DOI: 10.18372/2306-1472.71.11755

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COMPARATIVE ANALYSIS OF TECHNILOGIES OF CHITOSAN PRODUCTION FROM DEAD BEES

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Abstract

Objective: *The aim of this work is to study the characteristics of technology of chitosan obtaining from unconventional sources, namely from dead bees.* ***Methods:*** *The article considers three methods of chitosan obtaining from dead bees, namely the technology with the usage of dead bees with low degree of drying; the technology with the usage of dead bees with high degree of drying; the technology with the usage of dead bees with high degree of drying but without separation of deproteination and deacetylation stages.* ***Results:*** *It is proved that the technology with the usage of dead bees with high degree of drying but without separation of deproteination and deacetylation stages does not require high temperatures and long time. Yield of chitosan with the use of this technology is 21-24%.* ***Discussion:*** *The expediency of dead bees usage as raw material for the production of chitosan in Ukraine is shown. The technologies of chitosan obtaining from dead bees are compared, the most efficient one is chosen, which provide the highest yield of the finished product, so it is the most promising for the application in practice.*

Keywords: chitin; chitosan; deacetylation; dead bees; deproteination.

1. Introduction

With better appreciation of biopolymers such as polysaccharides derived from natural organisms, there is increased interest in their biomedical and industrial applications. Some of such important polysaccharides are chitin, chitosan, oligosaccharides and their derivatives, which attract significant interest in a view of their wide applications, in the biomedical, agricultural, food science, and other technological fields.

Eight thematic international conferences for the last 27 years and Russian Chitin Society creating in 2000 confirm great interest in problem of chitosan production and its usage.

In Ukraine, the researches in this area were conducted too. The problems of alternative sources of chitin for chitosan production, its usage in the production of organic foods and for cleaning of drinking and waste water were carefully studied by scientists of the Institute of Cell Biology and Genetic Engineering (ICBGE) of National Academy of Sciences of Ukraine. Bees cover structure has been determined and technology of chitosan from dead bees has been proposed. Dead bees usage is especially important for Ukraine through the absence of a sufficient number of traditional materials (shells of crabs) and limited catches of shellfish as a source of chitin for chitosan production [1].

Since in Ukraine dead bees are not used in industry and just rejected as waste, and Ukrainian annual raw resource of dead bees is about 900–1 200 tons, this allows considering the usage of dead bees as large-scale and perspective source of chitosan production.

C. Rouget has discovered chitosan in 1859, but current name has been proposed in 1894. Chitosanis polysaccharide is obtained from chitin by removing of acetyl group in C2 position in alkaline solution (deacetylation process) (Fig.1) Chemical name is 2–amino–2–deoxy–β–D–glucopyranose, molecular formula is $(C_6H_{11}O_4N)_n$[2],[3, 4].

Unlike chitin, chitosan is dissolved in water and diluted organic acids, for example, in aqueous solution of acetic acid. So, this characteristic results in extensive usage of this polysaccharide in different fields of application [3].

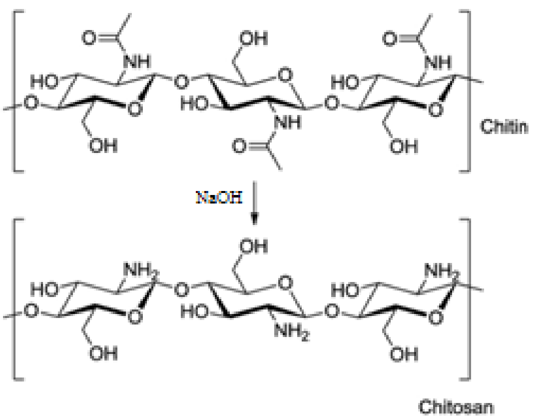

Fig. 1. Reaction of chitosan formation from chitin

2. Analysis of potential source for chitosan extraction

Chitosan has unique absorption properties and has positive effects on human organism: normalizes the function of many physiological systems of the body, stimulates wound and burns healing without scarring, shows antimicrobial action. Now more than 70 practical applications of chitosan are known [3].

Potential sources of chitosan are diverse and widely distributed in nature. Chitin in its natural state is present not only in the shells of crustaceans such as crabs, shrimp and lobster, but also in the external skeleton of marine zooplankton, including corals and jellyfish. Insects such as butterflies, ladybugs, bees and silkworm contain chitin in their wings. The cell walls of yeast, mushrooms and other fungi (*Aspergillus niger)* also contain chitosan (it performs a protective and supporting function, providing rigidity of cells). Usually the main quantity of chitin and consequently produced chitosan is obtained from shells of crustaceans. However, the volume of production is limited by catches of shrimps. In this regard, the need to find new source of raw materials is very actual. Therefore, as one of the alternative sources of chitin and chitosan can be the dead bees' bodies, the bees that died mainly during wintering and fell down in a beehive [4].

Complex process of chitosan production from chitinous cover of bees is performed for pharmacology needs, but it is expensive, so obtained drugs are also expensive and that is why are not always available [5].

3. Technology of chitosan production from dead bees

The potential sources of chitosan in bees are cuticles containing chitin. In bee's cuticles chitin is connected with melanin (Fig. 2).

During alkali deproteination, some of melanin parts are eliminated together with proteins and another part remain chitin linked. Due to melanin presence dark-brown color of chitin is preserved [6].

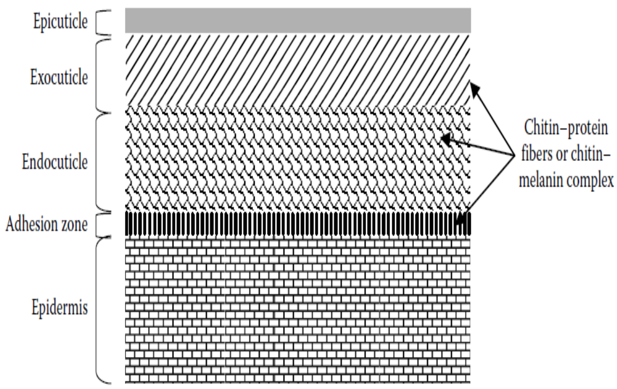

Fig. 2. Schematic interpretation of organic matrix of insect cuticle

Traditional technology of chitosan obtaining has been modified via the peculiarities of bee's cuticle structure. Bees cover is less mineralized than crustaceans cover so demineralization process is omitted.

The quantitative yield of chitosan from chitin of dead bees' bodies depends on the degree of grinding and drying of raw material and stages of production. In view of these factors we will consider three ways of chitosan getting from bodies of dead bees.

Technology of chitosan production from dead bees with low degree of drying

- Take dead bees consisting of entire bees and their parts, abdomena, wings with the storage humidity of 12 – 18 %, and crush.
- For the deproteination take 5 g of powdered sample and put into the flask with ground glass stopper, pour 10% NaOH alkaline solution in the ratio of 1:10 by weight and heat up to 78–82 ºC while stirring continuously with a magnetic stirrer during 1.5–2.0 hours.
- Filter obtained mass using Buchner funnel, filtered solution store for subsequent melanin isolation from it. Filtered mass is chitin with some melanin content as evidenced its dark – brown color.
- Wash the resulting chitin with distilled water till neutral reaction of wash water (pH 7).
- For chitin deacetylation, fill the obtained mass with 30 % NaOH solution in the ratio of 1:10. Heat the obtained mass up to 90–95 ºC without air and with stirring during 1.5 – 2.0 hours.
- Filter cooled after deacetylation mass using Buchner funnel, store filtered solution.
- Wash the obtained chitosan with some melanin content in it with distilled water till neutral reaction of washing water (pH 7).

- Conduct precipitation of melanin from stored solution with concentrated hydrochloric acid to change pH of the medium to the acid area and precipitate of dark flaky sediment.
- Carry out the separation of melanin precipitate from the solution by centrifugation with subsequent washing to neutral reaction of wash water (pH 7). Perform chitosan and melanin drying simultaneously without mixing at 50 – 60 ºC and humidity of 8 – 10 %.

Yield of chitosan in this case is 6–8 % of the original mass of dead bees and melanin yield is 62 – 69 % of raw weight of dead bees.

Technology of chitosan obtaining from dead bees with high degree of drying

Storage humidity of dead bees is 12 – 18 %. Dead bees of this humidity are poorly crushed and require drying up to humidity degree of 4–6 % at which it becomes brittle and easily crushed.

- The first stage involves drying. Drying is made in one of the following ways. The first way is to dry initial mass on a sheet of paper in the oven at 60 – 82 ºC; to spread it as the layer of 5 – 10 mm. The time of drying is 2.0 – 2.5 hours.
- By the second way, the initial mass is blown by the airflow at 60 – 82 ºC. Such drying process is completed after 15 – 20 minutes. The degree of mass dryness is determined by compressing of dead bees by the fingers. In this case, dead bees are the most crumble.
- The next stage is grinding of dried mass. Dead bees have a low density, so they are poorly crushed in a mill with knives. Therefore, in the laboratory it is necessary to grind them by chafing in laboratory mortar, especially for the low mass of material. If mass is big enough, the drum or cone mill shall be used obtaining the 0.2 – 0.3 mm particles. Ground mass has very large active surface contact with reagents that considerably accelerates chemical reactions and increases the completeness of treatment.
- The subsequent stages are performed similarly to the respective stages of the first method, namely, deproteination, filtration, washing, deacetylation, (chitosan obtaining), washing, melanin precipitation from filtrate.
- In this method, compared with the first one, the output of chitosan increases from 6 – 8 % up to 13 – 15 % of the original mass of dead bees; it indicates the necessity of initial material drying for the stage of crushing. This is achieved by a good grinding that increases the active surface of interaction of particles with reagents.

Technology of chitosan production from dead bees with high degree of drying and without separation of deproteination and deacetylation stages

Drying, milling and deproteination of the initial material is the same as in the second method. Unlike previous methods, deacetylation of the mass is made without filtration and separation of the solution, which contains melanin.

Chitosan output increases from 6 – 8 % for the first method and 13 – 15% for the second method up to 21– 24% for the last one due to the exclusion of it losses during the washing of chitin filtered mass which is provided both in the first and the second methods. Thus, the declared in the third method technology for chitosan obtaining may be considered as optimal [7], [8 − 10].

The general procedure of chitosan production by preferable method is represented on the Fig. 3 [7].

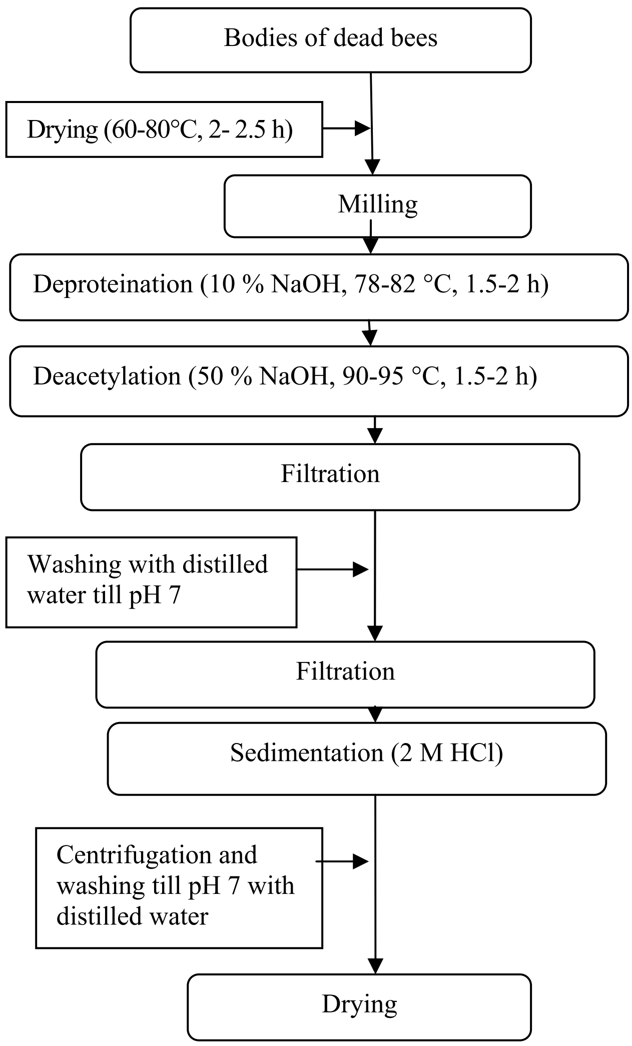

Fig. 3. Chitosan production procedure from dead bees

4. Conclusions

In the industry, the traditional large-scale sources for chitosan obtaining are crustaceans, microorganisms and fungi. These sources provide a high yield of chitosan, but the production process is costly, and raw materials are not always available in sufficient quantities.

Usage of dead bees for chitosan production is economically beneficial. Chitosan production from dead bees is much cheaper and easier than from crustaceans, mushrooms or insects. Since in Ukraine dead bees are not used in industry and just rejected as waste, and Ukrainian annual raw resource of dead bees is about 900–1 200 tons, this allows considering the use of dead bees as large-scale and perspective source of chitosan production.

In consideration of the fact that output of the desired product depends on the degree of grinding and the technological stage, a comparative analysis of three methods of chitosan obtaining from dead bees has been conducted. The yield of the final product increases from 6 – 8 % or 13 – 15 % up to 21 – 24 % when the third method is used. These figures indicate that technology of chitosan production from dead bees with high degree of drying and without separation of deproteination and deacetylation stages is the most appropriate technology for industries.

References

[1] Se-Kwon Kim. (1999) *Chitin, chitosan, oligosaccharides and their derivatives.* USA, Taylor and Francis Group, 633 p.

[2] Tang W.J., Fernandez J.G., Sohn J.J. (2015) *Chitin is endogenously produced in vertebrates.* USA, Pubmed,7−10 p.

[3] Hild S., Marti O., Ziegler A. (2008) Spatial distribution of calcite and amorphous calcium carbonate in the cuticle of the terrestrial crustaceans *Porcellio scaber* and *Armadillidium vulgare. Journal of Structural Biology*, vol. 23, no.2, pp.100–108.

[4] Hudson S.M., Smith C. (1998) *Polysaccharide: Chitin & chitosan: Chemistry and technology of their uses as structural materials*. New York, Springer Verlag, 96−118 p.

[5] Holovetska V.V. Bdzholy i mertvymy korysni liudstvu [Bees dead useful to humanity]. Available at: http://www.medolub.kiev.ua/pidmor.html (accessed 14.09.2016).

[6] Krivczov N.I., Lebedev G. M. (1999) Beekeeping. *Kolos*, vol.2, pp. 35.

[7] Tcjukhmestrenko G. A. Sposib otrymannja khitozan – melaninovogho kompleksu z pidmoru bdzhil [The process of chitosan – malanin complex from dead bees production]. Available at: http://findpatent.com.ua/patent/238/2382051.html (accessed 11.09.2016).

[8] Lebedev V. I. (2008) Ekology`cheskaya chy`stota produktov pchelovodstva [Ecological purity products of beekeeping]. *Pchelovodstvo*, no. 4, pp. 21–24.

[9] Bikov V.P. Furman D.I. (2000) Chitozan and hamarus production . *New prospect of chitin and chitosan research*, vol. 34, pp. 67.

[10] Bilyk E. V. (2005) *Vely`ky`j suchasny`j dovidny`k bdzholyara: 15 000 kory`sny`x porad dosvidcheny`x pasichny`kiv dlya pochatkivciv ta profesionaliv* [Large modern the beekeeper directory: 15 000 experienced beekeepers tips for beginners and professionals]. Donetsk, TOV BKF “BAO”, 528 p.

Received 10 May 2016

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Порівняльний аналіз технологій отримання хітозану з підмору бджіл

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Мета: Метою даної статті є дослідження особливостей технології отримання хітозану з нетрадиційного джерела, а саме з підмору бджіл. **Методи:** У статті розглядається три методики отримання хітозану з підмору бджіл, а саме: технологія за використання підмору з низьким ступенем висушування; технологія за використання підмору з високим ступенем висушування; технологія за використання підмору з високим ступенем висушування, але без поділу стадій депротеїнізації та деацетилювання. **Результати:** Доведено, що технологія за використання підмору з високим ступенем висушування і без поділу стадій депротеїнізації та деацетилювання не вимагає високих температур та багато часу. Вихід хітозану за використання цієї технології складає 21–24%. **Обговорення:** Показано доцільність використання підмору бджіл як сировини для виробництва хітозану в Україні. Порівняно технології отримання хітозану з підмору бджіл, виділено найефективнішу з них, що забезпечує найвищий вихід готового продукту, тому є найбільш перспективною для застосування на практиці.

Ключові слова: деацетилювання; депротеінізація; підмор бджіл; хітин; хітозан.

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Сравнительный анализ технологий получения хитозана из подмора пчел

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Цель: Целью данной статьи является исследование особенностей технологии получения хитозана из нетрадиционного источника, а именно из подмора пчел. **Методы:** В статье рассматривается три методики получения хитозана из подмора пчел, а именно: технология при использование подмора с низкой степенью высушивания; технология при использование подмора с высокой степенью высушивания; технология при использование подмора с высокой степенью высушивания, но без разделения стадий депротеинизации и деацетилирования**.** **Результаты:** Доказано, что технология при использование подмора с высокой степенью высушивания и без разделения стадий депротеинизации и деацетилирования не требует высоких температур и много времени. Выход хитозана при использование этой технологии составляет 21-24%. **Обсуждение:** Показана целесообразность использования подмора пчел в качестве сырья для производства хитозана в Украине. Были сравнены технологии получения хитозана из подмора пчел и выделено наиболее эффективную из них, которая обеспечивает высокий выход готового продукта, поэтому является перспективной для применения на практике.

Ключевые слова: деацетилирование; депротеинизация; подмор пчел; хитин; хитозан.

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