless, it is justified in prospecting identification analysis of objects with previously unknown composition. As it is going to be seen in the next section, each of the three fractions contains many tens of compounds belonging to different classes, the content of which differs considerably. Therefore, the use of "single injection" method unavoidably leads to more difficulties in identification due to overlapping of chromatographic peaks.

3.2. Chemical composition of extracts

Table 1 contains data on the average fractional composition of extracts from investigated beebread samples. About 8% of the mass was extracted by hexane, ca. 5.5% was transferred in ether, and ca. 50% in methanol extract. Overall it was 63.4 ± 5.9%, besides, the lowest yield was in the case of "fresh" beebread (sample BB-5). The residue, insoluble in the solvents used, made about 37% of beebread mass. It consists of unprocessed residues of pollen and possibly mechanical admixtures.

In hexane extracts about 40 "neutral" compounds were registered (Table 2). About 59.7 ± 7.5% of TIC in recorded chromatograms of these extracts consisted of C_{21}–C_{35} *n*-alkanes, 6.8 ± 5.4% of branched alkanes, and 6.0 ± 0.8% of alkenes. Higher alkanes are known to be one of the main components of natural waxes. In the homologue series the *n*-alkanes with an odd number of carbon atoms predominate considerably. In the investigated samples, the value of CPI (Carbon Preference Index) exceeded 12. Noticeable amounts (9.0 ± 4.0%) of C_{16}–C_{18} aliphatic acids and their esters were also identified in hexane extracts. The elucidation of the structure of unsaturated alcohols with LTPRI values 2875, 3077, and 3274 has not been possible. Two unsaturated alcohols, $C_{32:1}$-OH and $C_{34:1}$-OH, were detected in pure beeswax by Jiménez et al. (2003).

Table 1
Fractional composition of extracts from beebread samples (n = 3)

Sample	Hexane		Ether		Methanol		Sum of extracts	
	mg	%	mg	%	mg	%	mg	%
BB-1 (Poland)	263 ± 5	7.8 ± 0.2	181 ± 12	5.4 ± 0.4	1880 ± 120	55.9 ± 3.6	2324	69.1
BB-2 (Russia)	301 ± 13	8.8 ± 0.4	176 ± 14	5.1 ± 0.5	1809 ± 20	52.8 ± 0.6	2286	66.7
BB-3 (Latvia)	292 ± 19	8.3 ± 0.6	229 ± 10	6.6 ± 0.3	1819 ± 28	52.0 ± 0.9	2340	66.9
BB-4 (Latvia)	276 ± 10	8.5 ± 0.3	187 ± 17	5.8 ± 0.6	1445 ± 20	44.7 ± 0.7	1908	59.0
BB-5 (Latvia)	236 ± 16	7.8 ± 0.5	129 ± 9	4.3 ± 0.3	1315 ± 35	43.4 ± 1.2	1680	55.5
Average		8.2 ± 0.4		5.4 ± 0.9		49.8 ± 5.4		63.4 ± 5.9

Table 2
Chemical composition of *n*-hexane extracts from beebread.

Compound	LTPRI^{Exp}	LTPRI^{Lit}	Relative composition,%				
			BB-1	BB-2	BB-3	BB-4	BB-5
n-Nonadecane	1900	1900	0.09	0.02	0.06	0.05	0.07
Hexadecanoic acid	1966	1965	1.2	1.9	1.8	1.6	0.9
Ethyl hexadecanoate	1990	1991	0.02	Trace[a]	0.08	0.07	0.04
Methyl linolenate	2093	2098	0.04	0.2	nd [b]	0.2	0.3
n-Heneicosane	2099	2100	1.9	0.4	0.8	0.4	0.4
Linolenic acid	2142	2141	5.2	5.6	11.4	9.7	2.3
Ethyl linoleate	2155	2151	0.3	Trace	nd	Trace	nd
Ethyl linolenate	2161	2160	0.4	0.3	nd	1.6	nd
n-Docosane	2198	2200	0.3	0.1	0.2	0.2	0.2
2-Methyldocosane	2261	2264	0.03	0.2	0.4	nd	nd
9-Tricosene	2269	2271	0.3	Trace	Trace	0.6	0.2
n-Tricosane	2299	2300	5.1	3.3	4.7	4.4	3.6
1-Tetracosene	2393	2396	nd	nd	0.1	nd	nd
n-Tetracosane	2398	2400	0.7	0.4	0.5	0.2	0.6
2-Methyltetracosane	2462	2464	0.2	0.06	0.1	0.2	0.08
9-Pentacosene	2472	2476	0.6	0.4	0.7	1.1	0.5
n-Pentacosane	2500	2500	9.2	7.9	10.5	12.2	8.0
n-Hexacosane	2599	2600	1.2	1.0	1.0	0.5	1.5
2-Methylhexacosane	2661	2664	Trace	0.1	0.4	0.7	0.1
9-Heptacosene	2673	2676	0.4	Trace	0.6	0.7	0.4
n-Heptacosane	2701	2700	20.8	20.9	16.9	14.1	21.9
13-Methylheptacosane	2731	2772	0.6	0.6	0.3	0.6	0.5
2-Methylheptacosane	2762	2763	0.2	0.1	0.3	0.2	0.1
Tetracosanoic acid	2768	2774	nd	nd	0.4	nd	nd
n-Octacosane	2800	2800	1.3	0.9	0.9	0.5	1.2
Squalene	2816	2820	0.7	0.2	0.2	0.2	0.1
2-Methyloctacosane	2861	2863	1.8	1.5	9.7	13.9	0.2
Hexacosen-1-ol?	2875	–	nd	0.6	Trace	nd	0.8
n-Nonacosane	2901	2900	13.2	13.1	10.0	10.0	14.4
3-Methylnonacosane	2971	2972	nd	nd	0.7	0.8	Trace
n-Triacontane	2998	3000	0.9	0.7	0.8	0.3	0.8
2-Methyltriacontane	3063	–	0.2	0.5	1.0	0.8	0.3
9-Hentriacontene	3070	3077	4.8	5.1	3.3	3.0	5.1
Octacosen-1-ol?	3077	–	nd	4.5	2.7	3.0	4.6
n-Hentriacontane	3100	3100	9.4	9.9	6.0	5.1	10.4
n-Dotriacontane	3199	3200	0.5	0.4	0.5	0.1	0.4
1-Nonacosanol	3246	3242	nd	nd	1.2	nd	1.4
Tricosen-1-ol?	3274	–	15.3	15.7	8.9	8.7	14.5
n-Tritriacontane	3298	3300	2.0	2.0	1.4	1.1	2.1
n-Tetratriacontane	3397	3400	0.3	0.2	0.2	Trace	0.2
n-Pentatriacontane	3498	3500	0.5	0.1	0.4	Trace	0.2
n-Hexatriacontane	3597	3600	0.2	0.08	0.2	nd	0.1

[a] Below 0.02% of TIC.
[b] nd – not detected.

Table 3
Chemical composition of ether extracts from beebread samples.

Compounds, TMS	Identification parameters				Relative composition,%				
	RI^{Exp}	RI^{Lit}	Target ions,	M^+	BB-1	BB-2	BB-3	BB-4	BB-5
Ethylamine	950	–	174,100,73	189	1.9	0.8	0.6	0.9	2.8
NN	956	–	144,73,158	–	0.8	0.4	0.3	0.4	1.5
Ethanolamine	1051	1052	147,73,174	–	0.5	0.2	0.1	0.2	0.7
Lactic acid	1070	1066	147,73,117	–	0.5	0.5	0.7	0.8	0.8
Glycolic acid	1084	1080	147,133,177	–	Trace[a]	0.2	0.2	Trace	0.4
2-Furancarboxylic acid	1134	1134	125,169,95	184	Trace	Trace	0.1	Trace	Trace
β-Lactic acid	1151	1151	147,73,177	–	Trace	Trace	0.2	Trace	Trace
Benzoic acid	1245	1250	179,105,77	194	Trace	0.05	0.05	Trace	Trace
Nicotinic acid	1287	1292	180,136,106	195	Trace	0.06	0.1	Trace	Trace
Glycerol	1288	1293	73,205,147	–	5.9	6.0	8.5	12.2	11.0
Phenyl acetic acid	1291	1305	73,164,193	–	nd[b]	0.05	nd	nd	nd
1,2,3-Trihydroxybutane-1	1305	-	117,73,147	–	nd	Trace	0.1	0.2	nd
1,2,3-Trihydroxybutane-2	1308	-	117,73,147	-	nd	Trace	Trace	0.2	nd
Succinic acid	1316	1322	147,73,247	262	0.5	0.5	0.7	0.9	1.6
Glyceric acid	1344	1344	73,147,189	-	0.05	Trace	Trace	Trace	Trace
NN	1503	-	209,224,191	224	Trace	Trace	1.3	Trace	0.05
2-Deoxypentonic acid, γ-lactone	1508	-	73,147,103	-	nd	0.1	0.2	0.2	Trace
Pyroglutamic acid	1521	1527	156,73,147	-	nd	nd	nd	nd	0.05
5-Hydroxymethyl-2-furancarboxylic acid	1552	-	147,271,73	-	nd	0.06	Trace	Trace	nd
β-Phenyl lactic acid	1584	1591	193,73,147	370	Trace	0.4	0.2	Trace	1.1
4-Hydroxybenzoic acid	1632	1636	267,223,183	282	Trace	0.1	0.08	0.02	0.3
Arabinoic acid, γ-lactone	1641	1652	73,117,147	364	Trace	0.3	0.6	1.5	0.3
Dodecanoic acid	1645	1657	257,117,73	272	Trace	0.05	0.06	0.03	Trace
3-Deoxyribohexonic acid, γ-lactone?	1760	-	73,129,147	378	nd	nd	nd	0.2	nd
α-Fructofuranose	1842	1846	217,73,147	-	Trace	0.2	0.08	0.3	0.5
β-Fructofuranose	1850	1854	73,217,204	-	1.8	1.5	1.7	3.4	5.8
Gluconic acid	1919	1916	73,147,319	-	Trace	0.2	0.2	0.3	Trace
Galactonic acid	1929	1925	217,73,204	466	3.2	2.3	2.5	4.6	3.0
p-Coumaric acid	1944	1947	293,219,73	308	0.2	0.2	0.1	0.2	0.4
Pentadecanoic acid	1951	1953	299,73,117	-	Trace	Trace	0.05	Trace	Trace
Glucitol	1981	1982	73,319,205	-	nd	nd	nd	Trace	Trace
6-Hexadecenoic acid	2021	2022	73,75,79,117	-	Trace	0.1	0.09	nd	Trace
β-Glucopyranose	2030	2028	204,191,73	-	0.3	0.2	0.2	0.2	0.8
Hexadecanoic acid	2051	2052	313,117,73	328	17.4	16.2	17.7	15.7	13.7
Methyl linolenate	2100	2096	79,95,108	-	nd	nd	0.9	nd	Trace
(E)-Ferulic acid	2103	2104	338,249,308	338	Trace	Trace	Trace	Trace	Trace
Caffeic acid	2155	2155	396,381,73	396	nd	nd	Trace	Trace	nd
Linoleic acid	2218	2215	73,7,569,81	352	6.2	7.4	5.8	8.5	4.2
α-Linolenic acid	2225	2221	75,73,129	350	33.0	28.1	36.0	27.7	28.7
6-Octadecenoic acid	2230	2225	73,75,129	354	0.5	0.3	0.6	0.2	Trace
Octadecanoic acid	2251	2255	341,117,73	356	2.4	0.9	0.8	0.6	0.7
NN (cinnamic acid derivative)	2323	-	320,305,249	320	Trace	0.05	nd	Trace	nd
Unsaturated acid	2397	-	75,79,73,129	-	Trace	0.2	0.1	nd	nd
11,14-Eicosadienoic acid	2417	2413	73,75,67,129	-	nd	nd	0.1	nd	nd
Eicosatrienoic acid?	2424	-	73,75,363	378	nd	nd	0.2	0.3	nd
Eicosanoic acid	2448	2449	369,117,73	384	1.5	0.6	0.4	0.2	0.05
Benzyl *p*-coumarate	2515	-	73,91,326,219	326	Trace	Trace	Trace	Trace	0.05
bis-(2-Ethylhexyl)-phthalate	2547	2544	149,167,57	-	1.6	0.5	0.4	0.5	1.3
2-Monopalmitin	2579	2577	129,218,73	-	Trace	Trace	Trace	nd	0.05
1-Monopalmitin	2610	2611	371,73,147	-	1.2	0.7	0.4	0.3	0.05
NN	2636	-	73,131,233	528	0.8	0.8	0.6	1.0	Trace
Docosanoic acid	2646	2646	397,73,117	412	0.6	0.7	0.4	0.5	0.5
NN	2680	-	171,73,317	-	0.3	nd	nd	nd	nd
Sucrose	2715	2714	361,73,217	-	3.1	0.4	1.7	0.9	0.02
Benzyl (E)-caffeate	2723	2723	414,91,73	414	0.05	Trace	nd	nd	nd
Chrysin, di-TMS	2745	2745	383,73,398	398	Trace	Trace	Trace	Trace	nd
1-Tetracosanol	2755	2752	411,75,57	-	Trace	Trace	nd	0.05	Trace
Linoleic acis, α-glyceride	2776	2780	129,147,395	-	Trace	0.2	0.1	0.1	nd
α-Linolenic acid, β-glyceride?	2786	-	73,129,147	-	1.0	0.5	1.0	0.5	nd
NN	2804	-	383,73,147	-	Trace	0.5	0.3	nd	nd
Tetracosenoic acid ?	2821	-	423,73,117	-	Trace	0.1	0.1	nd	nd
Squalene	2829	2828	69,81,41	410	Trace	0.05	0.2	0.2	0.3
Tetracosanoic acid	2846	2845	425,117,73	440	1.9	7.7	1.4	1.6	4.2
2-Methyl octacosane	2864	2864	57,7,85,99	-	0.6	nd	0.2	nd	0.2
Naringenin	2894	2894	73,473,488	488	Trace	Trace	Trace	Trace	nd
NN	2996	-	411,73,147	-	0.7	1.0	1.5	Trace	0.5
Hexacosanoic acid	3043	3043	117,73,453	468	0.5	2.8	0.5	0.5	1.0
9-Hentriacontene	3074	3077	57,88,97	434	nd	0.4	nd	nd	0.4
7-Hentriacontene	3081	3083	57,71,85	-	nd	0.2	nd	nd	0.6
Kaempherol	3113	3114	559,560,73	-	0.4	0.3	0.4	0.1	0.08
α-Tocopherol	3147	3149	73,487,217	502	0.5	0.3	0.3	0.5	0.02
Apigenin	3158	3159	471,73,486	486	Trace	nd	Trace	Trace	nd
Isorhamnetin	3160	-	589,73,559	604	0.9	0.4	0.8	0.7	Trace
Cholesta-5,24-dien-3β-ol	3186	-	129,69,343	456	nd	nd	nd	0.3	nd

(*continued on next page*)

Table 3 (*continued*)

Compounds, TMS	Identification parameters				Relative composition,%				
	RI^{Exp}	RI^{Lit}	Target ions,	M^+	BB-1	BB-2	BB-3	BB-4	BB-5
Quercetin-1	3213	3212	575,73,662	662	Trace	Trace	Trace	Trace	Trace
Quercetin-2	3239	3240	647,73,662	662	Trace	Trace	Trace	Trace	Trace
Octacosanoic acid	3240	3245	481,117,132	496	0.5	2.7	0.7	0.6	2.7
25-Hydroxy-24-methylcholesterol	3247	3248	129,73,386	470	2.9	2.2	2.3	1.4	1.0
NN	3264		371,73,129	-	nd	nd	nd	0.4	nd
NN	3266	-	69,95,123	-	1.6	1.1	0.4	Trace	1.0
9-Tritriacontene	3276	3276	97,57,83	-	0.5	0.8	0.5	0.8	2.7
NN (glucoside?)	3295	-	73,204,589	-	Trace	nd	0.4	1.0	nd
NN	3317	-	217,73,219	-	nd	nd	nd	0.2	nd
NN	3326	-	217,73,625	-	nd	nd	0.1	0.3	nd
β-Sitosterol	3346	3342	495,357,75	486	0.8	0.9	0.3	0.7	0.5
Stigmasta-3,24(28)-dien-3β-ol	3363	3360	386,296,129	484	1.4	1.0	1.2	1.8	0.6
5α-Stigmast-7-en-3β-ol	3404	3404	255,486,75	486	1.0	0.7	0.3	nd	Trace
Triacontanoic acid	3440	3445	117,509,73	524	0.4	1.8	0.5	0.6	0.9
NN (glucoside?)	3450	-	217,204,393	-	nd	nd	nd	0.4	0.02
NN (glucoside?)	3470	-	73,217,204	-	nd	nd	nd	0.5	nd
NN (glucoside?)	3480	-	73,217,319	-	nd	nd	nd	1.0	0.02
1-Dotriacontanol	3528	-	523,75,57	-	nd	0.5	0.05	0.2	nd
Dotriacontanoic acid	3638	3638	117,537,145	552	0.3	1.3	0.4	0.4	0.5
Tetratriacontanoic acid	3838	-	565,73,117	580	0.05	1.0	0.3	0.4	0.6
Hexatriacontanoic acid	>4000	-	257,57,73	-	Trace	0.5	0.3	0.4	2.3

[a] Below 0.02% of TIC.
[b] nd – not detected.

Tables 3 and 4 list 180 compounds presented in ether and methanol extracts from beebread samples in amounts of not less than 0.02% of TIC. These tables contain some analytical parameters used for identification: literature LTPRI values, m/z of three more intensive ions in mass spectra of compound, and mass number of molecular ions (M^+), if it was detected in the mass spectra. There was some uncertainty when the literature values of LTPRI were absent. In these cases the component name is followed by a question mark indicating that its identification is tentative.

Ether extracts are characterized by a much more varied composition. Table 3 contains 95 compounds, 56 of which are registered in all five beebread samples, and only ten of them were found in one sample. Aliphatic acids are predominant components of these extracts (64.3 ± 9.0%), and unsaturated, α-linolenic and linoleic acids form more than a half of them. Relative content of other groups of organic compounds is not high: the contents of glycerol and glycerides, monosaccharides, and sterols are on the average 9.9 ± 2.3%, 4.6 ± 1.8%, and 4.3 ± 1.8%, respectively.

Phenol compounds are particularly interesting as their presence in some food products predetermines their medical properties. This group of compounds was in the focus of Baltrušaitytė et al. (2007) work. The authors identified by HPLC method *p*-coumaric acid, kaempherol, chrysin and apigenin in beebread samples. However, it is worth pointing out that in the cited work, eight samples out of nine were mixtures of beebread with honey (1:1) or with honey and comb, and only one sample was beebread after thermal processing. Unfortunately, these authors do not present any quantitative data (the concentrations were expressed by using peak area units), but they communicate that the main phenol compounds were *p*-coumaric acid and kaempherol, whereas chrysin and apigenin were present in the samples only in trace quantities. On the qualitative level this corresponds well to our results presented in Table 3. Apart from the compounds mentioned above, we also detected isorhamnetin in beebread samples as well as trace quantities of ferulic and caffeic acids, flavonoids naringenin and quercetin.

According to quantitative GC/MS analysis, the content of three phenol compounds in all five beebread samples was significant but ranged widely. Average concentrations of *p*-coumaric acid, kaempherol and isorhamnetin was 367 ± 101, 492 ± 350 and 1086 ± 720 μg/g, respectively. All these compounds exhibit antioxidant properties (Amić, Davidović-Amić, Bešlo, & Trinajstić, 2003; Furasawa et al., 2005), however, Baltrušaitytė et al. (2007) did not found correlation between the amount of identified phenolics and radical scavenging activity of beebread extracts. It is fairly possible that not all compounds having these properties have been detected in the preliminary studies. In particular, search for glycosides with phenol aglycones has not been carried out. Meanwhile, a wide range of glycosides have been isolated previously from pollen, with the most common type of flavonol-3-*O*-glycosides (Daguet, Bert, Dolley, Bekaert, & Lewin, 1993; Markham & Campos, 1996). In ether extracts (Table 3), a range of components with LTPRI values of 3295, 3450, 3470, and 3480, have been registered, which may turn out to be glycosides, however, studies with application of HPLC–MS technique are necessary to confirm this hypothesis.

Table 4 contains 92 compounds identified in methanol extracts from beebread. Among these only 13 compounds were also found in ether fraction of extracts. Expectedly, the main components of methanol extracts were carbohydrates, which account for 71.9 ± 1.4% of TIC on the average. The main part of this fraction (49.3 ± 2.4%) is constituted by monosaccharides, among which anomers of fructose and glucose are presented in the largest quantities. Carbohydrate alcohols and carbohydrate acids form the following groups in order of importance with the average ratio 13.8 ± 2.2% and 7.6 ± 1.1%, respectively. Cyclitols, inositol isomers, which are classified as vitamins (Angyal et al., 1959), were also included in the group of carbohydrate alcohols.

Generally, the composition of methanol extracts turned out to be similar in all the five beebread samples. The only exception was free aminoacids, which were detected in relative small quantities only in BB-5 sample. Aminoacids found in beebread are probably directly collected by bees from wild plants as part of pollen (Human& Nicolson, 2006; Rayner & Langridge, 1985) and therefore should have been present in all extracts. The most probable reason for the observed difference is a different type of pre-commercial preparation of beebread: only the sample BB-5, which cannot be subjected to long-term storage, was not exposed to thermal processing. In the drying process, the vast majority of free aminoacids can react with redusing carbohydrates according to the mechanism of the well-known Maillard reaction. Consequently, preliminary drying prolongs the expiry date of beebread but reduces to some extent its nutritive value.

Table 4
Chemical composition of methanol extracts from beebread.

Compound, TMS	Identification parameters				Relative composition,%				
	$LTPRI^{Exp}$	$LTPRI^{Lit}$	Target ions, m/z	M^+	BB-1	BB-2	BB-3	BB-4	BB-5
Alanine	1119	1119	116,73,147	-	nd[a]	nd	nd	nd	0.03
Valine	1222	1226	144,73	-	nd	nd	nd	nd	0.02
Leucine	1281	1285	158,73,100	-	nd	nd	nd	nd	0.02
Phosphoric acid	1287	1289	299,317,73	-	0.4	0.6	0.8	1.0	1.0
Glycerol	1291	1293	73,218,103	-	0.9	0.7	1.1	1.4	0.9
NN	1298	-	165,267,282	282	0.03	Trace[b]	0.1	0.1	nd
L-Proline	1299	1302	142,73,216	-	Trace	nd	nd	nd	1.0
Succinic acid	1322	1322	147,73,247	-	Trace	nd	Trace	nd	0.02
Glyceric acid	1344	1344	73,147,292	292	Trace	nd	Trace	nd	0.02
Serine	1373	1368	204,218,73	-	nd	nd	nd	nd	0.03
Malic acid	1502	1504	73,147,233	-	Trace	Trace	Trace	Trace	0.02
Pyroglutamic acid	1528	1527	156,73,147	-	nd	nd	Trace	nd	0.1
Threitol	1530	1529	73,217,147	-	Trace	Trace	Trace	Trace	Trace
L-Aspartic acid	1534	1543	232,73,147	-	nd	nd	nd	nd	0.03
L-Proline	1536	-	230,73,140	-	nd	nd	nd	nd	0.02
Erythronic acid	1565	1567	73,147,292	-	Trace	Trace	Trace	Trace	Trace
Phenylalanine	1635	1640	218,192,73	-	nd	nd	nd	nd	0.02
Xylofuranose	1635	1632	217,73,147	-	Trace	Trace	0.2	0.1	0,1
Arabinose	1638	1640	217,73,204	-	Trace	0.1	0.2	0.1	0.03
Arabinoic acid, γ-lactone	1648	1652	73,117,147	364	0.1	0.3	0.2	0.3	0.1
α-Ribofuranose	1667	1666	217,73,204	-	0.1	Trace	0.3	0.1	0.2
Ribonic acid	1694	1697	217,73,147	-	Trace	Trace	Trace	Trace	0.03
Xylitol	1736	1734	73,217,103	-	0.1	0.2	0.2	0.2	0.1
Atabitol	1760	1760	73,217,147	-	nd	Trace	0.3	0.1	0.04
Ribitol	1766	1766	73,217,365	-	nd	0.1	Trace	0.1	nd
α-Glycerophosphoric acid	1790	1794	73,357,299	-	nd	Trace	0.1	0.07	0.2
α-D-Methylfructofuranoside	1805	-	217,73,257	-	0.2	0.2	0.3	0.2	0.3
Arabinoic acid	1816	1812	73,292,147	-	0.1	0.2	0.1	0.2	0.1
Ribonic acid	1822	1823	73,217,147	-	0.1	0.3	0.3	0.3	0.6
α-Fructofuranose	1849	1846	217,73,437	-	13.6	16.2	14.2	17.6	6.2
β-Fructofuranose	1855	1854	217,73=204	-	10.3	8.9	11.3	11.1	23.1
Inositol, isomere 1	1870	-	73,260,147	-	0.6	0.7	0.3	0.5	0.3
1-Deoxyglucose	1876	1879	217,73,204	-	nd	0.1	0.8	0.7	0.9
α-Glucofuranose	1886	1889	73,217,204	-	0.9	1.7	3.4	2.6	1.0
α-Galactopyranose	1900	1899	204,73,191	-	1.5	0.2	0.9	0.5	0.2
D-Galctonic acid, γ-lactone	1923	1925	73,147,217	466	5.1	3.1	3.7	3.2	1.1
α-Glucopyranose	1933	1932	204,191,73	-	22.3	16.1	15.5	14.7	12.7
Galacitol?	1936	-	73,306,147	-	0.1	nd	0.1	0.1	nd
β-Mannopyranose	1947	1942	204,191,73	-	0.1	Trace	0.4	0.2	0.2
Inosose	1958	1953	305,73,306	-	-	Trace	0.1	Trace	0.5
Mannitol	1971	1970	73,275,147	-	9.9	3.7	3.9	4.6	6.1
Sorbitol	1975	1980	73,319,205	-	0.1	Trace	Trace	0.1	Trace
Glucitol + deoxyinositol	1985	1982	319,73,205	524	3.6	10.7	4.4	7.4	3.3
Inositol, isomere 2	1995	1993	73,217,147	-	Trace	nd	0.1	0.1	0.6
Inositol, isomere 3	1998	1994	318,305,147	612	0.6	0.5	1.3	0.1	0.7
Glucuronic acid 1	2008	2004	73,147,292	-	nd	0.1	0.1	0.1	0.1
β-D-Glucopyranose	2030	2028	204,191,73	-	12.4	13.6	12.2	11.0	12.6
Gulonic acid	2053	2056	73,333,292	-	2.3	4.3	3.9	3.7	3.6
Hexadecanoic acid	2054	2052	313,73,129	328	0.1	0.5	0.5	0.6	0.6
scyllo-Inositol	2070	2070	73,318,305	-	0.04	nd	nd	nd	0.03
Carbohydrate acid	2079	-	217,73,147	-	0.04	0.1	0.1	0.1	0.1
Glucuronic acid 2	2084	2083	217,73,147	-	-	Trace	Trace	Trace	Trace
myo-Inositol	2131	2128	305,217,73	612	0.7	0.5	0.6	0.7	0.7
Linoleic acid	2217	2218	73,75,129	352	0.03	0.3	0.3	0.2	0.4
α-Linolenic acid	2225	2221	73,75,129	-	0.1	0.7	0.8	1.0	1.0
α-D-Glucopyranose-1-phosphate?	2257	-	73,261,299	-	nd	nd	nd	nd	0.1
Monosaccharide	2276	-	217,73,147	-	nd	Trace	0.1	0.1	0.1
NN (glucoside?)	2295	-	73,437,520	-	0.1	Trace	0.2	0.3	Trace
Monosaccharide	2300	-	73,217,147	-	0.1	Trace	0.2	0.2	0.4
Monosaccharide	2319	-	204,73,147	-	0.04	0.2	0.1	0.1	0.1
NN (glucoside?)	2347	-	73,437,200	-	0.1	0.1	0.1	0.2	0.1
Monosaccharide	2358	-	204,73,147	-	nd	0.2	0.3	0.3	0.2
2-O-Glycerol-α-d-galactopyranoside?	2361	-	204,73,217	-	0.1	0.1	0.3	0.3	0.3
2-O-Glycerol-β-galactopyranoside?	2366	-	204,73,217	-	0.1	nd	Trace	0.1	nd
Uridine	2471	2469	217,73,259	-	0.05	0.1	0.1	0.1	0.1
Lactulose, anomer 2	2694	2695	217,73,437	-	0.04	0.1	0.1	0.1	0.2
Sucrose	2714	2714	361,73,217	-	0.1	0.4	0.1	0.1	0.1
Disaccharide	2722	-	217,73,361	-	0.03	0.1	0.1	nd	0.4
Disaccharide	2746	-	204,73,217	-	0.1	0.1	Trace	nd	0.4
Maltose, anomer 1	2759	2760	204,73,217	-	0.2	0.4	0.3	0.2	0.2
Maltulose, anomer 1	2781	2781	73,361,217	-	0.3	0.6	0.7	0.6	1.2
Maltulose, anomer 2	2786	2786	73,361,217	-	1.1	1.1	1.0	0.9	1.1
Turanose	2797	2795	361,73,217	-	1.2	1.5	1.3	1.1	1.6
Maltose, anomer 2	2805	2803	204,73,311	-	0.8	1.1	0.7	0.5	0.5

(*continued on next page*)

Table 4 (*continued*)

Compound, TMS	Identification parameters				Relative composition,%				
	$LTPRI^{Exp}$	$LTPRI^{Lit}$	Target ions, m/z	M^+	BB-1	BB-2	BB-3	BB-4	BB-5
Palatinose, anomer 1	2816	2811	217,73,204	-	1.2	1.3	1.5	1.1	2.2
Palatinose, anomer 2	2836	2835	217,73,204	-	1.2	1.4	0.5	0.4	1.2
Leucrose	2857	2855	361,204,191	-	1.2	0.9	1.2	0.9	1.6
Cellobiose	2885	2883	204,217,73	-	0.5	0.3	0.4	0.4	0.2
Disaccharide	2890	-	204,73,191	-	0.5	0.7	0.5	0.5	0.6
Disaccharide	2946	-	361,204,73	-	0.1	0.4	0.1	0.2	0.4
Disaccharide	2950	-	361,73,204	-	0.1	0.3	0.2	0.2	nd
Isomaltose, anomer 1	2957	2952	204,73,217	-	0.6	0.7	1.1	0.8	1.3
Disaccharide	2969	-	361,204,73	-	0.1	0.4	0.3	0.6	0.2
Disaccharide	2976		204,73,361	-	0.1	nd	0.3	0.3	nd
Gentibiose	2990	2991	204,73,191	-	0.2	0.2	0.2	0.3	0.3
Disaccharide	2998	-	204,73,361	-	0.2	0.2	0.2	0.2	0.4
Isomaltose, anomer 2	3008	3005	204,73,191	-	0.8	1.4	1.4	1.3	1.6
NN (glucoside?)	3079	-	487,204,73	-	0.1	0.1	0.1	0.1	0.3
Trisaccharide	3100	-	204,73,217	-	nd	0.1	0.1	Trace	0.1
25-Hydroxy-24-methylcholesterol	3249	3248	129,73,386	470	nd	nd	0.1	0.06	0.1
NN	3514	-	217,259,73	-	0.1	0.1	0.5	0.1	0.2
Panose	3694	3685	204,361,73	-	nd	nd	nd	nd	0.1

[a] nd – Not detected.
[b] Below 0.02% of TIC.

4. Conclusions

To our knowledge, this study is the first to compare the chemical composition of beebread samples from different countries. The results of the analyses show that beebread contains large quantities of unsaturated aliphatic acids (α-linolenic and linoleic acids) and digestible carbohydrates (fructose and glucose); it also contains free aminoacids, the content of which, however, depends on pre-commercial preparation of the product. This composition determines the high nutritive value of beebread.

All the analyzed samples also contain phenol compounds with antioxidant properties. High radical scavenging activity of phenol fractions of extracts from beebread was demonstrated by Baltrušaitytė et al. (2007); however, the absence of authentic correlation with the content of phenol compounds can indicate that other (unregistered) compounds are also responsible for this property, the most probable candidates being glycosides. Further work needs to be done to examine this assumption.

Acknowledgements

We are grateful to apiarist and owner of apicultural products shop Mr. Jānis Sulutaurs, Z/S "Dorites", Latvia, for a comprehensive consultation and for providing us with beebread not subjected to drying.

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