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Original Article

GC-MS INVESTIGATION OF THE CHEMICAL COMPOSITION OF HONEYBEE DRONE AND QUEEN LARVA HOMOGENATE

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Abstract

Honeybee larva homogenate appears to be underrated and insufficiently explored but this homogenate is an exceptionally valuable honeybee product. Drone larva homogenate is very nutritional due to its high content of proteins, free amino acids, lipids, and carbohydrates. Moreover, the biological characteristics of honeybee larvae indicate the presence of chemical substances that may be pharmacologically active. In spite of the above, the chemical composition of honeybee larva has not gained as much attention as that of other bee products. In this study, the chemical composition of honeybee brood homogenate has been investigated using gas chromatography/mass spectrometry. As a result, it was possible to isolate as many as 115 extractive organic compounds from 6 samples of crude queen and 9 samples of drone homogenate. The main groups of substances extracted from either type of homogenate were composed of free amino acids and carbohydrates. The relative content of amino acids in queen homogenate as well as the share of essential amino acids were found to be higher than in the drone homogenate. Disaccharide trehalose was the dominant sugar in the queen larvae, whilst glucose prevailed in the drone larvae. Comparative chemical analyses of honeybee queen and drone larva homogenates have allowed us to make a preliminary inference about a higher overall value of the former.

Keywords: chemical composition, GC-MS, homogenate, honeybee, queen and drone larvae

INTRODUCTION

The larvae of different insects is said to form a considerable part of the diet of many people from different cultural backgrounds (Schmidt & Buchmann, 1992). The various honeybee (*Apis mellifera* L.) families provide the greatest amount of easily accessible larva stored in one place. Hence, honeybee brood along with honey has always been a desirable trophy of honey hunters in Africa and Asia. It is considered a delicious, eagerly-consumed treat (Krell, 1996). Also medical applications of insect larva can be found in the literature on the subject. For example, in Chinese and Korean traditional medicine, the larva of the paper wasp (*Polistes dominula*, Vespidae), silk moth (*Bombyx mori,* Bombycidae), scarab beetle (Scarabaeidae) and hepialid moth (*Hepialus oblifurcus,* Hepialidae) have been commonly employed (Pemberton, 1999). Wasp larva is reported to have been used in the medicine of ancient Mexicans (Posey, 1986). The medical application of honeybee larva by different African cultures has also been documented (Mbaya, 1996; Meda et al. 2004). According to Narumi (2004) honeybee brood (mainly pupae) has been used in Chinese traditional medicine to treat a variety of health disorders.

For the last few decades, honeybee brood has been attracting ever greater attention as a valuable food (Schmidt & Buchmann, 1992; Krell, 1996; Narumi, 2004; Finke, 2005; Mutsaers et al., 2005; Bogdanov, 2015). Some recent investigations have also confirmed the medicinal properties of honeybee brood (Iliescu, 1993; Vasilenko et al., 2002; 2005; Gorpinchenko et al., 2004; Seres et al., 2013; Seres et al., 2014; Andrițoiu et al., 2014). The scientific confirmation of these valuable properties of honeybee brood have become a strong stimulus to commence its commercial production not only

as a dietary supplement in some European countries (Apilarnil Forte, Apivitas) but also as medicinal product (Apilarnil Potent®, S.C. Biofarm S.A., Romania; Apidron, The Ukraine). As a result, moves were made to standarise this non-traditional product of bee-keeping (Lazaryan et al., 2003; Budnikova, 2011, Krasovskaya, 2011). In spite of the above, the chemical composition of honeybee brood has not received as much attention as that of other bee products. The physico-chemical properties of honeybee larvae were solely determined by means of such general indexes as water content, ash, acidity, total protein, lipids, and sugar content (Burmistrova, 1999; Lasaryan, 2002; Bărnuțiu et al., 2013; Balkanska et al., 2014).

The main aim of this research work is to analyse and compare the qualitative and semi-quantitative chemical composition of honeybee queen and drone larva homogenates with the aid of high resolution gas chromatography coupled with mass spectrometry.

MATERIAL AND METHODS

Materials

Pyridine, bis (trimethylsilyl) trifluoroacetamide (BSTFA) with an addition of 1% trimethylchlorosilane and methanol, were acquired from Sigma-Aldrich (Poznan, Poland).

The 6 honeybee queen and 9 honeybee drone samples used in our experiments came from *Apis mellifera carnica* Poll. colonies maintained in the Białystok city region (north-eastern Poland). The cells with queen larvae were gathered in May–June 2009–2012, and transported directly from apiary to laboratory. Three larvae were carefully extracted from their cells and washed twice with methanol and then thoroughly homogonised in a fresh portion (3–5 mL) of methanol. After filtration through a paper filter, the solvent was removed on a rotor evaporator at 40°C.

Combs with drone larva were sampled in May–June 2009–2014. The drone larva homogenate was gathered by cutting it out from the combs and pressing. The resultant dense milky mass was directly refrigerated (-18°C) and transported to the laboratory for extraction with methanol, as described above. Additionally, the drone homogenate acquired in 2014 was lyophilised using a Donserv, model Alpha 1-2LDplus apparatus. For the preparation of lyophilised homogenate for further analysis, the methanol extraction process was followed by filtration and solvent evaporation on the rotor evaporator.

Sample preparation and GC-MS analysis

Dry residue of queen larvae or drone homogenate extracts (5–10 mg) were transferred to a vial which was 2 mL in volume. Next, 220 µL of pyridine and 80 µL of BSTFA were added and the vial was sealed and heated for 0.5 h at 60°C to obtain trimethylsilyl (TMS) derivatives.

The solutions of TMS derivatives were separated and analysed using the GC-MS apparatus with the mass selective detector MSD 5973 (Agilent Technologies, USA) and a capillary column HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness) (Isidorov, 2015). Helium flow rate through the column was 1 mL min^{-1}. Injection of 1 µL of the sample was performed by using HP 7673 autosampler. The injector (250°C) worked in split-splitless mode. The initial column temperature was 50°C rising to 300°C at the rate of 5°C/min. The detection was performed in full scan mode from 41 to 600 a.m.u.

A hexane solution of C_{10}–C_{40} *n*-alkanes was separated under the above conditions. Linear temperature programmed retention indices (I_T) were calculated from the results of the separation of this solution and brood homogenate extracts. For identification, mass spectral data and calculated I_T values were used. Registered mass spectra and I_T values were compared with the literature data (NIST Chemistry WebBook, 2013) and with our previously published data (Isidorov et al., 2012; Isidorov, 2015).

Other methods

The qualitative parameters of the crude and lyophilised drone homogenate (i.e. water content, ash, total protein, lipids, sugar content, and energy value) were determined in a

certified chemical laboratory of the Regional Sanitary-Epidemiological Station in Białystok, Poland in accordance with Polish standards. In the lyophilised samples, the determination of the fat content was carried out according to PN- EN- ISO 1736, whereas in the case of fresh homogenates the weight method in accordance with PB-14 was used. The protein content was designated by titration in accordance with PB-44 and the water content by PN-A-7911-3/1998. The amount of carbohydrates and the energy value were determined using PN-A-79011-6, and overall ash content by PN-A-79011-8.

RESULTS

Table 1 presents the general characteristics of the analysed honeybee drone homogenate. Of particular interest is the comparison of the data obtained for the lyophilised drone homogenate and lyophilised royal jelly. The lyophilised drone homogenate contains significantly larger amounts of lipids and carbohydrates and smaller amounts of protein and ash than the royal jelly.
Table 2 contains data on the chemical composition of honeybee brood homogenate. The chromatograms of both homogenates show 115 peaks of the substances whose share in the total ion current exceeds 0.01%. The identified components in raw homogenates do not fully overlap because of a different number of the registered peaks, namely 102 in the queen larvae chromatograms as opposed to 73 peaks found in the drone larvae chromatograms.

DISCUSSION

Quality parameters of honeybee brood homogenate

As can be seen from data in Table 1, honey bee larvae homogenate is an exceptionally valuable food product. For example, 100g of lyophilised drone homogenate has about twice as many kilokalories as 300 g of rump steak, or of 85 g of cream (35 % fat). The energy value of drone homogenate is explained by its high content of protein, lipids, and sugars. Some authors underline the similarity of the chemical composition of drone homogenate to that of royal jelly (Balkanska et al., 2014; Bărnuțiu et al., 2013; Bogdanov, 2015). In fact, the data shown in the last column of Table 1 indicate how similar these beekeeping products are. The energy value of the drone homogenate is even somewhat higher owing to a higher content of lipids and sugars. As can be seen, this is very much in agreement with the literature data results, as regards the crude homogenate (Bărnuțiu et al., 2013). However, a marked difference can be observed between our data concerning the total protein and carbohydrate content in lyophilised homogenate and those reported by Narumu (2004).

Table 1

Total characteristics of the crude and lyophilised honeybee drone homogenate and lyophilised royal jelly

Characteristics	Drone homogenate				Royal jelly
	crude		lyophilised		
	this work	literature[a]	this work	literature[b]	
Water, %	73.6±5.2	71.1±2.4	3.0±1.0	4.5	2.7±0.9
Protein, %	10.0±0.9	7.2±1.9	32.0±2.9	52.4	34.0±9.9
Lipids, %	3.5±0.1	3.8±1.5	24.2±1.0	21.9	13.5±7.8
Carbohydrates, %	12.2	-	38.9	17.8	26.8±5.8
Ash, %	0.7±0.3	0.9±0.1	2.7±0.8	3.5	3.5±2.1
Energy value, kcal/100g	120.3	-	501.4	-	364.7

[a] Bărnuțiu et al. (2013).
[b] Narumi (2004).

Table 2
Relative chemical composition (% of TIC) of methanol extracts of honeybee queen and drone larvae

Compound, TMS	I_T	Queen larvae			Drone larvae		
		Min	Max	Median (*n*)[a]	Min	Max	Median (*n*)[a]
Aminoacids							
Valine, O-mono-TMS	1090	-	-	-	0.02	0.2	0.1±0.1 (9)
Alanine, N,O-bis-TMS	1115	0.6	1.1	0.7±0.3 (6)	0.08	0.3	0.2±0.1 (9)
Glycine, N,O-bis-TMS	1133	0.2	0.5	0.3±0.2 (6)	0.1	0.4	0.3±0.1 (9)
Sarcosine	1147	0.1	0.2	0.1±0.1 (6)	-	-	-
β-Alanine, N,O-bis-TMS	1200	0.1	0.3	0.2±0.1 (6)	0.04	0.2	0.1±0.1 (7)
Valine, N,O-bis-TMS	1228	0.4	2.2	1.2±0.7 (6)	0.3	0.5	0.2±0.1 (9)
Leucine, N,O-bis-TMS	1284	0.2	1.2	0.5±0.4 (5)	0.06	0.4	0.2±0.1 (9)
Proline	1303	1.2	3.2	2.3±0.7	2.0	8.8	4.6±2.5 (9)
Isoleucine, N,O-bis-TMS	1306	0.2	1.4	0.7±0.5 (6)	trace	0.2	-
Threonine, O,O-bis-TMS	1306	0.2	0.8	0.4±0.2 (6)	-	-	-
γ-Aminobutyric acid	1315	0.05	0.02	0.1±0.1 (4)	-	-	-
Serine, N,O,O-tris-TMS	1380	0.1	0.7	0.5±0.2 (6)	trace	0.04	-
Threonine, N,O,O-tris-TMS	1407	0.2	0.8	0.4±0.2 (6)	0.1	0.3	0.1±0.1 (8)
Homoserine	1488	trace	0.04	-	-	-	-
Pyroglutamic acid, bis-TMS	1530	0.5	2.2	0.8±0.6 (6)	0.2	1.3	0.8±0.6 (9)
Aspartic acid	1541	0.04	0.2	0.1±01 (4)	trace	-	-
Phenylalanine, N,O-bis-TMS	1634	0.2	0.4	0.3±0.1 (4)	trace	0.1	-
Glutamine, N,O,O-tri-TMS	1640	0.4	1.7	1.0±0.5 (5)	0.1	0.2	0.1±0.1 (9)
Asparagine, N,O,O-tris-TMS	1691	trace	0.2	-	-	-	-
Lysine, N,N,O-tri-TMS	1726	trace	0.1	-	trace	-	-
4-Hydroxyproline	1543	trace	0.1	-	trace	-	-
Methionine, N,N,O-tri-TMS	1793	0.2	0.6	0.3±0.2 (5)	-	-	-
Tyrosine, N,O-di-TMS	1893	trace	0.3	0.2±0.1 (4)	trace	0.2	-
Histidine, N,N',O-tri-TMS	1947	-	-	-	-	0.2	-
Tyrosine, N,O,O-tri-TMS	1958	0.2	1.8	1.1±0.6 (6)	trace	0.5	0.2±0.2 (6)
Tryptophane, N,N,N',O-TMS	2212	trace	0.5	0.3±0.2 (4)	trace	0.2	-
Other N-containing compounds							
Urea	1254	trace	0.2	-	-	0.04	-
Uracil	1351	trace	0.05	-	trace	-	-
Uric acid	2143	trace	-	-	-	-	-
Uridine, tri-TMS	2469	0.1	0.5	0.3±0.2 (6)	-	-	-
Adenosine	2670	0.01	0.8	0.3±0.3 (6)	-	0.4	-
P-containing compounds							
H_3PO_4	1289	1.3	5.3	2.5±1.6 (6)	0.7	1.3	1.1±0.3 (9)
β-Glycerylphosphate	1763	trace	0.1	-	trace	0.05	-
α-Glycerylphosphate	1801	trace	0.2	-	0.05	0.3	0.1±0.1 (9)
Glucopyranosyl phosphate	2258	trace	0.4	-	trace	0.1	-
Aliphatic acids							
Lactic acid	1075	0.2	3.5	1.2±0.8 (6)	0.4	1.6	0.7±0.4 (9)
3-Hydroxybutyric acid	1174	trace	0.1	-	-	0.04	-
Succinic acid	1325	0.2	1.1	0.5±0.3 (6)	0.2	0.6	0.3±0.2 (9)
Glyceric acid	1348	trace	0.05	-	trace	0.04	-
3-Methylglutaconic acid	1461	trace	0.1	-	-	-	-
Fumaric acid	1357	trace	0.05	-	-	-	-
Malic acid	1510	0.1	0.4	0.2±0.1 (6)	0.06	0.1	-
Adipic acid	1517	trace	0.3	-	-	-	-

2-Hydroxyglutaric acid	1600	trace	0.1	-	trace	0.1	-
Hexadecanoic (palmitic) acid	2051	0.3	1.3	0.5±0.4 (6)	trace	1.2	0.3±0.4 (7)
Oleic acid	2222	0.9	4.3	1.7±1.3 (6)	trace	0.8	-
Octadecanoic (stearic) acid	2250	0.1	0.5	0.2±0.2 (5)	-	-	-
Carbohydrates and related compounds							
Erythritol	1536	0.03	0.2	0.1±0.1 (4)	trace	0.04	-
Threitol	1539	trace	0.07	-	trace	0.06	-
Xylitol	1731	trace	0.1	-	trace	0.05	-
Ribitol	1766	0.08	0.2	0.1±0.1 (4)	0.04	0.4	0.2±0.3 (7)
Arabinoic acid	1808	trace	0.1	-	-	-	-
α-D-Methylfuranoside	1817	0.02	0.1	-	trace	0.1	-
α-Fructofuranose	1843	trace	-	-	0.6	4.7	2.4±1.1 (9)
α-Mannopyranose	1845	0.2	03	0.2±0.1 (4)	-	0.2	-
β-Fructofuranose	1854	0.1	0.8	0.4±0.3 (3)	1.2	13.0	6.0±1.8 (9)
β-D-Methylgalactopyranoside	1861	trace	0.1	-	-	-	-
Pinitol, penta-TMS	1872	0.1	1.3	0.7±0.5 (5)	-	0.2	-
β-Fructopyranose	1887	-	-	-	0.04	0.5	-
β-Glucofuranose	1889	trace	0.2	-	trace	1.2	0.5±0.4(5)
α-D-Methylglucopyranoside	1918	trace	0.3	-	0.6	8.1	3.1±3.0 (9)
Galactonic acid, γ-lactone	1929	trace	0.3	-	trace	0.8	0.4±0.3 (6)
α-Glucopyranose	1931	trace	0.5	0.2±0.2 (5)	27.3	41.4	33.0±3.0 (9)
Galactopyranose	1941	-	-	-	0.2	0.7	0.5±0.3 (9)
Mannitol	1974	trace	0.2	0.1±0.1 (4)	0.6	0.9	0.7±0.2 (9)
Glucitol	1982	0.5	1.6	1.1±0.4 (6)	2.9	4.5	3.7±0.8 (9)
chiro-Inositol	1991	trace	0.1	-	-	-	-
Cyclohexanepentol, penta-TMS	2005	0.1	1.28	0.5±0.4 (6)	-	-	-
Hexitol	2027	0.1	0.9	0.4±0.4 (5)	-	-	-
β-Glucopyranose	2032	0.3	0.6	0.4±0.2 (5)	10.7	31.7	23.2±8.5 (9)
epi-Inositol?	2033	0.04	0.8	0.4±0.4 (3)	-	-	-
Gluconic acid	2047	0.1	0.7	0.4±0.3 (6)	0.4	0.6	0.5±0.1 (9)
scyllo-Inositol	2069	trace	0.1	-	trace	0.1	-
myo-Inositol	2128	trace	2.1	0.9±0.6 (4)	0.3	0.7	0.5±0.2 (9)
Disaccharide (217,361,73)	2686	4.4	9.7	6.3±2.6 (3)	-	-	-
Lactulose	2691	0.4	19.9	9.8±8.8 (4)	-	0.2	-
Sucrose	2714	trace	0.8	0.6±0.5 (3)	-	0.1	-
Disaccharide (204,73,217)	2737	-	-	-	0.3	0.5	0.2±0.1 (9)
α-Maltose	2750	-	-	-	0.2	0.3	0.2±0.2 (8)
Disaccharide (73,204,217,361)	2787	-	-	-	trace	0.7	-
β-Maltose	2800	-	-	-	0.3	0.7	0.5±0.1 (9)
Trehalose, octa-OTMS	2814	24.5	65.8	41.4±17.0 (6)	3.4	9.1	6.6±2.7 (9)
Disaccharide, hepta-OTMS	2829	trace	6.3	5.4±1.0 (4)	-	0.06	-
Disaccharide (73,204,217,361)	2844	-	-	-	0.4	3.2	0.9±0.8 (7)
neo-Trehalose	2850	trace	4.8	2.6±1.8 (3)	-	-	-
α-Isomaltose	2951	-	-	-	0.1	0.9	0.4±0.3 (9)
β-Isomaltose	3010	trace	-	-	0.2	1.2	0.5±0.5 (9)
Raffinose	3504	trace	1.2	-	-	-	-
1-Kestose	3515	trace	1.2	-	-	-	-
Melizitoze	3582	-	-	-	trace	0.4	0.3±0.1 (4)
Sterols							
Campesterol	3249	0.3	1.0	0.6±0.3 (6)	trace	-	-
β-Sitosterol	3342	trace	1.6	0.4±0.5 (5)	trace	-	-
Avenasterol	3355	trace	0.4	-	-	-	-

Glycerides							
2-Monopalmitoyl glycerol	2578	trace	-	-	-	-	-
1-Monopalmitoyl glycerol	2615	trace	0.4	-	-	-	-
2-Monooleoyl glycerol	2744	trace	0.9	-	-	-	-
1-Monooleoyl glycerol	2783	trace	2.6	1.0±0.9 (5)	-	-	-
1,2-Dipalmitoyl glycerol	3934	0.4	1.42	0.8±0.6 (5)	-	-	-
1,3-Dipalmitoyl glycerol	>4000	trace	1.1	0.6±0.4 (5)	-	-	-
1,2-Dioleoyl glycerol	>4000	0.2	1.1	0.7±0.4 (5)	-	-	-
1,3-Dioleoyl glycerol	>4000	0.2	0.5	0.4±0.1 (5)	-	-	-
1,3-Distearoyl glycerol	>4000	0.2	0.5	0.3±0.1 (5)	-	-	-
Other compounds							
Glycerol	1293	0.3	2.0	1.4±0.6 (6)	0.7	3.2	1.5±0.8 (9)
4-Hydroxybenzoic acid	1636	trace	0.1	-	-	-	-
Shikimic acid	1849	0.3	0.7	0.4±0.2 (4)	trace	-	-
Citric acid	1852	trace	0.8	0.2±0.3 (4)	-	-	-
Quinic acid	1902	0.1	0.5	0.3±0.2 (5)	trace	0.1	-
n-Tricosane	2300	trace	0.3	-	-	-	-
n-Heptacosane	2700	trace	1.1	-	-	-	-

[a] *n*, number of the homogenate samples with a component concentration ≥0.01% of TIC

It must be noted that royal jelly is a very expensive bee product and it is not very significant for human nutrition. Assuming a daily intake of 2 g per day (energy value ca. 7.3 kcal), the basic nutrients (i.e. proteins, lipids, and carbohydrates) contained in royal jelly do not play any significant role for their recommended use in the United States daily intake which is equal to 2000 kcal and 2600 kcal for women and men, respectively. However, the drone homogenate is incomparably cheaper and could be used in bigger quantities as a part of the human diet (Bogdanov, 2015).

The determination of the vitamin and mineral content in the homogenate was beyond our research scope, however the data presented by other authors (Burmistrova, 1999; Narumi, 2004) demonstrated a high content of water soluble B complex vitamins such as thiamine, riboflavin, niacin, pyridoxine, biotin, cobalamine, folic and pantothenic acids. On the other hand, there is almost a complete lack of lipid soluble vitamins, such as α-tocopherol. The lyophilised drone homogenate contains considerable quantities of potassium and phosphorus (10.4 and 8.0 g/kg, respectively) as well as such microelements as copper, iron, manganese, and what is most noteworthy, selenium (Narumi, 2004).

Every year, a typical honeybee family produces thousands of drones that consume a huge amount of food stuff stored by worker bees. Due to the above, and also due to the fact that *Varroa destructor,* a dangerous honey bee parasite that reproduces for the most part in drone cells, beekeepers are compeled to get rid of unwanted drone brood. Hence, in this case, any utilisation of drone larvae for the production of high quality food products appears to be a reasonable solution.

Chemical composition of queen and drone homogenate

Unlike in the case of drones, a typical bee family does not hatch new queens unless it begins preparation for swarming. But even when reproducing through swarming, honeybees create only a small number of queen cells. Yet, in the apiaries that specialise in royal jelly production, a large number of queen larvae are normally hatched. These larvae are usually treated as unwanted material or as a waste product to be thrown away. There is no reason to think, however, that queen homogenate is not valuable solely due to the fact that it is obtained in smaller amounts than the drone homogenate. Owing to the above, we decided to carry out a comparative study of the chemical composition of the extracts obtained from honeybee queens

and drones.

One of the most numerous groups of components identified in both types of the homogenate (Table 2), consists of free amino acids. The count included all 9 essential amino acids. Of the 20 proteinogenic amino acids in the test samples, no traces of either arginine or cysteine were found. Arginine as well as cysteine, however, were previously found in drone homogenates (Lazarryan, 2002) and queen larvae (Isidorov et al., 2012). The non-proteinogenic amino acids included sarcosine, β-alanine, homoserine, 4-hydroxyproline, γ-aminobutyric, and pyroglutamic acids. The relative content of free amino acids in the queen larvae turned out to be higher (an average of 11.8 ± 0.9% higher) than in the drone larvae homogenate (an average of 8.3±0.8%). At the same time, the content of essential amino acids in queen larvae homogenate amounted to 55.0±1.5% and drone larvae homogenate was equal to 41.0±14%. These data are well in agreement with previously obtained results (Lazaryan, 2002).

Noticeable differences in the relative content also apply to the other multi-component group of the homogenate, namely sugars. The queen larva homogenate contains small amounts of fructose (an average of 0.4 ± 0.3%) and glucose (6.0 ± 0.2%), whereas in the drone larvae their content reaches the values of 6.5 ± 1.8% and 56.7 ± 7.8%, respectively. The main disaccharide found in queen larvae homogenate is non-reducing trehalose (41.4 ± 17.0%), whereas in the drone larva homogenate there is considerably less (6.6 ± 2.7%). The latter homogenate also shows the presence of small quantities of maltose and isomaltose (0.9 ± 0.5 %). However, they are not found at all in the queen larva homogenate.

Aliphatic acids in the extracts of both homogenates include manly oleic, palmitic, and stearic acids in their free form as well as bound into mono- and diglycerides. Other substances found in queen larva homogenates worth noting for their relatively high content of isomeric inositols (about 1.5%) and also the nucleosides, uridine, adenosine and biomolecules with many physiological effects. We should also stress the presence of free phosphoric acid and its esters in the homogenates. However, neither of them contain any significant quantities of substances with considerable antibacterial properties. That is why the bee brood homogenate decomposes easily. Its long-term storage is possible only at low temperatures, in the lyophilised form or in mixtures with honey. The storability of honeybee brood homogenate can also be improved by binding fresh homogenate to a glucose-lactose adsorbent (Burmistrova, 1999).

Considering the presented material as well as the data included in Table 1, it is possible to classify the drone homogenate to nutraceutics, since apart from its nutritious protein content it also exhibits substantial health effects confirmed not only by recent *in vitro* and *in vivo* laboratory investigations (Seres et al., 2013; 2014; Andrițoiu et al., 2014), but also by clinical observations (Gorpinchenko et al., 2004). Furthermore, some investigations demonstrated the immunotropic and hepatotropic activity of drone homogenate (Vasilenko et al., 2002;2005).

In several cultures, honeybee larvae are used to treat male impotence (Mbaya, 1996; Meda et al., 2004). It had previously been wrongly assumed that the use of larvae for treating infertility was due to the high protein content in the larva. However, subsequent chemical analysis revealed the presence of sex hormones in drone larvae (Burmistrova, 1999). According to Burmistrova, fresh drone homogenate contains (in nMol/100g): 0.31±0.02 of testosterone, 51.3±8.7 of progesterone, 410.0±65.4 of prolactin, and 677.6±170.3 of estradiol. As can be seen, there is prevalence of female sex hormones. A more recent investigation (Budnikova, 2009) demonstrated the dynamics of the sex hormones in the course of drone development from larva to pupa. Five-day old larvae contained (in nMol/L): 8.2 of testosterone and 2745.0 of estradiol, however the homogenate from 15-17 day old pupa contained 15.6 of testosterone and 343.5 of estradiol. Drone larva homogenate has a more pronounced gonadotropic activity than royal jelly, allowing the rehabilitation of the blood concentration of testosterone and fructose (Burmistrova, 1999).

The positive effect of drone homogenate on the androgenic hormone content was dem-

onstrated by Gorpinchenko et al. (2004) in clinical treatments of male infertility. During the treatments, the lyophilised drone homogenate product under the name Apidron, was used. Similar effects were observed in animal *in vivo* tests (Yücel et al., 2011; Seres et al., 2013; 2014; Murav'ev & Kalachinskaya, 2014). Surprisingly, the raw drone homogenate increased not only the weight of the androgen-sensitive organs but also the plasma testosterone level in castrated rats (Seres et al., 2014). Yucel et al. (2011) also described the androgenic and anabolic effects of drone larva homogenate on male broilers.

In conclusion, we can state that honeybee brood is a valuable food product and also a prospective pharmaceutical raw material to be used for medical purposes. Comparison of the results of the chemical analysis of both queen and drone larva homogenates makes it possible to initially conclude that the former had a greater value. However, in order to better justify this conclusion further research is required. In particular, it is necessary to investigate the content and dynamics of hormonal changes in the development process of the queen larvae. Thus, honeybee brood has a variety of biological and health-promoting properties which make it an ideal additive to be used for prophylactic purposes.

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