Synthesis and Characterization of Chitosan from Fish Scales

Shekhar Pandharipande[1], Riya Jana[2], Akshata Ramteke[3]

Abstract— **Chitin is the most important natural polysaccharide found in shells of crab, prawn & other crustaceans after cellulose. However, it is not widely utilized for industrial application till now because it is insoluble in many solvents, relatively difficult to isolate from natural sources in pristine from and to prepare in a reproducible way under good economic condition. It's additionally arduous to characterize this polysaccharide. Chitosan has a number of commercial and possible biomedical uses. It is made by treating the chitin shells of shrimp and other crustaceans 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. Treatments with acid and alkali are carried out for better output & the analysis is done by FTIR of the product.**

***Index Terms*—Chitin, chitosan, fish scales, demineralization, deproteinization and deacetylation.**

1) INTRODUCTION

The last decade has seen tremendous growth in the biomedical applications of naturally occurring biopolymers. This has resulted in evoking a lot of interest amongst scientists & researchers in pursuing these biopolymers in exploring the potential further. Chitin & chitosan come under such a category. Chitin can be said to be one of the most abundantly naturally available biopolymer which is comprised of N-acetyl D glucosamine & derivative of glucose. Chitosan is obtained by removing number of acetyl groups from chitin. The chemical structure is given in figure 1. The common source of chitin is from shells of crustaceans such as prawns, crabs, fish scales etc.

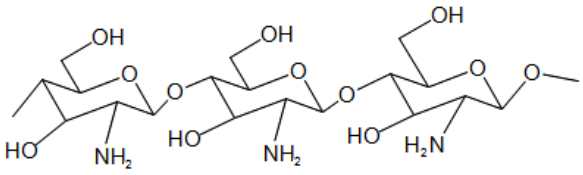

Figure 1. Chemical structure Chitosan

1) LITERATURE REVIEW

Tanvir Muslim et. al. extracted chitin from the fish scales of *Labeo rohita* and chitosan was successfully prepared from it by deacetylation reaction. The prepared chitosan was characterized by FT-IR spectral analysis and degree of 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. [1]

Suneeta Kumari and Pradip Kumar Rath extracted and characterized Chitin and chitosan from fish scales of Labeo rohita using chemical method involving following steps:

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

These three steps are the standard procedure for Chitin production. The waste shells of fish were used as raw material in the experiment and analysis is done by FTIR, XRD and SEM. [2]

Sumathi, Vignesh and Madhusudhanan extracted Chitosan from fish scales by chemical process including demineralization, deproteinization and deacetylation. The extracted chitosan was characterized using FTIR analysis. The anti-oxidant assay was studied using DPPH scavenging assay. The chitosan extracted from fish showed higher anti-oxidant activity. The anti-microbial assay was carried out by agar well diffusion method against microorganisms such as *E.coli*, *K.pneumoniae*, *P.aeruginosa*, *A.niger* and *A.terreus*. The chitosan extracted had also been studied for its effect on seed coating. The seeds treated with fish chitosan were found to be effective on seed germination. [3]

Javed Iqbal et. al, extracted Chitosan flakes from prawns and labeo rohita scales, with high adsorption capacity and were used to remove acid yellow dye from water. The results showed that adsorption capacity is dependent on pH, initial concentration of dye, surface area and pore volume of the adsorbent. They found in acidic conditions, the polymer amino groups were protonated (positively charged polymer chain), which showed attraction with negative ions

of anionic dye. Chitosan from prawn's scales showed higher dye adsorption under the same experimental conditions. Adsorption isotherms were developed and equilibrium data fitted to Langmuir and Freundlich isotherm models. [4]

2) PRESENT WORK

Material and Methodology

Raw materials and Chemicals: Fish Scales are procured from the local fish market for this project. HCL solution is used for demineralization, whereas NaOH solution is used for deproteinization. All chemicals used are of laboratory grade.

Process: The step wise procedure is given in figure 2.

Process Description: The raw fish scales are procured from local market. The scales are washed, solar dried for 3 days and then crushed. The next step is demineralisation which is carried out with soaking the scales in HCl solution for 2 hours, until the scales become squashy followed by rinsing with distilled water to remove acid & salt. The formed intermediate is washed with methanol and acetone & dried at 60^0C for 5 hr. The next step is deproteinization. It is done by slowly adding the demineralised scales to sodium hydroxide solution. The temperature is maintained at 60^0C and constant stirring was done for 5 hours. The residue is then washed until pH becomes neutral. The product obtained is chitin. Isolated chitin is added slowly into a flask containing a solution of sodium hydroxide for deacetylation step. The temperature is maintained at 100^0C and refluxed for 8 hours to remove some or all acetyl groups. The prepared chitosan is then dissolved in acetic acid to obtain acidic solution. The solution is centrifuged followed by drop wise addition of sodium hydroxide so as to obtain chitosan precipitate which is thoroughly washed with distilled water to get purified chitosan. The process conditions are maintained in each step; the typical details of a run are given in table 1, 2, and 3.

Sr. No.	Parameters	Description
1	Molarity of acid solution	1.0 M
2	Ratio of solid to acid solution	1:13(w/v)
3	Temperature of Oven	30^0C
4	Weight of Fish scales taken	10 Grams
5	Weight of intermediate product obtained	7.4 Grams

Table 1: Acid Treatment to fish scales

Sr. No.	Parameters	Description
1	Molarity of basic solution	1.0 M
2	Ratio of solid to alkaline solution	1:13(w/v)
3	Temperature of reaction mixture	60^0C
4	Stirring Period	5 hours
5	Weight of decalcified product taken	7.4 grams
6	Weight of Chitin obtained	5.2 grams

Table 2: Deproteinization details

Sr. No.	Parameters	Description
1	Ratio of solid to alkaline solution	1:15(w/v)
2	Temperature of reaction mixture	100^0C
3	Stirring period	8 Hours
4	Ratio of solid to acidic solution	1:10(w/v)
5	RPM of Centrifuge	1000
6	Time of Centrifuge	30 Minutes
7	Weight of Chitin taken	5.2 grams
8	Weight of Chitosan obtained	2.1 grams

Table 3: Deacetylation details

The photographs of step wise procedure followed in preparation of chitosan using fish scales are shown in figure 3.

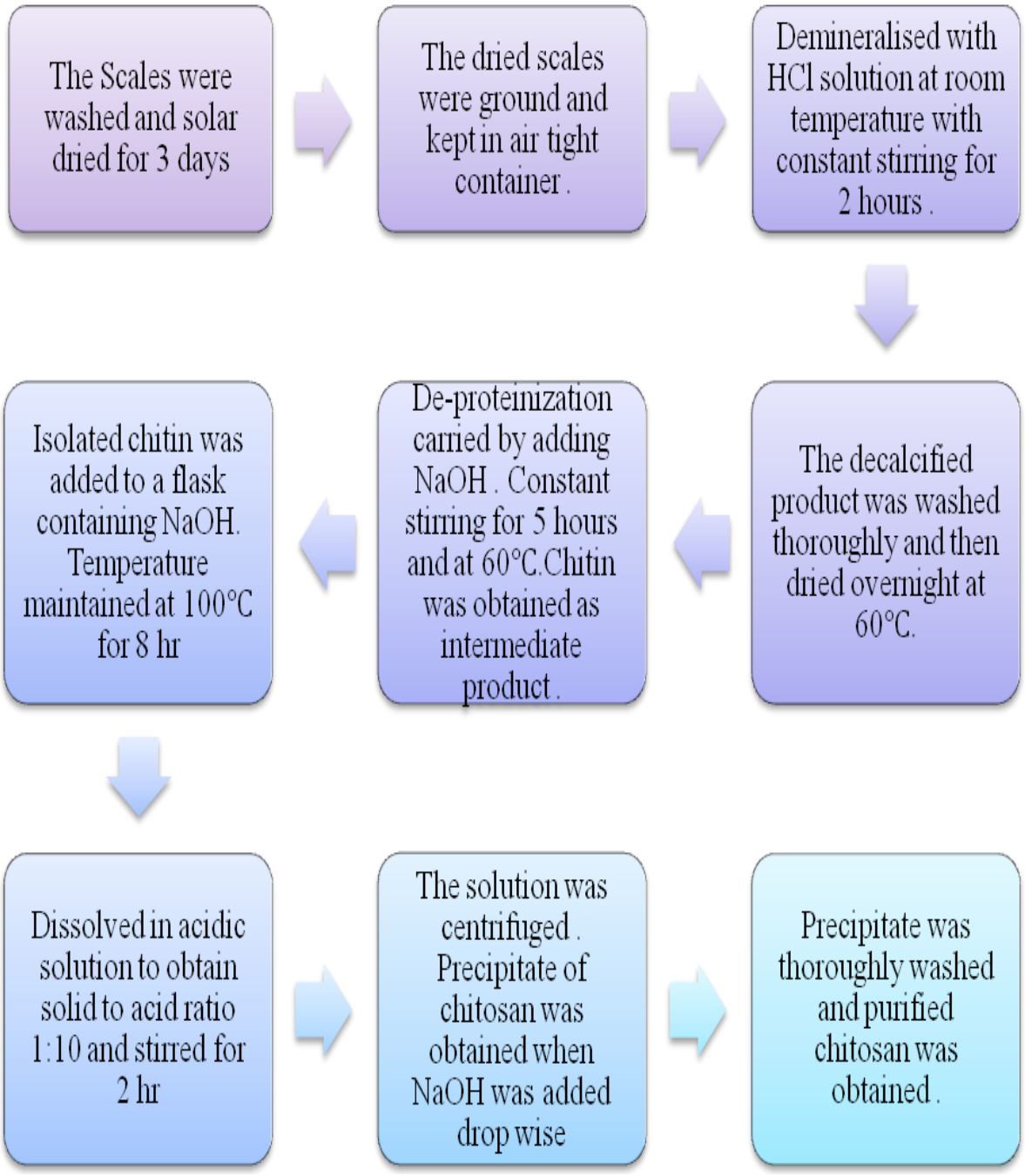

Figure 2: Flow chart depicting extraction of chitosan from fish scales

Figure 3: The photographs of step wise procedure followed in preparation of chitosan using fish scales

3) RESULT AND DISCUSSION

The product obtained after the experimentation was analyzed by FTIR in which the presence of functional groups of chitosan have been ascertained such as =C-H, C-O, C=C and –OH. The spectrogram & the interpretation details are given in figure 4 & table 4.

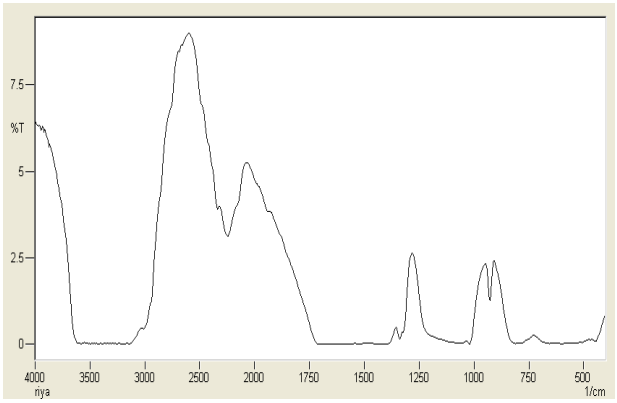

Figure 4: FTIR Spectrogram

Sr. No.	Peaks value	Functional group	Description
1	**3000-3500**	C-H O-H -NH	Stretch & medium stretch
2	**2250**	-C=C-	variable, not present in symmetrical alkynes
3	**1300**	C=O	Stretch & Strong
4	**875**	=C-H	Bending & Strong

Table 4: Interpretation of spectrogram [5]

4) CONCLUSION

The present work addressed to the problem of waste utilization of crustaceans with special reference to fish scales which is a rich source of Chitin and further of Chitosan. Several experimental runs had been conducted having three main process steps involving decalcification, deproteinization and deacetylation. The quality of the product is ascertained by carrying characterization 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 synthesised Chitosan from fish scales.

The work is demonstrate to more experimental runs with appropriate characterization methods need to be carried out to substantiate the claim further.

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