Chemical Composition and Biological Activities of Beebread – Review

Adriana URCAN[1], Liviu Al. MĂRGHITAȘ[1], Daniel S. DEZMIREAN[1], Otilia BOBIȘ[1], Victorița BONTA[1], Carmen I. MUREȘAN[1], Rodica MĂRGĂOAN[2*]

[1] Departament of Beekiping and Sericiculture, University of Agricultural Sciences and Veterinary Medicine Cluj- Napoca, Romania
[2] Departament of Micropropagation, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania
[*] corresponding author: rodica.margaoan@usamvcluj.ro

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Abstract

Beebread is a product of the hive obtained from pollen collected by bees, to which they add honey,digestive enzymes and subsequently is stored in the combs. The bees transform the bee pollen in beebread by an anaerobic fermentation process.A proper hive management promotes beebread collection, aimed at marketing it for human consumption since it can be considered a valuable food supplement due to its content of a wide range of nutrients. Its value is given by the content in protein, amino acids, fatty acids, carbohydrates, mineral salts, polyphenols and flavonoids, which depends on the botanical source of bee pollen. The nutritional and functional composition of beebread is widely reported; nevertheless, few studies on transformation processes of the pollen to improve the availability of the compounds present in this product were found. Overall, beebread is a recent collected and consumed bee product and at this stage it can be used as a food supplement.

Keywords: *beebread, biologic compounds, chemical composition*

Introduction

Beekeepers, until some years ago, do not collect beebread since the inherent difficulty of extraction that force the beekeeper to partially destroy the hive (Fuenmayor, 2009), and preferably they collected pollen through well-designed systems of traps and containers (Almeida-Muradian*et al.*, 2005), this bee product being more intended to human consumption. However, several reports have shown how beebread has and increased availability of nutrients and bioactive components with respect to pollen (Fuenmayor, 2009; Del Risco *et al.*, 2012). Currently, specialized materials and devices have been designed to extract beebread without any damage to the hive (Zuluaga *et al.*, 2015).

A proper hive management promotes beebread collection, which aims at marketing it for human consumption. Beebread can be considered a food supplement due to its content of a high range of nutrients. One of the contributions to their high nutritional value is the presence of significant amounts of proteins, carbohydrates, lipids, vitamins and phenolic compounds as natural antioxidants. The potential application of beebread as a food and as a nutraceutical supplement depends in a greater extent on its chemical composition which varies directly with the flora of the region and the time of collection by the bees (Markiewicz-Żukowska *et al.*, 2013).

Therefore beebread has become lately a product of high commercial value and a fair evaluation of chemical composition is needed to guarantee the quality. Nowadays, there are different proposals for standardization in several countries, but Russia is the only one who already has a standard for this product (GOST R 53408-2009,2009/1/1).

Beebread is in fact fermented and naturally preserved pollen. Pollen is gathered by bees and mixed with its own digestive enzymes, carried in the hive and preserved with a tiny layer of honey and bee wax. Some studies indicate that it is necessary the fermentation process to modify the outer layer overlying the pollen known as exine, made of sporopollenin, a compound that provides chemical resistance to pollen and preserves the compounds which are within it, and responsible for the limited capacity for absorbing nutrients and bioactive substances that are inside the pollen grain (Atkin *et al.*, 2011 cited by Zuluaga *et al.*, 2015). The bees are able to transform the bee pollen in beebread by an anaerobic fermentation process. Pollen transformation in beebread occurs as a result of successive interventions of different enzymes, some species of microorganisms such as *Pseudomonas*, *Lactobacillus*, *Saccharomyces* that are naturally present in pollen, moisture and temperature (35- 36º C) (Barene at al., 2015).During this fermentation, the wall of the pollen is disrupted and in this way the beebread has a better bioavailability.Comparatively with bee pollen, beebread is better tolerated by the human organism and has a lower pH (3.8-4.3) duo to its content of lactic acid which offers a good stability during the storage at room temperature (Berene *et al.*, 2015).

Aime: This bibliographic study aims to present a brief review of the most important scientific findings and research regarding beebread chemical composition and its biological activities such as antioxidant and antimicrobial action.

Beebread chemical compositiondepends on plants from where worker bees have collected pollen and can vary widely; even in the same apiary or bee hive, no identical samples of beebread may be found (Isidorov *et al.*, 2009). Beebread has a nutritional and compositional value that differs from bee pollen and has new nutrients, such as vitamin K (Nagari, 2004).

For honey bee colonies, the nutrients needed to grow colony populations and maintain their health come from nectar and pollen. Nectar provides carbohydrates and pollen supplies the remaining dietary requirements such as protein, lipids, vitamins, and minerals (Brodschneider and Crailsheim,2010).Nevertheless, bees do not consume either nectar or pollen directly; in both cases they induce biochemical changes, so nectar is transformed into honey and pollen into beebread (Krell, 1996).

Beebread is primarily composed of water, proteins, free amino acids, some bioactive compounds, fatty acids, and carbohydrates (Del Risco, 2012).

Proteins and amino acids

Beebread protein content is one of its most regarded nutritional features; According to the literature data this value ranged from 14.1 to 37.3 g/100 g (dry basis) evaluated by using factor N x 6.25, having a 23.1 (± 2.9) g/100 g mean. It is expected that the content of protein in beebread be similar to bee pollen (about 23.8 g/100 g) (Fuenmayor *et al.*, 2014), since the biochemical process induced by bees is aimed in degrading the outer layer of the pollen grain, without any damage of inner content (Zuluaga *et al.*, 2015).

The enzymes found in beebread include amylase, phosphatase and glucose-oxidase (Salazar-González *et al.*, 2016).

Beebread contains also amino acids as glutamic acid, aspartic acid, proline, found in the largest quantity, arginine, valine, histidine, leucine, isoleucine, lysine, methionine, tryptophan, phenylalanine, threonine, cysteine, tyrosine, alanine, glycine and serine (Berene *et al.*, 2015).

The presence of proline and glutamic acid is associated with bee pollen quality; glutamic acid concentration greater than 20 mg/g indicates freshness, whereas lower proline value indicatesaging and technological process (Dominguez-Valhondo *et al.*, 2011).

Lipids

According to the literature, the lipids content of raw beebread varies widely depending on the plant origin of pollen that is produced. Lipids are represented by fatty acid which contribute to the biological value of this bee product.

There are only few papers published on the fatty acids composition of the beebread. Human and Nicolson (2006) reported only 18 fatty acids in beebread from an indigenous South African bee. Čeksterytė*et al.*, (2008) identified twenty-two fatty acids in beebread (containing >45 % rape or willow pollen) collected in the spring and summer seasons, including five ω-3, four ω-6 and three ω-9 polyunsaturated fatty acids. Octadecenoic and icosa-tetraenoic acids were the most abundant unsaturated fatty acids, constituting around 15 % of total fatty acids.

In another study, Čeksterytė and Jansen in 2012 reported the highest content (27–43.8 %) of octadecatrienoic acid (ω-3) among 22 fatty acids identified in spring rape and willow beebread. Fatty acids are compounds of high importance in fertility and health of the honeybees.

Current literature suggests that beebread is a good source of polyunsaturated fatty acids (PUFAs) that are crucial for human nutrition. PUFAs cannot be synthesized in human body endogenously and must be obtained from food.

The ratio between unsaturated/saturated fatty acids in beebread and bee pollen is closely associated with the same indices obtained in raw and processed yolk where it reached values of 0.58 and 0.64, respectively. Comparison of PUFA composition of yolk with that of beebread and bee pollen evidenced that all these products possessed the same fatty acids (Čeksterytė *et al.*, 2016).

In this respect, beebread can be considered as a potential source of PUFAs in human diet. However, in particular, scientific research exploring various properties of beebread is scarce and additional research into this topic is highly required (Muammer *et al.*, 2016).

Carbohydrates

The beebread carbohydrate content varies very widely depending on the origin of the pollen. According to chemical analysis we can say that an average content of beebread in carbohydrates is between 24.40 – 34.80% (Berene *et al.*,2015). Fructose is found in the largest amount (57,51% of the total fresh weight), followed by glucose (42,59% of the total fresh weight) and maltose (3,37% of the total fresh weight) (Stanciu *et al.*,2008).

Sucrose accounted for 0.12% which reflect its hydrolysis decomposition in the monosaccharide's during lactic fermentation of the pollen and its transformation in beebread.The remaining disaccharides (trehalose, turanoe and isomaltose) represent about 1,82% of the total fresh weight.In the natural process of fermentation which occurs in the hive, *Lactobacillus sp*., uses carbohydrates as a source of oxygen and produce lactic acid up to a concentration of 3.2%, a fact which inactivates the *Lactobacillus sp*.(Stanciu *et al.*,2008).

Minerals

Considering chemical composition, besides the compounds mentioned, beebread also includes: minerals such as Ca, Mg, P, Fe, Na, K, Al, Mn, S, Cu (Anđelković *et al.*, 2012). In beebread, potassium content occurred in the highest concentration (0.74%) followed by calcium and phosphorus (0.65%). The content in iron (121.99 mg/kg) and zinc (44.09 mg/kg) is also high in beebread (Stanciu *et al.*, 2009).

In the study made by Anđelković, *et al.*, (2012) who compared bee pollen with beebread the results show that the ash content of beebread is increased with 7.54% compared to bee pollen. Also the content of calcium, potassium, phosphorus, magnesium and iron increased compared to pollen. However, zinc and manganese content decreased. This decrease can be explained by microbial activity and chemical reactions that occur in the transformation of pollen in beebread (Foote, 1957;Haydak, 1958).

However, other factors such as geographical conditions and soil also affect the mineral content of beebread.

Beebread biological activities and effects

There are many studies that aim to evaluate the antioxidant activity of beebread samples of different geographical origin and establishing a correlation with the content of phenols, botanical origin and other compounds. The results in most of the research show large variations and significant differences in the amount and content of phenolic compounds found in beebread (Carpes *et al.*, 2007). This is due to the variation of the floral sources origin of analyzed pollen(Campos *et al.*, 2010). Some variation can also be due to the different quantification methods that have been used in the different studies.

Polyphenols are part of the chemical composition found in beebread that varies according to the year, botanical origin and collection place (Markiewicz- Zukowska *et al.*, 201;Durán *et al.*, 2014).

Several authors tested different solvents for the preparation of the extracts, such as methanol, ethanol, hexane or water, in order to evaluate their

Tab. 1. Content of bioactive compounds in beebread using different solvents (Stanciu *et al.*, 2007)

Solvent	Total phenolics (mg GAE/gbee-bread)	Total flavonoids (mg QE/gbee-bread)	Flavanones (mgNAE/g bee-bread)
Methanol extracts	22.72	0.696	7.86
Water extracts	15.84	0.352	3.41
Ethanol extracts	8.32	0.168	6.04
Methanol70: water30	11.90	0.624	5.38
Ethanol70:water30	9.12	0.256	3.12

Tab. 2. Antioxidant activity of beebread using different solvents (Stanciu *et al.*, 2007)

Solvent	DPPH Inhibition (%)	TEAC (μmol Trolox / g)	FRAP (μmol Trolox / g)
Methanol extracts	90.35	0.43	0.404
Water extracts	62.30	0.23	0.343
Ethanol extracts	55.69	0.16	0.196
Methanol70:water30	70.80	0.25	0.327
Ethanol70:water30	56.80	0.14	0.254

influence on the concentration of the bioactive compounds and their bioactive proprieties (Table 1 and 2). The data show that this content depends on the methods used for extraction. In the paper of Stanciu *et al.*, (2007), the best extraction efficiency was obtained in methanol.

Using HPLC and GS-MS methodsdifferent biologic active compounds were identified in beebread (Table 3), such as p-coumaric acid, kaempferol, two forms of quercetin, apigenin and isorhamnetin (Baltrušaitytė *et al.*, 2007b; Isidorov *et al.*, 2009). It has been suggested that the pollen of different botanical origin have different antioxidant capacity, which is more related to the specific flavone and phenolic acid profiles than to the total content of flavones (Almaraz-Abarca *et al.*, 2004) for this reason flavonoids may serve as biochemical markers of the particular plant source (Čeksterytė*et al.*, 2016). Apart from the compounds mentioned above, Isidorov *et al.*, (2009) also detected trace of ferulic and caffeic acids, chrysin and naringenin.

Tavdidishvili *et al.* (2014) analyzed the flavonoids content in Georgian beebread and also identified naringin, rutin and quercetin in amount of 20% of full content of flavonoids.

Also in 2016, Hryniewick*et al.*, identified in beebread samples lipophilic antioxidants such as α-tocopherol and coenzyme Q10 which play essential regulatory and metabolic functions in each cell of living organisms. It is involved in a regeneration of tocopheryl radicals. The biological function of α-tocopherol, and coenzyme Q10 is not to be underestimated.

Radical scavenging activity of beebread phenolic extracts was assessed by Baltrušaitytė *et al.* (2007b). The authors reported that after thermal processing, beebread had comparable inhibition of $ABTS^{+}$ radical cation and higher antioxidant activity in the DPPH reaction system than samples of honey and beebread mixed with honey.

Japanese researchers found that total phenolic content yielded 0.24 mg/ml in Lithuanian beebread ethanol extract while total phenolic content extracted from beebread with distilled boiling water or with water at 20°C was 0.20 and 0.45 mg/ml, respectively (Nagai *et al.*, 2004).

According to numerous authors (Gulcin *et al.*, 2003; Baltrušaitytė *et al.*, 2007b; Markiewicz-Zukowska *et al.*, 2013) there is a significant antioxidant activity in beebread anda significant correlation between the biologic activity of this product and its botanical origin.

Many of the present studies showed that the bioactive compounds that can be found in

Tab. 3. Bioactive compounds of beebread

Compound	Value	References
DPPH * (%)	94.0-89.9	Baltrusaityte *et al.*, 2006
DPPH (mg TE/g)	1.14	Ceksteryte *et al.*, 2016
ABTS *+ (%)	73.2-92.2	Baltrusaityte *et al.*, 2006
ABTS (mg TE/g)	4.86	Baltrusaityte *et al.*, 2006
Total flavonoids, (mg eq-quercitine/g)	1.9-4.5	Zuluga *et al.*, 2015
	0.14	Stanciu *et al.*,2008
Flavonones(mg NAE/g)	12.99	Stanciu *et al.*,2008
Total phenols (mg eq-gallic acid/g)	2.5-13.7	Zuluga *et al.*, 2015
	13.92	Stanciu *et al.*,2008
	4.9-9.5	Stanciu *et al.*,2012
	33.4-36.5	Markiewicz-Zukowska *et al.*, 2016
	21.2	Ceksteryte *et al.*, 2016
FRAP (μmoltrolox/g)	35.0-70.1	Zuluga *et al.*, 2015
	52.0	Stanciu *et al.*,2008
TEAC (μmoltrolox/g)	46.1-76.3	Zuluga *et al.*, 2015
	21.0	Stanciu *et al.*,2008
L-ORAC$_{FL}$(μmol TE/g)	3.8-11.0	Stanciu *et al.*,2012
H-ORAC$_{FL}$(μmol TE/g)	9.34-13.0	Stanciu *et al.*,2012
ORAC(mg TE/g)	626.30	Markiewicz-Zukowska *et al.*, 2016
Total antioxidant capacity (μmol TE/g)	14.9-22.96	Stanciu *et al.*,2008
	5.6-11.1	Markiewicz-Zukowska *et al.*, 2016
p-cumaric acid (%)	0.1-0.4	Isidorov *et al.*, 2009
Kaempferol (%)	0.08-0.4	Isidorov *et al.*, 2009
Tocopherol, (%)	0.3-0.5	Isidorov *et al.*, 2009
Isorahamnetin, (%)	0.4-0.9	Isidorov *et al.*, 2009
Chrysin (%)	Trace	Isidorov *et al.*, 2009
Apigenin, (%)	Trace	Isidorov *et al.*, 2009
Provitamin A (mg/100g)	200-875	Del Risco, 2004
Vitamin E 9 Tocopherol) (mg/100 g)	43.6 - 103.6	Hryniewick *et al.*, 2016
	170	Del Risco, 2004
Coenzyme Q10 (μg/g)	8.7 - 14.6	Hryniewick *et al.*, 2016
Vitamin C (mg/100 g)	6-2000	Del Risco, 2004

beebread include carotenoids (provitamin A) and vitamins C, B, E, K (Del Risco, 2004).

There are various methods available in the assessment of the antioxidant capacity of beebreadsamples. It is recommended to use at least two methods to assess and compare the antioxidant capacity (Sakanaka and Ishihara, 2008). They provide useful data, however, they are not sufficient to estimate a general antioxidant ability of the sample (Filipiak, 2001). These methods differ in terms of assay principles and experimental conditions. Consequently, in different methods, particular antioxidants have varying contributions to total antioxidant potential. The enzymatic and non-enzymatic methods are used to determine the antioxidant capacity. From the non-enzymatic methods, indirect methods (DPPH, ABTS+, FRAP) and direct methods (ORAC method) are used mostly.

Nevertheless, the wide varieties in pollen species, the recognized link of pollen to geographical origin and the lack of knowledge in some aspects, restrict the generalization of the results (Zuluaga *et al.*, 2014).

Beebread contain enzymatic and non-enzymatic antioxidants: glucose oxidase, catalase, ascorbic acid, flavonoids, also phenolic and other organic acids, producing nutritional and biological effects: antimicrobial, antioxidant, anti-inflammatory (Baltrušaitytė *et al.*, 2007a, Baltrušaitytė *et al.*, 2007b, Čeksterytė, 2002).

Until now, there are only a few studies regarding the effects of beebread in human health. The benefits of beebread on the hepatic function and blood parameters in alcohol abuse patients suffering from chronic hepatitis was demonstrated in the study of Čeksterytė *et al.*, (2012). The results showed that beebread helps to regulate the lipid metabolism and exerts a positive effect on the immune system of patients suffering from chronic arthritis and cardiovascular diseases and type 2 diabetes. The conclusion of this study was that beebread used together with medicaments and Livosan supplement exerted a hepatoprotective effect and improved liver function.

Good results of treatment with pollen and bee bread were gained in geriatrics in the symptoms of early old age as well as in neurasthenic inertia in older people (Komosinska-Vassev *et al.*, 2015).

The detoxifying activity of pollen and bee bread in phenomena such as occupational diseases, heavy metal contamination, industrial gases and dusts, and drugs (antirheumatic and anti-inflammatory preparations and antibiotics) should also be mentioned (Eraslan *et al.*, 2009).

Regarding the biological effects of beebread on animals there is a study of Awad *et al.*, 2013 who used beebread as a growth promoter and natural antioxidant in the Sinaichickens diets.The results were encouragingenhancing growth performance and meat composition.

Future studies arerequired to demonstrate other benefic effects of bee bread on human and also on animal health.

Digestibility of beebread

The quality of bee pollen, in terms of nutrition, depends mainly on its digestibility and bioavailability, which appears to be closely related to some morphological characteristics of the outer wall of the pollen (Bogdanov, 2011; Cook *et al.*, 2003).

Previous researches suggest that the availability of nutritional and bioactive compounds of beebread is limited and, therefore, its use as food (Cook *et al.*, 2003). In the last years, there have been some doubts on the ability of the human digestive system to break the outer layer who overlying the pollenand to absorb nutrients and bioactive substances that are inside it.

Different simulations *in vitro* of human digestion suggest that the beepollen is partially digested between 48% and 59% (Campos *et al.*,2010) and anthropological studies found coprolites (dried human excretions) with intact pollens, which has been used by some researchers as evidence of the strength of the outer wall of pollen, even to gastric acid (Rimpler *et al.*, 2003).

This study reflects the need to find alternatives for transforming beepollen for an integral use of this product. Despite the absence of conclusive data, the common view of different researchers is that bee pollen is insufficiently digested and a breaking of this outer wall would improve the digestibility and bioavailability (Campos *et al.*,2010;Rimpler *et al.*, 2003).

The beebread has a better bioavailability because the outer layer who overlying the pollen is partly destroyed by the natural fermentation process, therefore, the functionally and energetically rich content of pollen can be assimilated and used easier by the human body (Mutsaers *et al.*, 2005).

Experiments regarding *in vitro* human digestion revealed that pollen is partially digested; specifically between 38.7% and 85.3% of protein was digested/100 g pollen, while the digestibility of beebread instead is until 94.7 % protein digested/100 g bee-bread (Zuluaga *et al.*, 2015).

Antimicrobial and antitumor activity

Increasing evidence suggests beebread's potential therapeutic benefits, including antioxidant (Leja *et al.*, 2007; Kroyer and Hegedus, 2001; Roldán *et al.*, 2001), and antimicrobial properties (Basim *et al.*, 2006; Carpes *et al.*, 2007; Morais *et al.*, 2011).

Beebread could be useful in prevention of diseases where free radicals are involved due to its antioxidant proprieties (Pascoal *et al.*, 2013, Audisio *et al.*, 2005; Mutsaers *et al.*, 2005;

Baltrušaitytė *et al.*, 2007a). It is considered to be a natural health food which constitutes a potential source of energy and functional components for human consumption (Silva *et al.*, 2006), with a wide range of therapeutic properties.

Abouda *et al.*, (2011) studied the antimicrobial activity of samples of beebread from different regions from Morocco on antibio-ressistent bacterial strains isolated from human pathology including *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. The results revealed that most of strains were inhibited by the dilution ½ and ¼. The results revealed that all the samples showed strong antimicrobial activities on the bacterial strains. Moreover, the Gram positive bacteria were more sensitive to beebread than Gram negativebacteria.

Sobral *et al.*,(2017) studied the antitumor activity of beebread collected in northeast Portugal against different human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma), and also against non-tumor liver cells (porcine liver cells, PLP2). Beebread samples showed moderate antitumor activity; however, none of the beebread samples have shown toxicity for normal cells.

Conclusions

Beebread is regarded as a valuable special food. Its value is given by the high content in proteins, essential amino acids, fatty acids, carbohydrates, mineral salts and bioactive compounds, which depends on the botanical and geographical origin. Beebread composition and bioactive proprieties have not been studies thoroughly until now and are only few publications which present detailed studies of its chemical composition and proprieties, but worldwide interest increases with highlighting chemical and therapeutic properties of beebread.

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