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Short Communication

ACTION AND FTIR ANALYSIS OF CHITOSAN FROM FRESHWATER CRAB *BARYTELPHUSA CUNICULARIS* AND FRESHWATER PRAWN *MACROBRACHIUM ROSENBERGII*

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ABSTRACT

Chitin is the fiber in shellfish such as crab, lobster, shrimp, and prawn. The shrimp industry generates annually a huge amount of shell waste. This waste gives environmental issues. This waste can be utilized as an economic source of chitin and its derivative chitosan. Freshwater crab *Barytelphusa cunicularis* and freshwater Prawn *Macrobrachium rosenbergii* found abundant in Nanded region. Freshwater crab *Barytelphusa cunicularis* have tremendous demand in local market because of its medical property. Keeping the significance of chitin and chitosan, the present study has carried out for extraction and FTIR analysis of Chitosan from freshwater crab *Barytelphusa cunicularis* and freshwater Prawn *Macrobrachium rosenbergii*.

Keywords: Chitosan, FTIR, *Barytelphusa cunicularis, Macrobrachium rosenbergii*

1. INTRODUCTION

Chitosan is linear polymer of α (1-4 linked-2- amino-2-deoxy- β-D-glucopyranose) [1]. Chitosan has three types of relative functional groups such as an amino groups and primary and secondary hydroxyl group at C-2, C-3 and C-6 positions respectively. Aranaz et al. [1] studied the physic-chemical behavioral and functional properties of chitin and chitosan and also studied specific applications in drug delivery, tissue engineering, functional food, food preservative, biocatalyst, immobilization, waste water treatment, molecular imprinting and metal nanocomposites. Chitin is mainly derived from shells of prawns, crabs, krills, squids, crawfish, shrimps, lobsters etc. and is also derived from the cell wall of fungi. Chemical composition of Chitin is β- (1-4) 2 acetamido-2-deoxy-β–D-glucose (N-acetyl glucosamine). The deacetylated derivative of chitin is chitosan. Chitin is insoluble in aqueous media while chitosan is soluble in acidic conditions due to the free protonable amino groups present in the D-glucosamine units.

Chitosan made from chitin is a white to light-red solid powder, insoluble in water, soluble in organic acids, but indigestible by human digestive enzymes. It does not dissolve in standard polar and non polar solvents. Chitosan is insoluble in most organic solvents and in water at neutral pH, whereas it dissolves in acidic solutions. Chitin and its derivative chitosan are of commercial interest due to their excellent biocompatibility, biodegradability, nontoxicity, chelating and adsorption power. With these characteristics especially chitosan has many attractive applications in Biotechnology, Food and Pharmaceutical industry, in Cosmetics, Environmental Engineering, in Agriculture and Aquaculture [2-5].

In India alone 60,000 to 80,000 tonnes of chitinous wastes are produced annually, from which a lot of chitin can be recovered from crustacean bio waste [6]. At present only a small quantity of shell waste is utilized for animal feed or chitin isolation [7]. We are also surveys before the starts of experiment to know how much crabs and prawns are sold in Nanded market. It was found that weekly Wednesday market has more sellers as compared to Friday weekly market. Hence present study carried out to extract the chitosan from the freshwater crabs and prawns which are abundant in Nanded region. Followed by FTIR analysis was also done for the characterization.

Keeping in view of significance and applications of chitosan, the present experiment has been taken up to extract and evaluate the difference in yield % of chitosan between the fresh water crab *Barytelphusa cunicularis* and fresh water prawn *Macrobrachium rosenbergii*.

2. MATERIAL AND METHODS

Adult freshwater crab *Barytelphusa cunicularis* and freshwater Prawn *Macrobrachium rosenbergii* irrespective of sex were procured from Purna region Tq. Purna Dist. Parbhani and Nageshwadi water tank near AundhaNagnath Dist. Hingoli respectively used for extraction of Chitosan.

They were starved for 48 hours before sacrificing and sacrificed crabs and prawns were dried in oven at 65±1°C for 48 hours. After drying crabs and prawns were powdered and chitosan was extracted as per the standard method described [8]. 10 gram of powder was treated with 4% of NaOHfor 1hr in order to dissolve protein and sugar to isolate crude chitin. Samples were boiled in 4% NaOH on hot plate and then it allows cooling for 30 minutes at room temperature. After cooling each sample was washed thoroughly with deionized water thrice. Supernatant was discarded and residue was demineralized by treating it with 20 ml of 1% HCl for 24 hours. The demineralized samples were then washed with deionized water. After washing, the residue samples were treated with 50 ml of 2% NaOH solution for 1 hour. It decomposes albumin into water soluble amino acids. The supernatant was discarded and remaining chitin was washed with deionized water. The chitin was converted into chitosan by process of deacetylation. The deacetylation process was carried out by adding 50 ml of 50% NaOH to all samples and then boiled at 100°C for 2 hours on hot plate. Sa... then cooled for 30 min at room temperature with ... cooling. Sample were washed continuously with ... NaOH and filtered in order to retain the solid ... This solid matter is further washed thrice with deio... water. This solid matter was dried in oven at 120°C ... 24hours.

The process of deacetylation of chitin to chitosan was confirmed using test as suggested [9]. 5ml of I_2 / KI solution was added to each test tube which gives yellow colour to solution, to this solution concentrated sulphuric acid was added, if colour changes from yellow/ brown to dark purple indicate presence of chitosan.

2.1. Characterization of chitin and chitosan

Yield of chitosan: The percentage of the yield of chitosan was calculated by dividing the weight of produced chitosan to dry chitin weight before deacetylation [10]. Yields were calculated as follows;

Yield of chitin (%) = [Extracted chitin (g)/Crab shells (g)] x100

Yield of chitosan (%) = [Produced chitosan (g)/Chitin (g)] x100

The FTIR Spectra of chitosan was measured with Shimadzu FTIR Spectrophotometer. The spectra was average scan of 45 scans recorded at resolution of 16 (1/cm) in the range from 4000 to 400 cm^{-1}.

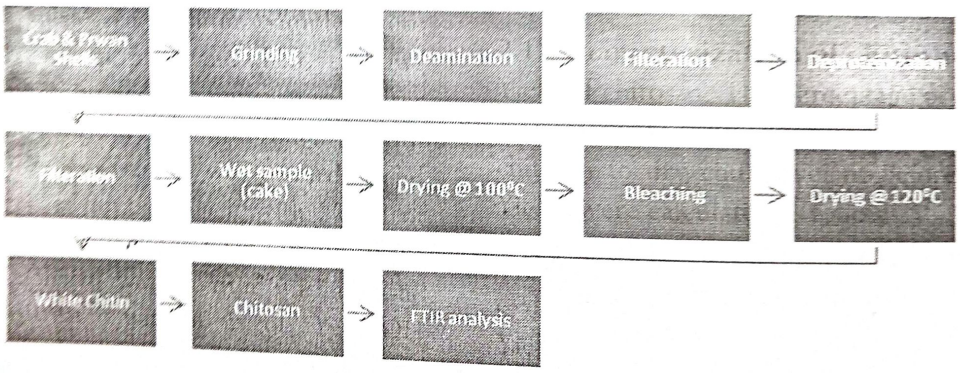

Fig. 1: Flow chart for extraction of chitosan from freshwater crab Barytelphusa cunicularis and freshwater Prawn Macrobrachium rosenbergii shells.

3. RESULTS AND DISCUSSION

Results of extraction of chitosan obtained from freshwater crab *Barytelphusa cunicularis* and freshwater Prawn *Macrobrachium rosenbergii* were shown in Table 1. The fresh water crab *Barytelphusa cunicularis* and fresh water prawn *Macrobrachium rosenbergii* are one of the potential species for chitosan production.

In the present study shells of *Barytelphusa cunicularis* goes to 12.6gms/100gm chitosan, whereas *Macrobrachium rosenbergii* produced 7.8 gm/100 gm of chitosan.

Fig 2 and 3 demonstrates the FTIR spectra of chitosan for *Barytelphusa cunicularis* and *Macrobrachium rosenbergii* respectively. The characteristics bands for chitosan can be observed in fig. 2 & 3 for freshwater crab and freshwater prawn respectively.

...action of chitosan from *Barytelphusa cunicularis* and *Macrobrachium rosenbergii*

	Chitin in gm/100gm	Chitosangm/100gm	% Chitosan
...husa cunicularis	23.7 gm	12.6 gm	53.16%
...achium rosenbergii	17.6 gm	7.8 gm	47.85%

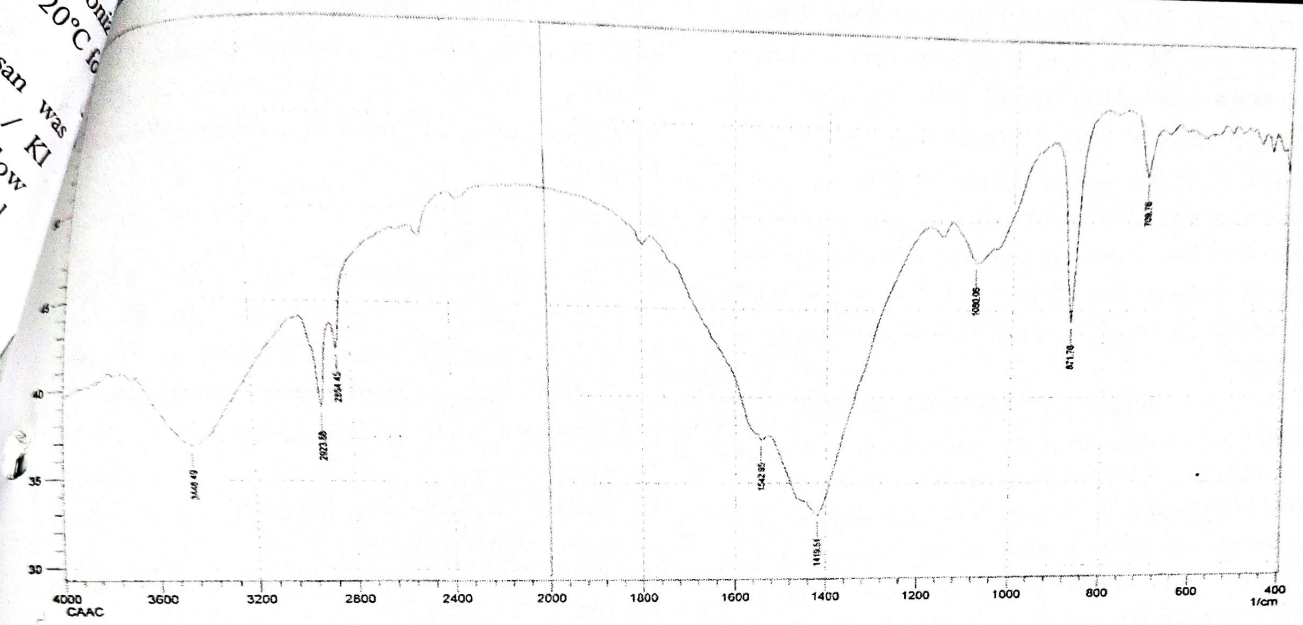

Fig. 2: FTIR Spectra of chitosan extracted fromfresh water crab *Barytelphusa cunicularis*

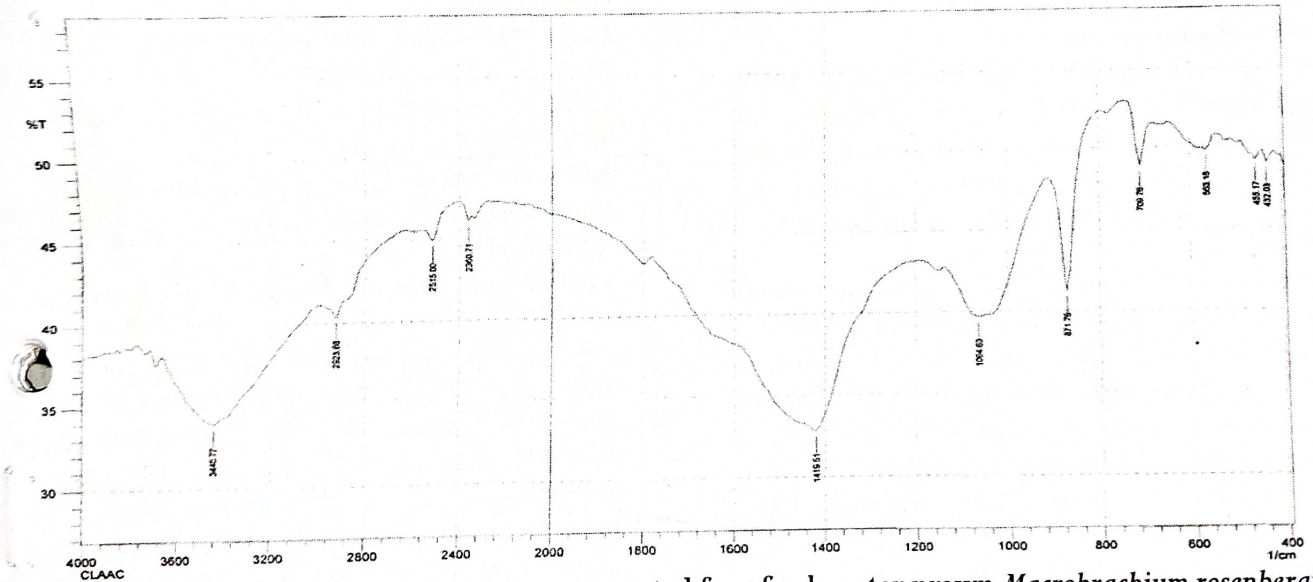

Fig. 3: Showing FTIR Spectra of chitosan extracted fromfresh water prawn *Macrobrachium rosenbergii*

The spectra showing the amine peak at 2923.88 cm^{-1} indicated the presence of CH stretch and the peak at 3448.49 cm^{-1}indicated symmetric stretching vibration of OH. In addition to this, the peak at 1542.95 cm^{-1} was due to C=O stretching, (amide I) the peaks at 1095.49 cm^{-1} and 1033.77 cm^{-1} show stretching. These bands are found in both spectra of chitosan for*Barytelphusa cunicularis* and *Macrobrachium rosenbergii* respectively [10, 11]. The wave length at 894.91 cm^{-1} represents a ring stretching a characteristics band for β-1-4- glycosidic linkage.

3.1. Chitin extraction and chitosan production

Yield of extracted chitin and produced chitosan: The yields have been calculated for extracted chitin and produced chitosan. The yield of chitin extraction from dry crab shells was 23.7% whereas the yield of chitin extracted from shells of freshwater prawn was 16.3%.

The yield of chitosan produced from extracted chitin of crab shell was 53.16% and from prawn shell it was 47.85% recorded. Our results are near to the results that were reported in the literature [2] produced 34.44 gm/kg chitosan from fresh water prawn *Macrobrachium rosenbergii* and 26.64 gm/kg chitosan from *Penaeus monodon* species. [4] the yield of chitosan produced from blue crab extracted chitin was 77.78%, [12] (76% yield of chitosan from *Callinectes sapidus* from İskenderun, Turkey) [13] (74.6% yield of chitosan of *Syllacerrata* from Mombasa, Kenya).The yields were above average in these studies and from our studies we concluded that freshwater crabs are one of the major resources of chitin and chitosan among the other crustacean group of organisms.

The chitin and chitosan are used in the preparation of materials like wound dressing, antiviral and antifungal agents, dialysis membranes Biomedical beads, Fabrics and gauzes [14]. Chitosan is a wound healing accelerator, and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation. It may cat as effectively against typhoid producing microorganism [15].The extracted chitosan from crab and prawn shells exhibits tremendous industrial, medical and pharmaceutical applications [16].

This experiment showed that chitosan can be generated using crab and prawn shells as starting materials. The Chitosan produced by deacetylation of chitin was observed to have many important properties like antibacterial, antifungal and radical scavenging activity as described below.

The use of the antimicrobial activity of chitosan has been used for development of antimicrobial films intended for use in packaging materials for foods, medical supplies and so on, or as laminated coating on items for which surface colonization is undesirable. Chitosan used as coating on fruits and vegetables is almost as effective as the fungicide TBZ at preventing spoilage during storage at proper conditions. Chitosan activity as anti-coagulant is useful in biomedical applications [17] like wound dressing, surgical sutures and for other treatments like reducing oxidative stress in live cells [18], Antitumor activity [19, 20], anti-inflammatory effect [21] HIV-1 inhibitors [22] .

This is baseline work carried out to know the chances of extraction of chitosan from freshwater crab and prawn. Further research is still needed in this area so that the biowaste in the form of crab and prawn shell was used for production of chitin and chitosan in future. It is also termed as Waste to Best Technology.

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