Preparation of Chitosan from Shrimp Shell and Investigation of Its Properties

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Abstract**-- Chitosan was prepared from shrimp processing waste (shell) using the same chemical process as described for the other crustacean species with minor modification in the treatment condition. The physicochemical properties, molecular weight (165394g/mole), degree of deacetylation (75%), ash content as well as yield (15%) of prepared chitosan indicated that shrimp processing waste (shell) are a good source of chitosan. The water binding capacity (502%) and fat binding capacity (370%) of prepared chitosan are good agreement with the commercial chitosan. FT-IR spectra gave characteristics bands of –NH_2 at 3443cm^{-1} and carbonyl at 1733cm^{-1}. X-ray diffraction (XRD) patterns also indicated two characteristics crystalline peaks approximately at 10° and 20° (2θ).The surface morphology was examined using scanning electron microscopy (SEM).**

Index Term**-- Shrimp waste, Chitin, Deacetylation, Chitosan, FT-IR, XRD, SEM**.

I. INTRODUCTION

Chitosan and its derivatives are examples of value-added materials. They are produced from chitin, which is a natural carbohydrate polymer found in the skeleton of crustaceans, such as crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes [1]. Insects, such as butterflies and ladybugs, also have chitin in their wings and the cell walls of yeast, mushrooms and other fungi also contain this substance [2]. Chitin (Fig.1), a homopolymer of 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) residues linked by ß-(1-4) bonds, is a common constituent of insect exoskeletons, shells of crustaceans and fungal cell walls [3]. Chitosan (Fig.1) is a polymer obtained from deacetylation of chitin, is a cationic polysaccharide with linear chain consisting of β-(1,4)-linked 2-acetamino-2-deoxy-β-Dglucopyranose and 2-amino-2-deoxy-β- D-glucopyranose [4]. Chitosan are used in dietary supplements, water treatment, food preservation, agriculture, cosmetics, pulp & paper and medicinal application [5]. There has been a large increase in chitosan research during the past decade. This is due to its biocompability, biodegradability, non-toxicity, and other unique properties such as film forming ability, chelation and adsorption properties and antimicrobial activity [6]. The functional properties of chitosan are reported to be dependent on its molecular weight or viscosity [7]. Earlier investigations demonstrated that chitosans with higher molecular weight (or viscosity) were more effective as food preservatives than those with lower molecular weight [8]. Due to its polycationic nature, chitosan can be used as flocculating agent and act as chelating agent and heavy metal trapper [5]. Chitosan has antibacterial activity [9] which can be prepared in the form of film or hydro-gel [10], [11] to be used in burn and wound dressing and also for fabricating suturing threads [12]. Zahed Hossain *et al.* reported [13] the preparation of chitin from shrimp shells and the common procedure for isolating chitin from shrimp shell involves demineralization, deproteinization and decoloration. Industrially, chitosan are normally prepared by alkaline de-N-acetylation of chitin [14].

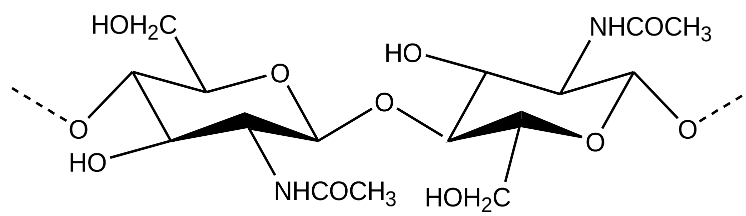

Fig. 1. Chitin

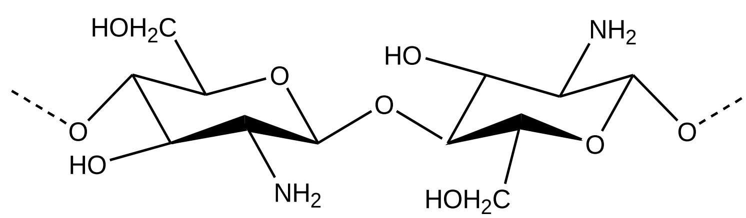

Fig. 2. Chitosan

The typical production of chitosan from crustacean shell generally consists of four basic steps: demineralization, deproteinization, decoloration and deacetylation [7]. Due to its simplicity, relative instrument availability, and independence of sample solubility, IR spectroscopy is one of the most studied methods for characteristics of chitin and chitosan [15]. The distinction of chitin and chitosan is somewhat blurred ; some maintain that chitin that is more than 50 per cent deacetylated is chitosan, whereas others define chitosan as soluble in 1 per cent acetic acid, chitin being insoluble [16]. Shrimps are in general sold headless and often peeled of the outer shells and tail. About 30-40% by weight, shrimp raw material is discarded as waste when processed shrimp is headless, shell on products [17]. The main aim of this present work was to prepare chitosan from fishery waste materials which are hazard and toxic for environment.

II. EXPERIMENTAL

Shrimp shell waste materials were collected from Khulna, Bangladesh. Shrimp shells were scraped free of loose tissue, washed with cold water and dried in sun for 2 days. IR was recorded on SHIMADZU, IR-8900 infrared spectrophotometer in the range of 4000-400cm^{-1}. XRD patterns were obtained on Philips PWO_4 XPert pro X-ray diffractometer. The X-ray source was Cu Kα with a voltage of 40 kV and a current of 30 mA. The measurement was in the scanning range of 5–70 at a scanning speed of 50 s^{-1}. A HITACHI S-3400N, Japan (BSE) scanning electron microscopic (VP-SEM) was used to examine the microstructure of chitosan with out any coating to the sample surface and the image was taken at accelerating voltage of 15.0kV. Chitin was extracted from shrimp shell as reported data [13], [18]. The process mainly involved the following steps: Demineralizations of Shells. In this step, the shells were suspended in 4% HCl at room temperature in the ratio of 1:14(w/v). After 36 hours, the shells were quite squashy and were rinsed with water to remove acid and calcium chloride. Deproteinization of Shells. The demineralized shells were then treated with 5% NaOH at 90°C for 24 hours with a solvent to solid ratio of 12:1(v/w). The residue was then collected and washed to neutrality in running tap water. Then it was dried in sun and the product is chitin. The preparation of Chitosan is simply deacetylation of chitin [5]. Removal of acetyl groups from the chitin was achieved by using 70% NaOH solution with a solid to solvent ratio of 1:14 (w/v) at room temperature for 72 hours. The mixture was stirred after some times for homogenous reaction. The resulting chitosans were washed to neutrality in running tap water and rinsed with distilled water. Then filtered and dried in sun. Intrinsic viscosity of the prepared chitosan was measured with a capillary viscometer using 0.5 M acetic acid – 0.2 M Na acetate as a solvent [19]. The intrinsic viscosity was determined from the Huggins equation:

$$\frac{\eta_{sp}}{c} = [\eta] + k[\eta]^2 c \qquad (1)$$

where η_{sp} is specific viscosity calculated from the solution and solvent flow time ratio; $[\eta]$ is intrinsic viscosity; c is chitosan concentration in the solution; k is a constant. From the intrinsic viscosity, the molecular weight was determined employing the Mark-Houwink equation:

$$[\eta]=KM^a \qquad (2)$$

where M is viscosity average molecular weight; K and a are constants, whose values depend on the polymer type and the chosen solvent. As was shown in [19], [20], for chitosan and the solvent 0.5 M AcOH – 0.2 M NaOAc, these constants are 3.5×10^{-4} and 0.76, respectively, and they do not depend on the deacetylation degree.

Moisture content of the prepared chitosan was determined by the gravimetric method [21]. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) was the difference between the weights of the wet and oven dry samples.

$$\text{\% of moisture content} = \frac{\text{(Wet weight, g – dry weight, g) x 100}}{\text{Wet weight, g}}$$

To determine the ash value of chitosan, 2.0g of chitosan sample was placed into previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 650^{o}C for 4 hr. The crucibles were allowed to cool in the furnace to less than 200ºC and then placed into desiccators with a vented top.

Calculation:

$$\text{\% Ash} = \frac{\text{(Weight of residue, g) X 100}}{\text{(Sample weight, g)}}$$

The deacetylation degree was calculated from the following relationships [22]:

$$DD=100 - \frac{(A_{1660\ cm^{-1}} / A_{3450\ cm^{-1}}) \times 100}{1.33} \qquad (3)$$

where DD is deacetylation degree; $A_{1660cm^{-1}}$ and $A_{3450cm^{-1}}$ are absolute heights of absorption bands of amide and hydroxyl groups. Water-binding capacity (WBC) and fat-binding capacity (FBC) of chitosan were measured using a modified method of Wang and Kinsella [23]. Water or fat absorption was initially carried out by weighing a centrifuge tube (50mL) containing 0.5 g of sample, adding 10mL of water or soybean oil, and mixing on a vortex mixer for 1min to disperse the sample. The contents were left at ambient temperature for 30min with shaking for 5 s every 10min and centrifuged at 3200 rpm for 25 min. After the supernatant was decanted, the tube was weighed again. WBC and FBC were calculated as follows: WBC (%) = [water bound (g)/sample weight (g)] x100; FBC (%) = [fat bound (g)/sample weight (g)] x100.

III. RESULT AND DISCUSSION

An attempt has been taken to investigate the physicochemical, functional as well as structural properties shrimp processing waste (shell) collected from Khulna, Bangladesh. The results of physicochemical and functional properties of the prepared chitosan are given in table. The prepared chitosan from chitin was confirmed as reported data [19].Chitosan [24] from brine shrimp shell contains moisture in the range 1.0-1.30% depending on the season, relative humidity and intensity of sun light. There is no significant difference in the % moisture content between the reported data else where 1-1.30% and the data obtained from the prepared chitosan (1.25%). According to KFDA [25] the moisture content of chitosan powder should be below 10%.

Chitosan sample had low ash content, is 1.20 %, indicating the effectiveness of the demineralization step in removing minerals. It is reported [23] that commercial chitosan contain ash about 1.18%. Molecular weight of the prepared chitosan is 165394 g/mole determined by the reported process [19], [20]. Several factors during production, including high temperature, concentration of alkali, reaction time, previous treatment of the chitin, particle size, chitin concentration, dissolved oxygen concentration and shear stress may influence the MW of CSs [26], [27].

TABLE I
PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF CHITOSAN

Yield%	Moisture%	Ash%	DD%	M.Wg/mol	FBC%	WBC%	Solubility
15.21	1.25	1.20	75	165394	370	502	1%CH_3COOH

Water binding capacity (WBC) and fat binding capacity (FBC) of the prepared chitosan is 502% and 370% respectively. According to Cho *et al*. [28], WBC and FBC of five commercial chitosan products ranged from 458% to 805% and 314% to 535% respectively. WBC and FBC is he functional properties of chitosan differ with preparation methods. The degree of deacetylation (DD) was calculated by using the equation no. 3 and FT-IR (infrared spectroscopic analysis) of the prepared chitosan [22]. The DD is an important parameter affecting solubility, chemical reactivity, and biodegradability. Depending on the source and preparation procedure, DD may range from 30% to 95% [29]. This study (Table I) revealed that, DD of the prepared chitosan is 75%. It is rare that the production of chitosan with 100% degree of deacetylation is achievable. Therefore, commercial chitosan with various degree of deacetylation in the range of 75–85% is commonly found.

The FTIR spectra of chitosan gave a characteristic band at 3450 cm^{-1} is attributed to $-NH_2$ and –OH groups stretching vibration and the band for amide I at 1652 cm^{-1} is seen in the infrared spectrum of chitosan [30]. The characteristic carbonyl stretching of chitosan at 1733 cm^{-1} is also observed. It is interesting that the absorption peak of chitosan at 1635 cm^{-1} corresponding to the chitosan NH_2 band exhibits broader peak [30].

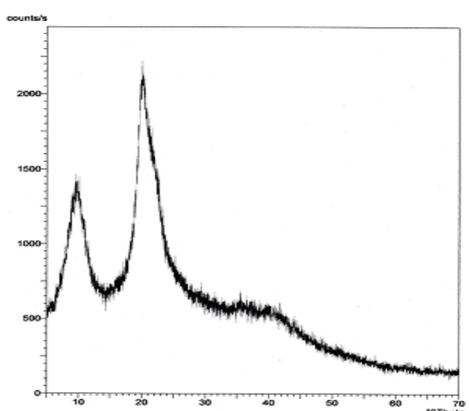

Fig. 3. XRD diffractogram of prepared chitosans

XRD patterns chitosans are illustrated in Fig. 3. The XRD pattern of chitosan exhibits broad diffraction peaks at $2\theta = 10^o$ and 21^o which are typical fingerprints of semi-crystalline chitosan [31]. Yen and Mau [32] found that fungal chitosan showed two crystalline reflections at 9.7º and 19.9º. Prashanth *et al*. [33] found that the WAXD patterns of shrimp chitosan showed two major characteristic peaks at 2θ = 9.9–10.7º and 19.8–20.7º. It is also reported that [34] the two characteristic crystalline peaks with slightly fluctuated diffraction angles found in the WAXD patterns indicated that two types of α- and γ-chitosans exhibited comparable degree of crystallinity and had two consistent peaks of 9–10º and 19–20º.

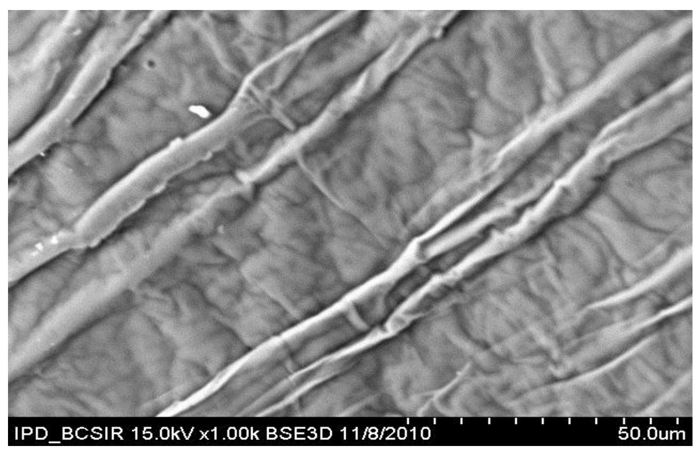

Fig. 4. The scanning electron micrographs of chitosan

Figure 4 presents the SEM micrographs illustrating the morphology of chitosan. Under the electron microscopic examination, chitosan showed non homogenous and non smooth surface with straps and shrinkage. ESAM A. EL-HEFIAN *et, al* [35] reported that chitosan exhibit some straps in surface.

IV. CONCLUSION

The present observations indicate that the prepared chitosan in this study is soluble in 1% acetic acid solution. The FTIR, XRD, physical and functional properties of the prepared chitosan confirmed that it can be used commercially in the different fields such as food supplement, additive, drug preparation as well as water treatment. The preparation of chitosan from shrimp processing waste (shells) would successfully minimize the environmental pollutants.

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