Engineering Science and Technology, an International Journal xxx (2017) xxx–xxx

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Contents lists available at ScienceDirect

Engineering Science and Technology, an International Journal

journal homepage: www.elsevier.com/locate/jestch

Full Length Article

Acetylation, crystalline and morphological properties of structural polysaccharide from shrimp exoskeleton

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ARTICLE INFO

Article history:
Received 7 December 2016
Revised 21 March 2017
Accepted 16 May 2017
Available online xxxx

Keywords:
α-Chitin
Acetylation
Crystalline index
Hydrogen bond
SEM/EDS

ABSTRACT

The extraction of a structural polysaccharide, α-chitin, from shrimp exoskeleton via chemical means using hydrochloric acid (HCl) and sodium hydroxide (NaOH) has been done. Concentrations of 0.4, 0.8 and 1.2 M for both HCl and NaOH were chosen to evaluate the acetylation degree (DA), crystalline structure and morphology of the chitin. The *N*-acetyl groups' content in the structural polysaccharide ranged between 65.6 and 99.4% in decreasing order of both acid and alkali concentrations combination used. The magnitude of chitin average hydrogen bond energy E_H was majorly influenced by OH(6)...OC intra and CO...HN intermolecular hydrogen bonds as they showed more predominance than OH(3)...O(5) and OH...OC intra and intermolecular hydrogen bonds. Chitin diffraction planes, crystalline index (CrI) and crystallite size (D_{hkl}) were investigated by X-ray diffraction (XRD) with reflections observed on (021), (110), (130) and (013) planes. The CrI occurred between 79.4 and 87.4%, while crystallite sizes were between 0.544 and 3.64 Å for the samples. Morphological study using scanning electron microscopy with energy dispersive spectroscopy SEM/EDS showed strong calcium and oxygen peaks. This established the shrimp shell surface to be composed of calcite and trace elements such as nitrogen and silicon. The observed α-chitin rough surfaces were attributed to the low degree deacetylation recorded during alkali treatment.

1. Introduction

Chitin is a linear polymer of amino sugars – β-(1→4) – linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and or β-(1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose (GlcN) units [31]. It is known to be a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine units linked with β-(1–4) glycosidic bond, having predominantly *N*-acetyl-D-glucosamine units in the polymeric chain [15]. Chitin ranks second in abundance to cellulose as natural polymers and serves as structural components supporting cell and body surfaces [16]. Its biological functions and chemical structure are similar to cellulose as a structural polysaccharide. However, it differs from cellulose as it contains an acetamide group instead of hydroxyl group at the C-2 position within the glucose unit and it is therefore regarded as a cellulose derivative with an acetamido group [27]. The chitin enhances the mechanical strength of fungal cell walls and exoskeletons of arthropods [20,18].

Crustacean shells are natural sources of chitin as they consist of (20–30%) chitin, (30–40%) proteins, (30–50%) calcium carbonate and the rest pigments (astaxanthin, canthaxanthin, lutein or β-carotene). The proportions of these constituents are dependent on the species and season in which the marine animal exists [5]. In skeletal tissue, chitin combines with protein to form protein-chitin matrix calcified to produce hard shells [30]. The shells may also contain lipids from the muscle residues and carotenoids of astaxanthin and its esters [3]. These constituents must be quantitatively removed to produce high purity biopolymer needed for biological applications. Two ways of doing this is either chemically or biologically. Biological extraction studies have reported that the use of proteolytic enzymes for protein digestion or using microorganism that allows digestion of both proteins and minerals in a fermentation process is a common method used [6]. Chemical treatment on the other hand, is a procedure [2] that entails the use of acid and alkali solutions for the removal of mineral and proteins respectively. The degree of acetylation (DA) is used to identify chitin or its derivative and it is the number of acetyl groups in the

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Peer review under responsibility of Karabuk University.

http://dx.doi.org/10.1016/j.jestch.2017.05.002
2215-0986/

polysaccharide. In another way, it is the ratio of *N*-acetylated group to *N*-de acetylated (amino) groups [21]. For DA >50%, chitin is formed [15] otherwise its derivative, chitosan is formed. Physical, chemical and biological properties of chitin are strongly dependent on its DA value. Therefore, DA is an important parameter that influences the performance of chitin in applications such as food component and medical fields [17,22]. Several studies have shown that chitin quality is dependent on its source, DA, treatment duration, temperature and type of reagent used [14,28]. In this present study, α-chitin is extracted from exoskeleton of shrimps via chemical treatment with the use of acid and alkali solutions at varying concentrations. Considering a constant treatment temperature and duration, the influence of this treatment on the crystallinity, DA and morphology of α-chitin are investigated.

2. Materials and methods

2.1. Chitin extraction

Shells of shrimp were scraped free of loose tissues, washed, dried and ground with the use of a steel ball mil. The ground particles were sieved to size $250\mu m$ using a standardized sieve of 60BSS. Demineralization was carried out at room temperature (32 °C) by soaking 100 g of ground samples in 0.4, 0.8 and 1.2 M HCl. For each concentration, the process was repeated several times until evolution of gas ceased. The demineralized samples were washed with distilled water to neutral (pH 7.0), filtered and dried in the oven at 70 °C for 4 h to constant weights. Deproteinization was carried out on each sample by heating mineral - devoid samples in concentrations of 0.4, 0.8 and 1.2 M NaOH solutions in a beaker at 100 °C for 1 h. At the end of this period, samples were filtered and soaked in fresh sets of alkali solutions (0.4, 0.8 and 1.2 M) for 18 h at 32 °C for effective protein removal. The samples were washed with distilled water to pH 7.0, filtered and oven dried at 70 °C. Depigmentization was carried out by soaking extracted chitin in 1 M H_2O_2 for 24 h at 32 °C. The chitin was washed in distilled water and dried for 4 h in an oven (70 °C) before characterizations.

2.2. Characterizations of extracted chitin

2.2.1. Acetylation degree measurement (DA) using Fourier Transform Infrared spectroscopy (FTIR)

A Nicolet 6700 M spectrometer in transmission mode was used in carrying out FTIR spectra of samples. Ten milligram of fine samples were dispersed in a matrix of KBr (500 mg), followed by compression at 22–30 MPa to form pellets. The transmittance measurements were carried out in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. This was carried out in Redeemers University, Nigeria. The DA was calculated using Eq. (1) [14]:

$$DA = [(A1650/A3450) \times 100/1.33] \quad (1)$$

where: A_{1650} is the absorbance of amide I vibration; A_{3450}, absorbance of OH vibration; 1.33 is a factor that represents the ratio of A_{1650}/A_{3450} for fully N-acetylated chitin.

2.2.2. X-Ray diffraction (XRD)

The XRD of each sample was conducted using PANanalytical X' Pert PRO MPD X-ray diffraction system PW3040/60 machine at Soochow University, China. Samples were exposed to a monochromatic Cu Kα radiation (k = 1.5406 Å), operating at 40 kV and 40 mA. Crystallinity index (CrI) determination for chitin was done using Eq. (2) [13].

$$CrI(\%) = [I_c/(I_c + I_a)] \times 100 \quad (2)$$

where I_c and I_a represent the intensities of the crystalline and amorphous regions respectively. Crystalline size normal to the h k l plane (D_{hkl}) was calculated from the full width of peak at half height of the source curve using Eq. (3) [2,28].

$$Dhkl = k\lambda/\beta\cos\theta \quad (3)$$

Where K is a constant (indicative of crystallite perfection and is assumed to be 1); λ(Å)is the wave length of incident radiation (1.5406 Å); β (rad) is the width of the crystalline peak at half height and θ (deg) is the diffraction angle corresponding to the crystalline peak.

2.2.3. Scanning electron microscopy (SEM) with energy dispersive X-ray Analysis (EDS)

An ASPEX 3020 model variable pressure SEM operated with an electron intensity beam 15 kV and equipped with Noran-Voyager energy dispersive spectroscope (situated in Covenant University, Nigeria) was used to observe the morphological features of all samples. The samples to be observed under the SEM were mounted on a conductive carbon imprint left by the adhesive tape prepared by placing the samples on the circular holder and coated for 5 min to enable it conduct electricity. For EDS analysis, samples were analyzed at an accelerating voltage of 15 kV.

2.2.4. Thermogravimetric Analysis (TGA)

Analysis of samples was carried out on TGA Q500 instrument where 2 mg of samples were heated to 750 °C at 10 °C/minute heating rate. In this test, the temperature for the onset of thermal decomposition (T_{onset}), temperature at the end of decomposition (T_{finish}), the temperature at which decomposition rate was rapid (T_{max}) and chitin content were deduced from the thermograms.

3. Results and discussion

3.1. Acetylation degree (DA)

Fig. 1 shows the DA of extracted chitin with concentrations of HCl and NaOH solutions. The DA has a maximum value of 99.4% when ground shrimp shells are treated with 0.4 M HCl and 0.4 M NaOH. This implies that the number of glucopyranose units with *N*-acetyl groups in this biopolymer is the highest compared to chitin obtained using 0.8 and 1.2 M NaOH at 0.4 M HCl (79.5 and 74.3%). The minimum DA (65.6%) is recorded for shrimp chitin obtained at 1.2 M HCl and 1.2 M NaOH. Here, the resistance of acetamide groups imposed by the *trans* arrangement of the C2-C3 substituents in the sugar ring is lowered by high concentrations of reagents. Thus, this study has shown that HCl and NaOH can induce partial removal of the *N*-acetyl groups resulting in DA decrease as concentration increases.

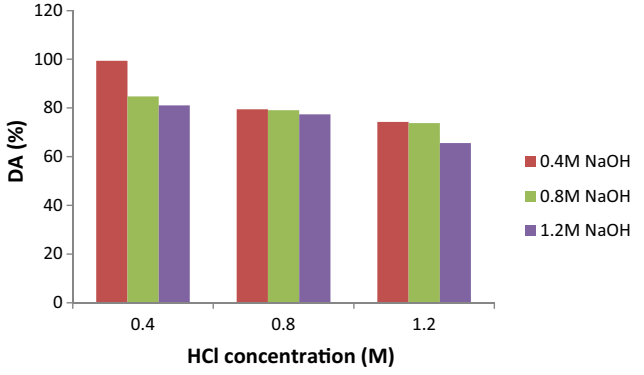

Fig. 1. DA of extracted chitin with HCl and NaOH concentrations.

Please cite this article in press as: O.P. Gbenebor et al., Acetylation, crystalline and morphological properties of structural polysaccharide from shrimp exoskeleton, Eng. Sci. Tech., Int. J. (2017), http://dx.doi.org/10.1016/j.jestch.2017.05.002