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## Extraction and Characterization of Chitin and Chitosan from (Labeo rohita) Fish Scales

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### Abstract

Chitin is the most important natural polysaccharide after cellulose found in crustaceous shell or in cell walls of fungi. However, it is not widely used for industrial application up to now because it is insoluble in many solvents, relatively difficult to isolate from natural sources in pure form and to prepare in a reproducible way under good economic condition. That is why it is also difficult to characterize this polysaccharide. The present study was undertaken to extract chitin and chitosan by chemical method. Several treatments with acid and alkali were taken into consideration to determine effective concentration for yielding optimum output. The waste shells of fish were used as raw material in the experiment and analysis by FTIR, XRD and SEM.

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*Keyword:* Chitosan, Demineralization, Deacetylation, FTIR, XRD.

### 1. Introduction

Chitin is usually distributed in marine invertebrates, insects, fungi and yeast. Chitin is a bio polymeric substance derived from crustaceous shell a homopolymer of  $\beta$  (1 $\rightarrow$ 4) linked N- Acetyl-D-glucosamine Austin et al. (1981). In spite of the presence of nitrogen it may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group Entsar et al. (2008). Like cellulose it functions a structural polysaccharides (Figure-1).

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Commonly the shell of selected crustacean consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate and 20-30 % chitin Knorr.D.(1984). The prime source is shellfish water such as shrimps crabs and crawfish Allan C.R and Hadwiger L.A.(1979). It is also obtained naturally in a few species of fungi. Chitin is formed a linear chain of acetylglucosamine groups while chitosan is recovered by removing enough acetyl groups ( $\text{CH}_3\text{-CO}$ ) from chitin therefore the chitin molecule and the resultant product is found to be soluble in most diluted acids. The actual variation between chitin and chitosan is the acetyl content of the polymer. Chitosan having a free amino group is the most useful of chitin No.H.K and Meyer S.P.(1992). Chitosan is a non toxic biodegradable polymer of high molecular weight. Chitosan is a one of the promising renewable polymeric materials for their broad application in the pharmaceutical and biomedical industrial for enzyme immobilization F.Nessa and Shahmd.Masum. (1992). Chitosan is used in the chemical wastewater treatment and food industrial for food formulation as binding, gelling, thickening and stabilizing agent No.H.K and Meyers S.P. (1989).

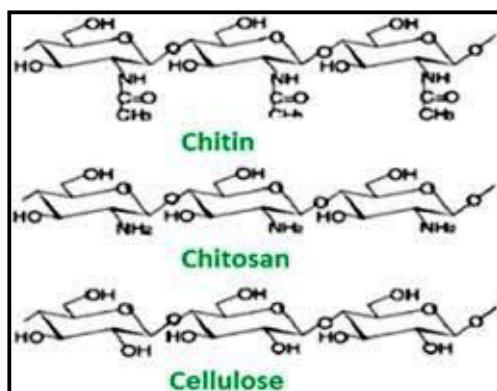


Fig.1. Chemical structures of chitin, chitosan, and cellulose

Commonly isolated of chitin from crustacean shell waste consists of three basic steps: demineralization (calcium carbonate and calcium phosphate separation),deproteinization (Protein separation),decolorization (removal of pigments) and deacetylation (remove acetyl groups).These three steps are the standard procedure for chitin production .The subsequent conversion of chitin to chitosan (deacetylation) is generally achieved by treatment with concentrated sodium hydroxide solution (40-50%) at 100°C or higher temperature to remove some or all acetyl group from the chitin No.H.K and Meyers S.P.(1995) or Galed G (1995) .Earlier studies by several authors have proved that the physicochemical characteristics of chitosan affect its functional properties which also differ due to crustacean species and preparation methods ChoY.I.H.K and Meyers S.P(1998) or No.H.K (1998).

Several methods have been developed and proposed by many researchers over the years for preparation of chitosan from different crustacean shell wastes. Some of these formed the basis of acid and base concentration for industrial production of chitosan. But most of the reported processes were carried out at 100° C or higher temperature with autoclaving Horton D and Lineback D.R.N (1965) or Domard A (1983). Therefore, the specific objectives of this work were to develop an optimum condition for production of chitosan from fish scales Prashanth R and Tharanathan R (2007 ) and Galed G. (2007)..

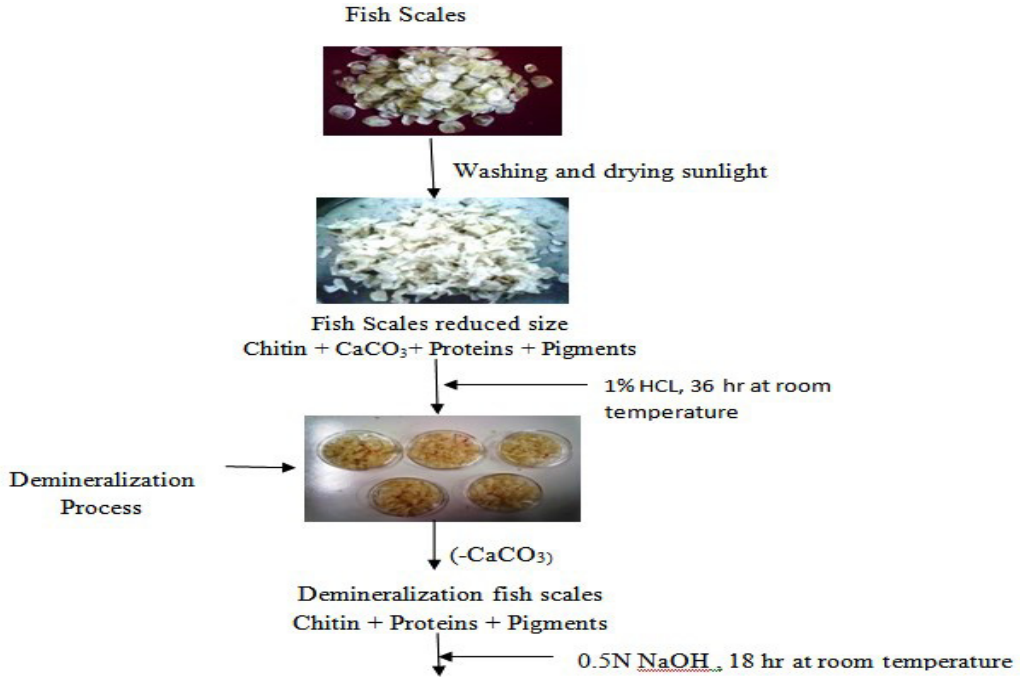
## 2. Materials and method

### 2.1. Raw materials and Chemicals

Fish Scales were taken from the local fish market for this project. Acid (HCL, 36.5g/mol) 1% solution of HCL was prepared for demineralization. Sodium hydroxide (NaOH, 40g/mol) 0.5 N solution of NaOH was prepared for Deproteinization. pH meter and distilled water were also used in the experimental process. All chemicals used were of laboratory grade.

### 2.2. Experimental Procedure for the Production of Chitin and Chitosan

Preparation of chitosan from fish scales was performed using the general method comprising of demineralisation, decolourisation and deacetylation. Raw fish scales were washed thoroughly with water, dried in oven and soaked in 1% HCL solution for 36 hours. It was then washed dried in oven and kept in 2N NaOH solution for 36 hours for demineralisation. Fish scales were then kept in Potassium permanganate solution (having composition 1g of  $KMnO_4$  in 100ml water) for 1 hour, followed by keeping it in Oxalic acid (having composition 1g of Oxalic acid in 100ml water) for the process of decolourization of the experimental sample. These processes resulted in chitin as the product which was further treated with 50% w/v NaOH for the process of deacetylation resulting in chitosan as the end product (Figure-2).



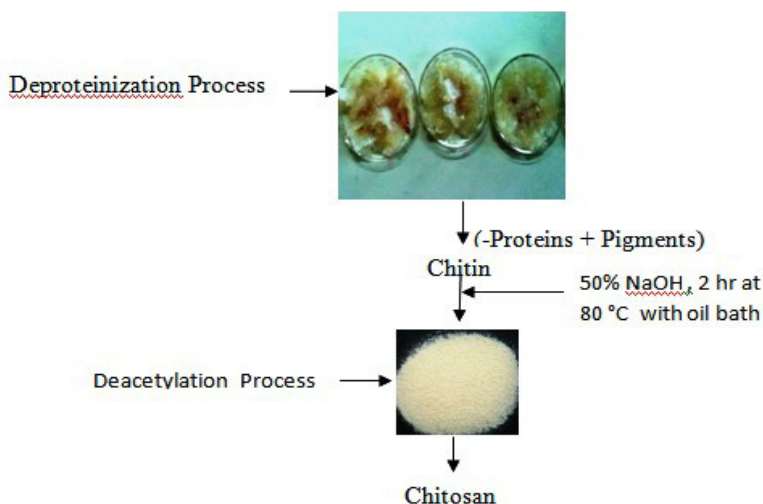


Fig.2. Overall Processes for preparation of chitin from fish scales

### 2.3. Degree of deacetylation

The DA of chitin / chitosan is the most important parameter that influences their various properties including biological, physicochemical and mechanical properties. The effectiveness and behaviours of chitin/chitosan and its derivatives has been found to be dependent on the DA. The expansion and stiffness of the macromolecular chain conformation and the tendency of the macromolecule chains to aggregate depend strongly on the DA. The determination of the DA for the two copolymers is essential for studying their chemical structures, properties, and structure- properties relationships. Knowledge on the DA is very important to maximize chitosan applications. If the DA is known many properties and applications can be predicated.

## 3. Characterization of chitin and chitosan

### 3.1. Fourier-Transform Infrared Spectroscopy Analysis

Infrared spectra of KBr chitin or chitosan mixtures were obtained over the frequency range of 400 to 4,000  $\text{cm}^{-1}$  at resolution of 4  $\text{cm}^{-1}$  using Prestige-21, FTIR Spectrometer Shimadzu. The sample was thoroughly mixed with KBr, and the dried mixture was then pressed to result in a homogeneous sample disk Mohammad R.Kassai.(2009).

### 3.2 .X-Ray Diffraction (XRD) Analysis

The wide-angle X-ray diffraction (WAXD) analysis was applied to defect the crystallinity of chitins and chitosan prepared and their patterns were recorded using a Rigaku III diffractometer (Rigaku Corp; Japan) with Cu radiation (40 kV, 30mA). Data were collected at a scan rate of 1°/min with the scan angle from 2° to 40° Ming Tsung Yen et al. (2009).

### 3.3. Scanning Electronic Microscopy (SEM)

The micrographs were produced at a scanning electron microscopy; model Philips XL 30, manufactured by Mems, Reston – Virginia, United States. The samples were fractured in liquid nitrogen, and the surface and cross section of chitosan were analyzed.

## 4. Results and discussion

### 4.1. Degree of deacetylation

Chitosan (0.2 gm) was dissolved in 20 ml 0.1 M hydrochloric acid and 25 ml of deionized water. After 30 minute of continuous stirring additional 25 ml of deionized water was added and stirring was continued for next 30 minutes. When chitosan was completely dissolved solution was titrated with 0.1 M sodium hydroxide solution. Degree of deacetylation (DA) was calculated using formula.

$$DA(\%) = 2.03 \times (V_2 - V_1) / [m + 0.0042(V_2 - V_1)] \quad (1)$$

### 4.2. FTIR

FTIR spectroscopic analysis of chitosan is shown in figure-3. The absorption bands of experimentally prepared chitosan were identical to those of standard chitins. Different stretching vibration bands were observed in the range 3425-2881  $\text{cm}^{-1}$  related to  $\nu$  (N-H) in  $\nu$  ( $\text{NH}_2$ ) assoc. in primary amines (Dilyana, 2010). The band at 3425-3422  $\text{cm}^{-1}$  could be assigned to  $\nu$  (N-H),  $\nu$  (O-H) and  $\nu$  ( $\text{NH}_2$ ) which present in chitosan in different amounts among which  $\text{NH}_2$  groups being the least. The presence of methyl group in  $\text{NHCOCH}_3$ , methylene group in  $\text{CH}_2\text{OH}$  and methyne group in pyranose ring was proved by the corresponding stretching vibrations of these groups in the range 2921-2879  $\text{cm}^{-1}$  (Figure-3). The band at 1597  $\text{cm}^{-1}$  has a larger intensity than at 1655  $\text{cm}^{-1}$ , which suggests effective deacetylation. When chitin deacetylation occurs, the band observed at 1656  $\text{cm}^{-1}$  decrease, while a growth at 1597  $\text{cm}^{-1}$  occurs, indicating the prevalence of  $\text{NH}_2$  groups. When the same spectrum is observed, in which the band from 1500-1700  $\text{cm}^{-1}$  is stressed, indicated that there was an intensification of the peak at 1597 and a decrease at 1656  $\text{cm}^{-1}$ , that suggests the occurrence of Deacetylation Dilyanaz.(2010).

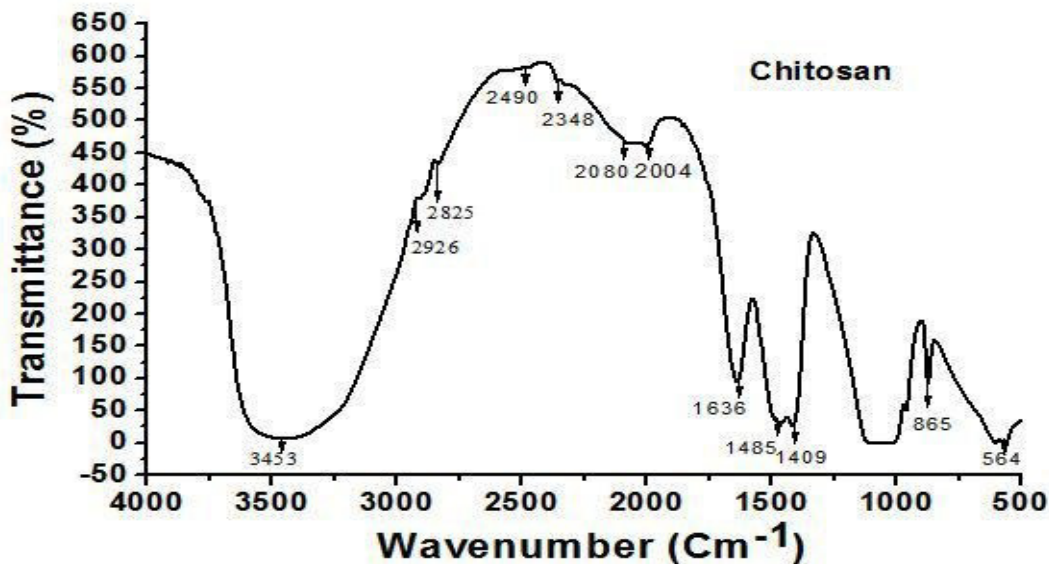


Fig.3. FTIR spectra of synthesized chitosan with using  $\text{KMnO}_4$

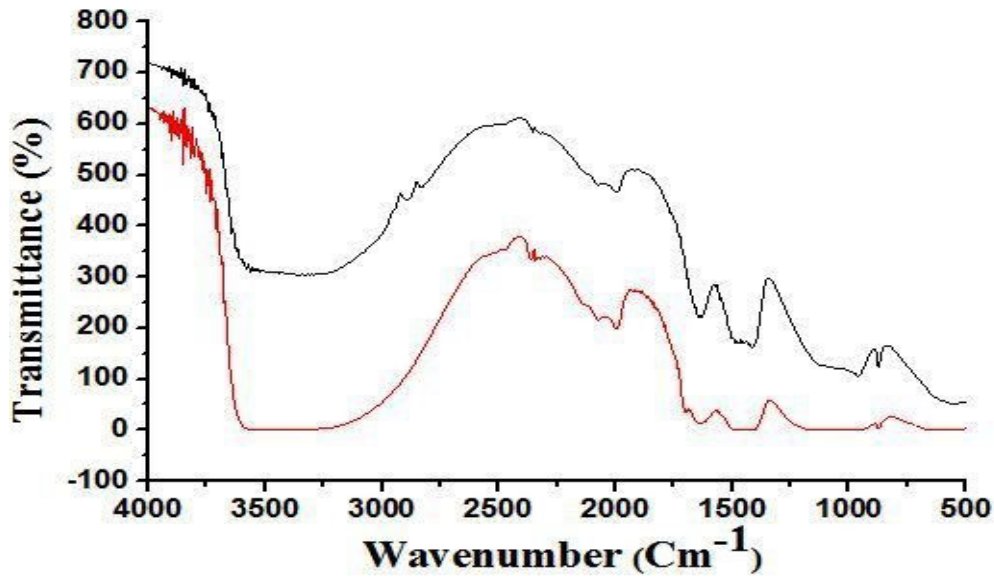


Fig.4. FTIR spectra of synthesized chitosan with different 50% of NaOH

#### 4.3. XRD

The X-ray diffraction patterns of the obtained chitin and the corresponding hydrolyzed chitosan are present in (Figure- 4). All chitin samples exhibited strong reflections at  $2\theta$  value of  $9-10^\circ$  and minor reflections at higher  $2\theta$  values. Generally, the sharpness of the bands is higher in the chitin samples than in their chitosan analogue with slight decrease in the crystalline percent S.G.Zaku et al. (2011).

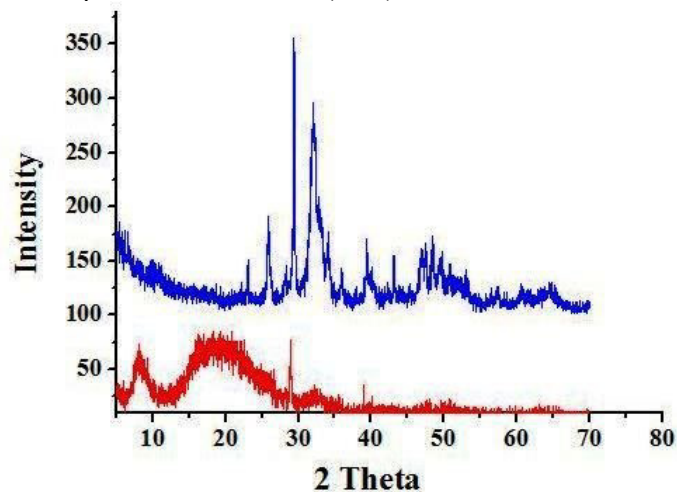


Fig.5. Comparison of X –ray power diffractograms of chitosan and fish scales

The XRD pattern of the fish scales sample is shown in figure. (4). Five crystalline reflections were observed in the



$2\theta$  range 10 to  $80^\circ$ . The peak intensity was 6.46, 24.42, 33.58, 37.42, 53.3 and 56.75 at  $2\theta$ . Chitosan sample crystalline reflections were observed in the  $2\theta$  range 10 to  $80^\circ$ . The peak intensity was 14.45, 20.02, 26.70, 37.32 and 54.32, respectively S.G.Zaku et al. (2011). Figure 4 shows that fish scales is amorphous region in structure and figure-5 chitosan is crystalline.

#### 4.4 SEM

Figure 7 (a) & (b) shows the SEM photographs of prepared chitin and chitosan from fish and chitin. Both samples exhibited rough and thick surface morphology under electron microscopic examination at 50 X magnification.

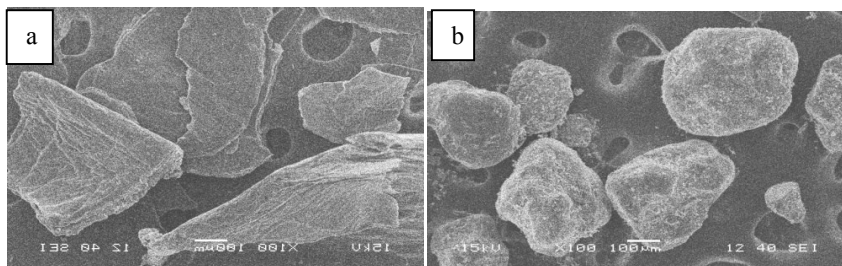


Fig.6. (a) Chitin picture (b) Chitosan picture

## 5. Conclusion

The chitin obtained from the scales of the fish could be used in variety of application especially when transformed into the more useful compound chitosan. Chitosan is prepared by using different sources 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 fish scales by employing FTIR spectroscopy, all functional groups in chitosan macromolecules are clarify. In the experimentally prepared chitosan, the bands were more pronounced and which proves the higher degree of morphological arrangement (higher degree of crystalline order) in the former.

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