BEES AS POTENTIAL SOURCE OF CHITOSAN

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Traditionally chitin and chitosan are produced from testa of Crustacea. In Russia chitin and chitosan are produced from testa of Far-Eastern crab and the production volume is limited because of the small amount of fishery. In this connection new sources of chitin and chitosan getting is to be found. One of them is small Crustacea; they are gammarids taletrids and insects. Domestic insects can provide considerable biomass of chitin due to their fast reproduction. To such insects belong a silkworm, a honeybee and a typhoid fly.

A honeybee belongs to Artropoda species, Tracheata subtype, Insect, Pterigota subclass, Hymenoptera class, Apocrida suborder, Aculeata type, Apoideal superfamily, Apidae class, Apis kind /4/.

In 1758 K. Linney described a honeybee and called it Apis mellifera (a bee that brings honey). But as it brings nectar he changed specific name into Apis mellifica (a producing honey bee). In Russia the first name is widely used.

A honeybee (Apis mellifera) is a social stinging insect. The normal quantity of a bee family is 50 – 80 thousand species. Bee family consists of a queen bee, working bees and drones. Working bees present a base of a bee family. They are females with underdeveloped genitals, 12 – 14 mm length and live 26 –40 days. Their main function is nectar and plant pollen gathering, which are necessary for family and larvae feeding, honeycombs building etc./1/.

Bee body is covered with hard integument - cuticle, which serves as a support for internals and a protection from external actions. There are outgrowths form on the cuticle and muscles fastened to them. All the diversity of the integument creates in the process of the insect development by means of cells that make cuticle. The cells have cubical and cylindrical form and present a thick layer, which is called hypoderm. Cuticle is very solid but thanks to chitin it is flexible. The percentage of chitin in cuticle is from 30% to 50 %. Chitin makes cuticle elastic and hardness and impenetrability provided by its complicated structure /4/.

The potential source of bee chitosan getting is cuticle, which contained chitin. Raw material for chitin and chitosan production can be dead bees, the bees that died mainly during wintering and fell down a beehive. In summer bees die more than in winter but it is less seen because they die out of a hive /6/. Bee's lifetime is determined by its organism state and the work it does. Queen bees live up to 5 years (at the most 8 years), drones 4 months. Working bees live not more than 1 year and in their active period, in summer is about 35 days. Autumn brood do not work hard passing the winter well and live 8 – 9 months /1/.

Due to wide development of beekeeping in this country there is an opportunity to get much more raw material for chitin. According to the data on 1.01.2000 there were 3457, 5 beehives in all types of rural economy of the Russian Federation. The weight of a bee family is about 3,5 – 4 kilograms. In summer during honey yield and in spring after wintering a bee family gets renewed for 60 – 80 % /7/. Thus we can get from 6 up to 10 thousand tons of dead bees yearly. Dead bees can be considered as a new potential source of getting chitosan from such insects as bees along with traditional raw materials.

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We took two kinds of dead bees for research.

- Dry dead bees, gathered after wintering and consist mostly of working bees. The raw material presents black – brown mass with specific odor. When carefully examining the bees we can see some safe bees and different parts of bees (heads, chests, legs, abdomens, wings and others). The length of a safe bee 10 – 12mm. There can be big bees (drones) too.
- Dry dead bees after CO_2 extraction. The raw material presents deep-brown mass and mostly consist of well viewed parts of the insect – head with proboscis, chest segments, abdomen with legs, legs and wings separately. Chemical composition of dead bees and bees after CO_2 extraction was determined and presented in the table № 1. There is a chemical composition of dry krill and hammarus testa given in the table for comparison.

Chemical composition of dead bees, dry kril and hammarus testa, %.

Table 1

Raw materials for chitin	Dampness	Mineral substances	Chitin	Protein	Lipids	Melanins
Dry dead bees	8-10	2 - 3	10-12	50-80	-	20-30
Dead bees after CO_2 extraction	8-10	2 - 3	20-22	45-50	-	20-22
Dry krill testa from protein isolate	8-10	25-30	20-22	25-30	1-3	-
Dried hammarus *	10,2-10,8	23-26	6,8-7,0	55-56	7,7-13	-

* Data on dried hammarus (2), protein content (5).

By the content of protein dead bees are very similar to hammarus, which pass two-phase processing by 2 – 3% alkali (2). However by the content of chitin dried bees are more like dry krill testa and that's why more concentrated solution of alkali 10% was used for its deproteinization (5). Dead bees practically do not contain mineral substances since insects' cuticle is demineralized. In this connection there is no need to demineralize bees chitin. After extracting lipids by Folch's admixture it turned out that the concentration of it in bees is too little therefore degreasing has not been done when chitin isolation.

When hydrolyzation bees by 10% of alkali (NaOH) there was the basic quantity of protein 80% isolated and a number of melonins. Alkali hydrolyzate is dark-brown viscous fluid. It is possible to precipitate protein from the fluid by means of titration when pH = 4,4 – 4,6. Chitin was washed out with plenty of distilled water up to pH = 7 and dried at 55± 5^0C. Bees' dry chitin is dark-brown mass with specific odor. There can be clearly seen separate parts of the insect: triangular form of heads 4-5 mm, round shape chests 4mm, legs and wings in the form of adhering nubbins and separate crushed parts and also belly pieces. The chemical composition of bees' chitin is presented in table №2.

Qualitative characteristic of dead bees' chitin

Table 2

Type of dead bees	Chitin output, %	Dampness, %	Mineral substances content, %
Dry dead bees	20-24	8,0	2,45
Dead bees after CO_2 extraction	12-17	8,2	2,08

In bee's cuticle chitin is fast binded with melanins (4) so that during alkali deproteinization some of its parts are eliminated together with protein but dark-brown color of chitin preserves. Thus the product can be considered as a chitin-melanotic system.

Deacetylation of chitin was made in 50% liquor of NaOH at 125± 5^0C. (5) At the same time melanins dissolve and give alkali black color. Further washing makes chitosan acid-soluble and its brown color indicates of melanins' remains. Indices of bees' chitosan are presented in table 3.

The chitosan was hydrolyzed by a system of chitinous ferments of microbiological origin to make it water-soluble (3). The new product is called "Pchelosan" ("Beetosan") with characteristics showed in the table below.

The main qualitative measure of "Pchelosan"

Table 3

Type of dead bees	**Chitosan from dead bees**			**"Pchelosan"**		
	Chitosan output from chitin, %	Viscidity	Diacetylene content, %	Output from chitosan, %	Viscidity	Diacetylene content, %
Dry dead bees	20	10,6	67,6	60	1,20	67,7
Dead bees after CO_2 extraction	30	7,83	67,8			

After freeze-drier "Pchelosan" is fine powder of hazel color, dissolved at pH 5,5.

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