Comparative Study on Quality Parameters of Royal Jelly, Apilarnil and Queen Bee Larvae Triturate

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Abstract

Given their beneficial effects in terms of health, the natural products, especially beehive products, have drawn the attention of consumers since long time ago. In order to guarantee the quality of these products on the market, their chemical composition needs to be analyzed. Thus, this current research had as objective the establishment of quality parameters for beehive brood food derived products: apilarnil and queen bee larvae triturate. These two products were compared with royal jelly which is the basis of brood food in the first 3 days of larval stage. The carbohydrates were determined by HPLC-IR and allowed the identification of seven carbohydrate compounds, predominantly glucose, fructose and sucrose. The lipid profile was analyzed by the Soxhlet method. The total protein content was determined by the Kjeldahl method. Free amino acids were analyzed by LC-MS. A total of 31 amino acids were identified of which nine are essential amino acids for humans.

Keywords: royal jelly, apilarnil, queen bee larvae, 10-HDA, amino acids

INTRODUCTION

Royal jelly (RJ) is a well-known beehive product with high nutritional values which promote human health thus it is used as a dietary supplement. It is a viscous secretion of the hypopharyngeal and mandibular glands of the young worker honeybees which take care of the brood in the hive. In their first 3 days all bee larvae are fed RJ and after this time point only the queen larvae receive exclusively RJ while the worker and drone larvae are fed with a modified food obtained from their glands secretion mixed with honey and pollen. Commercial RJ is obtained by harvesting the brood food from the queen cell in the 3rd day of larval development (ISO 12824:2016).

Diverse biological activities have been reported in the case of RJ: antibacterial (Fujivara *et al.*, 1990; Fontana *et al.*, 2004; Vezeteu *et al.*, 2016), antiviral (Zhang *et al.*, 2014) antitumor (Townsend *et al.*, 1961), antioxidative (Nagai *et al.*, 2001; Guo *et al.*, 2009; Buratti *et al.*, 2007; Delkhoshe-Kasmaie *et al.*, 2014; Malekinejad *et al.*, 2016), anti-inflammatory (Fujii *et al.*, 1990; Kohno *et al.*, 2004), immunoregulatory (Okamoto *et al.*, 2003; Vucevic *et al.*, 2007), insulin-like (Dixit and Patel, 1964; Kramer *et al.*, 1982), estrogen signaling mediating (Moutsatsou *et al.*, 2010), antiaging (Inoue *et al.*, 2003), reducing cholesterol levels (Kashima *et al.*, 2014), vasodilation (Tokunaga *et al.*, 2004).

The drones represent the males of honeybees which emerge from unfertilized eggs while the workers and the queen who are the female individuals of honeybees develop from fertilized eggs.

Ilieşiu (1991) described the process for obtaining a beehive product which has increased biological activity due to the sum of nutritive compounds found both in the brood food and the body of the larvae. This product, named Apilarnil, is obtained after taking the whole composition of the 7-days-old drone larvae comb cells which are afterwards subjected to trituration, homogenization, filtration and lyophilization in order to have a safe and better preserved product. Queen bee larvae triturate is obtained in the same manner having as starting material the whole composition of queen bee larvae comb cells 1 day prior to capping. The whole content of the larvae cell after harvesting has an unctuous consistency which after trituration changes to a homogeneous milky appearance with a slightly gray yellowish color which tends to get brownish in time. In table 1 the values for the main compounds in these two products are listed.

The drone larvae contain testosterone, progesterone, prolactin and estradiol (Bogdanov, 2015). Recent studies showed both estrogenic (Mishima *et al.*, 2005) and androgenic-like effect of drone food (Yucel *et al.*, 2011). The androgenic-

like effect seems to be related to the methyl palmitate and methyl oleate as active compounds found in the drone food (Seres *et al.*, 2013). The drone larvae triturate (known as Apilarnil) is traditionally used for treating neuro vegetative and sexual problems (Bogdanov, 2011). A more recent research showed values of 20 ± 2 µg/g of coenzyme Q-10 in drone homogenate and of 8 ± 0.2 µg/g in RJ (Hryniewicka *et al.*, 2016).

According to Ilieşiu (1991), in terms of organoleptic characteristics the product should have: viscous with fine granulation aspect, white with grey to brown shades colour, milky consistency and a characteristic brood food smell. Ilieşiu (1991) also analyzed the physico-chemical parameters for the obtained fresh Apilarnil (table 1).

In terms of chemical composition, there are various scientific articles and standards for RJ and Apilarnil, while for queen bee larvae triturate there is no literature data available until now, excepting a study from 2016 (Isidorov *et al.*, 2016).

Amino acids have health-promoting effects and a recent study showed that the concentration of free amino acids in RJ is dependent on the time of harvest: the abundant free amino acids in RJ are proline, phenylalanine, lysine, glutamic acid and tyrosine and their concentration increases with increasing larval stage from 4,30 mg/g in the first day to 9,48 mg/g in the 3rd day of

Table1. The physico-chemical composition of fresh Apilarnil and fresh royal jelly according to different references

	Fresh Apilarnil			Fresh royal jelly
	Ilieşiu, 1991; Stângăciu, 1999	Bărnăuţiu <i>et al.</i> , 2013; Narumi <i>et al.</i> , 2004	Isidorov <i>et al.</i> , 2016	Sabatini <i>et al.</i> , 2009
pH	4.8-6.7			3.4-4.5
Acidity		2.32		3.0-6.0
Water content (%)	65-75	71.1	73.6	60-70
Dry matter (%)	25-35			
Ash (%)	2	0.9	0.7	0.8-3.0
Proteins (%)	9-12	7.23	10.0	9-18
Lipids (%)	5-8	3.80	3.5	3.8
10-HDA (%)				> 1.4
Carbohydrates (%)	6-10		12.2	7-18
Glucose (%)	3.16-5	3.61		4-8
Fructose (%)	0.03-0.5	0.60		3-13
Sucrose (%)	0.03-0.5	0.14		0.5-2.0

larval development (Jie *et al.*, 2016). The major free amino acids found in RJ are proline, lysine, glutamate, b-alanine, phenylalanine, aspartate and serine and they have an average value of 7,3 mg/g (Boselli *et al.*, 2003).

Apilarnil has various important amino acids like lisine, histidine, arginine, serine, glutamic acid, alanine, valine, methionine, isoleucine, leucine, tyrosine, fenilalanine according to Ilieşiu (1991). Apilarnil is used as a biostimulating product that is beneficial for the stomach ulcer and congestive liver (Bogdanov, 2012).

Recently, a study revealed that drone bee larvae homogenate contains about 20 ± 2 µg/g coenzyme Q-10 (Hryniewicka *et al.*, 2016). This fat-soluble molecule is involved in cellular energy production and antioxidant mechanisms. Apilarnil and queen bee larvae triturate are good candidates for getting the most out of the effects of coenzyme Q-10.

MATERIALS AND METHODS

The samples were obtained from a beekeeper in the spring of 2015. The samples were taken directly from the hive and stored at -20 °C until further analysis.

Water content analysis was carried out by the gravimetric method according to Popescu and Meica (1997). The samples were dried in the oven at a temperature of 105°C until constant weight. The results were expresses as average percentage of three replicates $\pm$ standard deviation.

Total proteins in the samples were determined by the Kjeldhal method according to AOAC. The samples were digested using Büchi Digestion Unit K-424 coupled with Büchi B-414 Scrubber. The distillation step was done using the KjelFlex K-360 device. The titration was performed using an automatic titrator Schott Titroline using sulfuric acid of 0.25 M until a pH of 4.65. The conversion factor of 6.25 was multiplied with the total nitrogen content determined by titration and the result was then the total protein in the sample.

Carbohydrates were analyzed by HPLC-IR using the optimized method of Sesta (2006). The samples were weighed and homogenized, then a mixture of ultrapure water and methanol (v/v 3:1) was added, Carrez I and Carrez II reagent were used for precipitating the proteins. The centrifugation step was done at 4,000 rpm for 30 minutes. Afterwards the supernatant was washed

with dichloromethane in order total extract the lipids and afterwards filtered (Millipore 0.45 µm). The obtained filtrate was injected at a flow rate of 1 mL/min. and a 30 °C temperature inside a VP HPLC with 100Å Amino Altima column of 4.6 mm diameter, 250 mm length and 5 µm particle size. For the calibration curve glucose, fructose, sucrose, maltose, turanose, trehalose, erlose and isomaltulose were used as standards. The carbohydrate values were expressed as percentage.

The lipids were determined by the Soxhlet method extraction using diethyl ether on a Soxtherm extraction unit. The lipid content was calculated as average percentage of three replicates.

The 10-hydroxy-2-decenoic acid (10-HDA) content was analyzed by HPLC-PDA using the modified method of Liu *et al.* (2008). A mixture of ultrapure water and methanol of pH 2.5 was used as mobile phase. The samples were weighed and dissolved in a mixture (60:40, v/v) of methanol and ultrapure water of pH 2.5 and then sonicated for 30 minutes. Mobile phase was added and then the samples were centrifuged at 4,000 rpm for 10 minutes and filtered (0.22 µm filters). The analysis was done for a volume of injection of 20 µL at a flow rate of 1 mL /min. and 40° C temperature inside a LC-10 AD VP (Shimadzu) using a reverse phase column LC-18 Supelcosil of 4.6 mm diameter, 250 mm length and 5 µm particle size. The detection was at 210 nm. For the calibration curve concentrations of the standard 10-HDA were in the range 0.2-200 µg/ml with a coefficient of determination of 0.9998. The results of 10-HDA content were expressed as average percentage of three replicates.

The free amino acids were separated and quantified by LC-MS using a Shimadzu equipped with electrospray ionization spray and a positive mode of operation. The volume of injection was 1µL at a 35°C temperature inside a EZ: faast AAA-MS, 250x3.0mm column. The mobile phases were 10 mM ammonium formate in water and 10 mM ammonium formate in methanol. The free amino acids were purified and extracted using the sample preparation method described in the EZ:faast Phenomenex kit. The values were expressed as mg/100 g.

RESULTS AND DISCUSSION

Regarding the water content it can be stated that for RJ the results for this parameter were within the limits specified by the literature and standards. The quality standard proposed by Sabatini *et al* (2009) shows a water content percentage of 60-70% in fresh RJ. In our study the RJ samples had an average percentage of $66.03 \pm 0.03\%$ which is in the range proposed. For Apilarnil the water content limits proposed by Ilieșiu (1991) are 65-75%. The Apilarnil samples analyzed in our study had an average percentage of $73.25 \pm 0.02\%$. The average percentage of water content in the bee queen larvae triturate was $75.17 \pm 0.15\%$. For this parameter there is no information available in the literature for the queen bee larvae triturate.

Sabatini *et al.* (2009) established for protein content in RJ values of 9-18%. The RJ samples under study had an average content of $11.14 \pm 0.11\%$ which are within the limits proposed by the international standard. Ilieșiu (1991) determined for Apilarnil values of 9-12% protein content. The Apilarnil samples in our study had an average of $9.47 \pm 0.13\%$ total proteins. The queen bee larvae triturate had an average of $12.03 \pm 0.05\%$ total proteins which is superior to the protein content in RJ.

The identification and quantification of carbohydrate compounds was done in order to determine the quality and authenticity of

the samples. In the RJ samples the three major carbohydrate compounds (glucose, fructose and sucrose) were identified along with the minor carbohydrate compounds like turanose, maltose, trehalose and erlose. The three major carbohydrate compounds account for 7-8% in RJ according to Sabatini *et al.* (2009). In the RJ samples studied the major carbohydrates had a mean value of $15.39 \pm 0.33\%$. In the fresh Apilarnil samples, carbohydrates account for 6-10 % according to Stangaciu (1999). In the Apilarnil samples analyzed in this study glucose, fructose, maltose and trehalose were identified but no sucrose was found. In queen bee larvae triturate the same three major carbohydrate compounds were found. In table 2 the average values for the determined carbohydrates are given in the analyzed samples.

Literature studies found in RJ the lipids content having values of 3-8% (Sabatini *et al.*, 2009). The RJ sample analyzed in our study had an average percentage of $3.96 \pm 0.09\%$. For Apilarnil, Ilieșiu (1991) obtained a value of 5-8%. Our samples had an average percentage of $8.38 \pm 0.16\%$ total lipids. The queen bee larvae triturate presents an average percentage value of $10.30 \pm 1.74\%$. No literature data for the lipid content in queen bee larvae triturate was found.

For the RJ samples, the lipidic compound 10-HDA represents an authenticity marker as it can be found only in this product. Sabatini *et al.* (2009)

Table 2. Quality parameters as determined for royal jelly, Apilarnil and queen bee larvae triturate

	RJ	Queen bee larvae triturate	Apilarnil
Water content (%)	66.03 ± 0.03	75.17 ± 0.15	73.25 ± 0.02
Total proteins (%)	11.14 ± 0.11	12.03 ± 0.05	9.47 ± 0.13
Lipids (%)	3.96 ± 0.09	10.30 ± 1.74	8.38 ± 0.16
10-HDA (%)	1.96 ± 0.03	0.09 ± 0.01	-
Carbohydrates (%)	15.39 ± 0.33		
Fructose (%)	7.32 ± 0.17	1.25 ± 0.08	0.38 ± 0.01
Glucose (%)	7.37 ± 0.15	2.10 ± 0.03	3.55 ± 0.02
Sucrose (%)	0.7 ± 0.01	0.08 ± 0.01	-
Turanose (%)	0.01 ± 0.00	-	-
Maltose (%)	0.22 ± 0.01	0.78 ± 0.02	0.9 ± 0.05
Trehalose (%)	0.03 ± 0.01	0.11 ± 0.09	0.25 ± 0.12
Isomaltose (%)	-	-	-
Erlose (%)	-	-	-
Melesitose (%)	-	-	-

gives a minimum level of 1.4 % for RJ. The 10-HDA level in the RJ samples analyzed in this study had an average value of 1.96 ± 0.03 %. No 10-HDA was identified in Apilarnil while in the queen bee larvae triturate the average value was of 0.09 ± 0.01 %.

A number of 31 free amino acids were identified in the analyzed samples (Table 3) from which nine essential amino acids can be found which cannot be synthesized *de novo* by human organism and thus must be supplied by diet.

This is a very important observation, taking into consideration that all three types of bee products that were analyzed, could be used as food supplements in human diet.

Royal jelly contains eight out of nine essential amino acids, tryptophan is missing from the composition, but this amino acid is needed in the smallest amount in the human diet, according to World Health Organization. The total amount of essential amino acids in royal jelly was 247.35

Table 3. Free amino acid content determined in royal jelly, apilarnil and queen bee triturate by LC-MS

Amino acid	Royal Jelly (mg/100g)	Queen bee larvae triturate (mg/100g)	Apilarnil (mg/100g)
Arginine (ARG)	34.48	91.57	112.33
Glutamine (GLN)	177.59	-	-
Serine (SER)	2.72	105.70	86.84
Asparagine (ASN)	2.91	81.11	77.36
Proline-hydroxyproline (PHP)	-	-	-
3-methyl histidine (3-MHIS)	0.02	-	-
1-methyl-histidine (1-MHIS)	0.25	0.35	0.74
4-hydroxyproline (HYP)	2.28	-	-
Glycine (GLY)	3.91	71.84	114.72
Glycine-proline (dipeptide) (GPR)	-	1.53	1.16
Threonine (THR)	1.99	72.52	122.69
Alanine (ALA)	45.01	175.40	170.15
Gamma-aminobutyric acid (GABA)	15.09	11.29	13.35
Sarcosine (SAR)	2.96	11.47	6.72
Beta-aminoisobutyric acid (β AIBA)	2.70	1.91	2.20
Alfa-aminobutyric acid (ABA)	1.85	3.24	7.13
Ornithine (ORN)	3.35	4.68	3.33
Methionine (MET)	0.09	29.03	38.24
Proline (PRO)	234.10	162.10	277.51
Lysine (LYS)	221.07	201.28	120.79
Aspartic acid (ASP)	27.30	69.37	8.51
Histidine (HIS)	8.64	61.35	40.97
Valine (VAL)	3.67	91.16	81.35
Glutamic acid (GLU)	61.02	149.36	212.89
Tryptophan (TRP)	-	38.40	32.41
Leucine (LEU)	2.25	99.05	106.82
Phenylalanine (PHE)	7.55	72.47	63.12
Isoleucine (ILE)	2.09	36.58	49.47
Cystathionine (CTH)	-	3.19	1.22
Cysteine (C-C)	-	1.11	1.58
Tyrosine (TYR)	2.50	133.43	76.55
Total essential amino acids	247.35	701.84	655.86
Total free amino acids	867.39	1780.67	1830.07

mg/100 g. The highest amount of lysine is found in royal jelly (221.07 mg/100 g), this amino acid together with leucine are required in the highest amount in human diet, according to the same World Health Organization. Proline is a conditionally essential amino acid, meaning that its synthesis can be limited under special pathophysiological conditions, such as severe catabolic distress. Apilarnil has the highest content of proline from all the analyzed bee products (277.51 mg/100g), and for this reason this bee product is very important, as food supplement or ingredient in other type of natural supplements.

What is important to notice is that royal jelly is the only product where glutamine is found (177.59 mg/100g), another conditionally essential amino acid which is not found in queen bee larvae triturate and Apilarnil.

Queen bee triturate has a concentration of 701.84 mg/100 g essential amino acids (all nine) and 1780.67 mg/100 g total free amino acids. High amounts of non essential amino acids (the highest from the three bee products analyzed) were quantified in queen bee larvae triturate: 105.70 mg/100 g serine, 175.40 mg/100 g alanine, glutamic acid and also aspartic acid. From the essential amino acids present in this type of sample, highest amounts from all three types of analyzed bee products, were quantified as phenylalanine (72.47 mg/100 g), tryptophan (38.40 mg/100g), valine (91.16 mg/100 g) and histidine (61.35 mg/100 g). Beside this valuable composition in essential amino acids, queen bee larvae triturate is valuable for its high content of tyrosine, a conditionally essential amino acid.

Although Apilarnil has the highest amount of free amino acids content from all three types of bee products (1830.07 mg/100 g), the amount of essential amino acids was lower compared to queen bee larvae triturate, meaning that non-essential amino acids and conditionally essential amino acids might be higher in quantity in the chemical composition of Apilarnil. Indeed, Apilarnil has the highest amount of proline (277.51 mg/100 g), arginine (112.33 mg/100g) and glycine (114.72 mg/100 g).

What makes Apilarnil a valuable product from, is the high content of essential amino acids: threonine (122.69 mg/100 g), leucine (106.82 mg/100 g), isoleucine (49.47 mg/100 g) and methionine (38.24 mg/100 g). Comparing to royal

jelly, the amount of threonine in Apilarnil is more than 100 times higher (122.69 versus 1.99 mg).

These last two bee products are not very known and studied, thus very few literature data regarding their chemical composition is existing at this time (Bărnuțiu *et al.*, 2013, Andrițoiu *et al.*, 2014, Isidorov *et al.*, 2016), and this paper represent a step ahead in the characterization of these products.

CONCLUSIONS

Because insects are rich in proteins, lipids, carbohydrates, amino acids, vitamins and mineral elements they served and still do serve as food among some indigenous groups around the world. Thus edible insects have an important role in nutrition among people by supplementing the dietary needs and preventing malnutrition and other diseases.

This study showed that queen bee larvae triturate has high nutritive value when comparing with the other products analyzed. Queen bee larvae triturate has high lipid content, high protein content and high value of essential amino acids.

Information about the nutritive content of insect derived products is required in order to guarantee quality for consumers and provide the prospect for further exploitation for human health promoting food.

Therefore, the establishment of quality parameters for these brood food derived products makes possible the valorification in terms of nutritional and therapeutic value and contributes to the improved marketing of these products considering the increasing consumer demand for beneficial beehive products.

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