



EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM *LABEO ROHITA* SCALES

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ABSTRACT

Chitin is the most abundant polysaccharide in the marine ecosystem and second in nature after cellulose. Chitosan is a derivative of chitin after the process of deacetylation. Chitosan has a number of commercial and possible biomedical uses. Many biochemists have found that chitosan as biocompatible, biodegradable and non-toxic which made wide applicability in conventional pharmaceutics as a potential formulation excipient. It is made by treating the chitin sea waste like fish scales with an alkaline substance, like sodium hydroxide. The present study was undertaken to extract chitin & synthesize chitosan by chemical method. Chitosan is synthesized from waste fish scales by a sequence of chemical processes involving demineralization, deproteinization and deacetylation. It is analyzed for its physiochemical parameters & the analysis is done by FTIR of extracted chitosan.

Keywords: Chitin, Chitosan, HCL, NaOH, FTIR etc.

I. INTRODUCTION

Every year, some 6 million to eight million tons of waste crab, shrimp, and lobster shells are produced globally, about 1.5 million tons in South East Asia alone. In developing countries, wastage shells are often just dumped in landfills or at the sea. Yet, shells harbor use-full chemicals i.e. proteins, calcium carbonate and chitin (a polymer similar to cellulose, but which contains nitrogen) It is usually distributed in marine invertebrates, insects, fungi and yeast. Chitin or poly (β -(1 \rightarrow 4)-N-acetyl-d-glucosamine) is a natural polysaccharide of major importance; first identified in 1884¹. This biopolymer is synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline micro fibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast². So far, the main commercial sources of chitin are crab and shrimp shells. In industrial processing, chitin is extracted by acid treatment to dissolve the calcium carbonate followed by alkaline solution to dissolve proteins in addition a decolourization step is often in order to remove pigments and obtain a colorless pure chitin.

MATERIALS AND METHODOLOGY

2.1 Materials: Labeo fish scales were collected from local market, Knr., starch and other ingredients like acetic acid, sodium hydroxide, hydrochloric acid were analytical grade products.

2.2 Method: The extraction of chitosan obtained from chemical extraction method. The chemical extraction process was followed by three steps³.

1. Demineralization (calcium carbonate and calcium phosphate separation)
2. Deproteinization (protein separation), decolorization (removal of pigments)
3. Deacetylation (remove of acetyl groups)

The following steps are involved in the extraction process of chitosan:

- The fish scales waste was collected from local market at Karimnagar, Telangana and brought to laboratory for isolation of chitosan.
- Then, they were washed thoroughly with water to remove all other wastes. The scales were completely dried under sunlight.
- Extraction of chitosan the dried scales were demineralised with 1% HCl sample was allowed to soak for 36hrs to remove the minerals (calcium carbonate and calcium phosphate).
- Then, the scales are washed in water. The demineralised scales are then deproteinized using 0.5N NaOH at room temperature for 18 h. This step is to remove proteins from the scales to obtain chitin.
- The chitin further deacetylated to get the chitosan. The deacetylation process is carried out by adding 50% NaOH and then heated for 2 h at 80° C in water bath.
- Afterward, the samples are washed continuously with the water and filtered to obtain the solid matter, which is the chitosan.
- The samples were then left uncovered oven dried. The powder obtained from deacetylation is called chitosan.

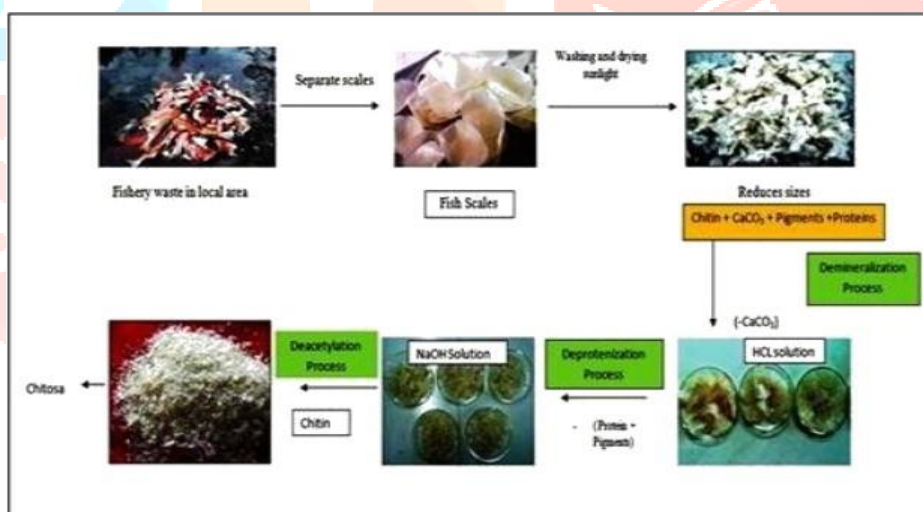


Figure 1: Chemical extraction process of chitosan from fish scales

2.3. Evaluation parameters:

1. Moisture content:

Moisture content of the prepared chitosan was determined by the gravimetric method. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass was the difference between the weights of the wet and oven dry samples⁴.

Percentage of moisture content = $(\text{wet weight, g} - \text{dry weight, g}) \times 100 / \text{wet weight, g}$.

2. Loss on drying:

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass determined by drying the sample to constant weight and sample after and before drying. The water mass (or weight) obtained showed the difference between the weights of the wet and oven dry samples⁵.

Percentage of loss on drying = $(\text{wet weight, g} - \text{dry weight, g}) \times 100 / \text{dry weight, g}$.

3. Ash value:

The ash value of chitosan was determined by taking the prepared chitosan sample which was previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 650° C for 4hrs. The crucibles were then allowed to cool in the furnace to <200° C and then were placed into desiccators with a vented top ⁶.

Percentage of ash = (weight of residue, g) x 100 sample weight, g

4. pH:

The pH measurement of the chitosan solutions was carried out using a digital pH meter.

5. Solubility:

The solubility of chitosan was demonstrated in various solutions like distilled water, acetone, ethanol, acetic acid and lactic acid. The chitosan obtained here got dissolved completely in acetic acid ⁷.

6. Degree of deacetylation:

Chitosan (0.2 gm) was collected in 20ml 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 by using formula ^{8,9}.

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

7. Characterization of chitosan:

The prepared biopolymer chitosan was analyzed by Shimadzu FTIR spectrometer is the wavelength between 400/cm and 4000/cm and in the solid state using potassium bromide ¹⁰.

RESULTS AND DISCUSSION

An effort had made to explore the physicochemical properties and structural properties of fish scales waste collected from Karimnagar market, Telangana. The results of physicochemical of the prepared chitosan are given in (Table 1)

Table 1: Physicochemical parameters of extracted chitosan

Physicochemical parameters	Extracted Chitosan
Moisture	4.3%
Loss on drying	2.42%
Ash value	2%
pH	6.8
Solubility	Acetic acid
Degree of deacetylation	48.5%

The prepared chitosan from chitin was confirmed as reported data. Chitosan from fish scales the moisture content obtained was in the range of 4.3%. Chitosan has very low ash value, 2%; indicates the efficiency of demineralization step followed in the preparation of the chitosan sample by removing the minerals. Commercial chitosan is reported to have ash value about 1.18%. The solubility of chitosan was checked with five different solvents such as water, ethanol, NaOH, acetic acid and lactic acid. It was not soluble in alkaline or neutral solution, but was soluble in acetic condition, whereas you compare with lactic acid, it was more soluble in acetic 90-95% solubility was seen. The pH values of chitosan also various from the range 6.2 to 8.0. The degree of deacetylation was found to be 48.5%.

Chitosan was extracted from chitin got from fish scales and further purified and confirmed by FTIR.

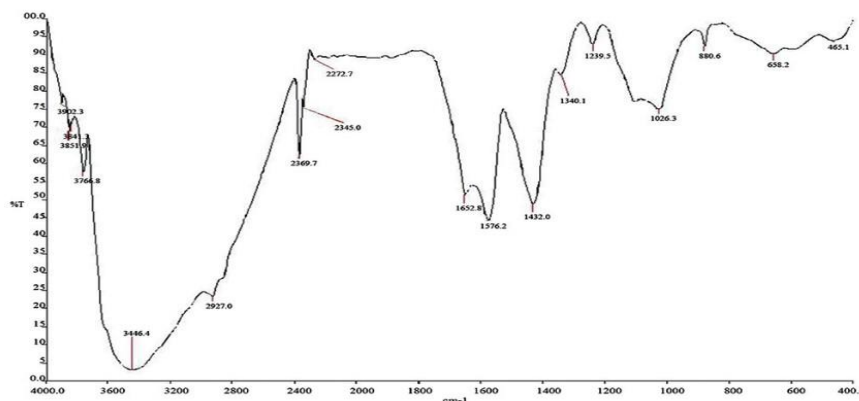


Figure.2: Fourier transform infrared spectroscopy image of extracted chitosan

FTIR studies of the chitosan from *Labeorohita* species. The band at 3425-3422/cm could be assign to (N-H), (O-H) and (NH₂) which presents in chitosan in different amounts among which NH₂ groups being the least. The presence of methyl group in NHCOCH₃, methyl group in CH₂OH and methine group in pyranose ring was proved by the corresponding stretching by vibrations of these groups in the range 2921-2879cm⁻¹ absorption band was observed between 1220/cm and 1020/cm which represents the free amino groups (-NH₂) at C2 position of glucosamine, a measure group present in chitosan. Further the sample showed the absorption bands at the various peaks 712, 880.6, 1026, 1432, 1576.2, 1652.8, 2927.0, 3446.4, which is similar to standard chitosan. This shows the confirmation of chitosan.

CONCLUSION

The present work addressed to the problem of waste utilization of crustaceans with special reference to fish scale which is a rich source of chitin and further chitosan. Several experimental runs had been conducted having three main process steps involving demineralization, deproteinization and deacetylation. The quality of the product is as certain by carrying characterization of study of one sample using FTIR as the analytical method. Based on the interpretation of the FTIR, it can be concluded that the present work has successfully synthesized chitosan from fish scales. The work is demonstrate to more experimental runs with appropriate characterization methods needs to be carried out to substantiate the claim further.

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