# ISOLATION OF CHITIN-GLUCAN COMPLEX FROM *ASPERGILLUS NIGER* BIOMASS, A BY-PRODUCT OF CITRIC ACID PRODUCTION

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**Abstract.** The excretion of radionuclides, toxic substances, pathogenic microorganisms from the human body contributes to the preservation of health and the duration of life. In the world, there are various natural biopolymers, in particular, biopolymer of the 21st century, chitin and its derivatives, which have a significant effect on the body. The daily recommended intake of chitin ranges from 3 to 7 g. In the food industry, chitosan (a chitin derivative) is used as a stabilizer, preservative, clarifier, emulsifier, and structurant. In medicine, based on chitosan, apyrogenic surgical threads and sutures are made. The sorption ability of chitin is used to purify water and localize leaks of radioactive substances. Scientific developments are under way to improve the technologies for the isolation of chitin and its derivatives from chitin-containing raw materials of various origins as well as expand the scope of application of these biopolymers. On the production scale of the Republic of Belarus, the isolation of the chitin-glucan complex from a by-product of citric acid production does not require significant raw material costs, and at the same time solves the problems associated with the processing of the by-product. The extraction of the chitin-glucan complex was carried out according to two developed technologies: having pretreated chitin-containing raw material with a solution of hydrochloric acid and an ozone-air mixture. The organoleptic evaluation and physicochemical analysis of the samples were carried out as well as the comparative evaluation with imported samples of chitosan. It has been established that the chitin-glucan complex has some advantages in terms of flavor characteristics as compared to imported analogues: anonymized odor and neutral taste, which makes it possible to use it not only as an independent food product, but also as a functional ingredient in other foods that do not alter taste of the main product. For the first time in the Republic of Belarus, a study of the morphological structure, chemical composition and X-ray diffraction analysis of the chitin-glucan complex were conducted. It has been established that the chitin-glucan complex has a porous structure and an amorphous-crystalline structure.

**Keywords:** chitin, chitosan, chitin-glucan complex, *Aspergillus niger L.* biomass, demineralization, deproteinization.

## Introduction

At present, there has been an increased interest in the problems of more rational use of renewable natural resources, both from the ecological and economic point of view.

In recent years, a special attention has been paid to the biopolymer chitin and its derivatives. Due to unique properties such as biocompatibility, biodegradability, nontoxicity, they have been applied in more than 70 different areas: food industry, ecology, agriculture, medicine, etc. The sources for the production of chitin can serve as the shell of commercial crustaceans, the biomass of fungi, and the cuticles of insects.

Sources for the production of chitin and its derivatives are the shell of commercial crustaceans, the calf's gladius, the seignior of cuttlefish, the silkworm pupa, the shell casing of cockroaches, the biomass of microorganisms, diatoms, and bee-pods. In the production scale of the Republic of Belarus, fungi, insect scum, earthworms can serve to isolate chitin (Camci-Unal at al. 2009). In the cell of fungi and diatoms, chitin is bound by covalent bonds with glucans and melanin, thus it is very difficult and expensive to extract pure chitin. One of the source of raw materials of the chitin-glucan complex can be the waste of the microbiological industry, in particular, the biomass of *Aspergillus niger*, which remains in the production of citric acid. The only enterprise in the Republic of Belarus, and now also in the post-Soviet space, the enterprise for the production of citric acid JSC «Skidelsky Sugar Refinery» annually recycles 1500 tons of raw mycelium of the mold fungus *Aspergillus niger*, which, according to various sources of literature, may contain up to 30% of chitin, strongly bound to β-glucan in the chitin-glucan complex. The presence of glucan changes the properties of this biopolymer providing it with a pronounced antitumoral, wound-healing characteristics, the selective sorption capacity for carcinogens, heavy and radioactive metals, prolonging the shelf life, which are valuable properties for the use of the chitin-glucan complex as a source of dietary fiber or an ingredient for therapeutic and prophylactic nutrition.

The aim of the study is to develop a scientifically based technology for isolating the chitin-glucan complex from *Aspergillus niger* biomass, a by-product of citric acid production, and its commodity evaluation.

At first, the initial analysis of scientific and technical literature was conducted, and the scientific data on the structure, chemical composition and properties of chitin and its derivatives were summarized. A detailed analysis of the technologies for the production of chitin and the chitin-glucan complex was carried out (Camci-Unal, 2009; Ballassa, 2017; Muzzarelli at al., 2004; Trimukhe, 2008).

It has been established that in the production scale of the Republic of Belarus, the use of crustaceans as a source of chitin for the Republic of Belarus is very costly. At present, a negative factor is that the contamination of the shells with heavy metals and other toxic waste products increases.

Thus, the isolation of the chitin-glucan complex from the raw mycelium of the fungus *Aspergillus niger* requires low costs and at the same time solves a number of problems associated with the processing of the by-product. Therefore, a very urgent task for the Republic of Belarus is to develop a technology for isolating the chitin-glucan complex from domestic raw materials, namely from the by-product of citric acid production.

## Methodology of research

Today, there are three main technologies for isolating the chitin-glucan complex: chemical, biotechnological and electrochemical. These methods are based on several stages of deproteinization and demineralization of chitin-containing raw materials. The quality of the final product directly depends on the order and completeness of these processes.

All chemical methods are based on the processing of chitin-containing raw materials in chemically aggressive media, which can lead to a partial destruction of the final product, require a special site for the preparation of solutions and a multiple purification system, which worsens the ecology of production and the quality of the final product.

Biotechnological methods allow to obtain chitin under more gentle processing conditions. However, they are more expensive, and the final product contains a large amount of protein.

As an alternative, in recent years, an electrochemical technology for isolating the chitin-glucan complex, based on the conversion of electrical energy into the energy of chemical reactions, has been developed. The process of electrolysis allows controlling the properties of the final product and chitin obtained by electrochemical technology, and increases the sorption ability, as evidenced by a significant number of literature sources.

Loss on drying was measured by the method of drying to constant weight. Lipids were measured by the extraction method. The ash was measured by drying at a temperature of 500-600 °C. The nitrogen-free extractives were calculated from the difference between 100% and the sum of percent hygroscopic water, raw ash, lipids, cellulose and proteins (Клишанец, 2018).

## Results of Research

As a result, two technologies for isolating the chitin-glucan complex from the by-product of the citric acid production of *Aspergillus niger* biomass have been developed: the retreatment of chitin-containing raw material with a solution of hydrochloric acid and an ozone-air mixture (Клишанец, 2018).

The method developed for obtaining a chitin-glucan complex by pretreatingthe chitin-containing raw material with a solution of the hydrochloric acid includes the following operations (Клишанец, 2018):

- preparation of chitin-containing raw materials (mixing the chitin-containing raw materials with the hydrochloric acid solution; heating; filtration; separation of a semi-finished product; mixing the resulting semi-finished product with glucose solution; filtration; separation of a solid fraction);
- mixing the resulting solid fraction with the previously obtained catholyte solution; ageing for several hours; heating; separation of the solid fraction, the chitinous semi-finished product;
- electrochemical treatment;
- heating and separating the finished product.

The presence of dark pigment melanin reduces the consumer characteristics of the final product. For this reason, the technology developed requires an additional stage, that is depigmentation. The depigmentation step was carried out using a hydrogen peroxide solution. The effect of the parameters of processing with a solution of hydrogen peroxide on the mass fraction of the yield of the final product and on the change of the colour of the chitin-glucan complex (20 samples) was studied. The technique of expressing colour with numerical coordinates has been adapted with respect to food products, which made it possible to determine the optimal technological parameters of an additional stage of processing the chitin-glucan complex with hydrogen peroxide solution.

The method developed for obtaining a chitin-glucan complex according to the technology with pretreatment of chitin-containing raw materials by the ozone-air mixture includes the following operations (Клишанец, 2018):

- preparation of chitin-containing raw materials (extraction of biomass, washing it to neutral reaction with distilled water);

- determination of the number of cyanides in *Aspergillus niger* biomass;
- mixing the biomass with the previously obtained catholyte solution;
- treatment of the mixture with an ozone-air mixture;
-stabilizing, filtering the mixture, washing up to neutral reaction with distilled water;
- separation of the solid fraction, that is, the chitinous semi-finished product;
- electrochemical treatment;
- heating and separating the finished product.

It should be noted that the technology developed by using an ozone-air mixture is fundamentally new. The finished product obtained applying this technology has a higher mass and, as a consequence, a larger mass fraction of dry matter and a smaller mass fraction of moisture. Moreover, it does not require an additional stage of bleaching with hydrogen peroxide solution in comparison with the technology developed for isolating the chitin-glucan complex by using a solution of hydrochloric acid. The technology developed by using an ozone-air mixture does not require the cost of reagents (hydrochloric acid) and the withdrawal of the site for the preparation of solutions, which will reduce raw material and time costs as well as decrease the production area. However, the chitin-glucan complex obtained by using the technology of the pretreatment of the chitin-containing raw material with the ozone-air mixture has a high mass fraction of the ash.

The comparative characteristics of the physicochemical parameters of the quality of the chitin-glucan complex obtained by the technologies developed are presented in Table 1.

*Table 1 - Comparative characteristics of the physicochemical parameters of the quality of the chitin-glucan complex obtained by the technology of pretreating the chitin-containing raw material with a solution of hydrochloric acid (1) and an ozone-air mixture (2)*

| № | Content in the finished product,% | | | | | |
|---|---|---|---|---|---|---|
| | loss on drying | ash | proteins | lipids | cellulose | nitrogen-free extractives |
| 1 | 13,0±0,20 | 3,77±0,05 | 1,99±0,02 | 0,29±0,02 | 49,65±0,11 | 31,30±0,38 |
| 2 | 10,10±0,15 | 8,16±0,05 | 13,71±0,19 | 0,10±0,03 | 51,83±0,26 | 16,10±0,63 |

## Conclusions

Therefore, having estimated the mass fraction of the ash of the final product as an indicator of the depth of demineralization, it is recommended to apply the technology of pretreating the chitin-containing raw material with a solution of hydrochloric acid for the production of chitin-glucan complex for further use in the food industry due to the low content of ash and protein, and the chitin-glucan complex. In other industries it is recommended to apply the technology of pretreating chitin containing its raw materials by an ozone-air mixture.

The resulting chitin-glucan complex presented for tasting has a pronounced brown colour. It should be noted that in the production environment, the colour of the produced chitin-glucan complex may vary depending on the purpose of the use by introducing an additional depigmentation step.

The studies of the morphological structure, chemical composition and X-ray structural analysis of the obtained chitin-glucan complex were carried out. It has been established that the surface of the chitin-glucan complex has an uneven structure. On the surface, there is a large number of different convexities and concavities. The shape and size of these formations differ, there is no regularity of these formations. The following chemical elements were found on the surface of the chitin-glucan complex: oxygen 82.32%; calcium 10.44%; aluminium 2.66%, magnesium 2.20%, silicon 1.41%, phosphorus 0.97% (Клишанец, 2018). However, the chemical elements such as sodium and potassium were not observed on the surface of the chitin-glucan complex. The study of the white formations found on the surface of chitin-glucan complex revealed that they are formed from the following chemical elements: calcium 50.81%, oxygen 47.41%, sodium 1.21%, magnesium 0.34%, aluminium 0.18%, phosphorus 0.05% [5]. Silicon and potassium in crystalline formations are absent.

The diffraction pattern of a sample of the chitin-glucan complex contains reflexes characteristic of human chitin: 9.2°, 12.6°, 26.2°. However, in the region of 19.2° [5], there is a sharp amorphous halo. It is the amorphous region that is responsible for the sorption properties of the final product. It should also be noted that the presence of glucan does not occur in the new structures.

Therefore, on the basis of the studies carried out, scientifically based technologies for isolating the chitin-glucan complex from the byproduct of citric acid production of *Aspergillus niger* biomass have been developed; and the effect of demineralization, deproteinization and depigmentation of chitin-containing raw materials on quality as well as the quantitative weight loss of the final product has been established. The optimal parameters of demineralization and deproteinization make it possible to achieve a significant economic effect by reducing raw material costs. The comparative organoleptic evaluation with imported analogues revealed that the chitin-glucan complex obtained by taste characteristics is significantly different from the imported one. It has an indistinguishable smell and neutral taste. For the first time in the Republic of Belarus, a study of the morphological structure, chemical composition and X-ray diffraction analysis of the chitin-glucan complex obtained from a byproduct of the citric acid production of *Aspergillus niger* biomass has been made (Клишанец, 2018).

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