

## Full Length Research Paper

# Chitosan from shrimp shell (*Crangon crangon*) and fish scales (*Labeorohita*): Extraction and characterization

Suneeta Kumari<sup>1</sup>, Rath P.<sup>1</sup> and Sri Hari Kumar A.<sup>2\*</sup><sup>1</sup>Department of Chemical Engineering, NIT, Rourkela-769008, India.<sup>2</sup>Department of Chemical Engineering, Defence Engineering College, Bishoftu, Ethiopia.

Received 30 November, 2015; Accepted 10 May, 2016

Chitosan is a naturally available biopolymer. It has been prepared by alkaline N-deacetylation process of shrimp (*Crangon crangon*) chitin and fish (*Labeorohita*) chitin. The physico-chemical properties such as the degree of deacetylation (DD), solubility, water binding capacity, fat binding capacity and chitosan yield have indicated that shrimp shell and fish scale waste are good sources of chitosan. The deacetylation value of shrimp shell chitosan, fish scales and commercial chitosan was found to be 76, 80 and 84%, respectively. The crystalline index (CrI) of fish and shrimp shell was 84 and 82%. Fat binding capacity of fish chitosan, shrimp chitosan and commercial chitosan was found to be 226, 246 and 446%, respectively. Fourier transforms infrared spectroscopy (FTIR) spectra presented a detailed structure of  $\alpha$ -chitin with O-H, N-H and CO stretching movements. Structural differences between shrimp chitosan and fish chitosan were studied by using FTIR, thermo-gravimetric analysis (TGA), X-ray powder diffraction (XRD) and scanning electron microscopy (SEM). FTIR spectra were used to determine the chitosan degree of deacetylation (DD). Characteristic properties of extracted chitosan were found to depend upon the source of origin and degree of deacetylation.

**Key words:** Chitosan, fish scales, shrimp shell.

## INTRODUCTION

All fish processing industries generate different types of wastes. Fish processing plants produce solid waste such as bones, shells, skin, head and meat. These waste materials generate pollution in coastal areas and contaminate the environment. Fishery wastes tend to get spoiled quickly by enzymatic and bacteriological processes which accumulate flies, rodents and other vermins. The Fish processing industry produces 30-40% of solid waste (Islam et al., 2004). Fishery waste is very

useful and it contains high amount of proteins, fats, minerals, oil and chitin. Chitin and chitosan are polysaccharide polymeric materials; chitin is the second most abundant renewable polysaccharide after cellulose (Salaberria et al., 2014). Shrimp, crab, squid, lobster, insect cuticle, fungi and yeast are the best naturally occurring sources of chitin (Figure 1). Chitin and its derivatives are biomolecules of great importance, having versatile biological actions, and they exhibit

\*Corresponding author. E-mail: [annamareddy360@gmail.com](mailto:annamareddy360@gmail.com). Tel: +91-8978600061.

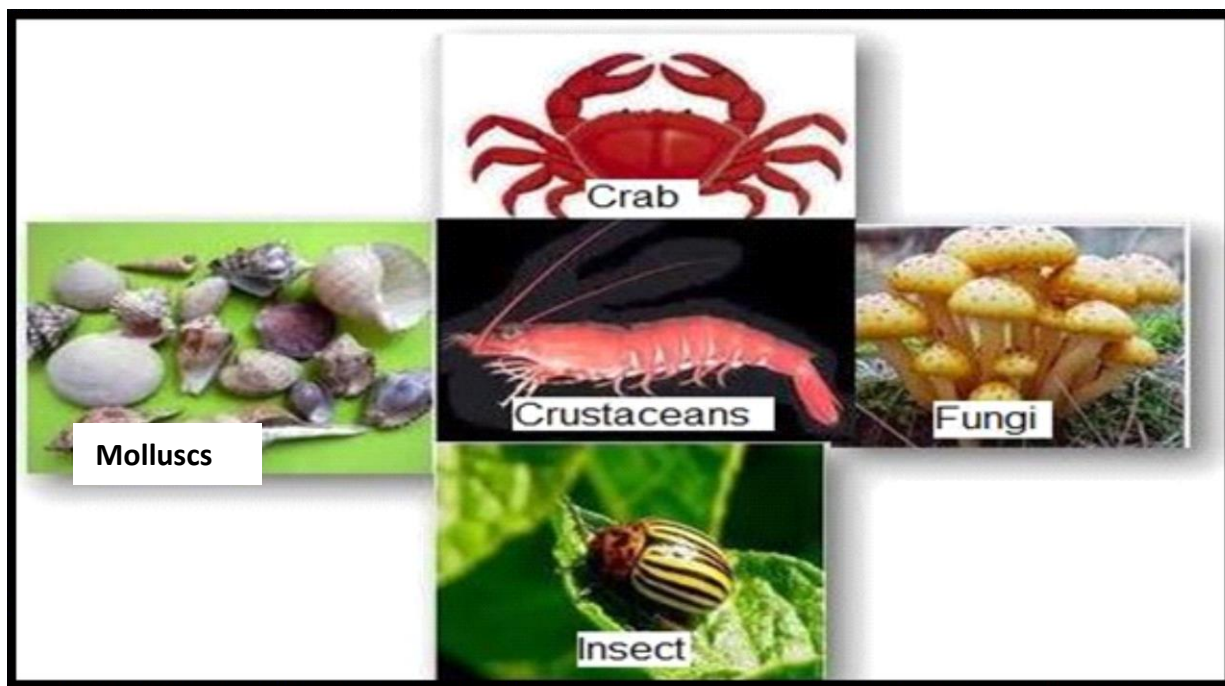


Figure 1. Sources of chitin.

biocompatibility and biodegradability. As a result, chitin and its derivatives are extensively used in pharmaceutical, cosmetics, feed additives, agriculture, semi-permeable membranes, food and textile industries and waste water treatment (Zeng et al., 2012).

Chitosan is prepared by deacetylation of chitin. During this reaction, the acetamide groups ( $-\text{NHCOCH}_3$ ) of chitin are converted into amino groups ( $-\text{NH}_2$ ) leading to chitosan formation. Depending on the source from where it is extracted, chitin can be found in three polymorphic conformations ( $\alpha$ ,  $\beta$  and  $\gamma$ ) as shown in the Figure 2. Chitosan and chitin have become materials of great interest not only as an under-utilized resource but also as a new functional biomaterial of high potential in various fields. Recent progress in chitin chemistry is quite significant. In India chitin, solid waste fraction has ranged from 60,000 to 80,000 tons per year. Chitin and chitosan are now produced commercially in India, Poland, Japan, US, Norway and Australia (Eijsink et al., 2010).

Naturally occurring polysaccharides such as cellulose, pectin, alginic acid and carrageenans are neutral or acidic in nature. Chitin and chitosan are examples of highly basic polysaccharides (des Rieux et al., 2006; Vinsova and Vavrikova, 2008). Chitosan is known for its properties such as being nontoxic, odorless, biocompatible in animal tissues and enzymatically biodegradable. The most important properties of chitin and chitosan include polyoxysalt formation, ability to form films, chelation with metal ions and optical, structural characteristics (Dash et al., 2011). Degree of deacetylation and molecular weight of chitosan have a strong impact on its physical

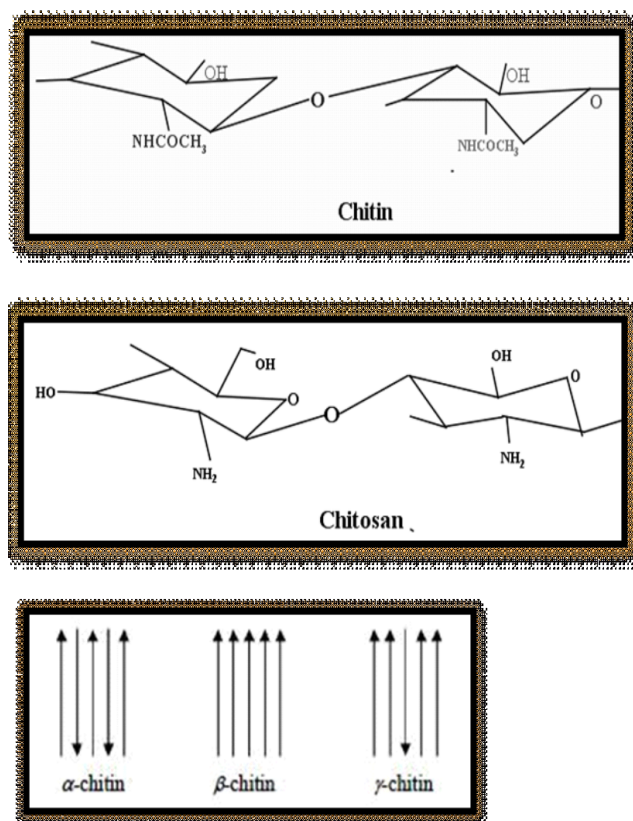


Figure 2. Chemical structure of the polysaccharide, (a) chitin (b) chitosan and (c) the polymer chains in different forms of chitin [3].

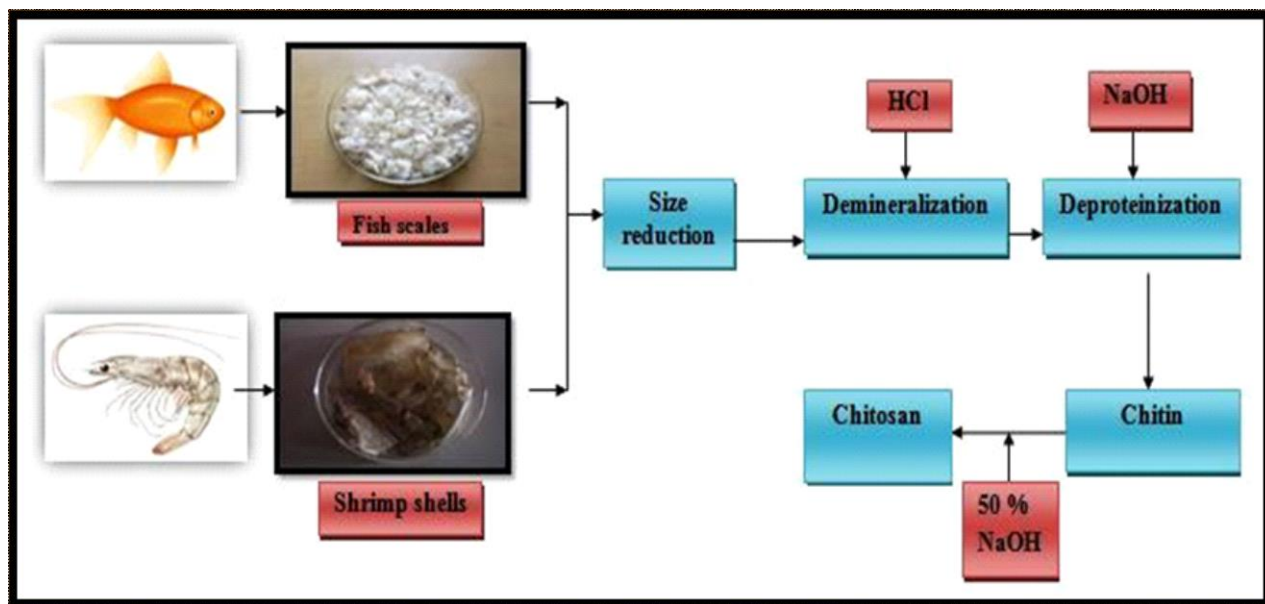


Figure 3. Isolation of chitosan from marine sources.

properties (Kumirska et al., 2010). Chitin is inimitable material for different applications because it has a high percentage of nitrogen (6.89%) as compared to that of synthetically modified cellulose (1.25%) (Hayes, 2012). Chitin has already found applications in various products that have reached the market. The industrial production and usage of chitin have been gradually increasing since the 1970's. The worldwide chitin production is estimated at approximately  $10^{10}$  - $10^{12}$  tons per annum (María and Roque, 2013). The major applications of chitin are focused on water treatment, food processing and metal ion chelation.

Previous studies have reported that molecular weight of chitosan affects its solubility (solubility decreases with increasing molecular weight), tensile strength, bacteriological properties, coagulant-flocculant performance of chitosan and crystallinity (Shukla et al., 2013). Chitosan is insoluble in water, and it is soluble in acidic solutions due to the protonation of its amine groups (Aranaz et al., 2009; Lee et al., 2011). Due to the presence of  $\text{NH}_2$  group, chitosan potentially has high attraction properties to absorb pollutants such as heavy metals and dyes (Peng et al., 2013). Crystallinity and availability of amine groups affect the adsorption capacity of chitosan (Miretzky and Cirelli, 2009).

The objective of the present work is to synthesize chitin from shrimp shell and fish scales using acid and alkaline treatments followed by decolorization with potassium permanganate and to prepare chitosan by further N-deacetylation treatment with concentrated sodium hydroxide solution. The percentage yields, degrees of N-deacetylation (DD) and molecular weights (Mw) have been determined. The physico-chemical properties of

chitosan thus prepared have been determined by using the techniques such as scanning electron microscopy (SEM), fourier transforms infrared spectroscopy (FTIR), X-ray powder diffraction (XRD) and thermo-gravimetric analysis (TGA). Physico-chemical properties of the prepared chitosan have been also determined.

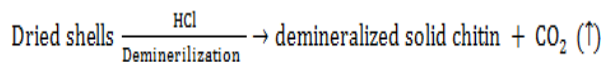
## MATERIALS AND METHODS

### Preparation of raw material

Shrimp shells (*Crangon crangon*) and fish (*Labeorohita*) scales were obtained in fresh conditions from a local fish market and thoroughly washed with tap water, desiccated at room temperature and subjected to size reduction followed by drying at room temperature. Hydrochloric acid (analytical reagents, Rankem), glacial acetic acid 100% (Merck) and sodium hydroxides pellets (Rankem) were purchased from Rankem and Merck chemicals. Commercial chitosan (86% deacetylated) was purchased from India Sea Foods, Kerala in India.

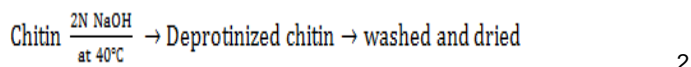
### Demineralization

Shells contain many inorganic components out of which calcium carbonate is the main. Dilute hydrochloric acid was used to remove calcium carbonate and to prevent the hydrolysis of chitin. The hydrochloric acid concentrations ranging from 0.25 to 2 N was used and the reaction was carried out for 36 h at 30°C under constant stirring (150 rpm) (Figure 3). The dry shell to acid ratio was maintained in the range of 1/10 to 1/30 (w/v). The product mass was washed with distilled water to attain neutrality and then oven-dried at 80°C for 12 h.



## Deproteinization

Deproteinization of chitin was carried out using 2 N NaOH (1:10 (W/V) ratio of chitin to NaOH solution) at 40°C. The treatment was repeated several times. The synthesized chitin was filtered and washed to neutrality using distilled water. The solid mass was dried in oven at 110°C.



## Deacetylation

The conversion of chitin to chitosan involves deacetylation and it was carried out using the process suggested by Kurita (2006). The prepared chitin was refluxed in aqueous sodium hydroxide (50% by weight) at 90 to 100°C temperature with constant stirring. After 6 h of reflux, the solid mass was filtered, washed with both water and ethanol (80% v/v) till the filtrate reached neutrality and then the material was oven dried at 80°C for 12 h (Sânia et al., 2012).

## Physico-chemical properties of chitosan

### Viscosity average molar mass of chitosan

The viscosity measurements were done using an Ubbelohde Viscometer and the efflux time of the solution was recorded at constant bath temperature (25 ± 0.1°C). Chitosan samples were dissolved in a solvent system of 0.3 M acetic acid/0.1 M sodium acetate. The intrinsic viscosity ( $\eta$ ) was obtained from linear plots of reduced viscosity ( $\eta_{sp}/C$ ) against concentration (C, g/ml), by extrapolating the plot to zero concentration. The viscosity average molar mass (MW) of chitosan was estimated using the Mark-Houwink relationship (Brugnerotto et al., 2001):

$$[\eta] = K(MW)^a \quad 3$$

Where  $K = 1.81 \times 10^{-3} \text{ cm}^3/\text{g}$  and  $a = 0.93$ . The mean of four replicates was taken from the viscosity measurements.

### Solubility

0.1 g chitosan powder (sample taken in triplicate) was placed in a centrifuge tube of known weight, and then dissolved in 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 240 rpm and 25°C. The mixture in the centrifuge tube was heated for 10 min using boiling water bath and then cooled to 25°C and centrifuged at 5000 rpm for 10 min. The supernatant was discarded, and undissolved particles were washed in distilled water (25 ml) and then undissolved matter was dried at 60°C for 24 h and by weighing the mass of particles, the percentage solubility was determined using the following equation:

$$\text{Solubility (\%)} = \frac{(\text{initial weight of (tube+chitosan)}) - (\text{final weight of (tube+chitosan)})}{(\text{initial weight of (tube+chitosan)}) - (\text{weight of empty tube})} \times 100 \quad 4$$

### Fat binding capacity (FBC)

FBC of chitosan was measured using the method suggested by Wang and Kinsella (1976). 10 ml of soya bean oil was added to a centrifuge tube with 0.5 g of prepared chitosan and thoroughly mixed. The contents were left at ambient temperature for 30 min

with intermittent shaking (5 s) for every 10 min and centrifuged for 25 min (3500 rpm). The supernatant was decanted, and the tube was weighed. FBC was calculated as follows:

$$\text{FBC (\%)} = \frac{(\text{fat bound})\text{g}}{(\text{initial sample weight})\text{g}} \times 100 \quad 5$$

### Water binding capacity (WBC)

WBC of chitosan was measured using a modified method of Wang and Kinsella (1976). 10 ml of water was added to a centrifuge tube with 0.5 g of prepared chitosan and thoroughly mixed. The sample contents were left at ambient temperature for 30 min with intermittent shaking (5 s) for every 10 min and centrifuged at 3,500 rpm for 25 min. The supernatant was decanted, and the tube was weighed.

$$\text{WBC (\%)} = \frac{(\text{water bound})\text{g}}{(\text{initial sample})\text{g}} \times 100 \quad 6$$

## Chitosan characterization

### X-ray powder diffraction (XRD)

The X-ray diffraction (XRD) powder patterns were recorded in transmission geometry with CuK $\alpha$  radiation in the 2 $\theta$  range of 10 to 80° on a Rigaku D max 2000 machine at 40 kV, 30 mA. Crystalline index (CrI) values were calculated by using the formula given below:

$$\text{CrI} = [I_{110} - I_{am}] \times 100 / I_{110} \quad 7$$

$I_{110}$  = The highest intensity at 2 $\theta$  value of 20°;  $I_{am}$  = the amorphous diffraction intensity at 2 $\theta$  value of 13°.

### Thermo-gravimetric analysis (TGA)

During TGA, 1 mg of sample was weighed and a warm-up operation was conducted from 10 to 600°C by ramping temperature input of 10°C per min. For TGA analyses, Shimadzu DTG-60H machine was used.

### Fourier transforms infrared spectroscopy (FTIR)

Infrared spectra were obtained using a Perkin-Elmer type FTIR 1000 spectrometer at room temperature and using KBr pellet scanning method. Pellets were scanned at room temperature (25°C) in the spectral range of 400 – 4000 cm<sup>-1</sup>. FTIR was used to confirm the formation of chitin and chitosan (synthesized from the fish and shrimp shells). The KBr pellets were prepared by thoroughly mixing KBr (200 mg) and sample (4 mg) and made into pellets. The degree of deacetylation (DD) of chitosan was calculated according to the method proposed by Yeul and Rayalu (2013) as follows:

$$\text{DD (\%)} = [A_{1650} - A_{3450}] / 1.33 \times 100 \quad 8$$

### Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDX)

Scanning electron microscopy coupled with energy dispersive X-ray



**Table 1.** Physico-chemical properties of fish chitosan, shrimp chitosan and commercial chitosan

Sample	Solubility (%)	FBC (%)	WBC (%)
Fish chitosan	75	226	492
Shrimp chitosan	70	246	358
Commercial chitosan	90	446	520

spectroscopy is the best known and most widely-used methods of the surface analytical techniques. High resolution images of surface topography, with excellent depth of field, are produced using a highly-focused, scanning (primary) electron beam. The primary electrons enter the surface with an energy of 0.5 to 30 kV (Shimadzu SSX-550 EDX) and generate many low-energy secondary electrons.

## RESULTS AND DISCUSSION

### Solubility

Chitosan is a semi-crystalline biopolymer; because of its rigid crystalline structure, it is not soluble in most of the solvents like water, alkali or aqueous solution (pH  $\approx$ 7) and common organic solvents. At certain pH values under continued stirring, chitosan is soluble in few acids such as hydrochloric, lactic, propionic, phosphoric, tartaric, citric, succinic, acetic and formic acids (Chung et al., 2005; Krajewska, 2004; Qin et al., 2006). Brine and Austin (1981) have stated that lower solubility values suggest incomplete removal of protein. In the present work, it was found that fish chitosan has more solubility as compared to shrimp shell chitosan (Table 1). Solubility values (Equation 4) of fish chitosan, shrimp shells chitosan and commercial chitosan are found to be 75, 70 and 90%, respectively.

### Fat binding capacity (FBC)

Fat binding capacity (FBC) of fish chitosan, shrimp chitosan and commercial chitosan samples were measured using soybean oil. FBC value depends on chitosan produced and its sources. Rout (2001) has observed that the average FBC of commercial crab chitosan and crawfish chitosan for soybean oil was 587% and 706% respectively. The FBC values were calculated by following the procedure explained earlier and it was found that fish chitosan (226%) had lower fat binding capacity as compared to shrimp shell chitosan (246%) and commercial chitosan (446%). Conducting demineralization prior to deproteinization and deacetylation results in increase in FBC than deproteinization followed by demineralization and deacetylation (Moorjani et al., 1975).

### Water binding capacity (WBC)

The WBC is generally involved with the molecular weight,

**Table 2.** Crystalline index (%), (DD %) and molecular weight (MW) of fish chitosan, shrimp chitosan and commercial chitosan.

Sample	Crystallinity index (%)	DD (%)	MW (g/mol)
Fish chitosan	84	80	5200.96
Shrimp chitosan	82	76	1263.11
Commercial chitosan	96	84	17,046.39

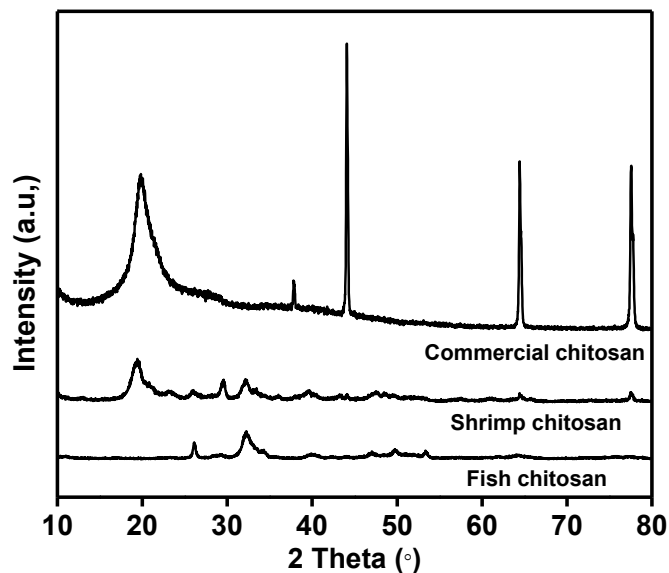
DD and degree of crystallinity of chitosan. The surface area increases due to the decomposition of chitosan; as a result, the area for binding with the  $-OH$  groups,  $-NH_2$  groups and end groups also increases. The increased DD provides more  $-NH_2$  groups to bind water, and the decrease in crystallinity increases the penetration of the water molecules (Rout, 2001). The FBC value of shrimp chitosan (358%) was found to be lower than that of fish scale chitosan (492%) and commercial chitosan (520%).

### Viscosity average molar mass of chitosan

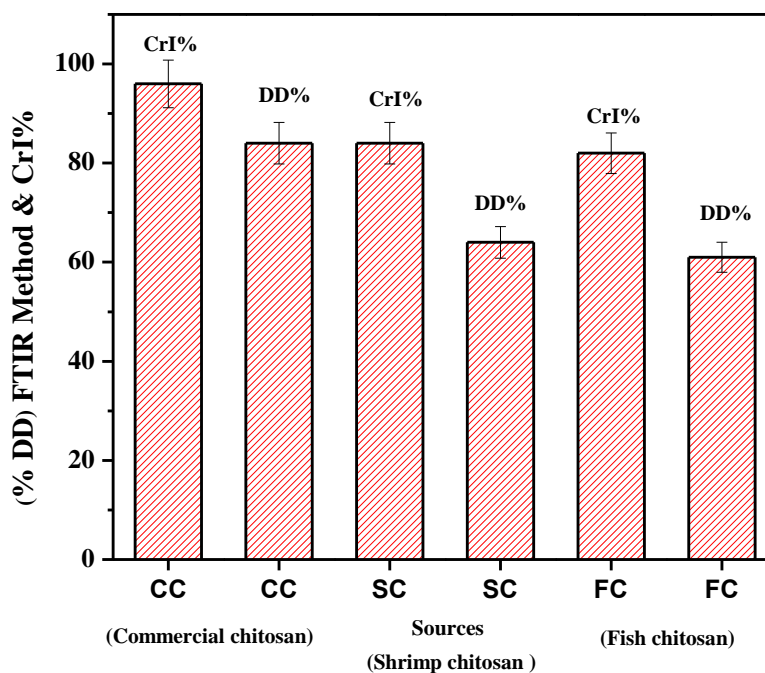
Viscosity average molar mass (Table 2) strongly depends on the sample polydispersity, especially on the constant K. For a given set of constants (K and a) and  $[\eta]^a$  values, it is known that the constant (K) is underestimated when the polydispersity of the sample increases, thus leading to overestimated molar mass. Viscosity average molar mass of chitosan varied with the sources, the extraction method and the residual aggregates in solution. In the present study, fish chitosan showed the higher viscosity average molar mass (5200.96 g/mol) whereas shrimp chitosan showed the lower viscosity average molar mass (1263.11 g/mol). For better comparisons, viscosity average molar mass of commercial chitosan sample, was also determined and it was found that commercial chitosan exhibited significantly high viscosity average molar mass (17,046.39 g/mol) as compared to fish and shrimp chitosan. According to Jia et al. (2001), chitosan viscosity average molecular mass decreases with increase in the hydrolysis time (demineralization, deproteinization and deacetylation). Due to a higher temperature (90-100°C) deacetylation process employed in the present study, viscosity average molar mass of prepared fish and shrimp chitosan is found to be significantly lower than that of commercial chitosan.

### X-Ray powder diffraction (XRD)

X-Ray diffraction is normally used to determine the polymorphic forms of a compound which has different crystalline structures for which distinct powdered X-ray diffraction patterns are obtained. The XRD patterns of chitosan samples extracted from two sources (shrimp shell and fish scales) exhibited strong reflections at 2 $\theta$



**Figure 4.** X-ray diffraction patterns of commercial chitosan, fish chitosan and shrimp chitosan.

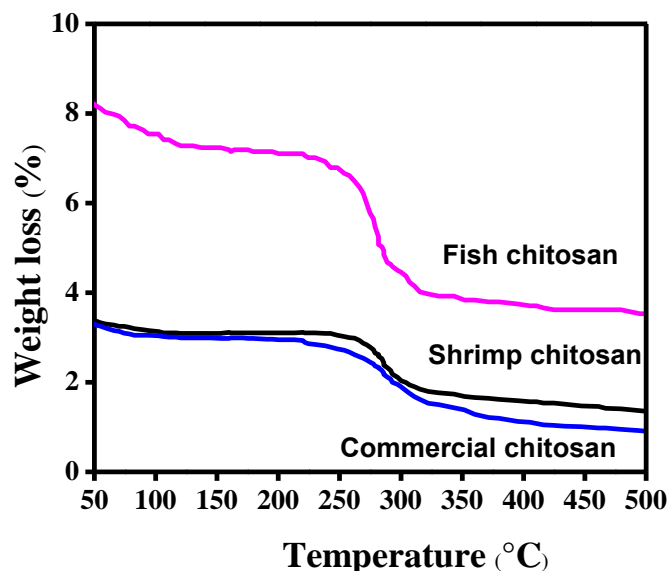


**Figure 5.** CrI% and DD%. The values represent means  $\pm$  SD  $n=3$ .

values around 19-25 and 29-32°, respectively (Figure 4). The XRD analysis of chitosan from shrimp shells displayed different characteristic peaks at  $2\theta$  values of 19.50, 29.52 and 32.21°, whereas chitosan from fish scales exhibited peaks at 25.79 and 32.02°. XRD pattern of commercial chitosan sample has also been recorded and presented for better comparison, where the

characteristic peaks were observed at  $2\theta$  values of 19.74, 37.82, 44.06, 64.43 and 77.92 (Kaya et al., 2014).

The crystallinity (Figure 5) was calculated on the basis of X-ray diffractogram. The two sharp peaks were observed for chitosan synthesized from fish scales chitosan at 25.0 and 32.9° whereas for chitosan synthesized from shrimp shell intense peaks were



**Figure 6.** TGA thermograms of fish chitosan, shrimp chitosan and commercial chitosan.

observed at 10.57 and 20.72°. The intense peak at 32.9° for fish chitosan sample indicates the presence of hydroxyapatite mineral content. A similar observation was made by Allison et al. (2014). Two peaks of chitosan (extracted from shrimp shell) at 10.57 and 20.72° are in good match with the chitosan XRD patterns (chitosan isolated from organisms such as shrimp, crab and insects) reported by Yen et al. (2009) and Krajewska (2004).

Zhang et al. (2005) have proposed a method to determine CrI % using XRD peak intensity (Equation 7). CrI value of the shrimp shell chitosan was calculated and it was found to be 82%, whereas the CrI values of chitosan extracted from fish scale and commercial chitosan sample were found to be 84 and 96% respectively (Table 2). Kaya et al. (2014) and Shaofang et al. (2012) have estimated the CrI values of chitosan isolated from organisms such as crab and insects and the CrI values were reported to be in the range 54 and 91%. Zhang et al. (2005) observed the linear relationship between  $CrI_{020}$  and DD, and suggested a possibility for XRD to determine DD of macromolecular chitin.

### Thermo-gravimetric analysis (TGA)

The thermogravimetric curves were obtained at a heating rate of 10°C min<sup>-1</sup> under a dynamic atmosphere of nitrogen in the temperature range of 10 – 600°C. The profiles of the thermal decomposition of chitosan samples are depicted in Figure 6. It is observed from the thermograms that fish chitosan has stage wise weight loss in the range of 50-150 and 250 – 300°C, whereas in the case of shrimp shell chitosan and commercial sample,

decomposition occurred in single stage (250 - 300°C). The initial weight loss in the range of 50-150°C corresponds to the removal of moisture content. It was observed from a TGA curve in the figure and the decomposition stage of chitosan occurred between temperatures of 250 – 300°C, which suggests that chitosan had a lower thermal stability (Sânia et al., 2012).

### Fourier transforms infrared spectroscopy (FTIR)

FTIR analysis of shrimp chitin and fish chitin are depicted in the Figure 7. Formation of two separate bands in the region of 1662 - 1630 cm<sup>-1</sup> (shown in inset for better visibility) confirms the presence of  $\alpha$  chitin in both shrimp and fish chitin. The two separate bands exhibited by  $\alpha$  chitin in the range of 1662 - 1630 cm<sup>-1</sup> correspond to the occurrence of the intermolecular hydrogen bond CO•••HN and the intra molecular hydrogen bond CO•••HOCH<sub>2</sub>, respectively (Focher et al., 1992). In the case of  $\beta$  chitin, only a single peak at 1659 cm<sup>-1</sup> could be observed, which corresponds to the stretching of CO group hydrogen bonded to amide group of the neighboring intra-sheet chain (Hajji et al., 2014).

The vibrational modes involved in intermolecular hydrogen bonding CO•••HN and the intramolecular bonds of NH groups exhibit characteristics bands at 3264 and 3110 cm<sup>-1</sup>, respectively. These bands can be seen clearly in the  $\alpha$ -chitin spectra, whereas these bands are not usually observed in  $\beta$  chitin. Sagheer et al. (2009) have observed the presence of a specific band at 1429 cm<sup>-1</sup> in the case of  $\alpha$  chitin and a strong well-defined band at 1436 cm<sup>-1</sup> (CH<sub>2</sub>) could be seen in  $\beta$  chitin. The nonexistence of bands at 1436 and 1659 cm<sup>-1</sup> conforms the absence of  $\beta$  chitin phase in both shrimp and fish chitin samples.

The FTIR spectra of chitosan samples are shown in the Figure 8. The peak noticed at 1555 cm<sup>-1</sup> corresponds to N–H bending of the secondary amide II band of –CONH– whereas, the amide I band is generally observed at 1655 cm<sup>-1</sup>. In the present case, the amide I band is not found. Further bands that are observed in the region of 1380–1460 cm<sup>-1</sup> are attributed to the symmetric and asymmetric bending vibrations of the methyl groups. Li et al. (1998) also found that the peak at 1415 cm<sup>-1</sup> indicates the C–H bending vibrations of –CH<sub>2</sub>.

A small peak around 2900 cm<sup>-1</sup> relates to –CH<sub>2</sub>–, –CH<sub>3</sub> functional groups. The C–O stretching vibrations of the structure are observed at 1075 cm<sup>-1</sup>. The peak near 3300 cm<sup>-1</sup> is usually attributed to intermolecular –H bands. Choi et al. (2007) have observed similar characteristic peaks for chitosan at 2940 (–CH<sub>3</sub>, –CH<sub>2</sub>), 1655 (C=O stretch vibration of secondary amide I band), 1555 (N–H bending vibration of amide II band), 1570 (N–H bending vibration of primary amides) and 1070 cm<sup>-1</sup> (C–O stretching). Strong amide II bands are not present in this case, due to the high degree of deacetylation of the produced chitosan. The present results are completely in line with

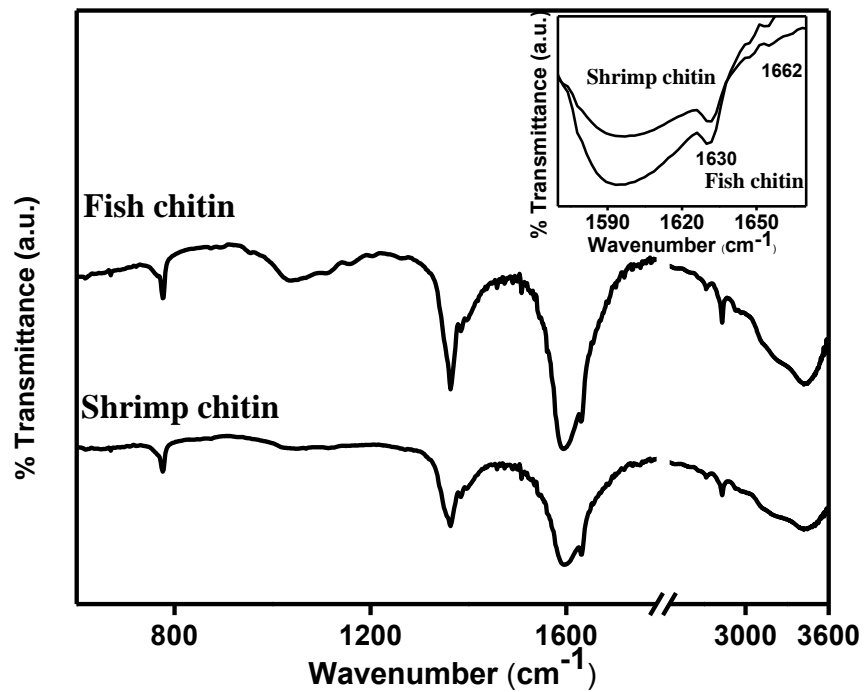


Figure 7. FTIR spectra of shrimp chitin and fish chitin.

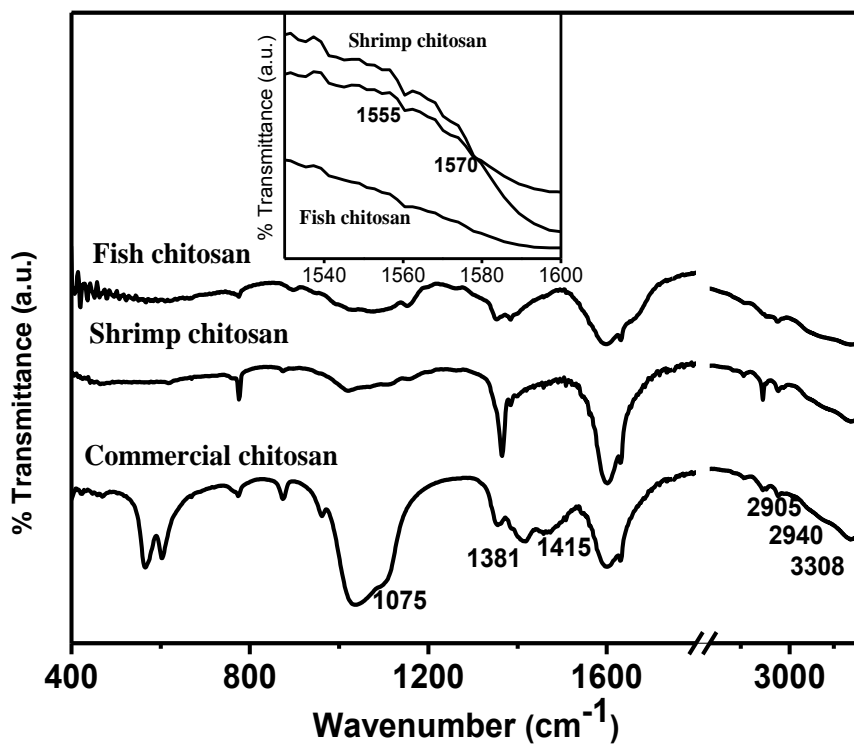
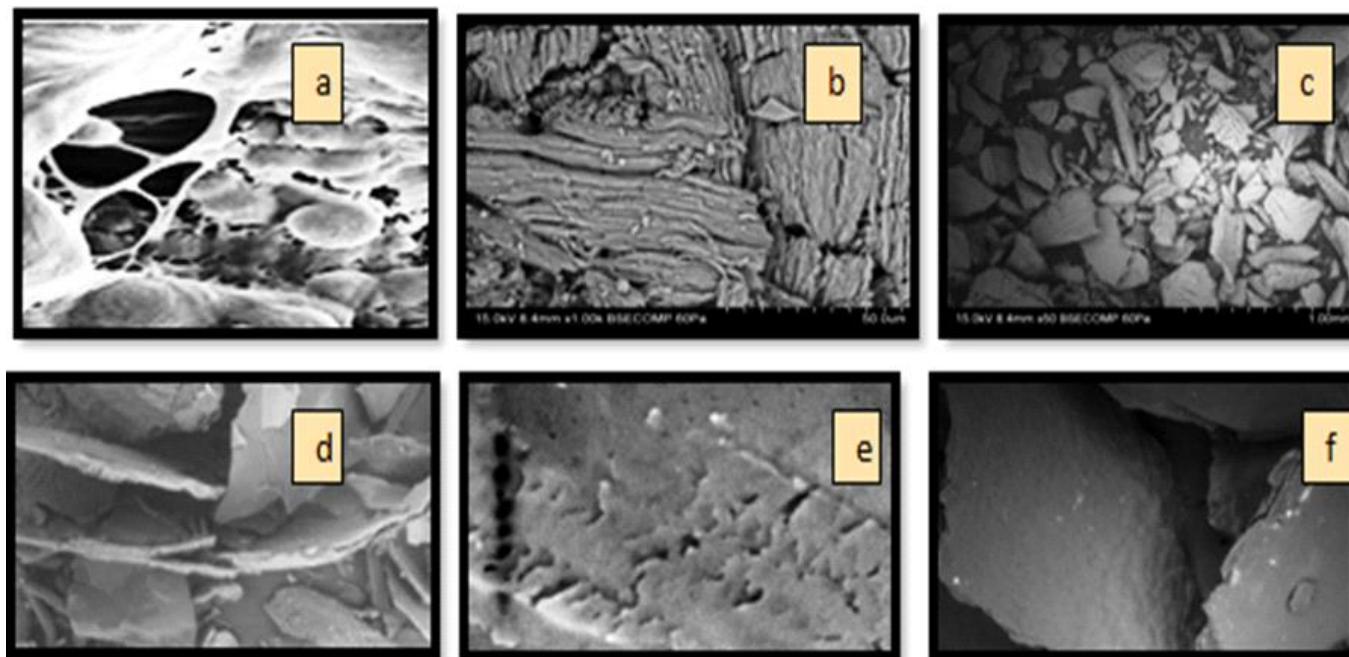


Figure 8. FTIR spectra of fish chitosan, shrimp chitosan and commercial chitosan.

reported literature and from FTIR patterns the formation of chitosan can be confirmed.





**Figure 9.** SEM image of (a) raw fish scales (b) fish chitin (c) fish chitosan (d) raw shrimp shells (e) shrimp chitin and (f) shrimp chitosan.

#### Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM/EDX)

The chitin and chitosan produced from fish scales and shrimp shell were selected for examination by SEM (Figure 9). SEM images of raw fish scales and shrimp shell showed fibrillary structure (Figure 9a and d) (Ikoma et al., 2003). The chitin demonstrated a noticeable organized microfibrillar crystalline structure in FESEM (Figure 9b and e) which was truant in the chitosan (Figure 9c and f), similar observation was reported by Yen et al. (2009), Arbia et al. (2013) and Muzzarelli et al. (2014). The extracted shrimp shell chitosan was observed to have layers of flakes, and porous nature could be seen in some areas. In some parts of chitosan, fibril structures can easily be distinguished. With the increased magnification, crumbling flakes were observed with fibril structures in some portions of chitosan, as in the study of Yen et al. (2009).

EDX analysis for fish chitosan and shrimp chitosan has been carried out and depicted in Figures 10a and b, respectively. The EDX results confirmed that the fish chitosan (Figure 10a) has small amount of Ca, whereas similar mineral contents could not be seen in the case of shrimp chitosan (Figure 10b). XRD patterns have also suggested the presence of hydroxyapatite mineral in the fish chitosan sample. However, shrimp chitosan is free of mineral content. Recent studies by Li et al. (2011) and Guan et al. (2015) suggested that purity of extracted chitosan depends on its original source, treatment method

(demineralization and deproteinization) and treatment time.

#### Conclusion

Chitin has been extracted from local sources, fish scales and shrimp shells. Chitin preparation involves demineralization, followed by deproteinization and deacetylation. Prepared chitosan was used to investigate the physicochemical properties such as viscosity average molar mass, solubility, fat binding capacity and water binding capacity. The physicochemical properties of prepared chitosan from fish scales (water-binding capacity (492%) and fat-binding capacity (226%)) and shrimp chitosan (water-binding capacity (358%) and fat binding capacity (246%)) are in total concurrence with commercially available chitosan. Solubility of chitosan from fish and shrimp shell was 75 and 70%, respectively. Using FTIR method, DD % of fish chitosan, shrimp chitosan and commercial chitosan was estimated and DD % was found to be 80, 76 and 84%, respectively. The XRD analysis has shown that commercial chitosan was more crystalline as compared to that of shrimp and fish scales. XRD analysis has also indicated that fish chitosan was more crystalline than shrimp chitosan. The CrI % of fish and shrimp scales was found to be 84 and 82%, respectively and commercial chitosan exhibited 96%. The results suggest that shrimp waste and fishery waste are most remarkable and good sources of chitosan.

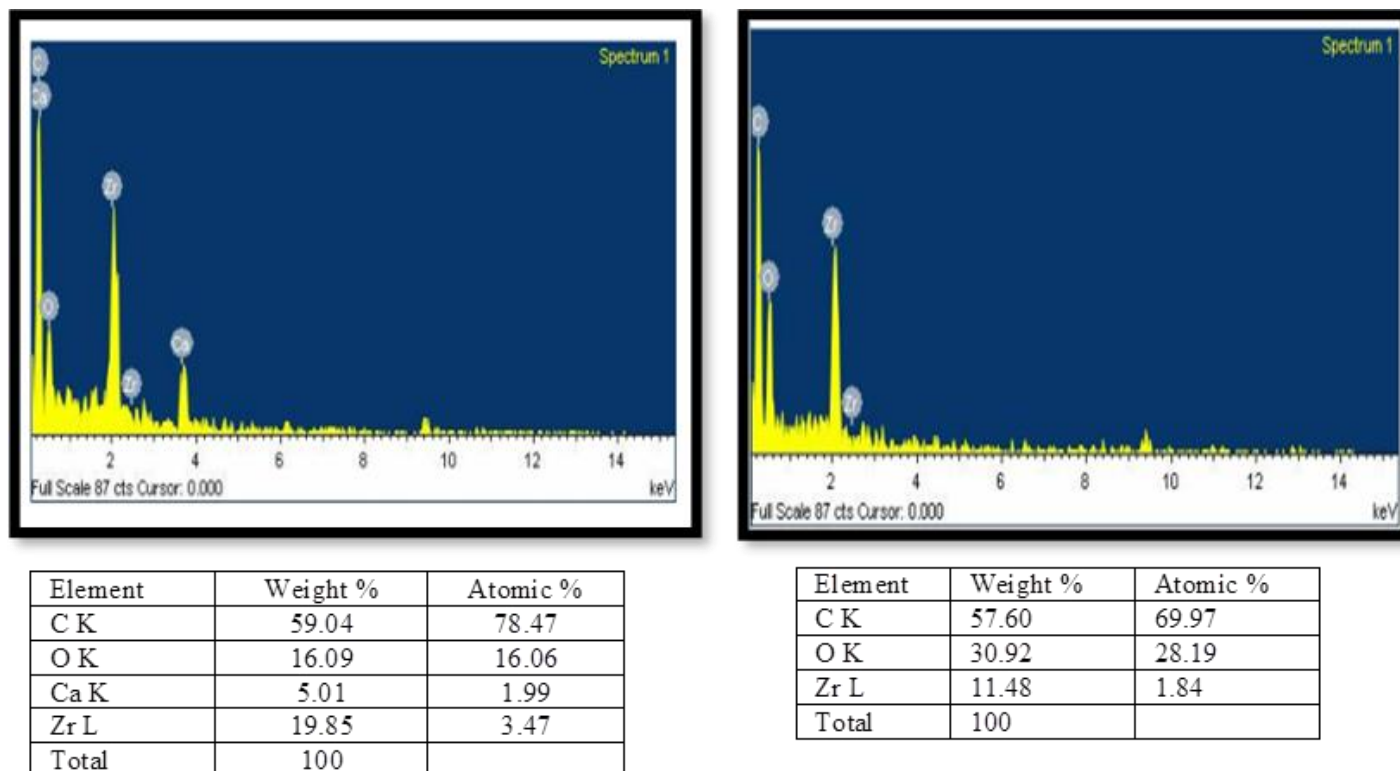


Figure 10. EDX spectra of shrimp chitosan and fish chitosan.

### Conflict of Interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENT

The authors are thankful to Prof. Sunil Kumar Sarangi (Director), National Institute of Technology, Rourkela, India, for providing the facilities to conduct the present research work.

### REFERENCES

- Allison PG, Rodriguez RI, Moser RD, Williams BA, Poda AR, Seiter JM, Lafferty BJ, Kennedy AJ, Chandler MQ (2014). Characterization of multi-layered fish scales (*Atractosteus spatula*) using nanoindentation, X-ray CT, FTIR, and SEM. *J. Vis. Exp.* 89:1-9.
- Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, Galed G, Heras A (2009). Functional Characterization of Chitin and Chitosan. *Curr. Chem. Biol.* 3(2):203-230.
- Arbia W, Adour L, Amrane A, Lounici H (2013). Optimization of medium composition for enhanced chitin extraction from *Parapenaeus longirostris* by *Lactobacillus helveticus* using response surface methodology. *Food Hydrocoll.* 31(2):392-403.
- Brine CJ, Austin PR (1981). Chitin variability with species and method of preparation. *Comp. Biochem. Physiol.* 69(2):283-286.
- Brugnerotto J, Desbrières J, Roberts G, Rinaudo M (2001). Characterization of chitosan by steric exclusion chromatography. *Polymer* 42(25):9921-9927.
- Choi CY, Kim SB, Pak PK, Yoo DI, Chung YS (2007). Effect of N-acetylation on structure and properties of chitosan fibers. *Carbohydr. Polym.* 68(1):122-127.
- Chung Y-C, Kuo C-L, Chen C-C (2005). Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction. *Bioresour. Technol.* 96(13):1473-1482.
- Dash M, Chiellini F, Ottenbrite R M, Chiellini E (2011). Chitosan—A versatile semi-synthetic polymer in biomedical applications. *Prog. Polym. Sci* 36(8):981-1014.
- des Rieux A, Fievez V, Garinot M, Schneider Y-J, Pr at V (2006). Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. *J. Control. Release* 116(1):1-27.
- Eijsink V, Hoell I, Vaaje-Kolstada G (2010). Structure and function of enzymes acting on chitin and chitosan. *Biotechnol. Genet. Eng. Rev.* 27:331-366.
- Focher B, Naggi A, Torri G, Cosani A, Terbojevich M (1992). Chitosans from *Euphausia superba*. 2: Characterization of solid state structure. *Carbohydr. Polym.* 18(1):43-49.
- Guan J-J, Tian B, Tang S, Ke Q-F, Zhang C-Q, Zhu Z-A, Guo Y-P (2015). Hydroxyapatite coatings with oriented nanoplate arrays: synthesis, formation mechanism and cytocompatibility. *J. Mater. Chem. B* 3(8):1655-1666.
- Hajji S, Younes I, Ghorbel-Bellaaj O, Hajji R, Rinaudo M, Nasri M, Jellouli K (2014). Structural differences between chitin and chitosan extracted from three different marine sources. *Int. J. Biol. Macromol.* 65:298-306.
- Hayes M (2012). Chitin, chitosan and their derivatives from marine rest raw materials: Potential food and pharmaceutical applications in: Hayes M (Ed.), *Marine Bioactive Compounds*. Springer US. pp. 115-128.
- Ikoma T, Kobayashi H, Tanaka J, Walsh D, Mann S (2003). Microstructure, mechanical, and biomimetic properties of fish scales from *Pagrus major*. *J. Struct. Biol.* 142(3):327-333.
- Islam MS, Khan S, Tanaka M (2004). Waste loading in shrimp and fish

- processing effluents: potential source of hazards to the coastal and nearshore environments. *Mar. Pollut. Bull.* 49(1-2):103-110.
- Jia Z, Shen D, Xu W (2001). Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydr. Res.* 333(1):1-6.
- Kaya M, Seyyar O, Baran T, Turkes T (2014). Bat guano as new and attractive chitin and chitosan source. *Front. Zool.* 11(1):1-10.
- Krajewska B (2004). Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. *Enzyme Microb. Technol.* 35(2-3):126-139.
- Kumirska J, Czerwicka M, Kaczyński Z, Bychowska A, Brzozowski K, Thöming J, Stepnowski P (2010). Application of Spectroscopic Methods for Structural Analysis of Chitin and Chitosan. *Mar. Drugs* 8(5):1567-1636.
- Kurita K (2006). Chitin and Chitosan: Functional Biopolymers from Marine Crustaceans. *Mar. Biotechnol.* 8(3):203-226.
- Lee H-S, Eckmann DM, Lee D, Hickok NJ, Composto RJ (2011). Symmetric pH-Dependent Swelling and Antibacterial Properties of Chitosan Brushes. *Langmuir* 27(20):12458-12465.
- Li LH, Zhao MY, Ding S, Zhou CR (2011). Rapid biomimetic mineralization of chitosan scaffolds with a precursor sacrificed method in ethanol/water mixed solution. *Express Polym. Lett.* 5(6):545-554.
- Li Q, Weng S, Wu J, Zhou N (1998). Comparative study on structure of solubilized water in reversed micelles. 1. FT-IR spectroscopic evidence of Water/AOT/n-Heptane and Water/NaDEHP/n-Heptane systems. *J. Phys. Chem. B* 102(17):3168-3174.
- María CG, Roque AH (2013). Biotechnological processes for chitin recovery out of crustacean waste: A mini-review. *Electron. J. Biotechnol.* 16(3):1-14.
- Miretzky P, Cirelli A F (2009). Hg(II) removal from water by chitosan and chitosan derivatives: a review. *J. Hazard. Mater.* 167(1-3):10-23.
- Moorjani M N, Achyuta V, Khasim T (1975). Parameters affecting the viscosity of chitosan from prawn waste. *J. Food. Sci. Technol.* 12:187-189.
- Muzzarelli R, Mehtedi M, Mattioli-Belmonte M (2014). Emerging Biomedical Applications of Nano-Chitins and Nano-Chitosans Obtained via Advanced Eco-Friendly Technologies from Marine Resources. *Mar. Drugs* 12(11):5468-5502.
- Peng Y, Chen D, Ji J, Kong Y, Wan H, Yao C (2013). Chitosan-modified palygorskite: Preparation, characterization and reactive dye removal. *Appl. Clay Sci.* 74:81-86.
- Qin C, Li H, Xiao Q, Liu Y, Zhu J, Du Y (2006). Water-solubility of chitosan and its antimicrobial activity. *Carbohydr. Polym.* 63(3):367-374.
- Rout SK (2001). Physicochemical, functional, and spectroscopic analysis of crawfish chitin and chitosan as affected by process modification. Louisiana State University: Baton Rouge, LA, USA.
- Sagheer FAA, Al-Sughayer MA, Muslim S, Elsabee MZ (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydr. Polym.* 77(2):410-419.
- Salaberria AM, Labidi J, Fernandes SCM (2014). Chitin nanocrystals and nanofibers as nano-sized fillers into thermoplastic starch-based biocomposites processed by melt-mixing. *Chem. Eng. J.* 256:356-364.
- Sânia MBdA, Rasiah L, Brismak G d R, Débora D B, Alcione O G (2012). The Use of Exoskeletons of Shrimp (*Litopenaeus vanammei*) and Crab (*Ucides cordatus*) for the Extraction of Chitosan and Production of Nanomembrane. *Mater. Sci. Appl.* 3(7):495-508.
- Shaofang L, Jie S, Lina Y, Chushu Z, Jie B, Feng Z, Mingjing Q, Chen J, Qingli Y (2012). Extraction and Characterization of Chitin from the Beetle *Holotrichia parallela* Motschulsky. *Molecules* 17(4):4604-4611.
- Shukla SK, Mishra AK, Arotiba OA, Mamba BB (2013). Chitosan-based nanomaterials: A state-of-the-art review. *Int. J. Biol. Macromol.* 59:46-58.
- Vinsova J, Vavrikova E (2008). Recent Advances in Drugs and Prodrugs Design of Chitosan. *Curr. Pharm. Des.* 14(13):1311-1326.
- Wang JC, Kinsella J E (1976). Functional properties of novel proteins: Alfalfa leaf protein. *J. Food Sci.* 41(2):286-292.
- Yen M-T, Yang J-H, Mau J-L (2009). Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydr. Polym.* 75(1):15-21.
- Yeul V, Rayalu S (2013). Unprecedented Chitin and Chitosan: A Chemical Overview. *J. Polym. Environ.* 21(2):606-614.
- Zeng J-B, He Y-S, Li S-L, Wang Y-Z (2012). Chitin Whiskers: An Overview. *Biomacromolecules* 13(1):1-11.
- Zhang Y, Xue C, Xue Y, Gao R, Zhang X (2005). Determination of the degree of deacetylation of chitin and chitosan by X-ray powder diffraction. *Carbohydr. Res.* 340(11):1914-1917.