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RESEARCH ARTICLE

ISOLATION AND EXTRACTION OF CHITOSAN FROM SHRIMP SHELLS

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Abstract

Chitosan is one of the versatile bio-polymer to be produced today. There has been increased inclination towards eco-friendly, biocompatible and biodegradable polymers, as most of the prevalent synthetic polymers lack these properties. Hence, the advantages of chitosan as a textile material were realized. In this paper chitosan has been extracted from shrimp shell waste by different methods. Each method included 3 distinct steps of demineralization, deproteinization and deacetylation of the shells. The quality of chitosan produced depends on the conditions and parameters maintained during the chemical extraction process. The result of extraction showed that 4% HCl and 4% NaOH were suitable concentrations for dimeralization and deproteinization respectively at ambient temperature (28 ± 2^0C). Chitosan with a higher degree of deacetylation (70%) was obtained with 60% NaOH when used during deacetylation for 24 hour at 60^0C. The formation of chitosan was characterized by elemental analysis and FTIR measurements.

Introduction:-

Sea food, found in a wide range of products is a delicacy in many coastal areas. The harvested products are packaged and processed by the sea food industries. During their processing, the meat is only taken, while the head and shells of shell fish are generated as waste. The consequence is the generation of a large amount of shell waste globally.

The fish industries, prominent in all coastal countries generate about 60,000 to 80,000 tons of waste. Although the wastes are biodegradable, the deposition of large quantities makes the degradation process to slow resulting in accumulation of waste over the time which leads to major environmental concern. The generated shell wastes thus obtained have only a low economic value and can be either used as animal feed or organic manure (Suchiva, Chandrkrachang et al. 2002, Parthiban, Balasundari et al. 2017).

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After cellulose the second largest supply of natural biopolymer is chitosan which commonly exist in the shells of crustaceans. Shell waste produced by the sea food industry is one of the most important problems contributing significant environmental and health hazards. Frequently the shells are disposed through burning which is not only costly due to their low burning capacity but also liberates hazardous gases in the atmosphere. In such a state, conversion of shrimp shell waste to chitosan, a commercially valuable product with diversified uses, could serve as an effective mode of converting shell waste for the better. Chitosan shows many unique physicochemical properties such as naturally renewable sources, non-allergenic, antimicrobial, biocompatible, non-toxic, and biodegradable. Due to these built-in interesting properties this biopolymer is attracting a great deal of interest over a broad range of research areas, including use in biomedical, food, textile and various chemical industries (Kumar 2000, Divya K 2014, Sen July 2012).

Even though chitosan is typically manufactured by de-acetylating chitin through chemical processes, it is also found naturally as a key component in fungal cell walls. Chitosan, the deacetylated chitin derivative, is more useful intrinsic biopolymer and chemically is a copolymer of α-(1-4) glucosamine ($C_6H_{11}O_4N$)n, with a varying content of N-acetyl groups. Being a natural cationic polymer it possesses a positive charge when in its dissolved state (pH<5-7), giving rise to versatile uses based on its chelating, anti- microbial, gelling and film-forming properties. Depending in the length of polymer, chemical sequence and purity chitosan can produce a family of products. Deacetylation of chitin can occur to various extents and is distinguished by the degree of deacetylation.

Chitin is estimated to be produced annually almost as much as cellulose. It has become of great interest not only as an under-utilized resource but also as a new functional biomaterial of high potential in various fields and the recent progress in chitin chemistry is quite significant.

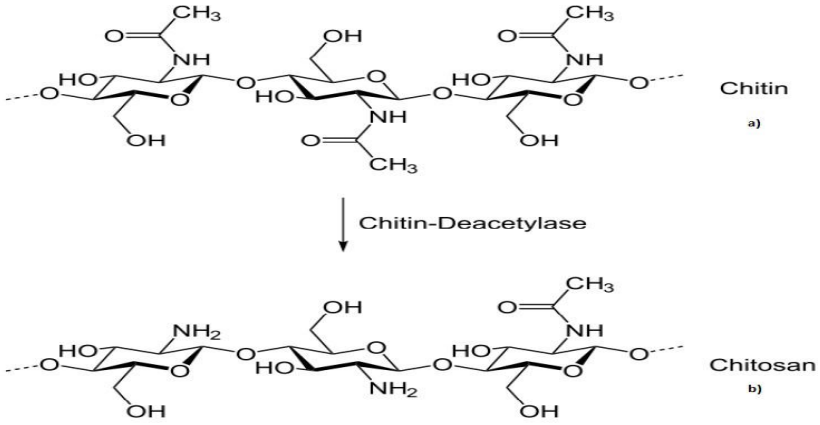

Figure 1: Chemical structural representation of chitin (a) and chitosan (b).

METHODOLOGY

Extraction of chitosan

Fresh shrimp was collected from local market. The head and shell was separated from shrimp using a sharp knife. The collected shrimp wastes were first scraped of all remaining skin and then washed with tap water, dried and crushed into small particles. Crushed shrimp wastage was kept in a polyethylene bag at ambient temperature (28±2°C) for 24 hours for partial autolysis to facilitate chemical extraction of chitosan and to improve the quality of chitosan. The subsequent 3 (three) steps, namely Demineralization, Deproteinization and Deacetylation are followed for the isolation of chitosan (Lamarque, Lucas et al. 2005). The details of the above three steps are discussed below:

Demineralization

Demineralization of shrimp shell has been carried out with four different concentration of HCI (2%, 3%, 4%, 5%) at ambient temperature (28±2°C) with a solid to solvent ratio 1:5 (w/v) for 16 hours. The residue was washed and soaked in tap water until neutral pH was obtained. Then the demineralized samples were dried and weighed (Huang, Khor et al. 2004).

Deproteinization

Deproteinization of shrimp shell was classically done with 4% NaOH at ambient temperature (28±2°C) with a solid to solvent ratio 1:5 (w/v) for 20 hours. The residue was washed and soaked in tap water until neutral pH was obtained. Deproteinized shrimp shell is called chitin. The purified chitin was then dried until it became crispy. Chitin flakes were further ground to smaller particle to facilitate deacetylation. The absence of color of the medium denotes the absence of protein (Al Sagheer, Al-Sughayer et al. 2009). The purified chitin was dried to a constant weight. The chitin content was determined from the weight differences of the raw materials and that of the chitin obtained after acid and alkaline treatments.

Deacetylation

Removal of acetyl groups from chitin was experimented using two different concentration of NaOH (40%, 60%) at 65°C temperature with a solid to solvent ratio 1:10 (w/v) for 20 hours. This experiment was designed for each deprotonized samples. That means total (4×2) = 8 deacetyled chitosan samples were produced. The residue was washed until neutral pH with tap water. The resultant 8 chitosan samples were then dried in oven for 4 hours at 65±5°C and prepared for characterization (Muzzarelli and Rocchetti 1985).

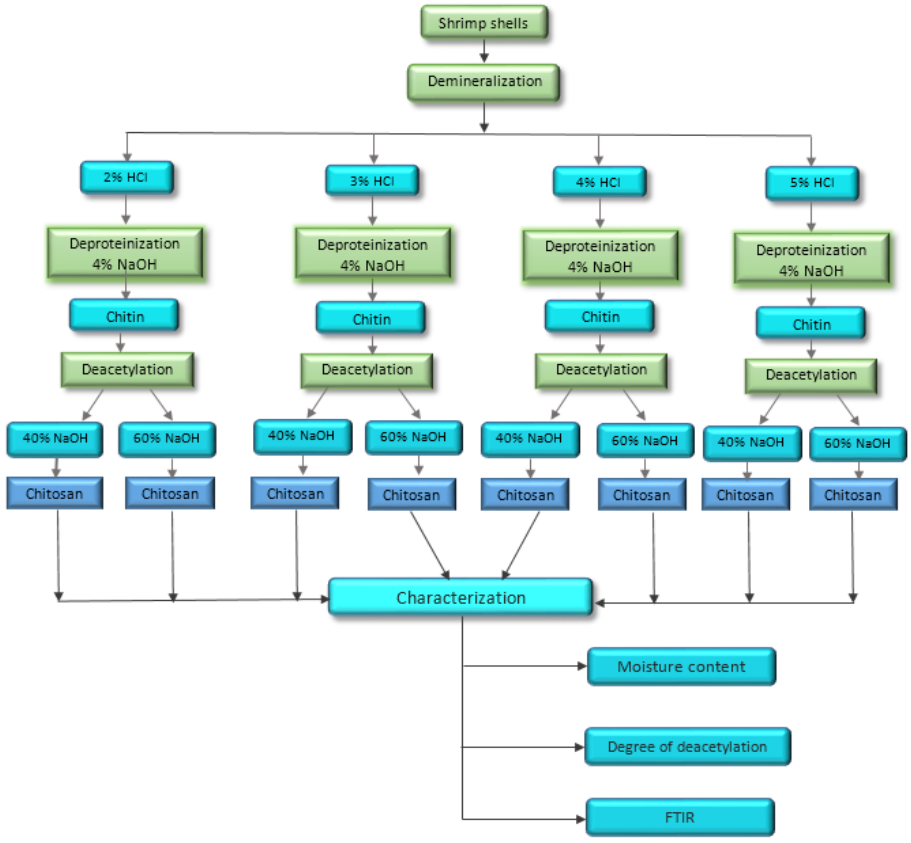

Figure 2: Schematic diagram of chitosan extraction

CHARACTERIZATION OF PREPARED CHITOSAN

Moisture content

The different physicochemical and functional properties were measured as per the standard methods, e.g., Moisture content was determined by the gravimetric method (Blake and Hartge 1965).

Procedure:

- At first the weight of a paper is taken and recorded this weight.
- 0.5 g chitosan sample was placed in paper and recorded this weight.
- The sample was placed in the oven at 110°C and dried for 3 hrs.
- Repeated the previous step until there was no difference between any two consecutive measurements of the weight.

$$\text{Moisture content \%} = \frac{\text{Initial weight} - \text{after drying weight}}{\text{Initial weight}}$$

Determination of degree of deacetylation

The degree of deacetylation (DD) was measured by the acid-base titration method. In brief, chitosan (0.1 gm) was dissolved in 30 ml HCl aqueous solution (0.1mol/l) at room temperature with 5–6 drops of methyl orange added. The red chitosan solution was titrated with 0.1mol/l NaOH solution until it turned orange. The DD% was calculated by the formula:

$DD\% = (C_1V_1 - C_2V_2) \times 0.016 \div (M \times 0.0994)$

Where, C_1= 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).

The number 0.016 (gm) is the equivalent weight of NH_2 group in 1 ml of standard 1 mol/l HCl aqueous solution and 0.0994 is the proportion of NH_2 group by weight in chitosan. The Degree of acetylation was calculated by subtracting the value of degree of deacetylation from 100% (Parthiban, Balasundari et al. 2017).

Solubility of Chitosan

Complete dissolution of chitosan is achieved in 1% acetic acid. 35ml 1% acetic acid was taken to which a few grams of chitosan were added. The beaker was kept in a magnetic stirrer for 30 mins. The sample was taken out and insolubles were removed by filtration through Whatmann No.1 filter paper and weighed.

Fourier Transform infra-red spectroscopy (FTIR)

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganics.

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000 – 600 cm-1.

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range (Domszy and Roberts 1985, Hirai, Odani et al. 1991).

RESULTS AND DISCUSSIONS

Moisture content

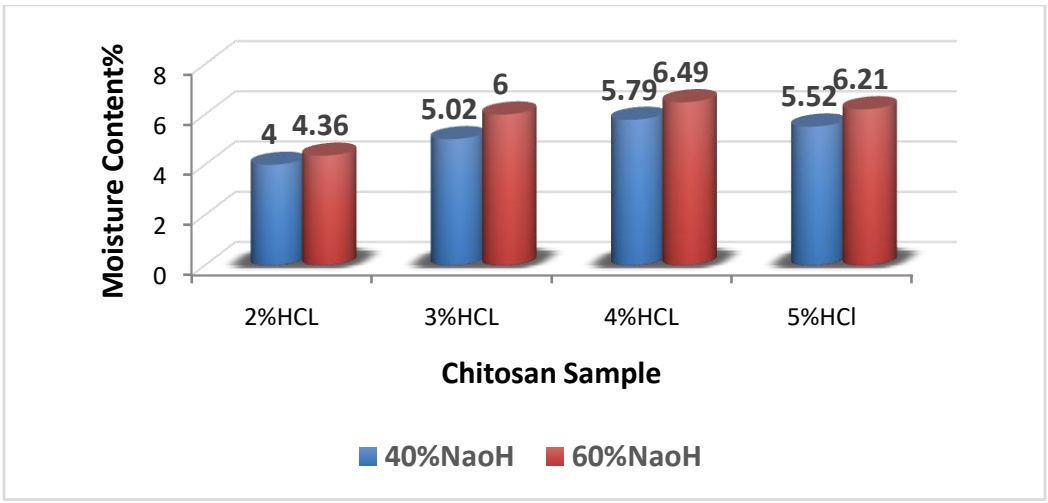

Figure 3: Moisture content of different chitosan sample

Chitosan is hygroscopic in nature hence it can be affected by moisture absorption during storage, relative humidity and light. The chitosan samples produced had a moisture content ranging from 4% to 6.49%.The highest moisture content% was obtained when treated with 4% HCL during demineralization process and 60% NaOH in deacetylation process. Although many previous reports states lower moisture content, the permitted level is below 10% (Kandile NG 20.07.2018).

Degree of deacetylation

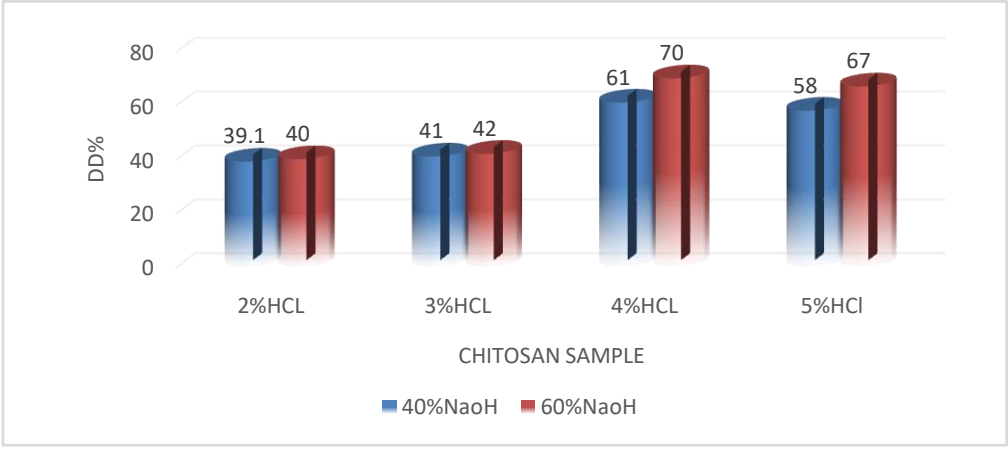

Figure 0: DD% of different Chitosan sample

The degree of deacetylation (DD) is influenced by NaOH concentration (Islam, Masum et al. 2011). Acetyl groups bounded in chitin is difficult to be removed. So, it needs high concentration of NaOH and temperature. In this case, the increase of NaOH concentration addressed to enhance the deacetylation grade where the highest deacetylation grade 70% could be reached at NaOH concentration of 60%.

FTIR of prepared Chitosan

IR spectra of chitosan obtained from different processes were recorded with a Tensor 27 Fourier transform infrared spectrometer FTIR (Germany). The spectral region between 4000 and 500 cm^{-1} was scanned. Specimens prepared as KBr pellets were used. Dried, powdery chitosan was mixed thoroughly with KBr and then pressed in vacuo to homogeneous disc with a thickness of 0.5 mm. The chitin concentration in the samples were 2%, calculated with respect to KBr (Zvezdova 2010).

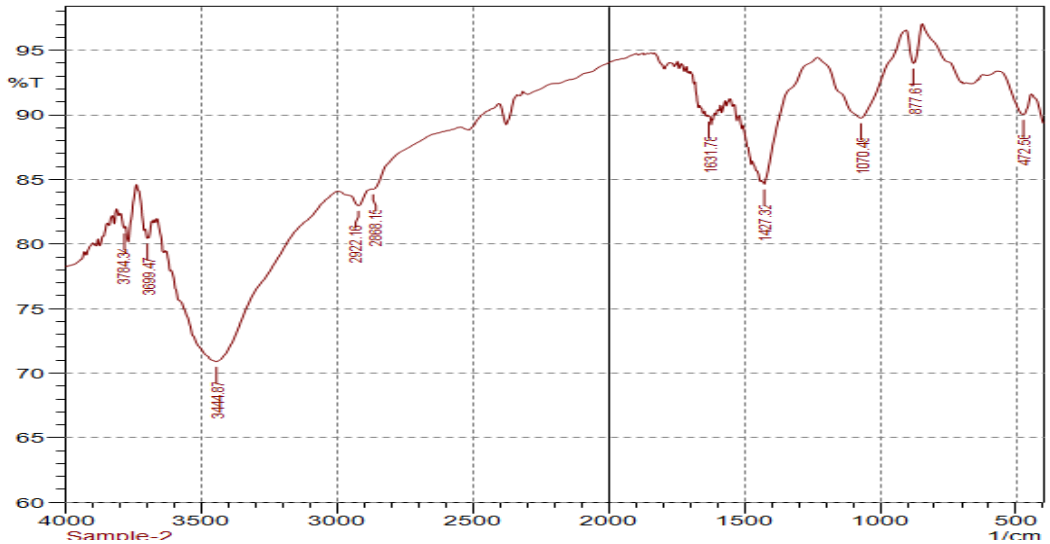

Figure 5: FTIR Spectra of prepared Chitosan with 40 % NaOH in deacetylation Process

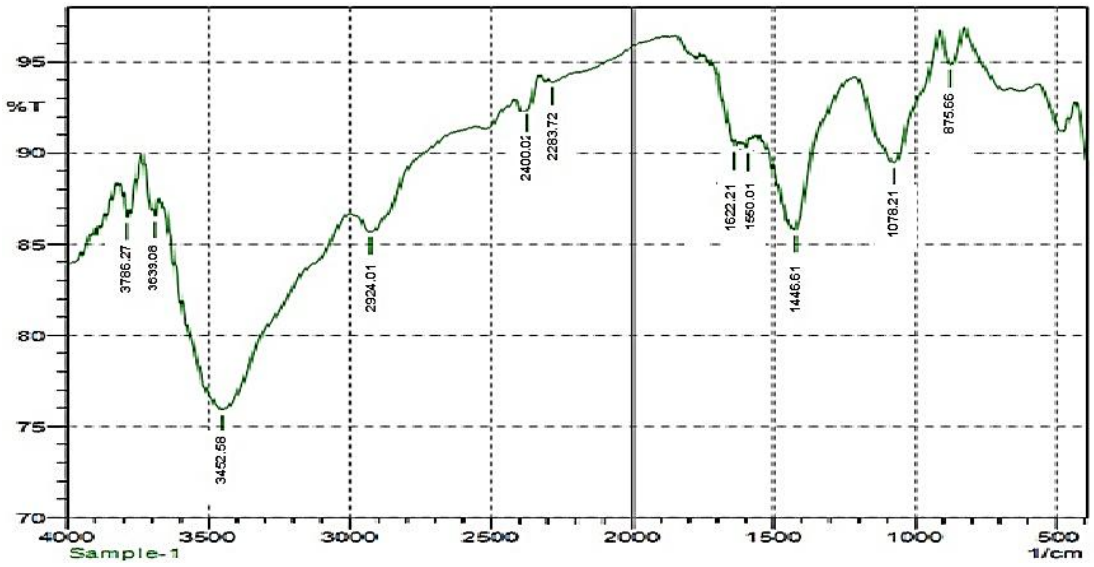

Figure 6: FTIR Spectra of prepared Chitosan with 60% NaOH in deacetylation Process

The FTIR Spectra of prepared Chitosan showed important absorption bands to identify the characteristic functional groups which were recorded in the middle infrared (4000 cm^{-1} to 500 cm^{-1}).The infrared spectra for chitosan biopolymers are shown in fig 5 and fig 6. Since the results are more prominent for the FTIR spectra obtained with 60% NaOH, this is explained below:

A strong band at 3452.58 cm^{-1} corresponds to N-H and O-H stretching. The peak is broader and stronger due to the overlapping of OH and NH bond. The absorption band at 2924.01 cm^{-1} can be attributed to C-H stretching. This band is characteristic of polysaccharide. The presence of residual N-acetyl groups was confirmed by the band at around 1622.21 cm^{-1} (C=O stretching of amide I). The small band at 1550 cm^{-1} corresponds to N-H bending of amide II. This is the second band characteristic of typical N-acetyl groups. The CH_2 bending was confirmed by the presence of band around 1446 cm^{-1}. The absorption band at 1078 cm^{-1} can be attributed to asymmetric stretching of the C-O-C bridge and 875cm^{-1} corresponds to bending out of the plane of the ring of monosaccharides (Paul, Jayan et al. 2014).

CONCLUSION

Chitosan was successfully extracted from shrimp waste in the laboratory by different chemical methods. The process parameters were identified for the optimized yield of chitosan. Moisture content & degree of deacetylation percentage of prepared chitosan were significantly higher when treated with higher concentrated NaOH in deacetylation process. The highest moisture content and degree of deacytilation was obtained when 4% hydrochloric acid (HCl) was used in demineralizaon process at ambient temperature and 60% sodium hydroxide (NaOH) in deaceytilation at 60^0C.

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