Influence of technology on the quality indicators of the composition of drone brood and royal jelly

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Abstract. Drone brood homogenate and compositions based on it are gaining popularity as functional food products and biologically active food additives. It has antioxidant, androgenic, anabolic, sedative, antidepressant, immunotropic, cosmetic and other properties. Royal jelly and drone brood have a pronounced biological activity, as they contain a large amount of decenoic acids, sulfhydryl groups, proteins, peptides and other biologically active substances. The possibility of expanding the spectrum of biological activity determines the relevance of developing a product containing a composition of drone brood and royal jelly. A significant superiority of the safety of biologically active substances in adsorbed drone brood and royal jelly in comparison with lyophilized ones was shown, which indicates the preferred method of stabilizing the combined product. The purpose of the work was to compare two technological methods for preparing a combined product. The first method consisted in the joint adsorption of royal jelly and drone brood, and the second - in separate adsorption and drying, followed by joint dry granulation of the components. It is shown that the differences in the physicochemical characteristics of products obtained by different methods are insignificant. The composition has intermediate values of physicochemical indicators between drone brood and royal jelly.

1. Introduction
Royal jelly and drone brood have high biological activity and contain a large amount of decenoic acids, sulfhydryl groups, proteins, which determines their prospects for human health improvement.

The amino acid composition of drone brood has been studied. A fairly high content of glutamic acid, proline, valine, alanine, tyrosine, lysine, leucine, arginine, serine, and histidine was found in it. The total content of amino acids in drone brood is from 37.6% to 40.6%, which indicates its high nutritional value and biological activity [1].

The stimulating effect of drone brood on the white blood system was noted, which leads to an increase in the number of leukocytes in experimental animals (pigs) by 9.5% compared with control animals. Also, the amount of protein in the blood serum of the experimental animals increased by 12.4%, and the albumin content increased by 18.8%. The concentration of total cholesterol in animals of the experimental group is 11.5% lower than that of the control group, and HDL is 20.7% higher. The anabolic effect of drone brood was manifested in an increase in the daily weight gain of the experimental animals by 6.8% compared to the control ones [2]. A statistically significant decrease in the concentration of testosterone in the blood of pigs at the age of 115 days was shown when drone brood was obtained with feed at a dose of 25 mg / kg. The weight of the animals and its daily gain were also greater than in the control group. Also, by the 115th day of the experiment, the
concentration of cortisol in the blood of the animals decreased, and by the 175th day it increased in comparison with the control [3]. It was suggested that the drone brood homogenate has an androgenic effect on sexually mature pigs, inhibits the development of follicles; therefore, it is not recommended to use it in animals of reproductive age [4].

An increase in the weight of pigs that received drone brood at a dose of 25 mg / kg compared to control animals was shown. At the same time, the thickness of the subcutaneous fatty tissue and the fat content in the muscle tissue decreased, which demonstrates the anabolic effect of drone brood [5].

The cytoprotective effect of drone brood and royal jelly on alveolar macrophages after two-hour incubation in 1% solution of the test product was revealed. In the drone brood solution, the cell survival was 41.5%, and in the royal jelly solution - 58.2% higher than in the control [6].

When comparing the weight gain of chickens, it was noted that at a dose of 6 g / kg of live weight, drone brood has the maximum stimulating effect, with an increase in the dose to 8 g / kg this effect decreases, and doses of 10, 12 and 15 g / kg inhibit the growth of chickens, which the authors associate it with an earlier completion of tissue maturation under the influence of biologically active substances contained in drone brood [7]. The doses tested seem to be unreasonably high, but the experiment demonstrates very low toxicity of drone brood. The low toxicity of the product based on lyophilized drone brood for white mice was shown, the LD50 for oral administration was 20 g / kg, with intraperitoneal administration - 7 g / kg [8].

An increase in the resistance of hamsters to swimming load under the influence of adsorbed drone brood and tincture of schizandra seeds was shown. On the first day of the experiment, swimming activity in the group of animals that received schizandra tincture, adsorbed drone brood, and their combination, swimming activity is insignificantly (less than 0.1%) lower than in the control. On the seventh day, swimming activity increased compared to the control by 7.7%, 11.5% and 10.2%, respectively, on the 14th day - by 9.8%, 17.7% and 20.3%, respectively, and on the 21st day - by 13.1%, 12.8% and 17.6%, respectively. Moreover, morphological changes in the heart muscle caused by intense physical activity were less pronounced in the experimental groups than in the control [9].

Some hypertrophy of skeletal muscles of hamsters was revealed during physical exertion both under the influence of schizandra tincture and under the influence of adsorbed drone brood. With the combined use of schizandra tincture and drone brood in response to physical activity, pronounced hypertrophy of skeletal muscles developed in combination with an improvement in muscle blood supply [10].

The stimulating effect of the veterinary drug SITR on the growth and bioconversion of feed during injection in young turkeys has been shown [11]. SITR is patented [12]. However, keeping drone brood in the refrigerator, proposed by the authors of the patent, can reduce its quality. The light gray color of the larvae is considered a sign of their spoilage, and according to GOST R 55324-2012 is not allowed. The use of phenol as a preservative is not desirable due to its high toxicity. Injection use can be dangerous due to the immunogenicity of a foreign protein, which, when re-administered, can cause hypersensitivity reactions, including fatal ones.

In an experiment on animals (rams), a positive effect of the drug Apistimul, based on lyophilized drone brood at a dose of 15 mg / kg * day, on blood and reproductive function parameters was established. In experimental animals, the volume of ejaculate increased by 30%, the concentration of spermatozoa in it increased by 14.3%, and the motility of spermatozoa also increased. The concentration of hemoglobin and erythrocytes in the blood of experimental animals increased. The physicochemical parameters of the obtained preparation were determined, characterizing the content of biologically active substances in it, as well as the content of hormones: testosterone, estradiol, progesterone, prolactin and cortisol. These data are in good agreement with the results of other studies [13].

Drone brood has been studied as a component of nutritional support in patients with age-related situational depression. When a drone brood is taken, depressive symptoms decrease or disappear, which the author associates mainly with the normalization of androgen metabolism [14].

An ointment for dilating the cervix during childbirth has been developed, containing 5% drone
brood. The developed ointment received a patent [15]. A product from a lyophilized homogenate of drone larvae “Bilar” has been developed and is being produced [16].

A review has been published on the homogenate of drone brood, in which the accumulated data on the chemical composition, methods of storage and stabilization of drone brood, its biological activity, and its use in nutrition and medicine have been systematized. Biological activity studies have been carried out in animals and humans. The ability of drone brood to stimulate the immune system, in particular, to increase the production of antibodies and enhance the response of T-lymphocytes, has been shown. The androgenic effect contributes to the normalization of reproductive function. Drone brood reduces the severity of oxidative stress and reduces the risk of death caused by disorders of the cardiovascular system [17].

In the USA, a method for restoring male libido with the use of dietary supplements for food containing drone brood has been patented [18]. A US patent was obtained for a dietary supplement containing drone brood, which helps to normalize androgen metabolism in women [19]. A method for the treatment of androgen deficiency in women [20] and a drug for the treatment of androgen deficiency has been patented [21].

When studying the possibility of obtaining drone brood as a source of food products in Danish apiaries, it was shown that from one bee colony during the season it is possible to obtain from 0.184 to 4.035 kg of combs with drone brood, on average, 1.776 kg, which corresponds to 1.064 kg of marketable biomass of drone brood for the colony. It is potentially possible to produce 80 tons per year of drone brood for functional nutrition, which will also increase the economic sustainability of beekeeping. To determine the amount of available product, pieces of combs with brood were frozen at -20 °C, weighed, broken into small pieces and frozen in liquid nitrogen, after which the wax was separated by rubbing the material with gloved hands, after which the amount of brood in each comb was calculated and its weight recorded. The duration of the brood harvesting season was determined, which was 15 weeks from April 28 to August 3. The largest quantities of brood were obtained on two dates: May 26 and June 23 [22].

Comparative studies of preparations of royal jelly, drone brood, and larvae confirmed a significant difference in their chemical composition. Seven carbohydrate components were identified and quantified by HPLC. Glucose, fructose and sucrose were predominant. Lipids were determined by the Soxhlet method. Protein content determined by the Kjeldahl method. 31 amino acids were identified by liquid chromatography with a mass selective detector, including all amino acids essential for humans [23]. The method of gel filtration identified two forms of cathepsin D of drone larvae - high molecular weight with a mass of 100-120 kDa and low molecular weight with a mass of 30-40 kDa. Both of these forms are contained in drone larvae at all stages of development, but their ratios change. A specific inhibitor of cathepsin D, pepstatin, has little effect on the studied enzyme. The optimum pH of both forms coincides and corresponds to pH 4.5 [24].

During the development of drone larvae from 4-5 days to 8-9 days, the content of proteins increases, while peptides, on the contrary, decreases [25]. A decrease in the mass fraction of decenoic acids by 10 times during the lyophilization of drone brood was noted [26].

In the hydrolysates of honeybee pupae obtained using alkalase, neutralase, trypsin and papain, peptides were found that have the ability to inhibit the angiotensin-converting enzyme. The neutral hydrolysates showed the highest ACE inhibitory activity. The effective concentration of the three most active inhibitors was 6.7, 47.8 and 223.9 μmol. The processes of cleavage of these peptides in the gastrointestinal tract have been modeled. These studies can be used to expand the field of application of hydrolysates of honeybee pupae in the food and pharmaceutical industries [27].

2. Materials and methods
There are several stabilization methods, one of which is adsorption. It is necessary to identify the optimal method of adsorption, most contributing to the preservation of the nutritional and biological value of the composition of royal jelly and drone brood.

The material for research was dry adsorbed drone brood, dry adsorbed royal jelly, compositions of
royal jelly and drone brood prepared in two ways. The following physicochemical parameters of adsorbed drone brood, royal jelly, as well as their compositions have been determined:

- humidity - refractometric in native samples, by drying to constant weight for wet and dry adsorbed;
- oxidizability index - by a method based on the ability of the test samples to discolor a solution of potassium permanganate in an acidic medium;
- hydrogen index (pH) - potentiometrically on a pH meter with a sensitivity of 0.01 for a 2% solution;
- mass fraction of decenoic acids - alkaliometrically after isolation of the fraction of decene acids;
- mass fraction of crude protein - by burning a sample in a Kjeldahl flask with subsequent titrimetric determination of the released ammonia;
- free acidity - by potentiometric titration of a solution of the test product to pH 8.3;
- acid number - alkaliometrically in an alcohol-ether medium, the indicator is phenolphthalein;
- iodine number - iodometrically in chloroform and glacial acetic acid, indicator - starch.

3. Results

The humidity of adsorbed drone brood and royal jelly was 0.62% for both products. With joint adsorption, the humidity is slightly higher - 0.71%, and with joint granulation - 0.99%. The oxidizability index of drone brood was 6.93 s, and royal jelly - 3.2 s. With joint adsorption and granulation 4.6 s and 3.53 s, respectively. The hydrogen index of drone brood was 5.52, royal jelly - 4.58, with joint adsorption 4.6, and with joint granulation 4.65 pH units. The mass fraction of decenoic acids in drone brood was 0.098%, in royal jelly - 0.58%, with joint adsorption - 0.195%, and with joint granulation - 0.178. The mass fraction of crude protein in the drone brood and royal jelly was 3.07% and 3.9%, respectively. With joint adsorption and granulation - 4.57% and 3.97%, respectively. The free acidity of drone brood was 55.34 mEq / kg, royal jelly - 100.62 mEq / kg, with joint adsorption - 73.85 mEq / kg, and with joint granulation - 62.28 mEq / kg. The acid number of drone brood was 5.47 mg / g, royal jelly - 6.93 mg / g, with joint adsorption - 6.62 mg / g, with joint granulation - 5.82 mg / g. The iodine number of drone brood was 3.77 g / 100g, royal jelly - 2.94 g / 100g, with joint adsorption - 2.89 g / 100g, with joint granulation - 2.26 g / 100g (table 1).

| Index                              | drone brood adsorbed | royal jelly adsorbed | composition   |
|------------------------------------|----------------------|----------------------|---------------|
| humidity, %                        | 0.62±0.091           | 0.62                 | 0.71±0.060    |
| oxidizability index, s             | 6.93±1.157           | 3.20                 | 4.60±0.346    |
| pH                                 | 5.52±0.413           | 4.58                 | 4.79±0.049    |
| mass fraction of decenoic acids, % | 0.098±0.015          | 0.58                 | 0.195±0.032   |
| mass fraction of crude protein, %  | 3.07±0.186           | 3.90                 | 4.57±0.706    |
| free acidity, mEq / kg             | 55.34±5.766          | 100.62               | 73.85±4.414   |
| acid number, mg / g                | 5.47±0.255           | 6.93                 | 6.62±0.236    |
| iodine number, g / 100g            | 3.77±0.731           | 2.94                 | 2.89±0.181    |

4. Conclusion

According to the main indicators, the composition of drone brood and royal jelly occupies an intermediate position between the original components. The tested technology options do not have a significant effect on physicochemical indicators.

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