ЕҢБЕК ҚЫЗЫЛ ТУ ОРДЕНДІ
«Ә. Б. БЕКТҰРОВ АТЫНДАҒЫ
ХИМИЯ ҒЫЛЫМДАРЫ ИНСТИТУТЫ»
АКЦИОНЕРЛІК ҚОҒАМЫ

Қазақстанның Химия Журналы

Химический Журнал Казахстана

Chemical journal of Kazakhstan

АКЦИОНЕРНОЕ ОБЩЕСТВО
ОРДЕНА ТРУДОВОГО КРАСНОГО ЗНАМЕНИ
«ИНСТИТУТ ХИМИЧЕСКИХ НАУК
им. А. Б. БЕКТУРОВА»

2 (66)

АПРЕЛЬ – ИЮНЬ 2019 г.

ИЗДАЕТСЯ С ОКТЯБРЯ 2003 ГОДА

ВЫХОДИТ 4 РАЗА В ГОД

АЛМАТЫ
2019

UDC. 615.03

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METHOD OF OBTAINING A CHITOSAN AMINOPOLYSACCHARIDE FROM BEHBAT *APIS MILLIFERA*

Abstract. The article presents the method of obtaining chitin and chitosan from *Apis Millifera* bees. The optimal conditions for the laboratory production of chitin and chitosan from bees submill were investigated. The composition and molecular structures of chitin and chitosan were studied using IR spectrophotometry, elemental analysis of chitosan, and microscopic images were taken using a scanning microscope. The resulting chitosan is a polydisperse molecular weight of D-glucosamine, containing 5-15% acetamide groups, as well as up to 1% of the groups, combined with amino acids and peptides. The biopolymers chitin and chitosan were obtained and characterized on the basis of a promising new source - the dead *Apis Mellifera* bees. This local raw material is of great importance as a starting material in medicine with unique properties, especially for the treatment of burn wounds.

Key words: chitin, chitosan, amino polysaccharide, IR spectroscopy, scanning microscope.

In recent years, renewable natural resources are becoming increasingly popular, among which chitin, the second most common polymer in nature after cellulose, and its derivative chitosan, occupy a special place. The substance chitosan is a part of many supplements, with a positive effect on lowering cholesterol and strengthening the immune system. Chitosan and its derivatives are also widely used in medicine [1], as well as in the chemical and food industries.

The most accessible for the industrial development of chitin production in the Republic of Uzbekistan is the silkworm pupae and the dead honey-bees. The raw material for the production of chitin from bees can serve as a dead bees [2].

A special type of chitosan - phelozan more potent biologically active substance than chitosan obtained from crabs and shrimps. A significant reserve of raw materials for the production of chitosan in Uzbekistan is represented by the local dead *Apis Mellifera* bees (figure 1).

In this regard, it is quite expedient to obtain reproducible biopolymers of chitin and chitosan from dead *Apis Mellifera*. Mainly bees dying during the wintering period and crumbling to the bottom of the hive. In summer, the death of bees is much more significant than in winter, but less noticeable, since they usually die outside the hive. Apizan or, as it is called, in a scientific way, a low molecular weight chitosan-melanin complex is obtained from dead bees.

Figure 1 – Honeybees Apismellifera (honeybee) and bee lizard

In the summer, during the period of active honey collection and in the spring after wintering, the bee family is renewed by almost 40-60%. The strength of a bee colony (the mass of working bees in a bee colony, measured in kg) is, on average, 4.5-6 kg. This makes it possible to consider the dead bees as a new promising source of chitin and chitosan along with traditional types of raw materials [3].

EXPERIMENTAL PART

We have used dead bees, collected during the spring renewal of the bee colony and containing a significant amount of chitin. Raw material is a black-brown mass with a specific smell. On closer examination, whole undisturbed bees and various parts of bees (head, chest, legs, abdomen, wings, etc.) are visible. The dead bees contain the minimum amount of mineral substances, since the insect cuticle is practically not mineralized [4]. A process of drying was carried out at a temperature of about 35 °C, laying out a thin layer. Dried raw material weight of 30 g was crushed and spent demineralization (DM), then deproteinization (DP) according to the following scheme (figure 2).

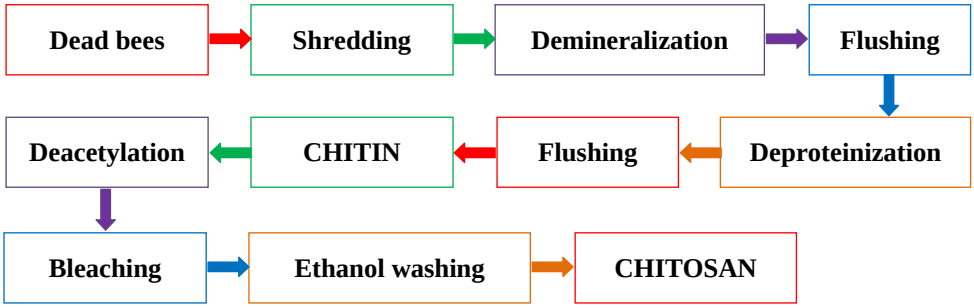


Figure 2 – The general scheme of obtaining chitosan from bee submarine

The DM was carried out according to the Hackman method [5] by treating dead bees with 2 M hydrochloric acid for 5 hours at room temperature. The DP was carried out by treating the crushed raw material with 1 N sodium hydroxide solution for 1 hour at 800 ° C. Next, the mass was filtered and dried at room temperature. Each process was accompanied by washing the raw material until neutral wash water (pH = 7).

Chitosan was obtained by deacelation (DA) of chitin with 35% aqueous solution of NaOH for 4 hours at a temperature of 850 °C and dried at 50-55 °C.

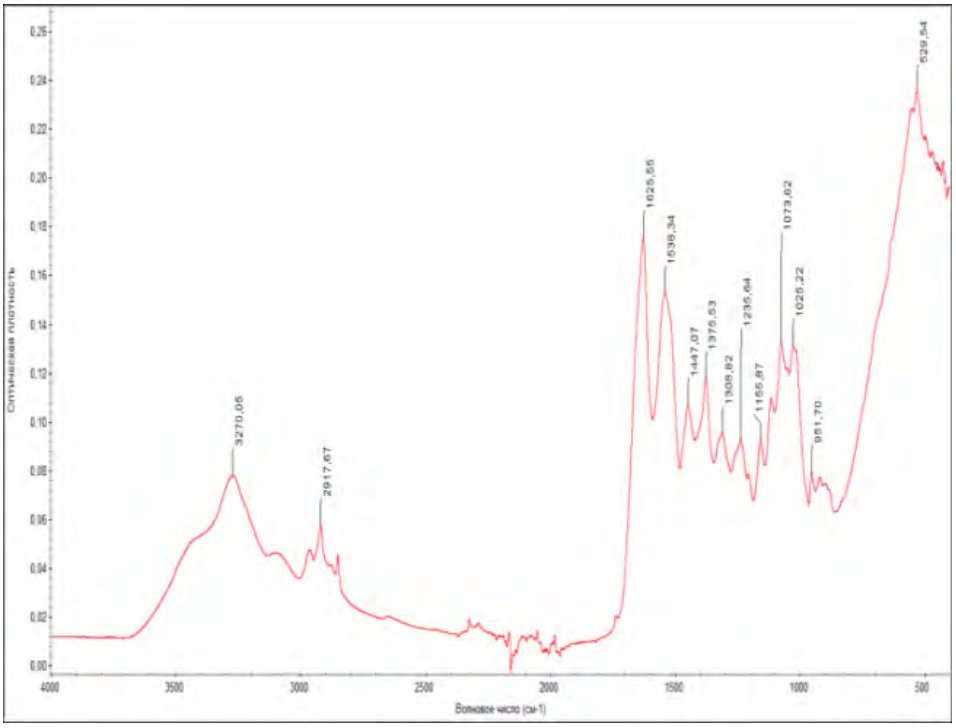
Reaction YES is accompanied by simultaneous breaking of the glycosidic bonds of the polymer. Chitosan is a polydisperse molecular weight of D-glucosamine, containing [1] 5-15% acetamide groups, as well as up to 1% of the groups associated with amino acids and peptides.

When drying at higher temperatures, chitosan condenses, darkens and loses solubility, which reduces the possibility of its use. Next, the resulting mass was decolorized with a 3% solution of hydrogen peroxide and washed with ethanol. The reaction product is a light beige mass with a specific smell.

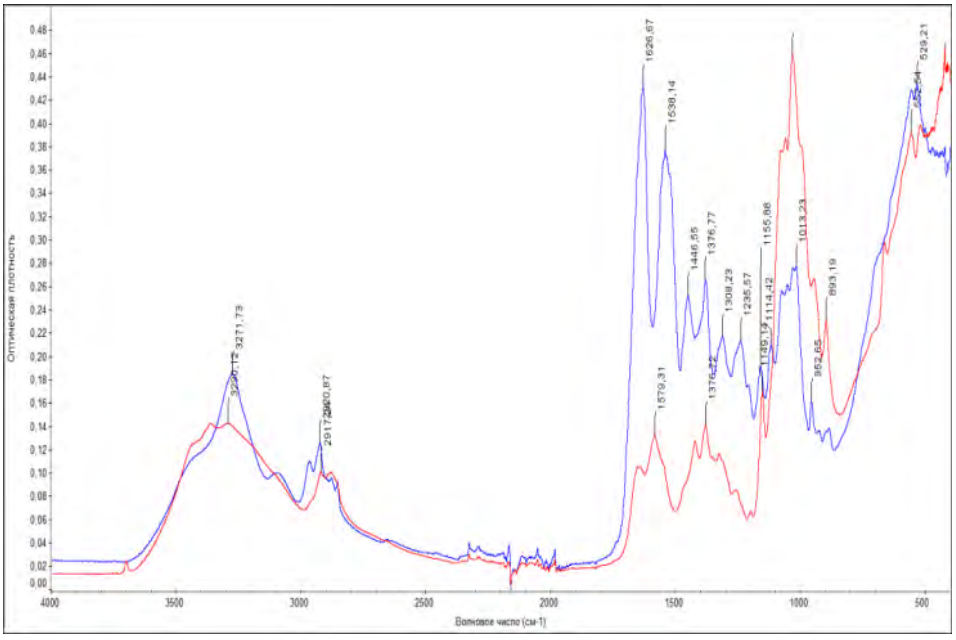
Interpretation of the obtained biopolymers was carried out by removing IR spectra on a Nicoleti S 50 FT-IR spectrometer (Thermo Fisher Scientific, USA), which are shown in figure 3 [6].

As can be seen from figure 3, in the IR spectrum of chitin (A), characteristic absorption bands are observed in the regions of 3290 cm^{-1}, the vibrations of the –N–H–bond, and also the absorption bands of 1371 cm^{-1}, which indicate the presence of the $–CH_3$ group Absorption in the region of 1579 cm^{-1} is characteristic of the C=O group. The IR spectrum of chitosan shows peaks in the region of 3272 and 1377–1028 cm^{-1}, which indicate the presence of the NH_2 group.

At this absorption, in the range of 1360–1000 cm^{-1}, all types of amines appear to have absorption bands caused by the participation of the C–N bond in the skeletal vibrations of the molecule. In the sample of chitin and chitosan (B), bands with peak at 1446 cm^{-1} of the deformation vibration of the CH_2 and CH_3 groups and 1373 cm^{-1} (inflection) of the deformation vibration of the OH bond were also recorded.



A

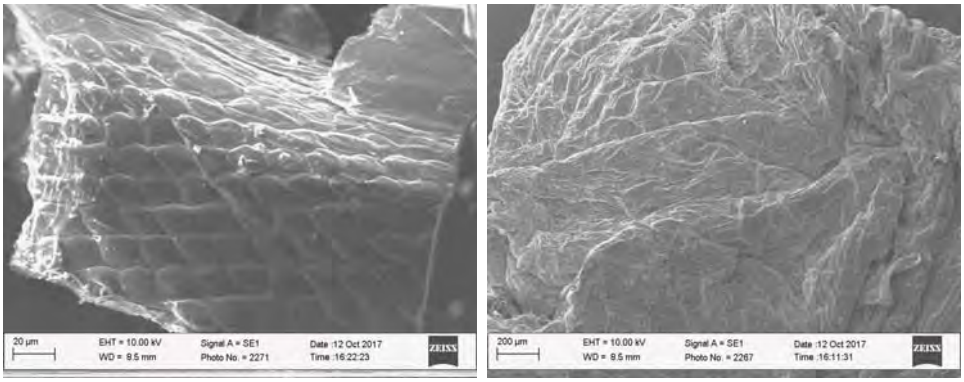


B

Figure 3 – Infrared Fourier spectra of chitin (A) and chitin-chitosan (B), obtained from bee submarine

In a sample of chitosan, a wide band of medium intensity is observed in the region of 1320–1387 cm^{-1}, corresponding to the oscillation of the OH bond.

An elemental analysis of chitosan – (CHN), obtained on a Termo-Elekton chromatograph with program from dead bees was also carried out, and microscopic images of the structure of chitin and chitosan were taken using a scanning microscope (figure 4).



a) chitin b) chitosan

Figure 4 – Electronic micrographs of chitin and chitosan from dead bees

The data in figure 4 shows that during the processing of chitin with a solution of alkali of its molecule, it is quick to pass into the amorphous state and then into the crystalline state, that is, amorphization occurs, the crystal lattices are gradually destroyed. It can be concluded from structural molecular and IR spectrometric data that chitin is converted to chitosan by deacetylation, i.e., molecular structures and chitosan crystals are different from molecular structures and chitin crystals.

Thus, we can conclude that this raw material is of great practical importance as a starting material in the production of biodegradable films for the treatment of burn wounds in medicine based on phelozan and its derivatives.

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Резюме

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APIS MELLIFERA АРАЛАРЫНАН ХИТОЗАН АЛУ ӘДІСТЕМЕСІ

Apismillifera араларынан хитозан алу әдісі келтірілген, сонымен қатар ИК-спектрометрия әдісімен хитин мен хитозанның құрамы мен молекулалық құрылымы, хитозанның элементтік анализі зерттелді. Сканерлейтін микроскоптың көмегімен хитин мен хитозан микроқұрылымдары алынды. Алынған хитозан құрамында 5-15% ацетамидті топтар, сондай-ақ амин қышқылдары мен пептид-термен біріктірілген 1%-ға дейінгі топтар бар d-глюкозамин молекулалық массасы бойынша полидисперсті болып табылады. Құрғақ *Apismellifera* араларының – жаңа перспективалық дерек көздері негізінде хитин мен хитозан биополимерлері алынды және сипатталды.

Түйін сөздер: алу әдісі, хитин, хитозан, аминополисахарид, ИК-спектроскопия, сканерлейтін микроскоп.

Резюме

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МЕТОДИКА ПОЛУЧЕНИЯ ХИТОЗАНА
ИЗ ПОДМОРА ПЧЕЛ *APISMILLIFERA*

Приведен метод получения хитозана из подмора пчел *apismillifera,* а также изучен состав и молекулярные структуры хитина и хитозана методом ИК-спектрометрии, элементным анализом хитозана. С помощью сканирующего микроскопа получены фото микроструктуры хитина и хитозана. Полученный хитозан представляет собой полидисперсный по молекулярной массе D-глюкозамин, содержащий 5-15 % ацетамидных групп, а также до 1 % групп, соединенных с аминокислотами и пептидами. Получены и охарактеризованы биополимеры хитин и хитозан на основе нового перспективного источника – сухого подмора пчел *Apismellifera*.

Ключевые слова: метод получения, хитин, хитозан, аминополисахарид, ИК-спектроскопия, сканирующий микроскоп.