iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** IV **Month of publication:** April 2019

DOI: https://doi.org/10.22214/ijraset.2019.4400

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue IV, Apr 2019- Available at www.ijraset.com

Extraction and Characterization of Chitosan from Aquatic Biowaste

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Abstract: Chitin is the second most abundant renewable natural source following cellulose and the main source of chitin is crustacean waste. Chitosan whichis a derivative of chitin after the process of deacetylation has multiple of commercial and possible medical uses based on its degree of deacetylation. The present study is aimed to extract chitosan from aquatic waste like shells of shrimp, crab and fish scales and to characterize the chitosan quality which includes parameters like ash, moisture, protein and lipid content and degree of deacetylation (DDA). Among the three aquatic biowaste selected, maximum quantity of chitin (459.66±25.25 %) and chitosan (418.33±28.32%) were obtained from shrimp shell waste and consist of relatively low protein contents of protein(7.9±0.51%), fat (3.4±0.08 %) moisture (2.0±0.06%) and ash (1.4±0.05%) on a dry basis compared to chitosan obtained from fish scales and crab shells. Based on these characteristics it can be concluded that shrimp chitosan appeared to have superior quality than crab and fish chitosan. Utilisation of shrimp shell waste for the production of chitin and chitosan will give more economical and biological value along with reduction of environmental pollution.

Keywords: Chitin, Chitosan, Aquatic biowaste, Quality parameters

I. INTRODUCTION

The shellfish processing industry in India generates 125,000 to 150,000 tons of shell waste per year (Ramyadevi, 2012). It is nearly about 45% waste of this processed seafood disposed on landfill, consequently leads to environmental pollution in terms of odor and aesthetic damage to the environment (Sagheeret al., 2009).

Crustacean shell wastes mainly consists of 30–40 % protein, 30–50 % calcium carbonate, and 20–30 % chitin, etc., which could be used to produce high value added materials if recycled (Nouri , 2015).

Chitin, a homopolymer of N-acetyl-D-glucosamine is the most abundant renewable natural resources and the main source of it is crustacean waste. (Pal et al., 2014). Chitosan, deacetylated product of chitin is a nontoxic biopolymer (Abdulkarim , 2013) and it is commercially produced from the crustacean shell wastes through different degrees of deacetylation, which attribute to a variety of properties (Rinaudo, 2006). Chitin and its derivative chitosan are of commercial interest due to their excellent biocompatibility, biodegradability, nontoxicity, chelating and adsorption power. (Szymanska, 2015).

Quality of chitosan is determined from several parameters, the degree of deacetylation is a quality parameter that indicates an acetyl group which can be removed from yield of chitosan. High deacetylation degree of chitosan means that the acetyl group contained in the chitosan is weak. Deacetylation degree of chitosan varies between 56-99% an average of 80% depending on the source and method of preparation (Hussain et al., 2013).

Nellore district of Andhra Pradesh is known as high rates of shrimp production and number of shrimp processing industries are located in and around Nellore. Considering the huge market potential and availability of raw material(shrimp shell waste) that can be sourced locally, the chitin and chitosan production will be a viable and innovative venture in the region. Keeping in view of significance and applications of chitosan, the present investigation has been taken up to evaluate the difference in yield % and in the quality parameters between the shrimp biowaste, crab shell waste and fish scales.

II. METHODOLOGY

A. Collection And Processing Of Aquatic Bio Waste

The shell waste (crab, shrimp and fish scales) was obtained from near by processing industries located at Rajupalem area of Nellore District. The sample were washed with tap water to remove any insoluble material on the shell then dried at 50°C in oven for 24h and homogenized in a laboratory mixer before using for further processing. The yield of dried shell was determined by weighting after being dried (Khanafari et al 2008).

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B. Chemical Extraction Of Chitin And Chitosan

1) *Deproteination:* The obtained crushed waste (100gr) was placed separately in 500 ml beakers and soaked in 100 ml sodium hydroxide(4%) for 24 hrs in room temperature in order to dissolve the proteins and sugars thus isolating the crude chitin. (Kumari and Rath,2014). Then solution was filtered and the samples were washed with distilled water.The shells were then further crushed to pieces of 0.5-5.0 mm using a meat tenderizer.
2) *Demineriliśation:* The grounded shells were demineralised using 1% HCl with four times its quantity. The samples were allowed to soak for 12 h to remove the minerals (mainly calcium carbonate) (Puvvada et al.,2012).The demineralized shell samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble aminoacids. The remaining chitin was washed with deionized water, which was then drained off.
3) *Deacetylation:* Chitosan was obtained from extracted chitin through deacetylation method (Kumari, 2014).The extracted chitin was dissolved in 100ml of NaOH (50%) at 60°C for 8h to obtain crude chitosan. After filtration, the residue was obtained, washed three times with hot distilled water at 60°C. The crude chitosan was obtained by drying in a hot air oven at 50°C overnight. Then 10% acetic acid was added to the residue and stored for 12 hours at room temperature. The dissolved sample was reprecipitated by adding 40% NaOH. The residuewas removed by filtration, then two fold volumes of ethanol was added to the filtrate. The crystal of water-soluble chitosan was liberated after incubation at ambient conditions overnight and dried in oven at 50°C.

C. Yield of Chitosan

The chitosan yield (%) was calculated as the dry weight of the chitosan flakes relative to the wet weightof shell waste (Nouri et al., 2015).

$$\text{Chitosan extraction yield (\%)} = \frac{\text{Dried extracted chitosan weight(g)}}{\text{Shell waste(g)}} \times 100\%$$

D. Proximate Analysis

Proximate analysis of extracted chitosan was carried out to determine moisture content, ash content, protein and lipid content. The samples were dried to a constant weight at 60 ^{o} C in an oven and the weight loss gives the amount of moisture in the samples. Samples were burned in a furnace at temperature of 555^{o}C and weighed to determine the ash content. The lipid and protein content were determined by standard method (AOAC, 1990).

E. Degree of Deacetylation

Degree of deacetylation in chitosan was determined by potentiometric titration. A homogenous chitosan solution was prepared by using HCl containing 0.01 M/L and it was titrated against 0.1 M NaOH. The end point was detected by the inflection of the pH values. Two inflections were mainly noted out of which first one corresponds to neutralization of HCl and second one to the neutralization of ammonium ions for chitosan chain. The difference between two points give the amount of the amino group in the chitosan chain (degree of deacetylation) DOD (Zhanga et al., 2010).

F. Fourier Transform - Infra Red Spectroscopy (FT-IR)

Extracted chitosan sample (10 mg) was mixed with 100 mg of dried potassium bromide (KBr) and compressed to prepare a salt disc. The FT-IR spectra were taken on an (FT-IR- 8300 instrument (Shimadzu)) (Szymanska-Chargot and Zdunek, 2013) accessory in the 400 to 4000cm-1 and repeated for three replicates. Standard chitosan was obtained from Himedia, Mumbai.).

G. Statistical Analysis

The tabulated values were analyzed by using SPSS 11.5 Computer based software programme.

III. RESULTSANDDISCUSSION

A total of three aquatic biowaste like shrimp shell, Fish scales and Crab shell were selected for the present study for the extraction and characterisation of chitin and chitosan. The yield of chitosan using chemical extraction method was shown in Table 1. Among the three aquatic biowaste selected, maximum quantity of chitin (459.66±25.25%) and chitosan (418.33±28.32%) were obtained from shrimp shellwaste where as minimum quantity of chitin (305.00±23.28%) and chitosan (268.33±25.17%) were obtained from fish scales. The crab shell has given reasonably good production of chitin (380.16±24.21%) and chitosan (315.00±22.57%) than fish scales. Proximate composition of shell waste varies with each species and many other factors. Chemical analyses showed that the chitosan obtained from shrimp waste consist of relatively low protein content of protein (7.9±0.51%), fat (3.4±0.08%), moisture

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(2.0±0.06%) and ash(1.4±0.05%) on a dry basis compared to chitosan obtained from fish scales and crab waste(Table 2). Degree of deacetylation (DOD) of chitosan plays a significant role for determining the specific applications of chitosan.Chitosan obtained from shrimp waste consist of relatively high degree of deacetylation 65±2.25% when compared to chitosan of crab waste (59±1.89%) and fish scales(61±2.22%)These findings show that shrimp shell waste can be considered as a good source of high quality chitosan compared to crab and fish scales.

FT-IR spectrum of extracted chitosan from shrimp waste showed peaks at 3418/cm that indicated stretching vibration of - hydroxyl group, -NH group of amines and hydrogen bonding which was comparable to spectrumpeak of standard, i.e.,3420/cm.1646/cm peak in extracted chitosan indicated the vibrations of carbonyl group (amide band I) and standard had this peak at 1654/cm. Peaks at 1594/cm (extracted chitosan) and 1580/cm (standard) showed the presence of amide band II (N-H bendings). For –CH groups in CH_2OH, peaks were observed at 2921/cm and 1422/cm in standard which overlapped with band spectrum at 2920/cm and 1421/cm in shrimp chitosan. -CH_3 group of NHCOCH (amide bond) was shown at 1380/cm in standard and at 1381/cm in shrimp chitosan. Oxygen stretching of glycosidic linkage was found at 1155/cm in standard but in shrimp chitosan it was found at 1151/cm. Pyranose ring was found at 895/cm in standard and in shrimp chitosan it was at 896/cm.The presence of the entire band stretching in the extracted chitosan compared with standard band stretching depicts that extracted material was chitosan.

IV. CONCLUSION

Now a days many industries like health care, biomedical, pharmaceutical etc. is in need of high quality, biocompatible and biodegradable materials like chitosan for addressing many health care issues. At the same time there are industries like shrimp processing plants generates huge quantity of crustacean wastes which causes environmental hazard. Fortunately, these bio wastes are considered as a potent source of chitosan. Keeping all these aspects, in the present work chitosan has been extracted from aquatic biowaste (shrimp shell, crab shell and fish scales) which form cheap and abundant functional raw materials. Chitosan was extracted by adopting modified process of previous methods and the yield was also high due to the repeated process of deprotination and demineralization steps. The physio-chemical parameters and structural characteristics are in agreement with commercial chitosan standard. The obtained chitosan had high deacetylation degree (DD),which has greater scope in various applications such as agriculture and horticulture, water and wastewater treatment, food industryand other industrial uses.

Table-1 Chemical extraction of chitin/chitosan from different aquatic bio waste

Source of Bio waste	Yield of chitin (mg/gm)	Yield of chitosan (mg/gm)
Shrimp shell	459.66±25.25	418.33±28.32
Fish scales	305.00±23.28	268.33±25.17
Crab shell	380.16±24.21	315.00±22.57

Mean ± standard deviation of triplicate determinations

Table 2: Proximate analysis of extracted chitosan

Constituents	Chemically Extracted Chitosan		
	Shrimp shell	Fish scales	Crab shell
Ash Content (%)	1.4±0.05	1.8±0.02	1.6±0.03
Moisture content(%)	2.0±0.06	3.1±0.09	4.0±0.09
Fat content(%)	3.4±0.08	4.2±0.05	4.7±0.06
Protein(%)	7.9±0.51	8.8±0.31	8.1±0.62
D.D(%)	65±2.25	61±2.22	59±1.89

Mean ± standard deviation of triplicate determinations

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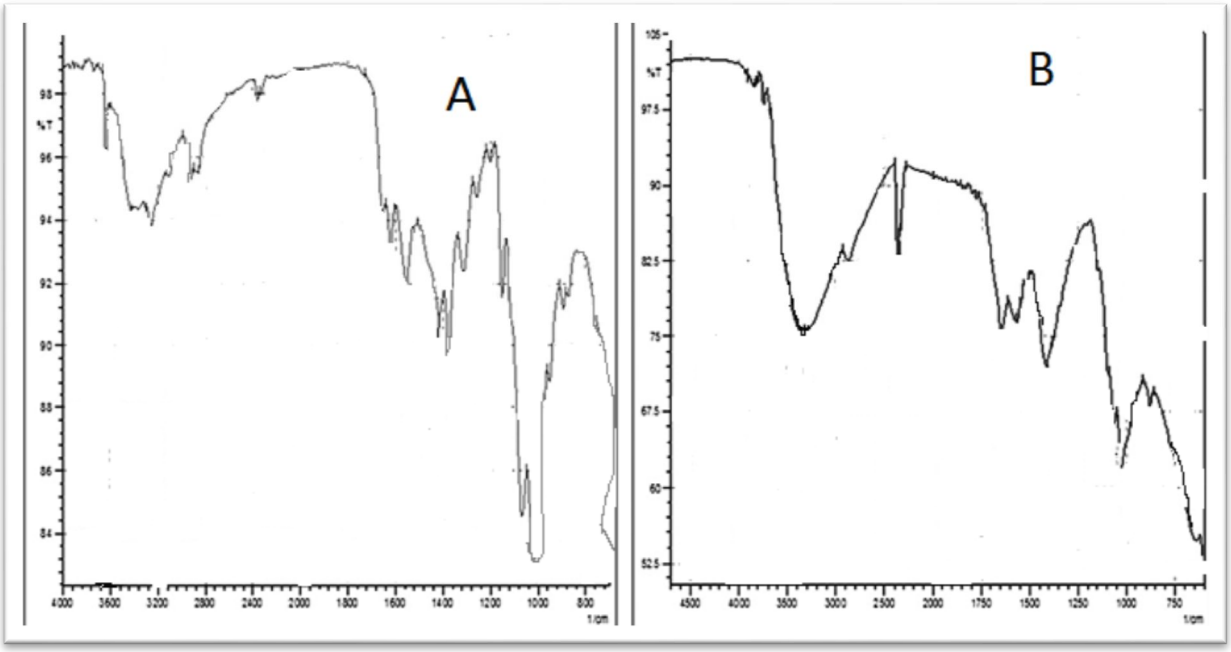


Figure 1: FT-IR spectra of A-chitosan extracted from shrimp shell, B-commercial chitosan

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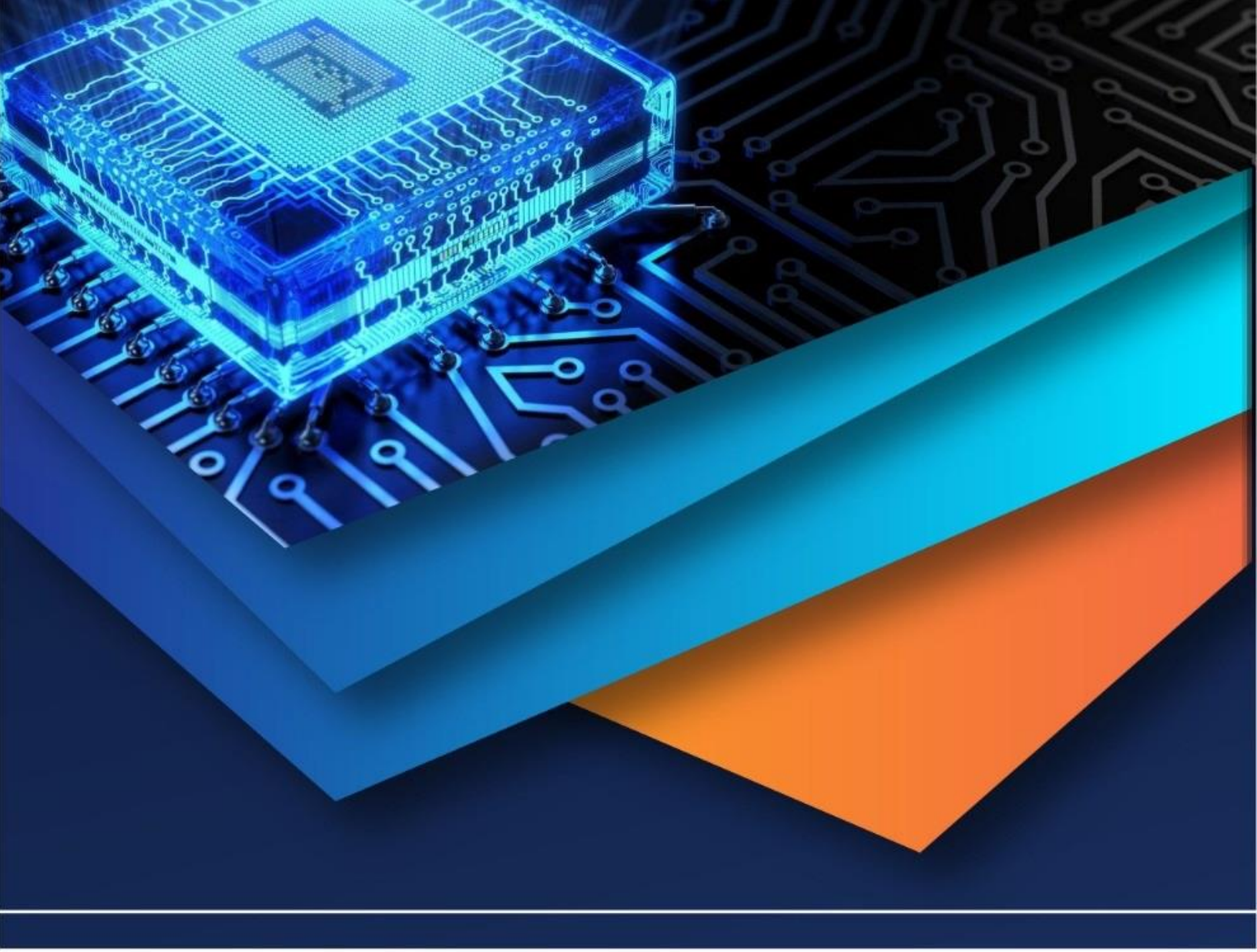
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