Prospects for processing bee bread as a natural adaptogen

Irina Prokhoda[1*], *Elena* Eliseeva[2], *Elena* Stratienko[2], *Nataliya* Katunina[2] , and *Nadezhda* Peshkova[2]

[1]Bryansk State Agrarian University, Bryansk, Russian Federation
[2]Bryansk State University named after Academician I.G. Petrovsky, Bryansk, Russian Federation

Abstract. In the work were studied complex lipids, amino and fatty acid composition of flower pollen (pollen) and bee bread and were scientifically substantiated it`s adaptogenic and antitoxic effects on a living organism. The results of the research showed that all the studied pollen samples collected in the Bryansk region contain high amounts of glutamic and aspartic amino acids; among the fatty acids are linoleic and palmitic. The quantitative composition of amino and fatty acids of canned dried parchment does not significantly change during storage for 1 year. The adaptogenic effect of bee bread is explained during intoxication with nitrates and fluorides.

1 Introduction

Bee products are the unique creation of nature: plants and bees. Bee products, as biologically active creations of bees (honey, propolis, flower pollen or pollen pellets, bee bread, royal jelly, bee venom, wax) combine both the nature of plant and bee origin, which ensures their uniqueness. These unique products are needed by bees, but people are considering the possibility of using them in a wider range. They are used for dietary and medical purposes, recently technologists and functionalists have begun to pay much attention to these products. Currently, the country has an epidemiological situation associated with covid-19, which has led to a decrease in human immunity and an increase in the incidence of the population.

The complexity of the situation is that, on the one hand, chemical agents that affect human health (such as nitrates and other fertilizers, heavy metals, pesticides, etc.) are necessary for the intensification of food and feed production, on the other hand, an excess of these substances leads to a number of undesirable consequences [1].

Environmental stress, high emotional stress, stress factors of everyday life require a search for adaptogens - substances that increase the body's defenses, vital activity, prevent aging of the body and adverse environmental impacts [2].

Bee products must always be of high quality. Each product must be of natural origin. Pollen and bee bread are necessary for bees, including young ones. With the appearance of the first pollen in the nest, the bees begin to grow "babies". They produce royal jelly and

* Corresponding author: irina.proxoda@yandex.ru

feed the brood, secrete wax and build honeycombs, produce enzymes, specific fatty acids and other substances that make up the biologically active secretions of their glands [1-2].

Recently, bee pollen has attracted great interest among beekeeping products. Bees harvest pollen from flowering plants and bring it to the hive during the season, turning it into pollen. It has a high humidity, up to 25-35%, which depends on the amount of nectar (honey) and the secretion of the glands that the bee uses to form the pollen. Freshly harvested pollen must be preserved.

The most common method of pollen stabilization is drying. Pollen dried at a higher temperature contains less sugar, free sulfhydryl groups (active amino acids and protein substances). Flower pollen collected by bees (pollen) has many useful properties and does not have a toxic effect on the body.

Studying the development of the food industry, a promising direction for processing plant raw materials may be pollen, as an important component of preventive human nutrition. In this paper, complex lipids, amino and fatty acid composition of flower pollen (pollen) and bee bread were studied from the point of view of their use as the strongest natural adaptogen and their adaptogenic and antitoxic effect on a living organism was scientifically substantiated.

2 Materials and methods

The object of the study was pollen from different plant species of both collection periods. The pollen was preserved by drying to 10-12%. Eight samples of pollen from different areas of the Bryansk region, stored for 3 and 15 months in the dark at a temperature of 3-5°C, were studied. Amino acids were determined using an automatic analyzer T 339 manufactured by Microtechna (Praha) by chromatography on ion exchangers, and fatty acids were determined by gas-liquid chromatography using a CHROM-5 chromatograph with a flame ionization detector.

The studies were conducted on 6 groups of white male rats with an initial body weight of 180±5 g, the animals were given a full diet for 60 days.

The following solutions were administered to them intragastrically (through a probe) - 1 series of experiments: group 1 (control): distilled water; Group 2: aqueous solution of $NaNO_3$ (490 mg nitrate ion/kg body weight). Group 3: aqueous emulsion of lipophilic extract of flower pollen (pollen pellets) (at a rate of 1 g pollen/kg body weight). Group 4: the same lipophilic extract of pollen in an aqueous medium containing nitrates (490 mg/kg). Group 5: aqueous emulsion of alcoholic extract of pollen pellets (1 g pollen/kg body weight). Group 6: the same alcoholic extract of pollen in an aqueous medium containing nitrates (490 ml/kg).

In series II of experiments to study the effect of bee bread on some biochemical indices of animal organisms, five groups of rats were treated under similar conditions: group 1 (control): distilled water; group 2: aqueous solution of NaF (9.4 ml fluoride ion/kg body weight); group 3: aqueous suspension of bee bread (at a rate of 1.2 g of bee bread/kg body weight); group 4: the same suspension of bee bread in an aqueous solution of NaF (9.4 mg of fluoride ion/kg body weight); group 5: forced feeding of crushed dry bee bread (1.2 g/kg) on an empty stomach.

All solutions were administered in a volume of 5 ml/kg of body weight. At the end of the experiment, the animals were decapitated. The following were determined in the animals' blood: concentration of methemoglobin, oxyhemoglobin and total hemoglobin, calcium, phosphorus, iron, total lipids, triglycerides, total protein and sulfhydryl groups of protein.

The activity of lactate dehydrogenase, gamma-glutamyl aminotransferase and alkaline phosphatase was determined in the blood serum. The concentration of cytochrome P-450,

vitamins A1, A2, B1, B2 and protein, as well as carboxylesterase activity were determined in the liver homogenates. During the experiment, the animals were weighed and carefully examined (every 7 days).

In each sample, amino acids were determined by acid hydrolysis at 105°C for 24 hours, followed by determination on a KLA-3B analyzer. Total nitrogen was determined by the Kjeldahl method [3], protein nitrogen - by the Barnstein method [4].

Crude and true protein were calculated by multiplying total and protein nitrogen by a factor of 6.25. As a basis for the nutritional value, calculations were made for essential amino acids in crude protein and the results were compared with literature data on amino acid requirements in bee feed.

3 Results and Discussion

According to many authors, all bee products are excellent natural adaptogens [5]. From this point of view, flower pollen deserves special attention. Along with studies of its chemical composition and biological activity, the results of experiments on experimental animals showed that hydrophilic extracts of flower pollen (pollen) exhibit anti-nitrate activity [6].

Pollen and bee bread are finding increasing application in medicine and dietetics. These bee products, rich in complete proteins, essential fatty acids, vitamins, and other biologically active substances, have a beneficial effect on the human body. Numerous researches have been conducted to study the biochemical composition of pollen. However, during the fermentation of bee bread, profound chemical changes occur in it [7-8].

Some authors consider [8] that the nutritional value of bee bread and pollen differs little, since the protein concentration of bee bread and pollen is similar. According to M.A. Atallah, the amino acid composition of pollen and bee bread differs significantly: the concentration of some amino acids decreases to 20%, the activity of enzymes changes dramatically.

During fermentation, bee bread is preserved by lactic acid [9], and the processes occurring in it should be stabilized [10].

However, there are few studies that analyze changes in the chemical composition of bee bread. This problem is the subject of that article.

As a result of our own research, 16 amino acids and 13 fatty acids were identified. The distribution of the quantitative composition of amino acids in the bee bread protein is presented in Table 1.

The obtained data showed that the amino acids glutamic (up to 29 mg/g) and aspartic (up to 30 mg/g) were found in the largest quantities in bee bread. The smallest quantities of methionine (up to 2 mg/g) and lysine (up to 4 mg/g) were found in the studied bee bread samples. The obtained data show that acidic (glutamic and aspartic) and subacidic (serine, threonine, tyrosine) amino acids prevail over alkaline (lysine, arginine, histidine) amino acids. The quantitative fluctuation of the concentrations of the same amino acids in different samples is not significant.

This is confirmed by the calculated dispersion ($0.05<G<0.2$). It should also be noted that the quantitative composition of amino acids in the protein of bee bread stored for 3 and 15 months does not differ significantly (Table 1).

Essential amino acids accounted for up to 42%. Comparing the amino acid concentrations of bee bread and pollen stored for 1 year, it can be seen that the amino acids of bee bread slightly prevail over the amino acids of flower pollen. This can be explained by the fact that during storage the humidity of bee bread decreases compared to pollen and during the recalculation process occurs a difference in results. On the other hand, it is possible that bee bread preserves amino acids better than pollen.

Table 1. Amino acid composition of the bee bread protein мг/г, (n=8, M±m).

No.	Amino acids	Bee bread		Pollen
		Shelf life: 3 months	Shelf life: 15 months	Expiration date. 12 months
1		2	3	4
1.	Glutamic	29.38±0.05	29.41±0.08	25.4
2.	Aspartic	29.92±0.02	29.85±0.04	20.5
3.	Leucine	18.70±0.04	18.68±0.02	16.0
4.	Alanine	12.69 ± 0.08	12.81±0.08	10.8
5.	Serin	13.31 ± 0.09	13.29 ± 0.06	10.5
6.	Glycine	11.13 ± 0.04	11.24 ± 0.07	10.2
7.	Threonine	11.49 ± 0.04	11.47 ± 0.05	9.4
8.	Valin	13.49 ± 0.08	13.08 ± 0.04	9.03
9.	Isoleucine	10.59 ± 0.07	10.47 ± 0.04	8.9
10.	Proline	9.04±0.06	9.16±0.03	8.7
11.	Phenylalanine	14.41±0.03	14.38±0.05	7.7
12.	Tyrosine	12.98±0.06	12.15±0.04	7.8
13.	Lysine	3.89±0.05	3.87 ± 0.05	3.7
14.	Histidine	12.21±0.05	12.17±0.08	4.3
15.	Arginine	6.61 ± 0.07	6.08±0.04	2.2
16.	Methionine	2.02 ± 0.08	1.98±0.04	-

The fatty acid composition of bee bread stored for 3 and 15 months was also studied. 13 fatty acids were determined and identified in the composition of bee bread lipids (Table 2).

Table 2. Composition of fatty acids in bee bread (% of total fatty acids).

No. p/p	Fatty acid	bee bread	
		Shelf life: 15 months	Shelf life: 3 months
1.	Lauric	0.27±0.09	0.23 ± 0.08
2.	Myristic	0.41±0.09	0.38 ± 0.09
3.	Myristoleic	1.11±0.07	0.76 ± 0.08
4.	Palmitic	35.92±0.04	25.76 ± 0.06
5.	Palmitoleic	14.36±0.09	17.11±0.08
6.	Stearic	0.93±0.09	0.85 ± 0.09
7.	Oleic	5.14±0.04	4.16±0.05
8.	Linoleic	8.11±0.06	11.71 ± 0.07
9.	Linolenic	28.26±0.08	27.55 ± 0.09
10.	Gadoleic	0.47±0.07	0.41 ± 0.09
11.	Arachidonic	4.08±0.06	4.35 ± 0.09
12.	Eruic	1.97±0.09	2.24 ± 0.06
13.	Clupinodonic	3.17±0.06	3.39 ± 0.06

The results showed that in all the studied samples the fatty acids linolenic (up to 34%) and palmitic (up to 30% of the total amount of fatty acids) were determined in the greatest quantity. The content of oleic, linoleic, arachidonic fatty acids fluctuates within 3-10%. No

reliable difference was found between the fatty acid composition of bee bread stored for 3 and 15 months, (p>0,05). Attracts attention the presence of a small amount of meristoleic and arachidonic acids in the lipids, typical of lipids of animal origin. The ratio (31%) of saturated and unsaturated (61%) fatty acids is 1: 2. Fatty acids, like amino acids, are divided according to their importance for the body into replaceable and essential (linoleic, linolenic, arachidonic), which in the studied bee bread make up to 48% of all fatty acids.

2. Scientific substantiation of the adaptogenic and antitoxic effect of pollen or bee bread, as well as their mixture, did not lead to any noticeable changes in the appearance and internal organs of rats. The body weight and liver of animals between groups did not differ statistically significantly. However, animals that received fluoride ion solutions were distinguished by more aggressive behavior. The results of biochemical studies in the blood and liver of animals reflect those changes in the body that can lead to a pathological condition of the animal, and are thus diagnosing chronic exposure to small doses of substances in the environment around us. In order to reveal the most effective antitoxic effect of pollen, we prepared its lipophilic and alcoholic extracts. The lipophilic complex was prepared by extracting flower pollen with diethyl (medical) ether under gentle conditions (without prolonged heating at a temperature of 37-40°C) and subsequent complete evaporation of the solvent. The lipophilic extract contains plant pigments - carotenoids, flavonoids, lipids, fatty acids, fat-soluble vitamins. After separating the ether extract, the same pollen was subjected to alcoholic extraction. The resulting alcoholic extract contained insignificant amounts of flavonoids and carotenoids. Analysis of the obtained data showed that both lipophilic (group 3) and alcoholic extracts (group 5) of pollen do not have a statistically significant - pronounced effect on many biochemical indices of the blood serum and liver of experimental animals. However, under the influence of the lipophilic extract, the concentrations of oxyhemoglobin and total hemoglobin in the serum of animals increased significantly (by 20 and 14%, respectively, compared to the control) and insignificantly - the concentrations of calcium and phosphorus (by 21 and 11%, respectively, Table 1). The increase in the concentration of the latter indicates a favorable effect of the components of the lipophilic extract of pollen.

Control values of such parameters as methemoglobin, cytochrome P-450, carboxylesterase, lactate dehydrogenase indicate that the studied lipophilic extract of pollen is non-toxic. It should be noted that the alcoholic extract unreliably increased the concentration of methemoglobin (by 10.3%), which suggests a slightly toxic effect of the alcoholic extract, although the activity of microsomal oxidation enzymes did not deviate from the norm.

Administration of nitrate solutions (as well as nitrites together with fluorides or fluorides alone) to animals leads to reliable shifts in almost all 14 of the 20 biochemical indices studied by us compared with the indices of the control group (Table 2). An increase in the concentrations of methemloglobin (by 47 and 18% for nitrates and fluorides, respectively), sulfhydryl groups of protein (by 70% for fluorides), a decrease in the concentrations of oxyhemoglobin and total haemoglobin (by 20 and 10%), cytochrome P-450 (by 18 and 26% for nitrates and fluorides, respectively) indicates the toxicity of the chemicals studied. Under their influence, the activity of alkaline phosphatase and carboxylesterase decreased to 81% (Table 1 and Table 2), and the activity of lactate dehydrogenase and the concentration of total lipids increased to 118 and 120%, respectively. An increase in the concentration of calcium, phosphorus and iron in the blood serum to 117, 122 and 178%, respectively, was also noted, indicating disturbances in the macroelement composition of the blood.

The introduction of extracts of pollen with nitrates was observed, a reliable decrease in the methemoglobin concentration to the control level and normalization of almost all the parameters that had changed under the influence of nitrates was observed (Table 1).

The concentration of total protein in the blood and liver, as well as the concentration of total lipids, vitamins B1, B2 and lactate dehydrogenase activity in all groups did not change significantly. A reliable increase in the concentration of vitamin A2 found in the group of animals administered an alcoholic extract of pollen (by 25% of the control).

The expected accumulation of fat-soluble vitamins in the liver of animals under the influence of the lipophilic extract of pollen was not confirmed. Along with studies of various pollen extracts, experiments were conducted on animals with naturally preserved flower pollen-perga. Crushed in the form of an aqueous suspension and dry perga were used. Fluorides, like nitrates, lead to disruptions in biochemical processes in the body (Table 3).

However, when introduced together with bee bread, they lose their toxic effect. The antifluoride effect of bee bread is clearly seen in the example of cytochrome P-450 and gamma-glutamyl aminotransferase activity: a decrease to 28% under the influence of fluorides and a partial restoration by 19% in the presence of bee bread. The increased concentration of sulfhydryl groups of protein (by 70% - 2nd group) reaches a normal value under the influence of bee bread. It should be noted that under the influence of both studied forms of bee bread, the concentration of triglycerides decreased (by 24 and 30%, respectively). A reliable increase in the concentration of oxyhemoglobin and total hemoglobin when introducing bee bread to animals is consistent with the effect of pollen. According to the study the concentrations of vitamins did not change.

Table 3. Changes in some biochemical parameters of blood (A) and liver (B) of rats under the influence of fluorides, bee bread and their mixture.

Group of rats	1st control	2nd fluorides	3rd solution of bee bread	4th solution of bee bread with fluorides	5th dry bee bread
Methemoglobin, % of total hemoglobin	0.77 ± 0.09	0.98 ± 0.06 118.2**	0.69 ± 0.06 89.6**	0.84 ± 0.06 109.1**	0.66 ± 0.06 85.7**
Oxyhemoglobin, mEq/L	0.083 ± 0.001	0.083 ± 0.001 100	0.087±0.001* 105	0.085±0.001 101.8	0.091 ± 0.001* 109
Total hemoglobin, g/l	134.8 ± 1.9	133.6 ± 1.3 99.1	141.4±1.1* 105	136.3 ± 1.7 101	146.8 ± 2.8* 109
Iron, mmol/l	48.1 ± 4.92	41.79 ± 7.56 87	34.5 ± 2.42* 72	45.01 ± 7.97 94	40.51 ± 3.95 84
Gamma-glutaminotransferase, E/l	329 ± 49	93 ± 16* 28	248 ± 30 75	154 ± 43* 47	272 ± 36 83
Triglycerides, mmol/l	140 ± 0.12	1.16 ± 0.20 83	1.06 ± 0.15 76	1.23 ± 0.18 88	0.98 ± 0.19 70
SH-group of protein, mmol/l	3.81 ± 0.32	6.46 ± 1.98* 170	3.53 ± 0.80 93	4.23 ± 1.12 111	4.49 ± 1.01 118
Cytochrome P-450, nmol/l	56.8 ± 1.7	42.0 ± 4.4* 74	58.2 ± 0.9 102	51.6 ± 2.0 91	61.0 ± 4.9 107
Carboxylesterase, mkmol/l /min./g	37.3 ± 2.3	30.1 ± 1. 9* 81	34.6 ± 2.5 93	35.6 ± 2.7 94	38.0 ± 1.4 102

* - Results differ significantly from the control; ** - % of control.

Based on the data from the analysis of biochemical indicators, we recommend using bee bread as a preventive adaptogenic agent.

4 Conclusion

The studies have shown that among certain amino acids of bee bread harvested in different areas of the Bryansk region, glutamic and aspartic amino acids are contained in the largest quantities; among certain fatty acids - linoleic and palmitic. The quantitative composition of amino and fatty acids of bee bread preserved by drying does not change during storage for 1 year.

The results of the studies have shown that lipophilic, alcoholic extracts and a hydrophilic solution of bee bread do not exhibit a toxic effect on the studied biochemical parameters of animals. In case of intoxication with nitrates and fluorides, the adaptogenic effect of bee bread is manifested.

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