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Extraction and characterization of chitin and chitosan from fishery waste by chemical method

Suneeta Kumari [a,*], P. Rath [a], A. Sri Hari Kumar [b], T.N. Tiwari [c]

[a] Department of Chemical Engineering, NIT, Rourkela-769008, India

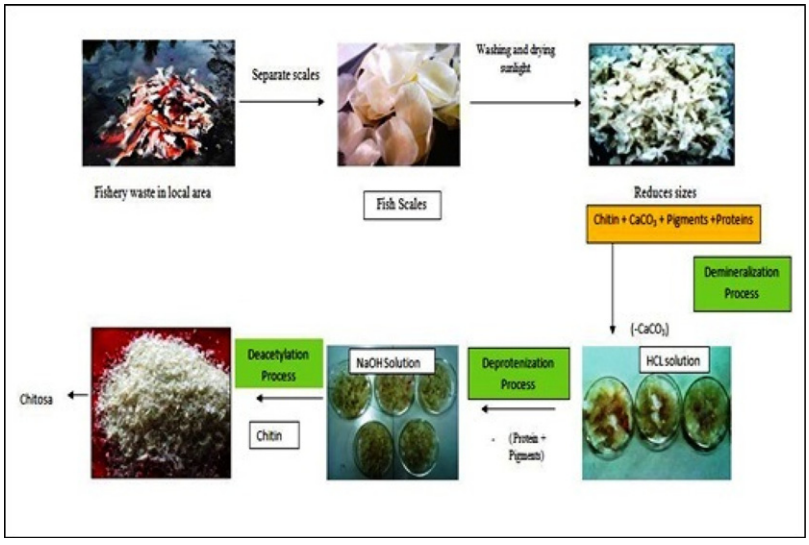
[b] Department of Chemical Engineering, Adama Science and Technology University, Adama, Ethiopia

[c] Unique Research Center, Rourkela-769014, India

HIGHLIGHTS

- Chitin and chitosan were extracted from the fish scales by chemical method.
- Investigated and characterized the extracted chitin and chitosan.
- FTIR analysis gave the most accurate DD value.

GRAPHICAL ABSTRACT



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ABSTRACT

After cellulose, chitin is the most widespread biopolymer available in nature. Chitin has economic value because of its biological activities, industrial and biomedical applications. There are three sources of chitin, namely crustaceans, insects and microorganism. The commercial sources of chitin are shells of crustaceans such as shrimp, crabs, lobsters and krill. In the present study, chitin has been extracted from locally available fish in Rourkela. The obtained chitin was converted into the more useful chitosan. The obtained chitin and chitosan have been characterized by using different techniques like spectral analysis, X-ray diffraction, Elemental analysis, Fourier transforms infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and Differential scanning calorimetry (DSC). XRD analysis indicated the crystalline nature of the chitin and chitosan. The FTIR patterns displayed the bands corresponding to stretching and vibration of O-H, N-H and CO bonds and conformed the formation of α-chitin. Degree of deacetylation (DD) value was calculated using elemental analysis, potentiometric titration and FTIR. Using FTIR analysis DD value was found to be 61%.

* Corresponding author. Tel.: +91 9438089731.
E-mail address: suneetak7@gmail.com (S. Kumari).

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1. Introduction

Fish wastes are available in a huge quantity in the environment, which have potential for the value added products. Globally 18–30 million tons of fish waste is being dumped every year. Fishery wastes are very hazardous because of their high biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), fat–oil–grease (FOG), pathogens, organic matters, other nutrients, etc. (Sapkota et al., 2008). According to the current research, fishery waste is a useful material which can be used for the preparation of chitin and chitosan. Chitin is nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates (Crini, 2006). Chitin and chitosan have high potential in new functional biomaterials in various fields such as cosmetics, agriculture, food and biomedical and textile industries as chelating agents, refinement of industrial effluents and in biotechnological applications (Lodhi et al., 2014). Chitosan is the de-acetylated chitin derivative, which is a useful bioactive polymer. Chitin and chitosan are naturally available polymers consisting of 2-acetamido-2-deoxy-β-D-glucose through a β $(1 \rightarrow 4)$ linkage and 2-amino-2-deoxy-D-glucose glucosamine ($GlcNH_2$) with β-D-$(1 \rightarrow 4)$ glycoside linkages (Jolanta et al., 2010) shown in Fig. 1. Usually, the shell of selected crustacean was reported by Abdulwadud et al. to consist of 30%–40% protein, 30%–50% carbonate and phosphate of calcium and 20%–30% chitin (Abdulwadud et al., 2013).

Exhaustive research works (Laka and Chernyavskaya, 2006; Yen et al., 2009; Mohanasrinivasan et al., 2014) have been done on the preparation of chitin and chitosan using shrimp, crab and fishery, etc. (Acosta et al., 1993) used Cuban lobsters, Sanlucar prawns, Norway lobsters, squills, Spanish crayfish, American crayfish and Fusarium oxysporum with a yield of 14%–25% chitin on a dry basis. The physico-chemical properties of chitin from the different sources were studied by IR spectroscopy and scanning electron microscopy and its degree of deacetylation was determined. Yen et al. (2009) and Khan et al. (2002) studiedchitin extracted from the fish scales of *Labeo rohita* and successfully prepared chitosan from it by deacetylation reaction. The prepared chitosan was characterized by FTIR spectral analysis and degree of the deacetylation was determined by pH-metric titration. The molecular weight of chitosan was estimated by viscometric method. Chitosan was converted into its carboxymethyl derivative using alkali and monochloroacetic acid. The prepared carboxymethyl chitosan was characterized by FTIR spectral analysis and the degree of substitution was estimated.

In the present work a systematic attempt to investigate and characterize the extracted chitin and chitosan from the fish *Labeo rohita* scales by XRD, SEM, FTIR, elemental analysis and DSC.

2. Material and method

2.1. Isolation of chitin

Fish scales were collected from different locally available sources to extract chitin are shown in Fig. 2. The scales of the species *Labeo rohita* were scraped free of loose tissue, washed, dried in sunlight for 4 days and then subjected to demineralization and deprotenization as shown in Fig. 2.

2.2. Demineralization

Demineralization was performed in dilute HCl solution. Minerals found in the fishery scales are not necessarily same for each species, hence different treatment methods were adopted for the chitin resources so obtained. Fish scales were treated with 1.0 M HCl for demineralization. The resulting solid was washed with distilled water until it was completely free of acid. Then, the demineralized samples were dried and weighed. The number of washings and their duration (15–180 min) were dependent on the species. The emission of gases, particularly CO_2 is an indication of the extent of mineral matter present and the degree of contact between the acid and the mineral matter which depends on the species used (Sagheer et al., 2009).

2.3. Deprotenization

Deprotenization of chitin was carried out using 0.5 N NaOH for 18 h at ambient temperature. After several treatments of the sample, complete deprotenization was finally indicated by the absence of any color of the solution even after leaving else it undisturbed overnight. After removal of the chitin from the liquid medium, the sample was washed several times with distilled water and rendered neutral. After that, purified chitin was dried in an oven at 110 °C and the chitin content was determined. Ash content of the dried chitin was also determined using standard procedure (Liu et al., 2012).

2.4. Deacetylation of chitin to chitosan

Preliminary experiments were carried out by refluxing chitin in strong NaOH solution in normal atmosphere. The experiments took more than 20 h producing low deacetylation content, and the reaction was accompanied by drastic degradation of the final chitosan. To avoid long heating times, the refluxing was done in alkaline solution. The heating lasted for several hours (5–6 h) and still the resulting chitosan was partially soluble in acetic acid indicating low deacetylation extent (Abdou et al., 2008). Kurita (2006) has observed that deacetylation of chitin can be highly facilitated by steeping in strong sodium hydroxide solution at room temperature before heating. This approach was then adapted and the effect of steeping time on the feasibility of deacetylation was investigated.

2.5. Determination of ash content

The ash content of chitosan was determined by placing 1 g. of chitosan into previously weighed crucible. The chitosan samples were heated in a muffle furnace at 600 °C for 2 h. The crucible was allowed to cool in desiccator for 30 min (Mohanasrinivasan et al., 2014). The crucible and ash were weighted as shown in Table 1. The % ash was determined using the relation:

$$\%\,\text{Ash} = \frac{\text{Weight of residue (g)} \times 100}{\text{Sample weigh (g)}}.$$