

Quality Parameters and Nutritional Value of Different Commercial Bee Products

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Abstract. The aim of this study was to determine the composition and microbiological quality of commercially honeybee-collected pollen, bee bread and royal jelly and to define the following physicochemical and microbiological characteristics: water content/dry weight, protein content, fat content, sugar spectrum and some microbiological parameters. The main sugars identified by high performance liquid chromatography were fructose, glucose, and sucrose, with small amounts of disaccharides. Among the microbiological parameters, a relatively high number of moulds and total aerobic counts were found in fresh pollen, but under the maximum admitted level.

Keywords: honeybee-collected pollen, beebread, royal jelly, composition, quality, microbiology, nutritional value

INTRODUCTION

Nutritional value, quality control and hazardous residues in foodstuffs represent a major topic of public interest (Ibáñez and Cifuentes, 2000). In the last decades, there is a growing interest in so-called “functional foods”, foods that can provide not only basic nutritional and energetic requirements, but also additional physiological benefits to the consumer. The term “functional food” was used for the first time in Japan in the 1980s and was applied to processed food that contains ingredients that conferred the benefits of some physiological functions. Nowadays a functional food is defined as a food that produce a benefic effect in one or more functional functions, increase well-being and decrease the risk of suffering from a particular medical condition (Gómez-Caravaca *et al.*, 2006).

Bee products are definitively high source of natural nutrients and also a rich source of biologically active compounds and in many cases might be considered as functional foods, or functional ingredients added to increase the nutritional value of other food products.

Bee pollen is frequently considered “the world's most perfect food” as stated on many advertisements. Bee pollen is usually composed of pollen species from various plants and can be collected from hives using different methods (Mărghitaș, 2005). It is a beehive product, which contains valuable substances like: all essential aminoacids, phenolic compounds (flavonoids and phenolic acids), vitamins, pigments (chlorophyll, carotenoids) which may act as potent antioxidants (Mărghitaș *et al.*, 2009; Kroyer and Hegedus, 2001). The major use of bee pollen today is as a food or, more correctly, as a food supplement.

Partially fermented pollen mixture stored in the honeybee combs, also referred to as “beebread” has a different composition and nutritional value than the field collected pollen pellets. The beebread has already been processed by the bees for storage with the addition of

various enzymes and honey, which subsequently ferments. One advantage is almost unlimited storability of beebread in comparison with dried or frozen pollen in which nutritional values are rapidly lost.

Royal jelly is part of the diet of honeybee larvae and plays an important role in the development of the queen honeybee (Osamu *et al.*, 2004). It is a most interesting healthy and functional food because it possesses several health-promoting and pharmacological properties (Nagai *et al.*, 2004).

Royal jelly is a complex matrix that contains water, crude protein, carbohydrates, lipids, traces of mineral salts and vitamins (Crane, 1990).

A large amount of royal jelly is sold and consumed as it is harvested. In its unprocessed, natural state, it is preferred by most producers, because it does not require any special technology, and by consumers because of its unaltered "naturalness". The fact that its taste is not very pleasant, instead of deterring consumers appears to enhance its image as a "medicine".

Moulds and yeasts spoil foods. It is therefore important to control raw materials and food products. Some strains produce mycotoxines such as ochratoxin and cause off flavors. Moulds can cause allergies and infections.

Our research aimed the analysis of chemical composition and nutritional value, together with some microbiological tests of three major commercially bee hives products: pollen loads, beebread and royal jelly.

MATERIALS AND METHODS

The research was carried out in the Laboratory for Quality Control of Bee Products, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, investigating commercially fresh bee pollen, beebread and royal jelly. Selective physicochemical parameters were determined according to Romanian and International Legislation for bee products or food samples. Water content was determined gravimetrically, drying the samples until constant weight in an oven (Binder GmbH Tuttlingen, Germany) and expressed as mg/100g; total protein content was determined spectrophotometrically (expressed as mg/kg) on a UV-1700 Shimadzu Spectrophotometer, using two different techniques (Lowry *et al.* 1951; Bradford, 1976; Seevaratnam *et al.*, 2009).

Each of the samples subjected to analysis were individually extracted three times with 5 ml ultrapure water solvent for Lowry determination, and with 5 ml phosphate buffer (pH 6,1) for Bradford determination. A centrifugation step is carried out at a speed of 4000 rpm for 25 minutes, obtaining two distinct layers: supernatant and pellet. Supernatant is collected carefully and stored until analysis (4°C).

Lowry method for protein determination, consist in the reactivity of peptides nitrogen with copper ions under alkaline conditions and the reduction of phosphomolibdenic-phosphotungstic acid (Folin Ciocalteu reagent) to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids (Dunn, 1992). Each sample is added together with protein reagent forms a coloured complex, whose intensity is directly proportional to protein content. The calibration curve was made in accordance with Lowry method using standard solutions (bovine serum albumin (BSA) stock solution) with concentrations in the range of 0.05-1 mg/ml (in 14 points), dissolved in ultrapure water (w/v) 1:1. The quantifications were performed using the calibration curve of BSA having the following equation: $y = -0.1742x^2 + 1.0229x + 0.041$, $r^2 = 0.9961$; briefly, the reference blank contained ultrapure-water, while sample contained either Lowry/Folin solution reagent. Bovine serum albumin is stored in the

freezer at a temperature of -20°C as the moisture content of solid protein can vary during storage. Three independent determinations were made for each concentration. The absorbance was measured at 750 nm.

Bradford assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. The color development is virtually complete in 2 minutes and the color is stable for about 1 hour (Pandey and Budhathoki, 2007). For determination of total proteins in pollen, beebread and royal jelly, Bradford method was used, readings were performed in a UV-VIS spectrophotometer (Shimadzu Instruments), at 595 nm. Standard protein was bovine serum albumin (BSA) used at a concentration of 1 mg/ml (100µg/ml) in distilled water as a stock solution 1:1. A standard curve and protein sample was prepared following the Bradford protocol and the following equation for the quantification was obtained: $y = 0.3404\ln(x) + 0.9985$; $r^2 = 0.9291$.

The content of total lipids was determined using Soxhlet method (Soxtherm, Gerhardt, Germany). An accurately weighted amount of sample was placed in filter paper tubes and glass bickers (previously weighted). The extraction solvent was petroleum ether, extraction time 3 hours, evaporation time 1.5 hours and cooling time 0.5 hours.

After drying until constant weight, the glass bickers were weighted again and total lipid content was calculated.

Sugar profile was determined by HPLC on a Shimadzu system equipped with a LC-10AD pump, DGU-14A degasser, SIL-10AV VP auto sampler, RID-10A refractive index detector, thermostatted at 30°C with CTO-10AS VP temperature controller of separation column (Altima Amino 100 Å 5 µm, 250 mm x 4.6 mm) with a mixture of acetonitril/water as mobile phase with 1.3 ml/min flow rate.

For the quantification of main sugars, a calibration curve in the range 4–0.5 g/100g, with regression coefficient of $R^2 = 0.9982$ for a mixture of 9 standards (glucose, fructose, saccharose, trehalose, maltose, turanose, isomaltose, erlose, melezitose) was used. Results were expressed in g/100g honey.

Microbiological and bacteriological parameters determined were total number of aerobic germs (TGN) and number of yeasts and moulds. TGN represent the number of microbial colonies grown on agar media after inoculation and incubation with the analyzed sample.

All chemicals and reagents were analytically grade purity. The experimental data were expressed as mean values \pm standard deviation SD. Statistical differences were estimated among different samples (pollen, beebread and royal jelly) at $p = 0.05$.

RESULTS AND DISCUSSION

Water content in the bee collected pollen samples was high (18.0%), because the product is commercially sold as fresh (crude) bee pollen. This of course needs special storage conditions and limited shelf life. Commercially beebread has a mean of 5.91% of water content and fresh royal jelly (usual for this product), ranged from 59.47 and 63.88%. Again, special storage conditions and limited shelf life is required for this product.

Lipid content of pollen consists of internal cytoplasm lipids and external lipids of the pollen kit. This fraction of all the tested bee products is represented by saturated, monounsaturated and polyunsaturated fatty acids. Total lipid content was found in highest quantity in beebread, expressed as wet weight sample (WW) (7.79 g/100g), but referring to

dry weight of each product, we can observe that, royal jelly is the product that has the highest amount of total lipids (10.73 g/100g).

The total protein content of the analyzed samples ranged from 16.03 g/100g in royal jelly samples to 22.1 g/100g in pollen loads when analyzing with Lowry method and between 10.28 g/100g and 22.15 g/100g when analyzing with Bradford method. For pollen loads and royal jelly there were detected higher quantities of total proteins when using Lowry method, and for beebread, higher quantities of total proteins were obtained when using Bradford method. Referring to dry weight of each bee product, royal jelly exhibits the highest quantities of total proteins, almost twice as much as the bee pollen and beebread (Tab.1).

Total protein content for collected bee pollen was in accordance with Szezęsna (2006) and with earlier studies (Kim, 1986; Serra Bonvehi and Escola Jorda, 1997). From the three commercially bee products tested, royal jelly is the richest product in respect of total proteins.

Comparable results obtained Carpes *et al.*, (2009) in Brazilian bee pollen.

In the tested multifloral pollen samples, beebread and royal jelly, monosaccharides – fructose and glucose and disaccharides – sucrose, turanose, maltose, trehalose and erlose were determined. For bee pollen and beebread, fructose was found to occur in the highest concentration (19.31 and 18.95 g/100g), while in royal jelly, fructose was not the highest monosaccharide (6.15 g/100g)(Fig.1). The content of glucose was 17.86 g/100g in bee pollen and lower in beebread (11.54 g/100g). Higher level of glucose towards fructose was found in royal jelly samples (6,54 g/100g). Sucrose was found neither in pollen samples, nor in beebread. In royal jelly, 1.45 g/100g sucrose was quantified. Other disaccharides present in the analyzed bee products were turanose, maltose, trehalose and erlose.

Carbohydrates represent an essential part of the bee collected pollen and beebread dry weight. Monosaccharides represent 94.28% from the total sugar content in bee collected pollen, 93.96% from total content in beebread and 83% from the total sugar content in royal jelly. Our findings were in accordance with those ones obtained by Szezęsna (2007), excepting sucrose content. Our investigations did not found sucrose in collected bee pollen as well as in beebread samples. Dissacharides were found in similar quantities.

There is a substantial difference in bee collected pollen, beebread and flower pollen and nectar in the carbohydrate composition (Solberg and Remedios, 1980). Royal jelly presents a low quantity of carbohydrates, the highest quantity being obtained for glucose, followed by fructose, sucrose, maltose trehalose, turanose.

Not all carbohydrates present in the bee products, mostly bee pollen and bvrbread, are nutritionally useful. An important part of the carbohydrates is represented by pectin (important structural component of the cell wall and essential in plant growth, but not known nutritional value for bees)(Aouli *et al.*, 2001).

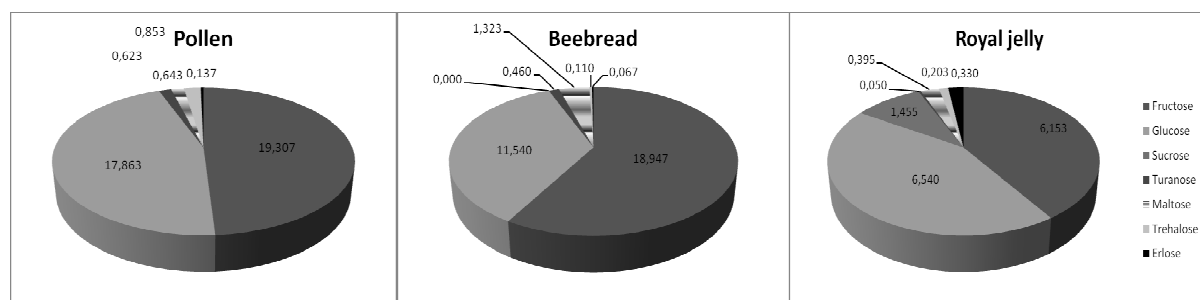


Fig. 1. Carbohydrate concentration in the tested bee products

Tab. 1

Water content, dry weight, total proteins and total lipids
of commercially fresh pollen loads, beebread and royal jelly

Sample	Water (%)	Dry weight (%)	Total lipids (g/100g)		Total proteins (g/100g)			
			WW	DW	Lowry method		Bradford method	
					WW	DW	WW	DW
Pollen	17.8	82.2	1.28	1.56	22.12	26.91	21.75	26.46
Pollen	18.2	81.8	1.32	1.61	22.15	27.08	21.59	26.39
Pollen	18.0	82.0	1.30	1.59	22.14	26.99	21.67	26.43
Mean	18.0	82.0	1,3	1.6	22.1	27.0	21.7	26.4
SD	0.2	0.2	0.0	0.0	0.0	0.1	0.1	0.0
Bee bread	5.97	94.03	7.98	8.49	21,96	23.35	22.15	23.56
Bee bread	5.84	94.16	7.59	8.06	21,89	23.25	22.09	23.46
Bee bread	5.91	94.10	7.79	8.27	21,93	23.30	22.12	23.51
Mean	5.91	94.10	7.79	8,27	21.93	23.30	22.12	23,51
SD	0.06	0.06	0.19	0.21	0.04	0,05	0.03	0,05
Royal Jelly	63.88	36.12	3.58	9.91	13.23	36,63	10.28	28.46
Royal Jelly	63.28	36.72	3.60	9.80	13.87	37.77	10.72	29.19
Royal Jelly	59.55	40.45	4.67	11.55	18.44	45.59	18.15	44.87
Royal Jelly	59.47	40.53	4.73	11.67	18.56	45.79	17.96	44.31
Mean	61.55	38.46	4.15	10.73	16.03	41.45	14.28	36.71
SD	2.36	2.36	0.64	1.01	2.87	4.92	4.37	9,11

WW – Wet weight; DW – Dry weight

Results in mycological and bacteriologic analysis show under 10^{-1} aerobic mesophilic germs/ml of diluted samples; yeast and moulds absent in bee collected pollen and beebread.

Pollen sample (crude pollen) present less than 50.000 aerobic mesophilic germs/ml diluted pollen and 100 yeasts and moulds/ml. Although, in pollen samples we find a higher number of germs, moulds and yeasts than in the other samples, the values were under the minimum level stated in Romanian Legislation.

CONCLUSIONS

There are six nutrients essential to our health: - three provide energy from calories: carbohydrates, fats and proteins and other three which do not provide calories but are important to human health: vitamins, minerals and water. All investigated bee products have a high nutritional value and benefic effects on human health. The high content of simple sugars, together with high amount of proteins, essential amino acids and monounsaturated fatty acids make from these products real natural supplements, which provide immune protection, fight against bacteria, providing energy or rebuilding tissues.

Acknowledgments. This study has been financed by the Ministry of Education and Research through the Grants 51-070/2007 and 390/2007.

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