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Chitosan Technology from Crustacean Shells of the Northern Seas

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Abstract

Technological schemes for the production of chitin and chitosan from the crustaceans of the Barents Sea have been developed. We used shells of king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) as chitin-containing raw materials, which are waste from the processing of crabs and contain 5.5 and 4.9 wt.% chitin, respectively. Technological schemes are developed taking into account the chemical composition of the used raw materials containing a large amount of residual protein (up to 26 wt.% in the king crab shell) and mineral substances (up to 17 wt.% in the snow crab shell). A chemical method for chitin production has been used. The technological scheme includes the stages of the first deproteinization, demineralization, the second deproteinization and depigmentation of the raw materials using chemical reagents - acids, alkalis, etc. The deacetylation reaction in an alkaline medium was used as the main method for chitosan production from chitin. Technological solutions have been found to significantly reduce the consumption of alkali, to form a circuit of alkaline solutions. This leads to the reduction of pollution of wastewater generated during the production of chitin and chitosan. The resulting polysaccharide chitosan has a degree of deacetylation of 80--85%. Such a product is considered as a valuable ingredient for high-quality functional foods.

Keywords: chitin, chitosan, crustacean processing technology, deacetylation reaction

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1. Introduction

Functional ingredients with high nutritional and biological value are of great importance for the development of high-quality food products including functional ones. Ingredients derived from marine biological resources are the most promising. Among them, polysaccharide chitosan derived from crustacean chitin occupies a special place. Chitosan is called the "biopolymer of the 21st century" for its complex of unique properties [1--3] which are determined by the structure of its macromolecules.

Chitin (poly-N-acetyl-D-glucose-2-amine) is the second most abundant (after cellulose) polysaccharide, the macromolecule of which is built from residues of N-acetylglucosamine linked by β- (1 → 4) glycosidic bonds. Chitosan is a deacetylated chitin derivative, which is a biopolymer consisting of β-D-glucosamine units. The production of chitosan is based on the reaction of cleavage of the acetyl group (N-deacetylation) and its replacement by an amino group in the structural unit of chitin [4--8]. The deacetylation reaction may be accompanied by a simultaneous breaking of the glycosidic bonds of the biopolymer. As a result, chitosan has a structural heterogeneity due to the incomplete completion of the deacetylation reaction and the breakdown of the polymer chain (polydispersity) [9]. Chitosan has a complex of unique properties; they determine its demand in agriculture, biomedicine [10], the cosmetic industry, the food industry [11]. In recent years there has been a rapid increase in the number of publications on chitosan [see monographs 12--15]. Among them, works on the technological aspects of chitosan production and the features of the deacetylation reaction [8, 16, 17] occupy a special place, which is the basis of technological solutions.

The history of the study of chitin and chitosan [18] cannot be complete without a mention of a scientific and technological school established in the Murmansk region. The main research of this school is related to the development and improvement of the technology of chitin, chitosan and their derivatives [19]. This is due to the proximity to the seas and coastal enterprises engaged in the processing of crustaceans. The composition and properties of chitin-containing raw materials influence technological solutions in the production of chitin and chitosan. Initially, in the mid-80s of the twentieth century, research conducted in Murmansk was associated with the use of shells of arctic krill and northern shrimp. Currently, in the Barents Sea, the king crab population has grown to an industrial scale. Therefore, the task of complex waste-free processing of the king crab is extremely important.

The work is devoted to the creation of technologies for chitin and chitosan from the shells of crustaceans of the northern seas using the king crab and snow crab. These technologies are central to the complex processing of crustaceans.

2. Materials and Methods

2.1. Materials

Shells of king crab (*Paralithodes camtschaticus*) and snow crab (*Chionoecetes opilio*) caught in the Barents Sea were used as raw materials for chitin and then chitosan

production. The shells were cleaned of muscle fragments, washed in running water, frozen and stored at −20 ˚C until the experiments. The chemical composition is given in Table 1. Chitin content is 5.5 and 4.9% in the shells of the king crab and snow crab, respectively. Raw materials contain not only chitin but also other valuable natural substances that could find practical application.

All chemical reagents used in the work had analytical-grade purity (Pro Analysis).

Table 1: Chemical composition (in wt.%) of king crab and snow crab shells.

Raw materials	Water	Protein	Chitin	Ash	Lipids
Shell of King crab	68.0±2.0	8.6±0.5	5.5±0.2	17.0±1.5	0.9±0.1
Shell of Snow crab	53.8±5.0	25.7±1.5	4.9±0.2	15.1±1.5	0.5±0.1

2.2. Methods

There are several ways to obtain chitin: chemical, biotechnological, physico-chemical and others. We used a chemical method consisting in carrying out the stage of deproteinization, demineralization, and depigmentation of the raw material using chemical reagents - acids, alkalis, etc. The deacetylation reaction was used as the main method for producing chitosan from chitin. The structural properties of chitin and chitosan are shown in Figure 1. FTIR spectroscopy was used to determine the degree of deacetylation of chitosan [20]. FTIR spectra were obtained using an IRTracer-100 FTIR spectrometer (Shimadzu, Japan); the frequency range was from 4000 to 800 cm−1 with a resolution of 4 cm−1 (the number of scans was 250).

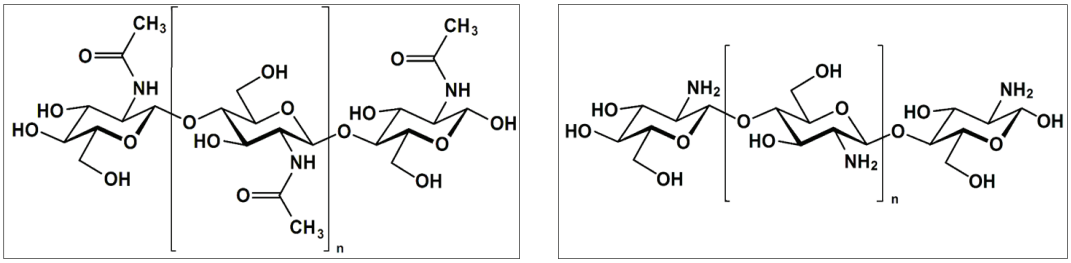

Figure 1: Structural formulas of chitin with a degree of deacetylation of 0% (a) and chitosan with a degree of deacetylation of 100% (b).

3. Results and Discussions

3.1. Chitin production

The technology of chitin is not difficult in general. It consists in the separation of lipids, proteins (deproteinization), and mineral salts (demineralization) from insoluble chitin. We have developed a technological scheme taking into account the composition and structure of the used raw materials; the scheme is presented in Figure 2.

In the scientific literature, as a rule, they use technology in which the first stage is demineralization [18, 20]. But the raw materials, we used, contain up to 50% of residual protein and 20-30% of mineral substances even when manually cutting crabs (Table 1). It is advisable to remove first the largest proportion of impurities - proteins. Therefore, we propose to use deproteinization as the first stage (Figure 2). In addition, protein selected as a by-product can be used in the future.

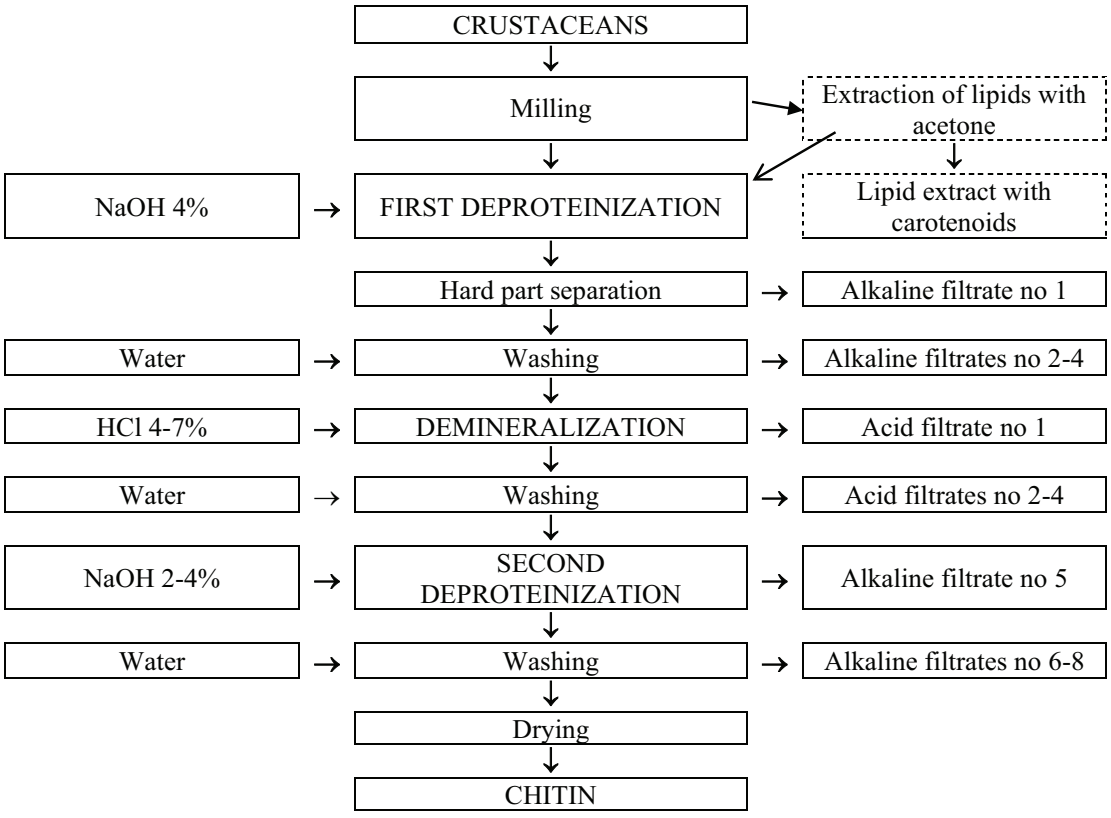

Figure 2: A flow diagram showing the technology of chitin.

The deproteinization was performed with a dilute NaOH solution. The degree of protein extraction and its quality can be controlled by changing the concentration of the alkali solution, temperature and time. The deproteinization was carried out at low temperatures (20-50 °C), a low concentration of NaOH solution (1-4%). Within 30-60

minutes of this treatment, about 90% of the proteins go into a solution; while protein hydrolysis is minimal. Proteins (up to 85%) were precipitated by neutralizing the protein solution to the isoelectric point (pH 5-5.5). Such precipitation allows a considerable reduction in wastewater pollution with protein. Then the protein precipitate was dried. In the future, it can be used for feeding purposes or to obtain protein hydrolysates [19].

The next stage of the technological scheme is demineralization (Figure 2), that is the removal of salts that make up the shell. The chemical composition of the mineral part of the shell is the carbonates and phosphates of calcium and magnesium, usually in the form of calcite and hydroxyapatite [21], like the bones of land animals. In addition, the mineral part includes various trace elements, but their presence does not affect the regimes of demineralization.

Diluted solutions of mineral acid are usually used for demineralization, for example, HCl, which gives water-soluble calcium and magnesium salts. The acid concentration is selected based on the mass fraction of minerals in the shell of the crustacean. Under a hydronic module of 8:1-12:1, ensuring good mixing of the reaction mixture, the concentration of HCl solution for demineralization of the deproteinized crab shell is about 2 mol/dm^3 (7.2%). The reaction effectively proceeds at room temperature for 30 minutes with the release of carbon dioxide. To avoid acidic destruction of chitin, sometimes demineralization is carried out at a low temperature (down to 0 °C).

The result of demineralization is a solution of calcium and magnesium chlorides. In the first versions of the technology, this solution was mixed with an alkaline solution after deproteinization for their mutual neutralization. The resulting precipitate was separated, dried and used as a feed additive. But this method did not allow all mineral salts and all proteins to be precipitated therefore the wastewater was heavily polluted. In the developed technological scheme, we propose to use the soda solution (Na_2CO_3) for neutralization the solution after demineralization. As a result, almost 90% of all salts are precipitated.

After the first two stages of processing the shell, chitin is obtained, which is still contaminated with residual protein inside the chitin fibrils. In addition, the shell is strongly colored by carotenoids, which are practically not extracted in the first stages. Therefore, we added the second deproteinization (Figure 2), the so-called alkaline washing. The regimes of this treatment are: the concentration of NaOH solution is 2-4%, the temperature is 95-100 °C, the duration is 20-30 minutes.

The output is pure white chitin. The residual amount of proteins is less than 0.5%, ash is less than 0.1%. Such chitin is a finished product that can be used on its own or to produce chitosan.

3.2. Chitosan production

The reaction underlying the production of chitosan from chitin is the reaction of deacetylation in an alkaline medium (Figure 3). To obtain chitosan, it is necessary to remove most or all of the acetyl groups attached to the amino groups of chitin. In this case, a polymer is formed with free amino groups, which, after protonation, impart solubility and determine the polycationic character of chitosan.

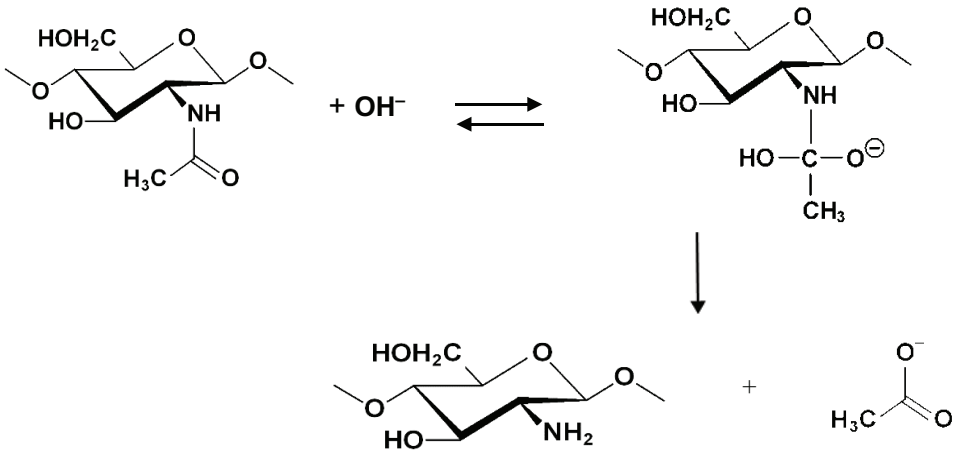

Figure 3: Scheme of the reaction of chitin deacetylation in alkaline medium.

The only industrial method that has become widespread in the world is alkaline deacetylation in concentrated solutions of NaOH or KOH at high temperature [18, 22]. Recently, there have been attempts to develop enzymatic technology [23] using deacetylase [24], but there is no information about large-scale production.

Deacetylation is a fairly simple process (Figure 4): preparing a NaOH solution (50%), heating it to 100 °C, loading dry chitin, mixing for 30-60 minutes, separating the obtained chitosan by filtration and rinsing with hot water. The difficulties of the process are primarily due to the use of hot concentrated alkaline solutions and the utilization of waste solutions.

The first problem with the proper organization of production and compliance with safety measures is solved quite easily using the necessary equipment and the transfer of the reaction mixture through pipelines using a vacuum.

We have taken a definite step back from the technology developed in the 70s and 80s. At that time, trying to reduce the consumption of expensive alkali and the amount of recycled alkaline solutions, they tried to reduce the hydronic module: the ratio of the mass of the alkali solution and chitin was adjusted to 10 or even 5. As a result,

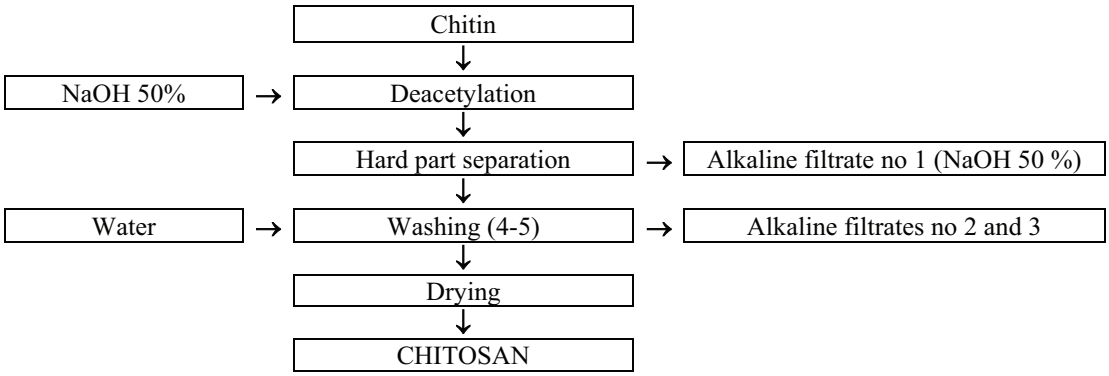

Figure 4: A flow diagram showing the technology of chitosan.

the consumption of alkali decreased, but the reaction mixture was high viscous, mixed poorly, resulting in heterogeneous deacetylation of chitin.

We increased the hydronic module to 20 or even 30. This made it possible to easily mix chitin with an alkaline solution, mix it in the process of deacetylation, and send it through the pipelines without diluting it to the filter. After filtration, a practically 50% NaOH solution mixed with sodium acetate was obtained. Part of the alkali was naturally lost with chitosan precipitate. But after the chitosan washes, the wash solutions remained with an alkali concentration of about 4%, which can be used at the deproteinization stages. The concentrated filtrate was adjusted to the initial concentration and volume for reuse during deacetylation.

It should be noted that such a reverse use of alkaline solutions is the more effective the cleaner chitin. Practically the only pollution of the NaOH solution is sodium acetate, which as shown by our research does not affect the deacetylation and deproteinization reactions. Chitosan, obtained by the developed technology, was characterized by a degree of deacetylation of 80--85%.

Using this technology, we reduced the consumption of alkali to a minimum, which was determined only by the degree of extraction of the chitosan precipitate after separation of the concentrated alkaline solution. Considering the further use of wash solutions for deproteinization, this consumption was about 2.5 kg NaOH per 1 kg of chitosan, although the use of vacuum filters could reduce this consumption. But in this case, the alkali could not be enough for the first stages of processing the shell.

4. Conclusion

As a result of research and experimental work, complex technological schemes for chitin and chitosan production have been developed. These technologies allow maximum

utilization of all components of the crustacean shells. An integrated approach was applied to technological processes in the preparation of chitin and chitosan, taking into account the specific properties of the raw materials - crustaceans of the Arctic seas. Research is associated with the development of environmental management, the creation of waste-free technology of marine crustaceans and the creation of high-quality functional food products based on chitosan.

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Conflict of Interest

The authors have no conflict of interest to declare.

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