EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM WASTE SCALES OF *LABEO ROHITA*

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Abstract- Chitosan has been produced from waste fish scale by chemical method involving demineralization, deproteinization and deacetylation. The quality of chitosan depends on the conditions of the chemical extraction process. The results showed that 2% HCl and 4% NaOH were suitable concentration for demineralization and deproteinization, respectively at ambient temperature (28 ± 2° C). Chitosan with a high degree of deacetylation (87.80%) was obtained by deacetylation with 4% NaOH for 24 hours at 60±5°C. The characterization of the obtained chitosan was efficiently done by using Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. The obtained chitosan from waste fish scale can be effectively used as biodegradable filler in packaging industries.

Keywords: chitosan, demineralization, deproteinization and deacetylation.

1. INTRODUCTION

Fish scale is one of the important fisheries products worldwide including India. The fish scales are discarded daily as waste materials from fish markets, canteens, fish processing industries or kitchens. This abundant waste may pose environmental hazard due to the easy deterioration. The use of this waste to produce valuable and biologically sustainable materials is a challenge for current research and development. The basic content of fish scale is 30-40% protein (type I collagen and ichthylepidin), 30-50% calcium carbonate, 30-50% calcium phosphate and 20-30% chitin [1]. The various sources of chitosan are Shrimp, Crab**,** Lobster, Squid, Crawfish, Krill, Squilla (mantis shrimp), Silkworm chrysalides, Fungi, Insects wings [2].However, this bio-waste can be used to produce value-added products such as chitosan. Chitosan [Figure 1(a)] is formed from N-deacetylation of chitin and has a chemical structure of linear chain consisting β-(1, 4)-linked 2-acetamino-2-deoxy-β-D-glucopyranose with 2-amino-2-deoxy-β-D-lucopyranose [3]. It is well known for having common properties of polysaccharides such as biocompatibility, biodegradability, non toxicity while possessing some unique properties like film forming ability, chelation and absorption properties as well as antimicrobial characteristic. In near decade, chitosan has been involved practically in dietary supplements, water treatment, food preservation, agriculture, cosmetics, pulp, paper, and medical applications [4]. Chitosan [Figure 1(b)]is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide [5]. After cellulose, chitin is the most widespread biopolymer in nature. Chitin and its derivatives have great economic value because of their biological activities and their industrial and biomedical applications. Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae [5]. Extraction of chitin involves two steps, demineralization and deproteinisation. Chitosan is a non-toxic, biodegradable polymer of high molecular weight and is very much similar to cellulose, a plant fiber. The only difference between chitosan and cellulose is the amine (-NH_2) group in the position C-2 of chitosan instead of the hydroxyl (-OH) group found in cellulose. However, unlike plant fiber, chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules [6]. In this respect, chitin and chitosan have attained increasing commercial interest as suitable resource materials due to their excellent properties including biocompatibility, biodegradability, adsorption, and ability to form films, and to chelate metal ions.

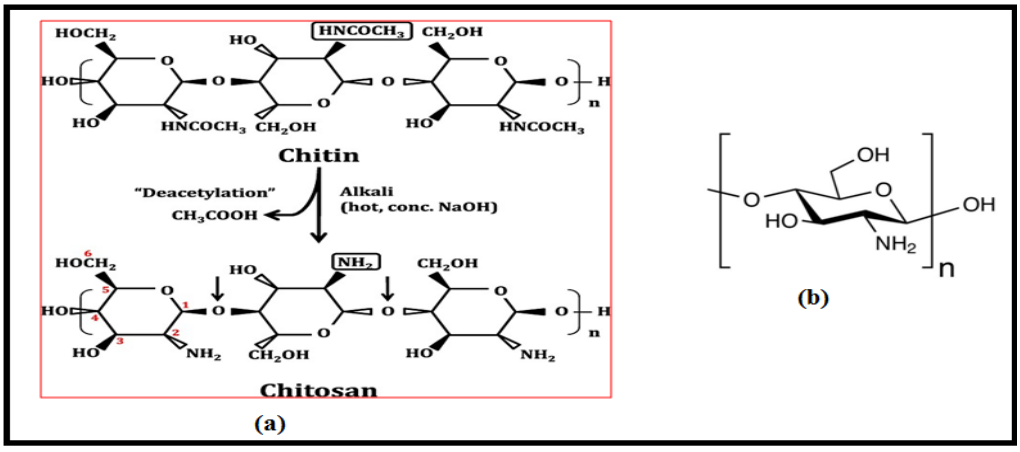

Figure 1(a) Deacetylation of chitin to chitosan [7], (b) Structure of chitosan [3]

2. MATERIALS AND METHODS:

Materials:

Fish (*Labeo Rohita*) scales freshly removed was obtained from local fish market in Bhubaneswar, Odisha. Laboratory quality distilled water prepared from steam distillation was used for the purpose. HCl and NaOH were purchased from E.Merck, Mumbai India Pvt. Ltd.

Method:

For chitosan to be extracted from fish scales it is necessary to convert it first to chitin. Generally, extraction of chitin from raw fish scales consists of three steps including demineralization for removal of calcium carbonate/phosphate, deproteinisation for removal of protein and then, chitin can be converted into chitosan by N-deacetylation which partially removes the acetyl group from the polymers chain composition [8].

Fish scales was washed to remove the muscles residue attached to it and then dried in intense sunlight and left for autolysis for 24 at room temperature which improve the quality of chitosan (Figure 3)

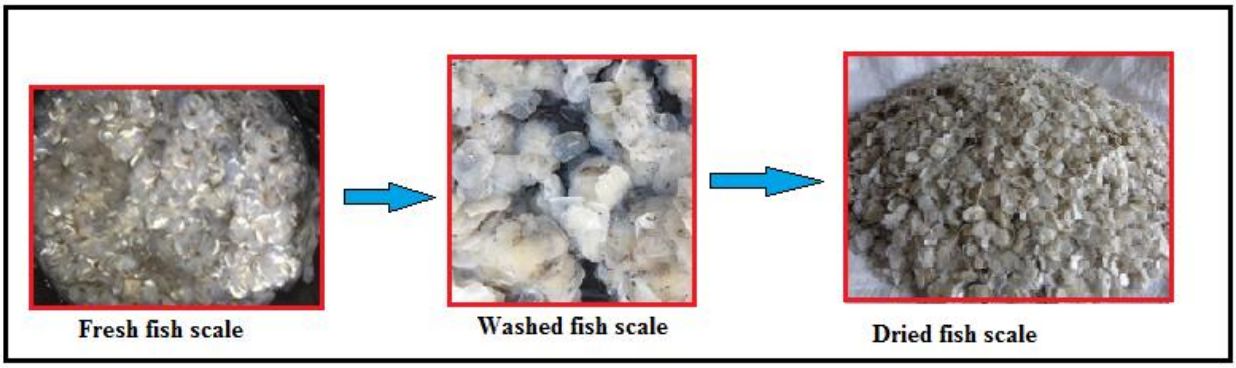

Figure 3: Pretreatment of fish scales

STEP 1*: *Demineralization

Demineralization is carried out with 2% HCl at room temperature with a solid to solvent ratio of 1: 5 (w/v) for 16 hr (Figure 4) for eliminating the organic matter specifically calcium carbonate ($CaCO_3$) in dilute acidic medium [9]. The residue was then washed till neutral pH and then dried in sunlight.

Figure 4: Demineralization process

STEP 2*: *Deproteinization

The Deproteinization was carried out with 4% NaOH at room temperature with a solid to solvent ratio of 1:5 (w/v) for 20 hr (Figure 5). for removal of protein content of fish scales [9]. The residue was washed until neutral pH and then dried in sunlight.

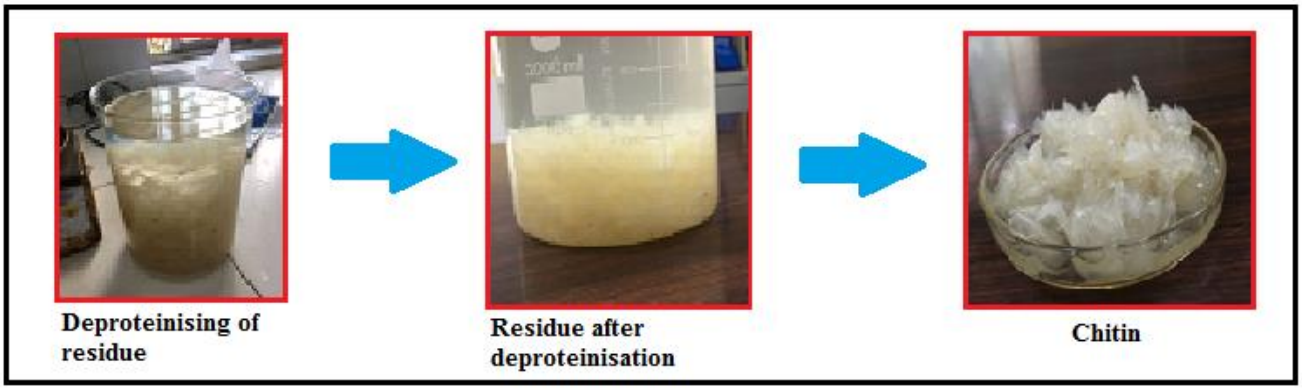

Figure 5: Deproteinisation process

STEP 3: Deacetylation

Deacetylation was carried out with 4% NaOH at 60 ± 5^{0}C with solid to solvent ratio of 1:10 (w/v) 20 hr (Figure 6). this process involve the partial removal of acetyl groups from chitin structure to convert chitin to form chitosan.

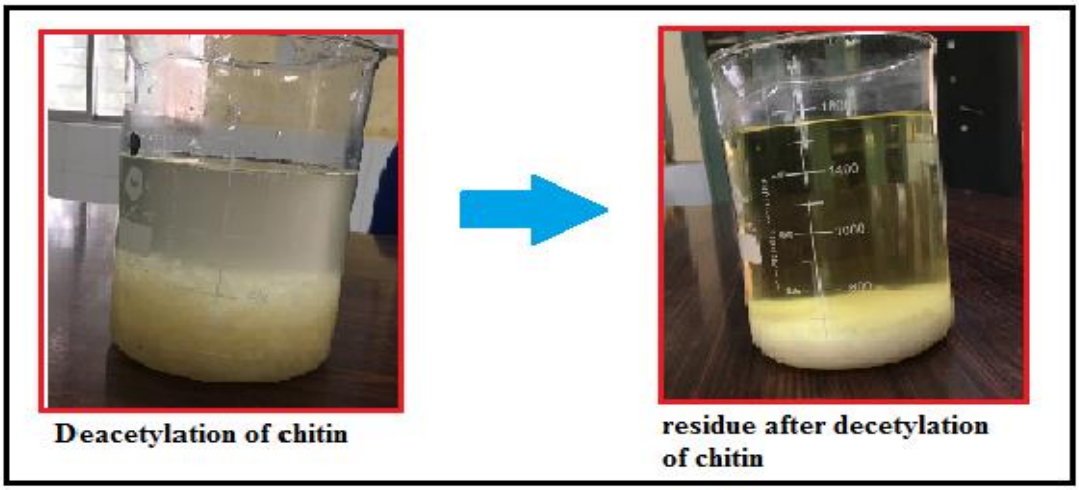

Figure 6: Decetylation of chitin

The residue was washed and dried at oven for 4 hr at 40±5^{0}C (Figure 7) and prepared for characterization.

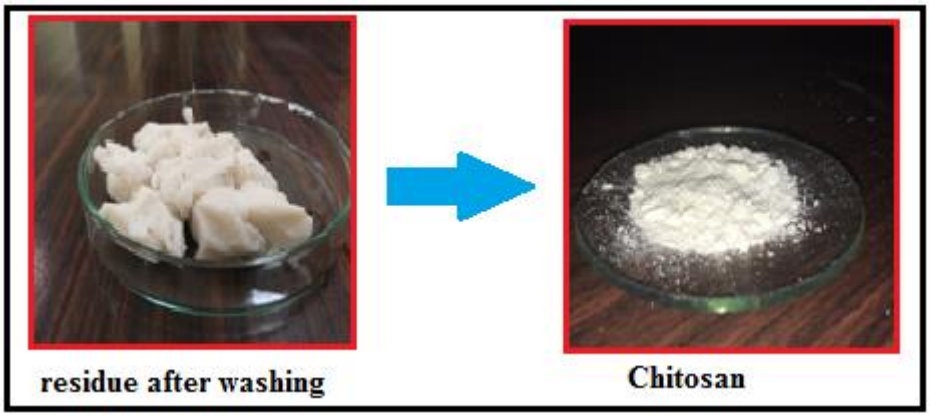

Figure 7: Residue after drying to get chitosan

3. CHARACTERIZATION AND RESULTS

3.1 Yield:

The percentage yield was obtained by the equation (1)

Yield (%)= (Amount of chitosin obtained / Amount of fresh fish scale used) ×100% (2)

Yield is found to be 22.4% which is quite high considering the conversion of waste raw material into a value added product.

3.2 Determination of degree of deacetylation:

Properties of chitosan to a varying extent are strongly dependant on degree of N-deacetylation of chitin. It is an essential factor to study the structure-property relationship. Degree of eacetylation was determined by using FT-IR spectroscopy [9]. It was reported that chitin with a degree of deacetylation of above 50 % can be considered as chitosan which it was soluble under 1% of acetic acid [10].

The degree of deacetylation was calculated by the equation (2):

$$DD(\%) = \frac{C_1V_1 - C_2V_2}{M \times 0.0994} \quad (2)$$

Where, C1 = concentration of standard HCl aqueous solution (mol/l), C2 = standard NaOH solution (mol/l), V1= volume of the standard HCl aqueous solution used to dissolve chitosan (ml), V2= volume of standard NaOH solution consumed during titration (ml), and M= weight of chitosan (g). We find that degree of deacetylation of our product is 87.80%

3.3 Water binding capacity:

Water binding capacity can be calculated by equation (3):

$$\text{WBC (\%)} = \frac{\text{water bound (g)}}{\text{Initial sample weight (g)}} \times 100 \quad (3)$$

Water binding capacity of the extracted fish scale chitosan was found 580.66%. Work done by various researchers was also found to tally with the obtained result .Cho *et al*., (1998, 2 to 4)[13] reported the WBC for five commercial chitosan from shrimp and crab hell range of 458% to 805%. On the other hand, Rout (2001, 22 to 24)[16]found that WBC for chitosan ranges between 581 to 1150% with an average of 702%. However, (Rout 2001) also commented that reversing the sequence of steps such as demineralization and deproteinization had a pronounced effect on WBC.

3.4 Fat binding capacity:

Fat binding capacity was calculated by equation (4):

$$\text{FBC (\%)} = \frac{\text{Fat bound (g)}}{\text{Initial sample weight (g)}} \times 100 \quad (4)$$

Fat binding capacity of fish scales extracted chitosan was measured using soybean oil[12]. Extracted chitosan sample showed 465.56% of fat binding capacity.

***3.5 Fourier Transform Infrared Radiation (FTIR)*:**

Infrared spectroscopy is one of the most common characterisation method for chitin and chitosan (Figure 8) due its simplicity, relative instrument availability and independence of sample solubility. Absorption band observed at 3423.83 was attributed to OH stretching. Absorption band observed at 1417.69 was attributed to CH_2 bending. Similarly, band 873.89 and 1032.53 attributed toward the CO stretching.

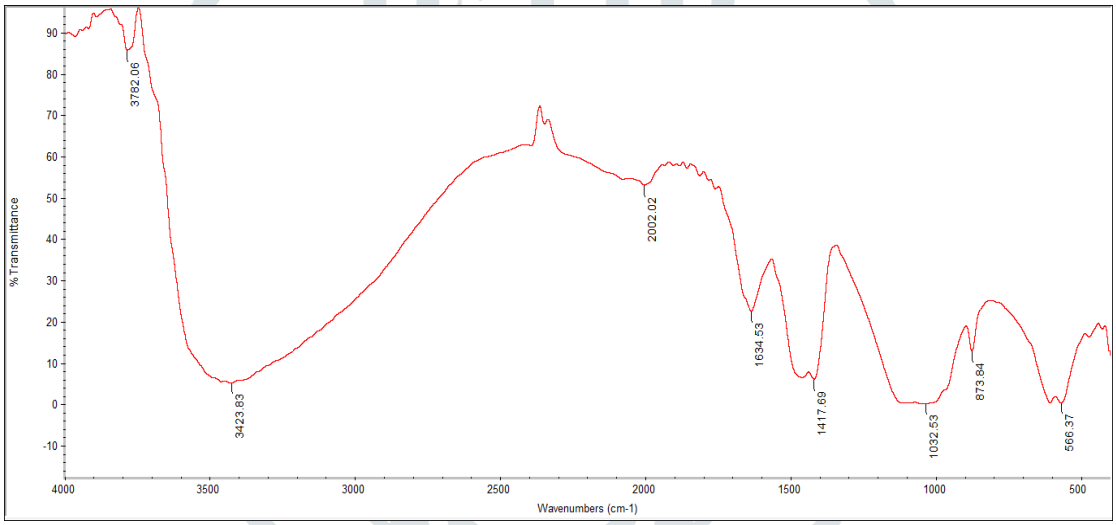

Figure 8 : FTIR of chitosan form fish scale

***3.6 X-ray Diffraction (XRD)*:**

The XRD pattern of Chitosan exhibits broad diffraction peaks at 10° and 34° which are typical diffraction peaks of amorphous chitosan. It is reported that, the characteristics crystalline peaks with slightly fluctuating diffraction angles were found. WAXD patterns indicated that two types of alpha-Chitosan and gamma-Chitosan exhibited comparable degree of crystallinity and had a consistent peak between 30-40°.

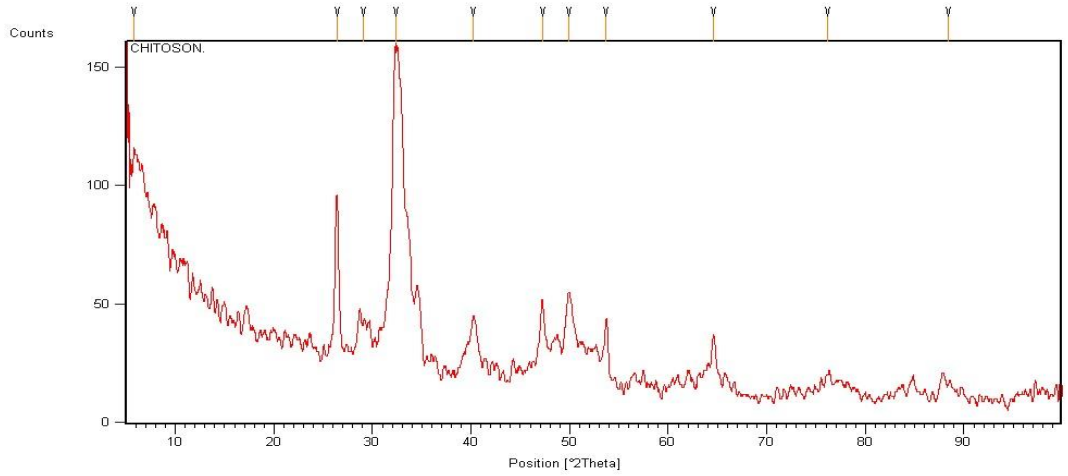

Figure 9 :XRD of chitosan from fish scales

4. CONCLUSION

Chitin extracted from fish (*Labeo Rohita*) scales by chemical method using 2% HCl , 4%NaOH.Chitin was converted to chitosan by demineralization followed by deproteinization and finally deacetylation process. Deacetylation of chitosan from chitin using 4%NaOH at 60±5^{0}C gave the yield of 22.4%. Degree of acetylation was found to be 87.80%. The water binding capacity and fat binding capacity was found to be 465.56% and is 580.67% respectively.The characterization of the obtained product by XRD and FTIR analysis gave relevant peks confirming the quality of the product. Thus the obtained product can be used as a good biodegradable filler which could efficiently meet the thrust of the present scenario considering the huge amount of waste generation due to the enormous use of synthetic plastic materials

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