Synthesis and characterization of chitosan from marine sources in Black Sea

Dilyana Zvezdova

Abstract: Chitosan has been extracted from different marine crustacean from the Black Sea of Bulgarian Gulf. The contents of the various exoskeletons have been analyzed and the percent of the inorganic salt , protein, chitinand chitosan was determined. Deacetylation of the different chitin produced was conducted by the conventional thermal heating method. The chitosan was characterized by elemental analysis, FTIR measurements.

Key wards: *Chitosan , demineralization, deprotenization,deacetylation, FTIR spectroscopy*

INTRODUCTION

Chitosan is chemically defined as a copolymer of *α*-(1,4) glucosamine $(C_6H_{11}O_4N)_n$, with a varying content of N-acetyl groups. It is produced by deacetylation of chitin [1,8,12]. Figure 1 indicates the chemical structure of glucosamine, which is the building unit of chitosan.

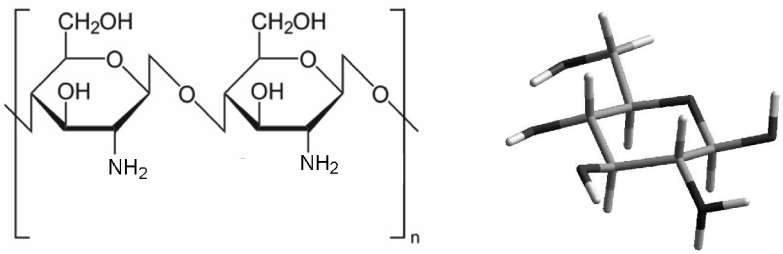


Fig. 1 Chemical and 3D-structure of N-Dglucosamine

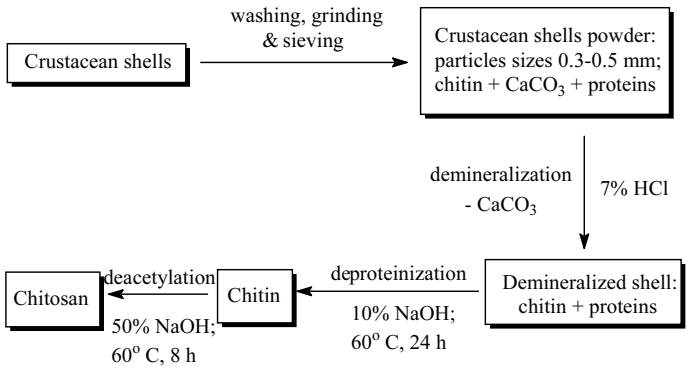
As a naturally occurring polysaccharide, chitosan shows many unique properties, such as biocompatibility, biodegradation, biological activity and low-toxicity [18]. Consequently, chitosan and its derivatives have drawn the attention of multidisciplinary research groups. The characteristics of chitosan in solutions depend on the degree of deacetylation (DD), the distribution of acetyl groups, the chain length, the molecular weight distribution, and the solubility [2]. Among the most important features of chitosan in solution is the conformation of this polymer at the surfaces and in the bulk phase. Consequently conformational studies may contribute to the understanding of different phenomena related to the interaction between chitosan and the solvent. The conformational flexibility of chitosan was previously reported, and is attributed to the presence of the free primary amino groups [9,13,14,22]. It was concluded that conformational changes occur from looser chain to coiled sphere upon increasing concentration of chitosan solutions [13]. However, these studies were carried out on widely dispersed high molecular weight samples. Because of low molecular weight chitosans are more suitable for incorporation in liquid drug delivery systems, it becomes necessary to understand their conformational behavior in aqueous solutions.

Several techniques to extract chitosan from different sources have been reported. The production of chitosan from crustacean shells obtained as a food waste is economically feasible, especially if it includes the recovery of carotenoids. The shells contain considerable amount of astaxanthin, a carotenoids that have so far not been synthesized, and which are marked as a fish food additive in aquaculture.

Chitosan itself was directly extracted from fungi by alkaline and acid treatment [4]. Some authors [15,23] have developed methods to use microorganisms or proteolytic enzymes

for the deproteinization of the crustacean chitin wastes in this way a more economic production of chitin and chitosan can be achieved.

The most common method is referred to the chemical procedure [Sheme 1]. The chemical method for isolation of chitosan from crustacean shell biomass involves various major steps: elimination of inorganic matter (mainly calcium carbonate) in dilute acidic medium (demineralization) and chitin deacetylation. Usually demineralization is accomplished by using HCl followed by extraction of protein matter in alkaline medium (deproteinization). The later is traditionally done by treating shell waste with aqueous solutions of NaOH [Sheme 1]. The effectiveness of alkali deproteinization depends on the process temperature, the alkali concentration, and the ratio of its solution to the shells.



Scheme 1. Isolation of chitosan from marine sources

The aim of the present work is to isolate the useful polymer chitosan from the marine sources in the Black Sea shelf of Bulgaria. The experimentally prepared chitosans were characterized by means of FTIR spectroscopy.

METHODS

1. Extraction of chitin

1.1. Raw materials preparation

The different local resources used to extract chitosan are shrimp shell (CS-Ss) and crab shell (CS-Cr). The shells of these species were scraped free of loose tissue, washed, dried, and grounded to pass through a 0.315-0.5 mm sieve. Then they were subjected to demineralization, deproteinization and deacetylation (Scheme 1).

1.2. Demineralization

Demineralization was carried out in dilute HCl solution. The mineral content in the exoskeleton of crustacean is not the same for each species treatment. All species were treated with 7% HCl solution at ambient temperature with a solution to solid ratio of 10 ml/g. The resulting solid fraction was washed with distilled water until neutral pH was achieved. Then the demineralized samples were dried and weighed. The treatments with hydrochloric acid and their durations (24-72 h) depend on the nature of species. It was observed that the emission of CO_2 gas depends upon the mineral content of different species and penetration in shell's mass.

1.3. Deproteinization

Deproteinization of chitin was carried out using 10% NaOH at 60 °C. The treatment was repeated several times. The absence of proteins was indicated by the absence of color of the medium at the last treatment, which was left overnight. Then the resulting solution was

washed with water to neutrality and with hot ethanol (10 ml/g). The purified chitin was dried at 50 °C to constant weight. The chitin content was determined from the weight differences of the raw materials and that of the chitin obtained after acid and alkaline treatments.

1.4 Deacetylation of chitin

The chitin (10 g) was put into 50% NaOH at 60°C for 8 h to prepare crude chitosan. After filtration, the residue was washed with hot distilled water at 60°C for three times. The crude chitosan was obtained by drying in an air oven at 50°C overnight.

2. Fourier transform infrared spectroscopy (FTIR)

IR spectra of chitosans obtained from different sources were recorded with a Tensor 27 Fourier transform infrared spectrometer FTIR (Germany). The spectral region between 4000 and 400 cm^{-1} was scanned. Specimens prepared as KBr pellets were used. Dried, powdery chitosan was mixed thoroughly with KBr and then pressed in vacuo to homogeneous disc with a thickness of 0.5 mm. The chitin concentration in the samples was 2%, calculated with respect to KBr.

3. RESULTS AND DISCUSSION

3.1. Chitosan composition of raw material of crustacean shells

Chitosan was isolated from two sources (crab shell, shrimp shell), all from the Bulgarian shelf of Black Sea. The experimentally established chemical composition of the source materials from CH-Ss is 79.80 % and from CH-Cr is 65.89 %.

3.2. Chitosan characterization

Various absorption bands within the 4000-400 cm^{-1} range were recorded in the FTIR spectra of chitosan, prepared from srimp shell (CH-Ss) and crab shell (CH-Cr) [3,5,6,11,16,17,19,20]. These bands were compared with those of standard chitosans (CH-Ss and CH-Cr) from Sigma-Aldrich. As can be seen from Table 1, the absorption bands of experimentally prepared chitosans were identical to those of standard chitins. Different stretching vibration bands were observed in the range 3425-2881 cm^{-1} related to ν(N-H) in $\nu(NH_2)$ assoc. in primary amines [5,19,20]. The band at 3425-3422 cm^{-1} could be assigned to ν(N-H), ν(O-H) and $\nu(NH_2)$ which present in chitosan in different amounts among which NH_2 groups being the least. The presence of methyl group in $NHCOCH_3$, methylene group in CH_2OH and methyne group in pyranose ring was proved by the corresponding stretching vibrations of these groups in the range 2921-2879 cm^{-1} (Figure 1, Table 3). The band at 1597 cm^{-1} has a larger intensity than at 1655 cm^{-1}, wich suggests effective deacetylation. When chitin deacetilation occurs, the band observed at 1656 cm^{-1} decreases, while a growth at 1597 cm^{-1} occurs, indicating the prevalence of NH_2 groups.

When the same spectrum is observed, in which the band from 1500-1700 cm^{-1} is stressed, indicated that there was an intensification of the peak at 1597 and a decrease at 1656 cm^{-1}, that suggests the occurrence of deacetylation.

The doublet mode of Amide I band is attributed to the occurence of intermolecular hydrogen bonds from the type C=O…HN and also intramolecular ones such as C=O…$HOCH_2$ [3,7,19]. In the experimentally prepared chitin, this doublet was more pronounced than in the standard one, which proves the higher degree of morphological arrangement (higher degree of crystalline order) in the former. The second band (Amide II) is located at 1559 cm^{-1}, which corresponds to the statement that, for the *trans*-secondary amides, this band can be detected within the 1570-1530 cm^{-1} interval, depending on the degree of intermolecular association between C=O and N-H groups. Moreover, within the 1300-1200 cm^{-1} region, the absorption bands at 1262 cm^{-1} and 1205 cm^{-1} were observed, which could be assigned to complex vibrations of NHCO, with characteristic frequencies for secondary amides only [7]. As presented in Fig. 1, the area between 1000 and 1158 cm^{-1} was saturated, which is due to the presence of three distinct vibrational modes of C-O-C, C-OH, and C–C ring vibrations [10].

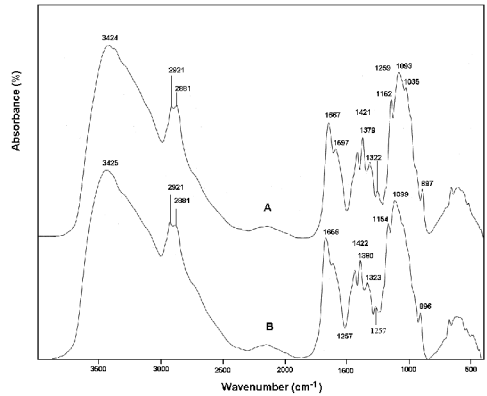


Fig. 1. FTIR spectra of experimental chitins prepared from Srimp shell-CH-Ss (A) and from Crab shell-CH-Cr (B)

Table 1. Characteristic absorption bands in the FTIR spectra of standard and experimentally prepared chitosan

Wave number, ν (cm^{-1})				Vibration modes
Chitosan from CH-Ss		Chitosan from CH-Cr		
Standard	Experimental	Standard	Experimental	
3423	3424	3422	3425	$\nu(NH_2)$ assoc. in primary amines ν (OH) assoc. in pyranose ring
2923	2921	2923	2921	$\nu_{as}(CH_2)$ in CH_2OH group
2880	2881	2879	2881	ν (C-H) in pyranose ring
1667-1629 (shoulder)	1667-1597 (shoulder)	1655-1627 (shoulder)	1656-1628 (shoulder)	ν (C=O) in $NHCOCH_3$ group (Amide I band)
1422	1421	1422	1422	$\delta(CH_2)$ in CH_2OH group
1380	1379	1382	1380	$\delta_s(CH_3)$ in $NHCOCH_3$ group
1322	1322	1322	1323	δ (C-H) in pyranose ring
1262	1259	1257	1257	complex vibrations of NHCO group (Amide III band)
1155	1152	1077	1158	ν_s(C-O-C) (glycosidic linkage)
1077	1093	1030	1154	ν_{as}(C-O-C) (glycosidic linkage)
1074	1074	1073	1099	ν (C-O) in secondary OH group
1031	1035	1029	1027	ν (C-O) in primary OH group
897	897	897	896	pyranose ring skeletal vibrations
664	665	662	665	δ (NH) out of plane
616	614	605	603	δ (OH) out of plane

The changes in the region 1667–1597 cm^{-1} were due to the differences in IR absorption characteristics of the free amine and the amine salt. A medium to strong band in the 1650–1590 cm^{-1} region arises from the NH_2 scissoring vibration. In contrast, NH_3^+ are characterized by N–H bending vibrations in the 1600–1400 cm^{-1} region [7]. In addition, the characteristic spectra of the original chitosan (amine) at 1600.9 cm^{-1} and 1647.19 cm^{-1} were assigned to the N–H bending mode of $–NH_2$ and amide 1 band, respectively [21]. The absorption bands within the 1422-603 cm^{-1} region confirmed the presence of CH_3, CH_2

and CH groups as well as the primary and secondary OH groups, attached to the pyranose ring, and the oxygen atoms in ether groups (Figure 1, Table 1).

CONCLUSIONS

Chitosan is prepared by using different local marine sources from Black Sea as starting materials, and applying an appropriate treatment with diluted HCl and NaOH. The relative content of chitosan in these sources is determined. The most convenient raw material sources for preparation of chitosan are found to be crab shell (CH-Cr) and shrimp shell (CH-Ss). By employing FTIR spestroscopy, all functional groups in chitosan macromolecules are elucidated. In the experimentally prepared chitosan, the bands were more pronounced than in the standard one, which proves the higher degree of morphological arrangement (higher degree of crystalline order) in the former.

REFERENCES

[1]. Alexandre, T., Paulino, J.I., Simionato, J.C., Garcia, J.N, Carbohydr. Polym., 2006, 64, 98–103.

[2]. Birth, G., Dautzenberg, H., Carbohydr. Polym., 2002, 47, 39–51.

[3]. Brugnerotto, J., J. Lizardi, F. M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, Polymer*,* 2001, 42**,** 3569.

[4]. Chatterjee, S., Adhya, M., Guha, A.K., Chatterjee, B.P., Process Biochemistry, 2005, 40, 395–400.

[5]. Dunkan, A. B. F., W. Gordy, R. N. Jones, F. A. Matsen, C. Sandorfy, W. West, Chemical Application of Spectroscopy*,* Interscience Publ., London, 1956, 448-466.

[6]. Focher, B., A. Naggi, G. Torri, A. Cosani, M. Terbojevich, *Carbohydrate,*1992, 18**,** p. 43.

[7]. Furniss B., Hannaford A., Smith P., Thatchell A.: Text Book of Practical Organic Chemistry, 5th edn., pp. 695–698. Longman Scientific and Technical, Harlow, 1989.

[8]. Gao, Q.,Wan, A., Carbohydr. Polym., 2006, 64, 29–36.

[9]. Kasaai, M.R., Carbohydr. Polym., 2007,68, 477–488.

[10]. Kolhe P., Kannan R., Biomacromolecules, 2003, 4, 173–180.

[11]. Kurita, K., K. Sugita, N. Kodaira, M. Hirakawa, J. Yang, Biomacromolecules*,* 2005, 6, 1414.

[12]. Majeti, N.V., Kumar, R., React. Funct. Polym., 2000, 46, 1–27.

[13]. Ming Larng Tsaih, M. L., Chen, R. H., Int. J. Biol. Macromol., 1997, 20, 233–240.

[14]. Ogawa, K., Yui, T., Okuyama, K., Food Ingred. J. Jpn., 2004, 4, 311–319.

[15]. Oh, Y. S., Shih, I. L., Tzeng, Y. M., Wang, S.L., Enzyme and Microbial Technology, 2000, 27, 3–10.

[16]. Pawlak, A., M. Mucha, Thermochimica Acta*,* 2003, 396, 153.

[17]. Rinaudo, M., Progress in Polymer Science, 2006, 31, 603.

[18]. 4. Sabnis, S., Block, L.H., Int. J. Biol. Macromol., 2000, 27, 181–186.

[19]. 18. Shigemassa, Y., H. Matsuura, H. Sashiwa, H. Saimoto, International Journal of Biological Macromolecules*,* 2006, 18**,** 237.

[20]. Spasov, S., A. Arnaudov, Application of Spectroscopy in Organic Chemistry*,* Nauka i Izkustvo, Sofia, 1978, 107-115, 151-156.

[21]. Tian, F., Liu, Y., Hu, K., Zhao, B., Carbohydr. Polym., 2004, 57, 31–37.

[22]. Tsukada, S., Inoue, Y., Carbohydr. Res., 1981, 88, 19–38.

[23]. Yang, J.K., Shih, I.L., Tzeng, Y.M., Wang, S.L., Enzyme and Microbial Technology, 2000, 26, 406–413.

За контакти:

Гл. ас. д-р Диляна Звездова, Катедра “Органична химия”, Университет “Проф. д-р Асен Златаров”-Бургас, тел.:056-858-254, e-mail: dzvezdova@yahoo.com

Докладът е рецензиран.