Stabilization methods and biochemical parameters of drone brood

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Abstract. The range of practical use of bee products is constantly expanding. Among the large assortment of these products, in recent years, drone brood larvae have received great attention, which can be obtained without harming the bee colony during the active period of the beekeeping season. Drone brood contains a complex of biologically active substances, including unique fatty acids, vitamins, essential amino acids, steroid hormones. The content in the drone brood of a large amount of nutritious and biologically active components serves as the basis for its inclusion in the diet of animal feeding and the diet of people in order to improve the body's health. Drone brood homogenate is a new beekeeping product, which is a biomass obtained from homogenized drone larvae. This product is a biologically active raw material with a rich composition necessary for the normal functioning of the body. Once removed from the hive, the brood must be stabilized for further use. In this work, the following methods of stabilization of the drone brood homogenate have been studied: freezing, adsorption using a lactose-glucose adsorbent, and lyophilization. The use of stabilization of drone brood by means of freeze-drying (freeze-drying) allows preserving some biologically active compounds in the brood. But freeze-dried products actively adsorb moisture from the environment, which stimulates the development of reactions that lead to a decrease in the quality of the product. Adsorption, in comparison with freeze drying, makes it possible to better preserve biologically active substances in a homogenate obtained from drone larvae. Stabilization of drone brood by freezing provided the most complete fixation of the most important components of drone brood in contrast to other stabilization methods.

1. Introduction

The honey bee is the scientific name for one of tens of thousands of zoological species of Hymenoptera. The composition of the bee colony is unstable throughout the year. During the active period, bee and drone brood appears in the family [1]. In many countries of the world, in recent years, there has been an interest in the brood of honey bees, and specifically in the drone brood, which is grown in sufficient quantities in bee colonies during the active period of their growth and development.

This interest in natural biologically active beekeeping products is not accidental. According to a number of authors, drone brood is primarily a source of proteins rich in essential amino acids, fats and carbohydrates [2, 3]. It also includes enzymes, sterols, vitamins, macro- and microelements, hormones and other physiologically important components [4, 5]. The content in the brood of a large amount of nutritive and biologically active components serves as the basis for its inclusion in the diet of people and the diet of animals, as well as for the purpose of improving health. The presence of these
substances helps to quickly restore the fund of necessary substances, in case of violations caused by the pathological process, and to ensure the normalization of the activity of physiological systems [6].

From the drone larvae, by pressing the combs or by homogenizing the extracted drone larvae, a homogenate of drone brood is obtained - a new product of beekeeping. Drone brood homogenate is an opaque biomass, white or slightly creamy, astringent taste with a specific aroma. The processing of bee brood has been supplied only at individual enterprises. However, producers need to carefully monitor its quality, since after being removed from the hive, the brood dies within a short time. Being an absolutely natural product, the homogenate from drone larvae oxidizes very quickly upon contact with oxygen, which leads to its deterioration. Storage temperature and duration also affect the quality of this biologically active beekeeping product [7]. The creation of technologies and equipment that ensure the long-term preservation of the native properties of biological objects has been and remains one of the most important tasks of many industries. As a rule, objects of living nature contain a significant, about 75%, amount of water, which results in a rapid decrease in their quality without the use of various methods of stabilization [8]. Therefore, in order to preserve this valuable beekeeping product, stabilization issues are the primary tasks in its use.

2. Materials and methods

The material for the study was a homogenate of drone larvae. To study the effect of stabilization methods on the content of physiologically active components, we studied drone brood, selected from one group of bee colonies. Groups of bee colonies were selected according to the principle of analogous colonies, equal in the number of bees in the nests, feed, combs and brood, as well as in the age and origin of the queens. Drone brood for experimental purposes was obtained by collecting drone combs with sealed cells from colonies, and placing empty combs in colonies every 12 days. The drone brood delivered to the laboratory was removed from the combs and homogenized.

The following methods of stabilization of the drone brood homogenate have been studied: freezing, adsorption and lyophilization of the brood. As known, freezing provides fixation of the most important native properties of products. For freezing, the homogenate was placed in dark glass vials, placed in a freezer, the temperature of which was set in the cooling mode -18 ± 2 °C.

Another part of the homogenate samples for evaluating the efficiency of product preservation was stabilized by adsorption and lyophilization. For adsorption, a mixture of 96% lactose and 4% glucose was used as an adsorbent. The ratio of adsorbent - product is selected as follows 5: 1. The adsorbed homogenate of drone brood was dried under vacuum at a temperature of + 35 °C to a residual moisture content of the product of 0.7-1.2%. The main advantage of vacuum drying units is that drying in them is carried out in an accelerated mode, this is ensured by boiling water at a lower temperature, which in turn is achieved by lowering the pressure in the drying chamber.

The next stabilization method is lyophilization (sublimation). In the food industry, lyophilization is the removal of moisture from frozen foods in vacuum devices (sublimators). Lyophilization is based on the ability of ice to evaporate under certain conditions, bypassing the liquid phase [9]. Drone homogenate samples were lyophized by sublimation on a Crist L-1,4 LD apparatus. To remove moisture, the homogenate was frozen in liquid nitrogen for a few seconds to a temperature of 35–40 °C and then sublimated in a vacuum. In the process of such drying, a lyophilized or freeze-dried drone homogenate was obtained.

The quality assessment of the stabilized homogenate of drone brood was carried out according to organoleptic and physicochemical indicators:
- oxidizability index, characterizing the total amount of unsaturated compounds - by the redox method of decolorization of 0.1 mol / dm³ of potassium permanganate solution;
- mass fraction of decenoic acids (%) - alkalimetricaly after sequential isolation of decenoic acids with diethyl ether;
- hydrogen index (pH) - potentiometrically on a pH meter with a sensitivity of 0.01 for a 2% solution;
- mass fraction of proline - photometrically, in an aqueous solution of the homogenate with, carrying out the reaction of proline with ninhydrin with the formation of a colored complex compound, and
measuring the optical density of the solution;
- mass fraction of crude protein - by burning a sample in a Kjeldahl flask with subsequent titrimetric determination of the released ammonia;
- antioxidant activity - amperometric, in terms of galic acid.

Determination of the antioxidant properties of drone brood was carried out on a Tsvet-Yauza-01-AA liquid chromatograph. The work was carried out in an accredited laboratory of the Federal State Budgetary Scientific Institution "Federal Research Center of Beekeeping".

3. Results
Unsaturated fatty acids are essential for the growth and development of the body, for the normal functioning of many organs and systems, participate in cholesterol metabolism, control blood pressure, muscle function. The content of unsaturated fatty acids was controlled by the amount of decenoic acids and the oxidizability index. The research results showed that the content of decenoic acids remained practically unchanged when the homogenate was frozen. During the adsorption of the homogenate, the unsaturated compounds are retained better than during its lyophilization. This is confirmed by a decrease in the amount of unsaturated compounds (a decrease in decenoic acids by 14.9%) in the lyophilized brood samples as compared to the adsorbed drone brood homogenate, where the decrease in decenic acids was 5.6% (Fig. 1).

The amino acid proline is responsible for the production of collagen, an essential component of skin tissue. Proline is characterized by the fact that, unlike other amino acids, its amine nitrogen is bound here not to one, but to two alkyl groups. Due to this, proline is referred to as the so-called secondary amines. It is noted that during freezing of the homogenate of drone brood, as a stabilization method, the proline content practically did not change. Upon lyophilization of the brood, a slight decrease in proline concentration by 7.5% is observed. In the adsorbed brood, the content of proline also changed, but less than in the lyophilized brood, the decrease was 5.0% of the initial product.

Crude Protein refers to the amount of total nitrogen found in the sample. The nature of the stabilization has a marked effect on the protein content of the product. Thus, in the adsorbed brood, the protein content decreased by 22.3% from the initial homogenate of the drone brood. Perhaps, in this case, the decrease in protein is due to the filler - the adsorbent. In the lyophilized product, the decrease in the protein content was 34.2% of the original homogenate. Consequently, in the process of
sublimation, a more significant decrease in the protein fraction of drone brood occurs. Perhaps during storage, this indicator may decrease even more in the lyophilized brood, since it has greater adsorption properties than the adsorbed brood, which can negatively affect the quality of the product during storage.

**Table 1.** Change in biologically active compounds in drone brood homogenate during its stabilization

| sample characteristic | oxidizability index, s | mass fraction of crude protein, % | mass fraction of proline, mg / g | pH       |
|-----------------------|------------------------|----------------------------------|---------------------------------|---------|
| fresh                 | 10.67±0.257            | 24.31±0.152                      | 0.0121±0.0009                   | 6.15±0.026 |
| frozen                | 10.85±0.131            | 22.88±0.061                      | 0.0120±0.0010                   | 6.07±0.022 |
| % of the original     | 101.69                 | 94.12                            | 60.17                           | 98.70    |
| adsorbed              | 14.46±0.271            | 18.64±0.106                      | 0.0115±0.0024                   | 5.98±0.018 |
| % of the original     | 115.78                 | 76.68                            | 95.04                           | 97.24    |
| lyophilized           | 15.08±1.003            | 15.52±0.511                      | 0.0112±0.0011                   | 5.08±0.031 |
| % of the original     | 141.33                 | 63.84                            | 92.56                           | 82.60    |

The minimum change in the protein substances of the homogenate was shown by the stabilization method - freezing. The decrease in this indicator was about 5.0%.

Hydrogen index (pH) - characterizes the concentration of free hydrogen ions in an aqueous solution of drone brood homogenate and expresses the degree of acidity or alkalinity of drone brood. Lyophilization of the homogenate contributed to a significant decrease in the hydrogen ion index by - 17.4%, and in the samples using the lactose-glucose adsorbent this index changed very little, only by 2.8%. After freezing the product, the active acidity of the drone brood homogenate remained almost unchanged.

Antioxidant activity has recently received great attention. Preparations with an antioxidant effect are increasingly used in medicine to correct the processes of free radical oxidation in various diseases. We found that the homogenate of drone brood is characterized by significant antioxidant activity (Fig. 2).

![Figure 2. Dynamics of changes in the content of antioxidant activity in the homogenate of drone brood during its stabilization, mg / g](image)

Product adsorption resulted in a 33.3% decrease in antioxidant activity. But as a result of lyophilization, the antioxidant activity of the product decreased significantly by 50.0%. Therefore, lyophilization is not the best way to stabilize the antioxidant properties of the studied beekeeping product. Dehydrated products actively adsorb moisture and oxygen from the environment, which leads to intensive development of oxidative processes. This can be the reason for the deterioration in the quality of lyophilized products. The frozen homogenate also showed a decrease in the studied indicator, but already minimal, the decrease in this case was 1.8%.
4. Conclusion

Stabilization of the drone brood of bees by freezing ensured the fixation of the most important biologically active substances of the drone brood. We have found that the adsorption proposed for royal jelly is also one of the effective stabilization methods for drone brood homogenate. As a result of stabilization by lyophilization, the nutritional value of the drone brood decreased, since after this stabilization method, the drone brood acquired a porous structure that has a high absorption capacity, intensively absorb oxygen and moisture from the environment. Thus, freezing at low temperatures and adsorption are promising methods for stabilizing drone brood.

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