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Production and Characterization of Chitosan from Shrimp Shell Waste of *Parapeneopsis stylifera*

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A B S T R A C T

Chitosan was prepared by utilizing the *Parapeneopsis stylifera* shrimp shell waste generated from shrimp processing industries around Veraval region, Gujarat. Proximate composition, structural analysis and functional properties were carried out to understand the quality of the chitosan. Yield of the prepared chitosan was 4.41%. Proximate composition *viz*., moisture, protein and ash content of the chitosan were 6.24%, 1.99% and 0.39% respectively. Solubility in 1% acidic acid was 98.34% and pH was 7.9. Structural property of chitosan was studied using fourier transform infrared spectroscopy (FTIR) analysis. Degree of deacetylation was calculated from the FTIR spectral data and it was found 76.43%. Functional properties such as water binding capacity and fat binding capacity were 637.33% and 331.28%, respectively. This study reveals the chitosan prepared from *P. stylifera* shell wastes could be utilized for commercial purposes by food and other industries.

Introduction

Chitin is the most abundant natural polysaccharide. It gives strong structure to the exoskeleton of living organism. Marine resources especially, shells of crustacean such as those from shrimp, crab and lobster are the most important chitin source for commercial purposes. It is a linear homo-polysaccharide and chemically it has several units of n acetyl D- glucosamine linked by β 1-4 linkage (Younes and Rinaudo, 2015). Chitin is not readily dissolved in water and mild acids due to the acetamide group. Though the chitin showed a various functional property, their application in many fields were restricted due

to its high molecular weight and less solubility. This drawback leads to the finding of the chitosan from chitin in late 1850s (Crini, 2019). The important derivative of chitin is chitosan and it is partial deacetylated form of chitin. Chitosan is a rigid linear chain heteropolymer consisting of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) joined by β-(1, 4)-linkage. Commercial chitosan has the molecular range of 50-2000 kDa. The ratio between the glucosamine and N-acetyl glucosamine is called degree of acetylation. This degree of acetylation determines the chitosan's biological property. Generally the Degree of deacetylation (DD) for commercial processed chitosan was around 80-85%. It is the cationic polysaccharide due to the presence of positive charges on amino group. Chitosan are dissolved in mild acids. Among the chitin derivatives, chitosan is considered as the most important derivative because of it solubility. Chitosan offer a wide range of application from the agriculture to pharmacy industry due to its specific properties like bioactivity, biodegradability, chelating ability, absorption capacity and environmental friendly (Hamed *et al.*, 2016). Because of its cationic property it has wide range of application in industrial usage. Chitosan also possesses a number of functional properties such as antioxidant, antibacterial, cholesterol reduction agent, edible film packaging material, texturizing, flocculating agent, binding, emulsifying and clarifying agent.

Ever increasing demand of shrimp consumption in the local as well as international markets, Indian shrimp industry gained a great importance in the world seafood trade. In Indian waters there are 85 species of shrimp are recorded. Among them 55 species are considered as commercially important species which having considerable demand in market. In 2017-18, India has exported 13,77,244 tons of marine products, in which frozen shrimp contributed 65,980 ton. Along the Indian coast 581 seafood freezing plants are present, with a built in capacity of 31,006.07MT (MPEDA, 2019). *Parapeneopsis stylifera* is commonly called kiddi shrimp mainly available in the west of India. The shrimp processing produces a large quantities of head and shell waste accounts approximately to 40-50%. In India, shrimp processing plants produces more than 1 lakh tons of shrimp wastes annually (Kumar & Suresh 2014). The waste generation from seafood processing especially crustaceans creates the practical difficulties in disposal and environmental pollution. Shrimp's head generally constitutes 34-45% and body shell constitutes 10-15%. These wastes contain 35-40% protein, 10-15% chitin, 10–15% minerals and carotenoids (Sachindra and Bhaskar, 2008). Proper usage of shrimp shell wastes for the conversion of chitin and chitosan leads to economic gain to the producer. Apart from that, it reduces the unwanted environmental pollution by dumping the waste in open environment. So this present study was intended to study the structural and physicochemical properties of chitosan prepared from the *Parapeneopsis stylifera* shrimp shell waste.

Materials and Methods

Preparation of chitosan from shrimp shell waste

Chitosan from the shrimp shell was prepared using the method described by Benhabiles *et al.*, (2012). The shrimp shell wastes were thoroughly washed in potable running water and cooked for 1 h to remove the adherent tissues and impurities. The washed shells were dried and ground into powder. Demineralization of shell was done using 1.5 M HCl (1:10 w/v) for 30 min at room temperature. The decalcified shell were repeatedly washed with water to remove the

acid and dried at 80^{o}C. Deproteinization was done using 2N NaOH (1:10 w/v) for 2 hr at 40^{0}C followed by washed with water and dried. This chitin was converted into chitosan by deacetylation using 50% NaOH at the chitin and NaOH ratio of 1:10 at 90^{0}C for 3 hr. Finally the solid was filtered, neutralized by washing with water and 80% alcohol and dried at 80^{0}C overnight.

Moisture, protein, ash content and pH of Chitosan

Moisture content was analysed as per AOAC method (2000) using hot air oven. Crude protein content of chitosan estimated using the Kjeldhal method (AOAC, 2000). Ash content was determined as per the standard method (AOAC, 2000). The pH was determined using a digital pH meter.

Fourier transform infrared spectroscopy (FTIR) analysis

FTIR spectral data of the chitosan was obtained using FTIR spectrometer (iS5 NICOLET, Thermo Scientific, USA). The absorbance spectra of pellet were measured from 400 - 4000 cm^{-1}. Spectrum signals were collected in 36 scans at a resolution of 4 cm^{-1}. Spectral data analysis of was done with the help of OMNIC 9.0 software programme.

Determination of degree of deacetylation (DD)

The degree of deacetylation (DD) was determined by Fourier transform infrared spectroscopy (FTIR) data. The degree of acetylation (DA) was calculated by following Moore and Roberts (1980) equation:

$$DA = [(A_{1655} / A_{3450})x100]/1.33$$

where A_{1655} and A_{3450} are the absorbance intensity of respective wavelengths; 1.33 is the ratio of the absorbance at 1655 cm^{-1} to that of 3450 cm^{-1} for fully N-acetylated chitosan.

Degree of deacetylation (DD) was calculated using the following formula:

$$DD\ (\%) = 100 - DA(\%)$$

Determination of molecular weight of chitosan

The viscosity-average molecular weight of the chitosan was determined by viscometric method described by Roberts and Domszy (1982). Chitosan was dissolved in 0.2M acetic acid with various concentrations (0.5mg/ml, 1mg/ml and 1.5mg/ml).

The viscosity was measured at 25^{o}C using an Ostwald glass viscometer (Brosil, India). The molecular weight of chitosan was calculated using the following Mark–Houwink equation:

$$\text{Intrinsic viscosity}(\eta) = K(MW)^{a}$$

where K =1.81×10^{-3}, and a =0.93

Solubility

Solubility of chitosan in weak acidic solution was measured following the method of Ahing and Wid (2016). One gram of chitosan was dissolved in 1% acetic acid solution to make 1% chitosan solution. This mixture was stirred using magnetic stirrer for 2 h at room temperature. The mixture was filtered through a pre-weighed Whatman No. 1 filter paper (W_i). Then, filter paper was dried at ambient temperature and re-weighed (W_f). The solubility percentage was calculated by using the following formula:

$$Solubility = 100 - [\left(\frac{w_f - w_i}{w_s}\right)x100]$$

where, (W_f - final weight of filter paper (g), W_i - initial weight of filter paper (g) and W_s - chitosan weight (g).

Water Binding Capacity (WBC) and Fat Binding Capacity (FBC) of chitosan

Water binding capacity (WBC) and fat binding capacity (FBC) of chitosan were determined according to the method described by Wang and Kinsella (1976) with slight modification. For WBC or FBC, weight of 50 ml capacity centrifuge tube with 1 g of sample was measured followed by 20 ml distilled water or vegetable oil was added. Both tubes solutions were mixed for 5 s at every 10 min. After one hour, the resulting solutions were centrifuged at 3200 rpm for 25 min at room temperature. The supernatant was removed and the tube weight was measured. WBC and FBC were determined by using the following formula:

$$WBC\ (\%) = \frac{Water\ bound\ sample\ (g)}{Initial\ weight\ of\ the\ sample\ (g)} x100$$

$$FBC\ (\%) = \frac{fat\ bound\ sample\ (g)}{Initial\ weight\ of\ the\ sample\ (g)} x100$$

Results and Discussion

Moisture, protein, ash and pH of chitosan

The efficiency all the process involved in chitosan preparation from the shrimp shell waste viz., demineralization, Deproteinization and deacetylation was determined by the biochemical quality analysis. The moisture, protein, ash content and pH of the chitosan are shown in the Table 1. The yield of chitosan was 4.41%. The moisture content of the chitosan was reported as 6.24 %. Better shelf life of chitosan was achieved by maintain the moisture content below 10% (Nouri *et al.*, 2016; Li, 1992). In this study the ash content of chitosan was 0.39%. The limit of ash content in high quality chitosan was less than 1% (No and Meyers, 1995). Moreover, ash content of the chitosan directly correlates with the effectiveness of demineralization process. Protein content of the chitosan was 1.99%. It indicates the effectiveness of Deproteinization during the chitosan production. pH of commercial chitosan is around 8 and in our present study it is noticed that the pH of chitosan prepared from shrimp shell was 7.9.

FTIR analysis

FTIR spectrum is based on the vibration of the atoms in the molecule. The FTIR spectrum of chitosan is shown in Figure 1. The absences of sharp band above the region of 3500 cm^{-1} explained the presence of intra-inter molecular hydrogen band in the hydroxyl group in C_2 and C_6 position of chitosan (Kumirska *et al.*, 2010).

The peak above 3000 cm^{-1} in this chitosan was centred at 3444 cm^{-1}. This higher frequency shift revealed a higher order structure present in this chitosan sample. According to kumar *et al.*,2004 the appearance of single broad peak at around 3371cm^{-1} conform the presence of β structure in the chitosan.

The peak around 2882 cm^{-1} was representing the presence of glucosamine in chitosan. Amide I peak present at 1653 cm^{-1} was denoting the C=O stretching in chitosan and it was mainly due to hydrogen bonding (Nouri *et al.*,2016). Amide II peak represents the N-H bending and it was present at 1596 cm^{-1} in this chitosan sample. Generally amide III peak in chitosan is around 1320 cm^{-1}. This peak indicates the presence of n acetyl glucosamine in chitosan molecule (Brugnerotto *et al.*, 2001). In this study the amide III chitosan was at 1323 cm^{-1}. The peak at 1379 cm^{-1} represented the presence of asymmetrical C-H bending of the CH2 group in chitosan. In

polysaccharides CH_2 bending is represented by the presence of peak near 1429 cm^{-1}. In this sample it was present at 1421 cm^{-1}.

Degree of Deacetylation (DD) of chitosan

The ratio between the glucosamine and N-acetyl glucosamine is called degree of acetylation (DD). The conversion of chitin into chitosan was determined by the amount of formation of glucosamine content. Therefore, the higher the glucosamine content higher the degree deacetylation of chitosan. The study revealed that DD of the chitosan using FTIR method was 76.43%. When the degree of deacetylation reaches 50 %, chitosan is readily dissolved in weak acids. DD is mainly influenced by the concentration of NaOH and temperature used for Deacetylation (Hargono and Djaeni, 2003). DD determines the molecular weight, viscosity, solubility and chemical reactivity. As per the previous studies, the DD may varies from 30% to 95% depends on the species and method of preparation. It is rare to achieve the 100% DD (Islam *et al.,* 2011).

Molecular weight of chitosan

Average molecular weight of the chitosan estimated by using intrinsic viscosity was 110.64 KDa (Table 1). Chitin identified as a higher molecular weight polysaccharides. Generally molecular weight of the chitosan is above one million, while commercial chitosan products have 100 KDa to1200 KDa (Struszczyk *et al.,* 2002). Molecular weight of chitosan determines the functional properties of chitosan. The molecular weight of chitosan influenced by the concentration of NaOH, time temperature and substrate solvent ratio (Rout,2001). Moreover, viscosity of the chitosan is proportional to the molecular weight of chitosan.

Solubility of chitosan

Solubility plays a major role in the determination of chitosan quality. Good quality of chitosan has higher solubility. The prepared chitosan showed 98.4% solubility in 1% acidic acid solution. Solubility of chitosan was influenced by degree of deacetylation because the solubility is related to the elimination of acetyl group from the chitosan. Apart from that several factors such as time and temperature of deacetylation, alkali concentration and ratio of deprotenization, alkali concentration and ratio of chitosan production from chitin and size of the chitosan particle are also influence on the chitosan solubility (Hossain and Iqbal, 2014).

Water Binding Capacity (WBC) and Fat Binding Capacity (FBC) of Chitosan

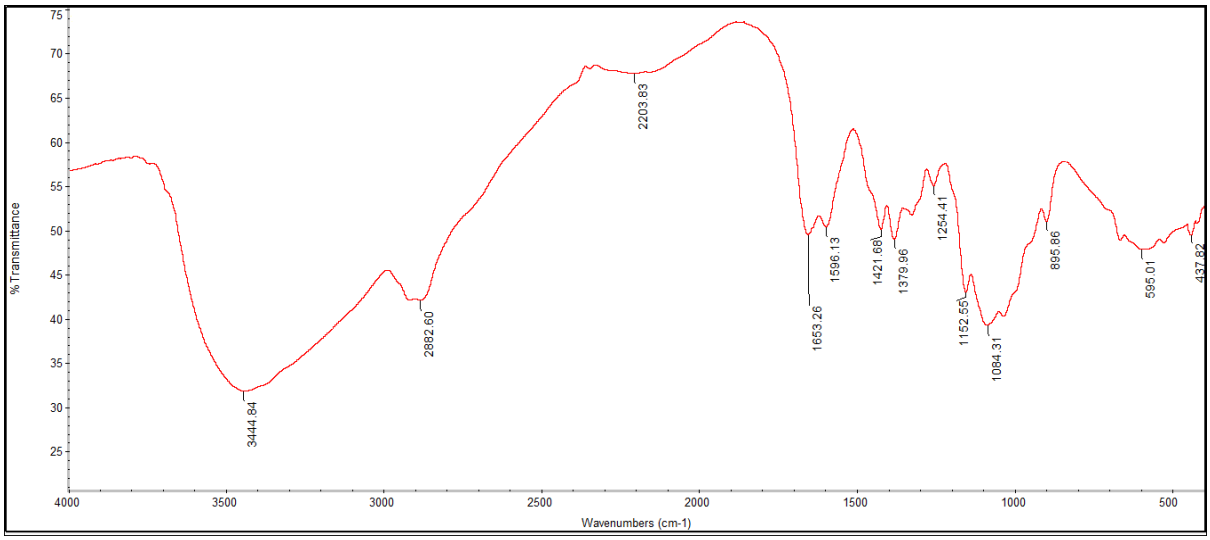
The molecular weight of plays a major role in the functional properties of chitosan. In the present study the fat binding capacity of chitosan 331.28% whereas the water binding capacity was 637.33%. WBC of commercial chitosan ranges between 581 to 1150% and the fat uptake of chitosan ranges from 315 to 170% (Rout, 2001; Knorr, 1982). WBC of chitosan was also influenced by the dissimilarities in crystallinity, availability of salt forming groups, and the residual protein present in the chitosan (Knorr, 1982). Viscosity may influence on the FBC of chitosan. The decreased viscosity of chitosan may be responsible for the lower fat binding capacity (Rout, 2001).

Shrimp shell wastes generated from shrimp processing industries consist of several valuable compound like chitin, chitosan and pigments and are generally discarded as waste into the environmental cause severe environmental problem.

Table.1 Parameters analyzed for chitosan extracted from shrimp shell wastes

Parameters	Chitosan
Yield	4.41±1.22
Moisture	6.24±1.01
Protein	1.99±0.29
Ash	0.39±0.14
pH	7.9±0.11
DD	76.43±0.91
Molecular weight (KDa)	110.64±1.65
Solubility	98.40±0.54
WBC	637.33±2.54
FBC	331.28±3.24

Fig.1 Caption: FTIR spectra of chitosan prepared from shrimp shell waste



By utilizing these wastes for the production of chitin or chitosan pave a new path to better use of shrimp wastes and lead a way to cleaner environment. In this study shrimp shell wastes generated from the processing factories in Veraval region was utilized for the production of chitosan and their characteristics were studied. In summary, the physiochemical, functional and structural characteristic of chitosan properties were accordance with the chitosan studied by several authors. Hence, the chitosan could be a raw material for food, agricultural, medical or other industrial applications.

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