Original Scientific Paper
DOI: 10.7251/AGRENG1603089G
UDC 639.512:544.032.52

PREPARATION AND PROPERTIES OF CHITOSAN FROM CRAB SHELL CONTAINING RAW MATERIAL BY ELECTROPHYSICAL PROCESSING

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ABSTRACT

Traditional technologies of chitosan producing involve the use of hard alkali-acid treatment of crab shell raw materials, which negatively affects the main quality parameters of chitosan (molecular weight, the degree of deacetylation). We propose an alternative technical approach. It involves the use ofelectrohydraulicshocks,whichuseextra-long bits.Theproposed approach has the following advantages:the stages of grinding and deproteinizationof the raw materialsare combined,the additional use ofalkaliis excluded at the stage of deproteinization.For comparative characteristics of the structure of the polymer the IR-spectra of the samplesof chitosanwere removed. Chitosan, obtained using electrohydraulic treatment is not inferior in its physico-chemical parameters of chitosan, obtained by using alkaline reagents. It is possible to organize the process of chitosan production on the base of the enterprises for shrimp processing. Specific requirements for physico-chemical and functional properties of chitosan-containing substances make the actual means and methods of control of the target parameters, the key of which are qualitative identification and comprehensive determination of chitosan in the composition of film-forming compositions and films coatings. As the chromophore to measure the surface potential of the chitosan substances, we used 1-aniline-8-naphthalenesulfonate (ANS). The maximum fluorescence of the dye in chitosan films is shifted to longer wavelengths compared to chitosan gels, because of the increased polarity of the medium of films on the attitude to gel-like chitosan substances. The data obtained by thefluorimetric studies can be used in the development of methods for the detection of chitosan.

Keywords: *chitin, chitosan, electro-shocks, the degree of deacetylation*, *shrimp shell.*

INTRODUCTION

The issues of intensification of processes for the production of structural biopolymers – chitin and chitosan, occupy a central place in the works of domestic

and foreign researchers(Cho et al, 2000; Viarsaghl et al, 2009; Gartman et al, 2013). This trend reflects the global direction of development of all processing industries, including food and pharmaceutical production. The traditional technology of obtaining the chitosan involves the use of the shell crustaceans. The main role in the traditional technologies played by the transfer of the matterfrom one phaseto another.

The technology of obtaining the chitosan from chitin-containing raw material includes the following main stages: grinding of raw materials; removing the protein fractions (deproteinization); translation the mineral components of raw materials in the soluble form (demineralization); deacetylation of chitin with obtaining the chitosan.

The priorities in this area are the technological solutions that reduce the consumption of aggressive reagents at the stage of the deproteinization of the shell crustaceans. For example, the replacement of the sodium hydroxide solution to the ammonium hydroxide solution is allowed to obtain the volatile components as the reaction products (Kasyanov, 2013).

The purpose of the work is to develop the technique and technology of obtaining chitosan with the use of electrophysical processing of chitin-containing rawmaterials of the crustaceans.

MATERIALS AND METHODS

We used crab shell containing raw material (SCRM) obtained in the industrial processing of freshwater crayfish, Arctic shrimp (*Ledovo* Company,Schelkovo, Moscow region). The catch season was from March to October, 2015.Shrimps have been caught in Okhotsk Sea (the Far East fishery basin).

We received and investigated the experimental samples of chitosanin the laboratory of Technology and Merchandizing Faculty in Voronezh State Agricultural University from April to December, 2015.

The fluorimetric studyof chitosan substances we carried out in the laboratory of Institute of Cell Biophysics of the Russian Academy of Sciences(Pushchino, Moscow region) in May, 2015.

The quality of the obtained chitosan was adjusted on a complex of indicators. The content of minerals was established by dry ashing.

The molecular weight of chitosan was determined by a standard viscometric method. The measurements were carried out at 250 °C in a capillary viscometer Ubbelohde, the diameter of which is equal to 0.54 mm. A sample of chitosan we previously dispersed in succinic acid. Calculation of molecular weight was carried out according to equation of Mark-Kuhn-Houwink (Gartman*et al*, 2013).

The degree of deacetylation was determined by potentiometric titration on universal ionometer EV-74 using a glass electrode. The method is based on the titration of the chloride hydrogen connected with a molecule of a chitosan. The researches were carried out by the titration of solution of a chitosan by sodium hydroxide solution.

Microbiological parameters were determined according to standard procedures.

The comparative evaluation of structural changes of products of chitin-containing raw material subjected to various types of preliminary treatment with chemical reagents was carried out with the use of a method of IR-spectroscopy (Vasilyev*et al*, 2007). The IR spectra of chitosan are removed on the spectrophotometer Vertex70 (*Bruker, Germany*) in the rangeof 4000-400 cm^{-1}. The preparations were prepared by drawing a thin film of a sample on a silicon substrate.

Spectral properties of chitosan dispersions and films was studied using the fluorescent double-beam scanning spectrophotometer PERKIN ELMER Lambda 800. We recorded the fluorescence spectraat 20 °C in a mirrored cuvette with optical path length 1 cm in the range of 400-550 nm (upon excitation at 370 nm) and 430-500 nm (upon excitation 380 nm). The light-transmitting slit was set at 8 mm. The samples for fluorescence studies containing chitosan and hydrophobic dye with the concentration of $1*10^{-6}mol/dm^3$, were incubated for 2-3 hours at 20 °C. Fluorescence ranges of dye solutions and chitosan containing compositions weresubtracted from the fluorescence spectra of the samples. In determining the degree of polarization we used the wavelength of excitation 380 nm and emission of 430 nm.

The magnitude of light scattering was measured on a spectrofluorimeter similar to the previous tech experience (in the mirror cells) in the crossed monochromators: at the same wavelength of 560 nm (slit of 8 nm in the first monochromator and 1 nm in the second).The channel got ambient light in proportion to the size of the particles and their number.

Chitosan films were prepared from chitosan substances with the addition of 1-aniline-8-naphthalenesulfonate (ANS) with a concentration of $1.56*10^{-6}mol/dm^3$by the method of spreading on the glass substrate with subsequent evaporation of the acid in the air. The films were kept to evaporation of the acid at the ambient temperature for 36 - 48 h. To study the films were deposited on cover glasses and placed in a glass cuvette on the diagonal. Fluorescence spectrum was removed in the range of 430-500 nm, at the excitement wavelength310 nm. The light-transmitting slit size was set at 5 nm for excitation and 2.5 nm for emission.

RESULTS AND DISCUSSION

Electrohydraulic shock allows transformingthe electrical energy into mechanical energywithout the intermediate mechanical links. In the case of implementation of electrohydraulic shock in the volume of the liquid which is in a tank under the influence of specially created pulse electric spark discharge around a zone of its education there are extreme hydraulic pressures capable to make the useful mechanical operation and followed by a complex of the physical and chemical effects.

The technological capabilities of electrohydraulic shock are provided at the expense of superlong discharges in the carrying-out liquids by the limit reduction of the activearea of the positive electrode (that is adjoining to liquid). At the same time increasing the active area of the negative electrode(Yutkin, 1986).

For reproduction of the electrohydraulic shocks in the volume of the compound consisting of the the shell of crustaceans and water in the ratio 1:15 we used the installationwhich is turning on the source of energy with the condenser as the accumulator of electrical energy (Figure 1).This scheme is implemented in the original technical solution of the apparatus for producing chitin and chitosan(Figure 2).

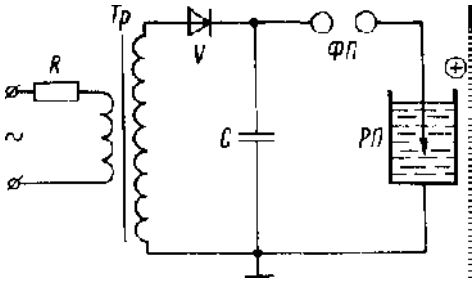

Figure 1. An electric circuit of installation for reproduction of electrohydraulic shocks: R - charge resistance; Тр - the transformer; V - rectifier; ФП - the creating spark interval; РП – a work space; С – the capacitor

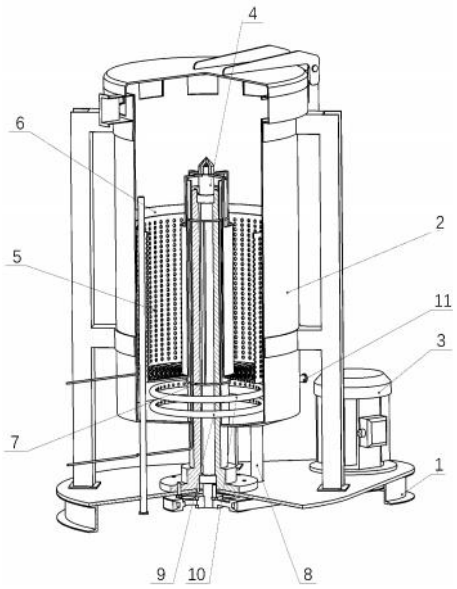

Figure 2. General view of the installation for obtaining of chitin and chitosan: 1 – base box; 2 – reactor tankage; 3 - electric motor; 4 - vertical shaft; 5 - perforated removable container; 6 –externalnegative electrode; 7 - central positive electrodes; 8 - drain pipe; 9, 10, 11 -the nozzles for supplying process media

We established experimentally that the processing of shell crustaceans is advantageously to carry out with an average mode of operation of the electro-hydraulic setup, the capacitance of the capacitor (C) = 0.1 μf; voltage U= 50 kV; the distance between electrodes (l) = 25 mm; the temperature of the mixture (t) = 20 °C. In the result there is a grinding shellcrustaceans in suspension to a particle size of 0.05-0.1 mm. The resulting suspension is passed through a suction filter, the precipitate is placed inthe reactor with a mixer and a shirt. The precipitate is treated with hydrochloric acid with volume fraction of 2-4% at the hydraulic module of 1:10, a temperature of 20-25 ^{o}C and stirring for 2 h.Further the solid and the liquid fraction were separated, the precipitate was washed with distilled water to pH 7.0. Depending on the kind of used shell crustaceans, next a solution of sodium hydroxide with a mass fraction of 35-45% was added to the obtained chitin and the mixture was incubated at a temperature of 95-98 °C during 1-2 hours. Table 1 presents the physico-chemical characteristics of chitosan which was obtained by the proposed method in comparison with the the traditional way.

Table 1. Physico-chemical characteristics of chitosan samples obtained by different methods

Physico-chemical characteristics of chitosan	The chitosan from the carapace of a crab(the producer is "Bioprogress", Schelkovo, Moscow region)	The samples of chitosan obtained by the proposed method	
		from the shell of a shrimp	From the carapace of the freshwater crayfish
Characteristic viscosity (in the 2 % solution of acetic acid), dl/g	25.0	24.1	22.9
Molecular mass, to	260	300	270
Degree of deacetitisation, %	82	92	90
Mass share of ash, %	0.7	0.4	0.5
Protein residue,%	0.05	0.05	0.03
Mass share of moisture , %	9	9-10	8-10
Particle size (granulometry-cal composition), mm	0.1-0.2	0.05-0.1	0.05-0.1

The microbiological characteristics of chitosan obtained by the proposed technology under laboratory conditions, were determined. Data are presented in the table 2.

Table 2. Microbial attributes of chitosan

The nameof an indicator	The threshold value	Actual measure value
Mesophilic aerobic and facultative anaerobic microorganisms, Units forming colonies /g	$4 \cdot 10^4$	$3.9 \cdot 10^4$
Coliform bacteria	Prohibited	Not discovered
Pathogenic microorganisms	Prohibited	Not discovered
Mold fungi, Units forming colonies /g	$2 \cdot 10^4$	$1.7 \cdot 10^4$

Organoleptic characteristics of chitosan derived from shrimp shell by the developed technology in comparison with the commercial sample are presented in the Table 3.

Table 3. Organoleptic parameters of chitosan

Indicator name	Characteristics for the samples of chitosan	
	from the carapace of a crab (the producer is "Bioprogress", Schelkovo, Moscow region)	from the shell of a shrimp
Appearance	Fine-fibrous particulates	Fine powder
Colour	Yellow	White
Smell	No smell	No smell

Organoleptic characteristics of chitosan from shrimp shell is almost identical to the biopolymer obtained by the traditional method. The contrast of chitosan according to the traditional technology is the white color due to the presence in the production stage of bleaching.

For comparative characteristics of the structure of the polymer was removed IR spectra of chitosan samples (Figure 3) obtained from the shell of shrimp (the proposed technology) and crab (traditional technology).

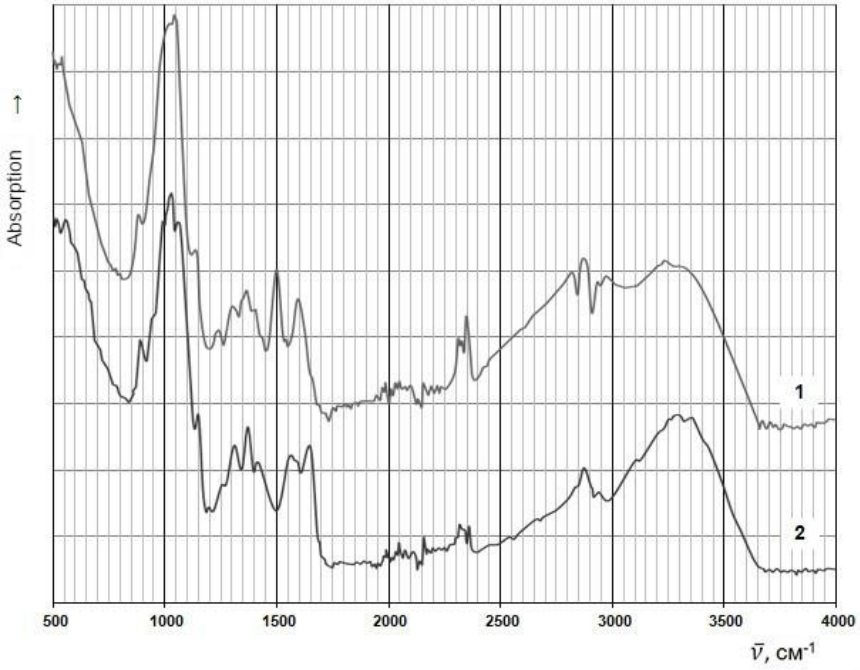

Figure 3. IR-spectra of chitosan from the carapace of a crab, the manufacturer is the closed joint-stock company "Bioprogress" (1) and from the shell of shrimp (2)

In the IR-spectra of chitosan samples presented in the Figure 3, pronounced absorption bands 1010-1050 and 1310-1360 cm^{-1}. They characterize the deformation fluctuations of hydroxyl group of primary and secondary alcohols.In the range of 3250-3350 cm^{-1} there is a broad absorption peak corresponding to the stretching vibrations of the hydrogen atoms in hydroxyl and amine groups (Otto, 2003; Tarasevich, 2012).Absorption peaks in the 2880-2950 cm^{-1} correspond to the stretching vibrations of methylene groups.

Analysis of IR spectra, in general, showed both the identity of the chemical structure of the chitosan samples. But attention is drawn to the presence of a second sample in the spectrum absorption bands 1550-1560, 1650 cm^{-1} characteristic to a greater degree the oscillations NH-links the primary amino groups, and their displacement in the long wavelength region for the first sample to 1510 and 1600 cm^{-1}, corresponding to vibrations of secondary amide linkages. The presence of additional absorption peaks of 2820, 2980 cm^{-1} in the spectrum of the first sample may be due to variations in the CH-bond methyl acetyl groups of chitin fragments. Thus, these data indicate incomplete deacetylation of chitin in the preparation of the sample 1 by the acid-alkali treatment.

To use the chitosan substances in various branches they have to possess the specific physical, chemical and functional properties. In this regard, the actual task is to develop the means and methods of control of target parameters, key of which are qualitative identification and quantitative determination of chitosan in the composition of the functional compositions and products with their use.As the chromophore to measure the surface potential of the chitosan substances, we used 1-aniline-8-naphthalenesulfonate (ANS). The maximum fluorescence of the dye in chitosan films is shifted to longer wavelengths compared to chitosan gels, because of the increased polarity of the medium of films on the attitude to gel-like chitosan substances(Vekshin, 2015).The data obtained with the use of fluorometric studies can be used in the development of methods for the detection of chitosan.

CONCLUSIONS

We have developed an alternative technical approach to obtaining chitosan from crustacean shell.It is designed to combine the stages of grinding and deproteinization, avoids the use of alkali at the stage of deproteinizationthrough the use of electro-hydraulic shock is carried out using extra-long bits (Balabaev, 2015; Glotova, 2015). The proposed method of obtaining chitosan has the following advantages: the possibility of organizing the recycling process of shellscrustaceans on the production base of processing of the main raw material; reducing the consumption of alkaline and sewage the volume of waste waterthrough the use of electro-shock on the stage of the deproteinization. Thus it can be concluded that the chitosan obtained by electro-processing is not inferior in its physical and chemical indicators from the samples of chitosan obtained with traditional alkaline reagents. By the combining of the processes of grinding and deproteinization of the shellsofcrustaceans,it is possible to reduce the total duration and labor input of the process, to improve the ecological state of the production.

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