# Extraction of Chitosan from Prawn Shell Wastes and Examination of its Viable Commercial Applications

**Abhrajyoti Tarafdar & Gargi Biswas**

National Institute of Technology, Durgapur
E-mail : abhra@outlook.com, sunshine.gargi@gmail.com

***Abstract* – Chitin is the second most abundant natural polysaccharide after cellulose and is present in the crustacean exoskeleton, insects and fungi. The shell fish industry generates a huge amount of shell waste per processing which usually cause environmental nuisance. Alternatively this waste can be utilized as an economic source of chitin and its derivative chitosan. Chitin and chitosan are considerably versatile and promising biomaterials. Chitosan, the deacetylated chitin derivative, is a more useful and interesting bioactive polymer. Despite its biodegradability, it can be chemically modified to produce derivatives which have varied applications in biomedical field. These derivatives are easy to produce and can be made commercially available easily. This study aims at the procedures of chitosan extraction from prawn shells and the use of this chitosan in successfully carrying out various applications in laboratory.**

***Keywords – Chitosan, Chitin, Environment, Health.***

## I. INTRODUCTION

Environmental pollution is a natural consequence of human activities. It is also the result of natural processes. Volcanoes erupt and discharge gases; rains erode and transport silts and dissolved compounds; winds move dirt particles, salt particles and a wide variety of other gaseous and solid materials. In the course of human development, industrialization has made possible higher standards of living in our modern society. Such "progress" has created increased problems with wastes from processing operations and their ultimate disposal – creating water pollution, air pollution and land pollution [1].

The nature of wastes is wide and varied, being broadly classified into: agricultural, municipal and industrial. The latter source tends to produce waste of the most polluting types – the majority being chemicals (37.6% of the total) and metals (29.1%). The remainder of the industrial wastes includes such diverse materials as paper (4.6%), petroleum (2.4%), stone, rubber, leather and textiles. Of this wide range of industrial waste, some 3% is generated by the food processing industries [2]. Such waste is, however, amenable to forms of treatment because of its organic nature. Organic food waste contains proteins, vitamins, carbohydrates and fat and such components are highly polluting due to what is termed their high BOD (Biochemical Oxygen Demand). They are also, however, extremely valuable components which can be recovered.

The shell fish industry is operative among all the costal countries and contributes hugely to the food delicacies. During the processing of prawns, shrimps and lobsters mostly the meat is taken, while the shell and head portions are generated as wastes. This results in the generation of a huge amount of waste throughout the world. It is estimated that the shell-fish industry produces about 60,000-80,000 tons of waste. The disposal of such an enormous amount of waste has become a serious environmental concern [3]. Although these wastes are biodegradable but the rate of degradation of a large amount of waste generated per processing operation is comparatively slow [4]. This results in accumulation over time and the ads to environmental concerns as they not only produce obnoxious smell but also attract pathogenic insects, flies and rodents, thus creating an unhygienic atmosphere.

The immediate solution to this problem seems to be quick recycling of the crustacean shells generated and extraction of commercially viable substances to be further used in other applications [5]. As we know the shell and head wastes of crustaceans contain chitin, proteins and minerals. So by demineralising and deproteinizing the wastes chitin can be obtained [6]. Chitin can be used for various economical applications. Moreover the chitin can be further deacetylated to

produce chitosan, a valuable chemical substance having a wide range of viable uses [7]. Various derivatives of chitin and chitosan can also be manufactured which diversify the fields of application of these two chemicals [8].

## II. MATERIALS AND METHODS

### *A. Extraction of Chitosan from Prawn Shells:*

Process I:

- Materials:

*Penaeus monodon*, commonly known as ―Giant Tiger Prawn, generally has a light pink hue and *Penaeus indica* or Indian prawn was obtained from the local market at Bidhannagar, Durgapur.

*P. monodon* and *P. indica* inedible parts including head, body shells and tails were removed from the whole body for extraction of chitosan.

The chemicals used in the extraction process consists of-

*1. Hydrochloric acid (1.25N to 1.5N):* for 100ml stock solution, 10.8ml HCl is measured and the volume made up to 100 ml with distilled water. 2. Sodium Hydroxide (0.5% w/v): 0.5 gm NaOH per 100 ml distilled water. 3. Sodium Hydroxide (3% w/v): 3gm NaOH per 100 ml distilled water. 4. Sodium Hydroxide (42% w/v): 42gm NaOH per 100 ml distilled water. 5. 1% Acetic Acid: 1ml acetic acid per 100 ml distilled water.

- Method (Process I):

To extract Chitosan, 10 grams of prawn shell waste as raw material was collected. After washing it properly, the prawn shells were under sunlight. Then we proceeded with the demineralization process by adding 1.5N HCl at room temperature for 1hour. The spent acid was discarded and the shells were repeatedly washed with distilled water until the pH is neutral. The de-mineralized shells were then de-proteinized with 0.5% NaOH at 100°C for 30 minutes. This method helped to weaken the protein tertiary structure of the shells. Protein solution was removed and washed thoroughly with distilled water and the pH was checked. The de-proteinization process was again repeated for the removal of the remaining protein from the shells, for that 3% NaOH was added to the sample at 100°C for 30 minutes. After draining the residual proteins along with the effluents, the sample once again washed and the pH was observed till it was approximately near to neutral. This step also helped in decolourization of the shells. Hence the chitin slurry was obtained. The excess water was removed and chitin cake was formed. The Chitosan was prepared by deacetylation of chitin by treating with 42% aqueous NaOH at 95°C for 1.5 hour [9]. After deacetylation the alkali was drained off and washed thoroughly with distilled water until the pH is less than 7.5 and then dried at ambient temperature (30 ± 2°C).

Process II:

- Materials:

Raw material used was *Penaeus monodon*, commonly known as Giant Tiger Prawn and *Penaeus indica* or Indian prawn, obtained from the local market at Bidhannagar, Durgapur. The inedible parts including head, shells and tails were removed from the whole body for extraction of chitosan.

The chemicals used in this extraction process consists of

1. 4% Sodium Hydroxide (w/v – 1:4.5)
2. 4% Hydrochloric Acid (v/v – 1:4.5)
3. 50% Sodium Hydroxide (w/v- 1:20)
4. 1% (v/v) Acetic Acid

- Method (Process II):

Chitin and chitosan were prepared from prawn shell waste. Biomass of shrimp waste collected was 5grams.The shell waste was washed with tap water and dried for further use. It was then de-proteinised in 4% aqueous sodium hydroxide (1:4.5; w/v) at room temperature (25°C) for 21 hours. After draining the alkali, for the removal of residual protein from the shell, it was washed with distilled water repeatedly unless the pH drops to neutral. The de-proteinised shell was de-mineralized by 4% HCl (1:4.5; v/v) at room temperature for 12 hours. The acid was drained off and washed thoroughly with distilled water. The chitin was dried at ambient temperature (30± 2°C). The Chitosan was prepared by deacetylation of chitin by treating with 50% aqueous sodium hydroxide (1:20; w/ v) at 40°C for 3 days. After deacetylation, the alkali was drained off and washed with distilled water thoroughly until the pH is less than 7.5. Finally, the chitosan was dried at ambient temperature (30 ± 2°C) [10][11].

### *B. Verification of the Chitosan produced:*

1. Quality of the chitosan produced was checked by a solubility test with 1% Acetic Acid. Chitosan dissolves completely in 1% Acetic Acid. For the estimation of chitosan produced we took the sample out of the storage and weighed few flakes of prawn shells. Then the sample was put inside a clean beaker and 10 to 20 ml of 1% acetic acid was added to it. The solution was kept in BOD shaker for 30 to 40 minutes. Then the sample was taken out and weighed, carefully.

2. It is known that highly benzoylated chitin is soluble in (DMSO) Dimethyl Sulphoxide. Chitosan being

organic in nature should be completely soluble in DMSO. We also analyzed the solubility of chitosan in DMSO as a quality check parameter. We observed that 15mg of grinded shells were completely dissolved in 30 ml of DMSO solution.

3. The quality check parameters however confirmed that the chitosan obtained from deacetylation of P. monodon shells was of superior activity and quality than that obtained from *P. indica* shells and hence was used in the corresponding experiments devised [12].

## III. VARIOUS APPLICATIONS OF THE CHITOSAN EXTRACTED:

### 1. ANTIBACTERIAL ACTIVITY

- Materials:

The natural antibacterial characteristics of chitosan and its derivatives have resulted in their use in commercial disinfectants. According to the literature, chitosan possesses antimicrobial activity against a number of gram negative and gram positive bacteria. Chitin extracted from different prawn species exhibited important antibacterial activity against *E. coli, B. Subtilis, B. cerevisea*. The antibacterial activity of chitosan is influenced by its molecular weight, degree of deacetylation, concentration in solution, and pH of the medium. The protection of the host against bacterial infection is stimulated by chitosan. The mechanism underlying the inhibition of bacterial growth is thought to be that cationically charged amino group may combine with anionic components such as N-acetylmuramic acid, sialic acid and neuraminic acid on the cell surface, and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition meal ions and inhibiting enzymes. Due to the positive charge on the C-2 of the glucosamine monomer below pH 6, chitosan is more soluble and shows better antibacterial activity [13]

1. Compounds tested: chitosan extracted employing both the extraction processes viz. chitosan-I and chitosan-II were tested for the presence of anti-bacterial activity.

2. Media used: In this application also agar media was used. Composition: peptone -1gm, sodium chloride (NaCl)- 0.5gm, Beef extract- 1gm, agar- 2gm. For this particular media the pH should be neutral i.e. 7- 7.5 for 100ml of solution.

- Method:

The method used for this particular experiment was the Agar Cup Diffusion method. In this method, first the media was prepared, poured into three culture plates and left overnight for solidification. The three bacterial species *E. coli, B. subtilis* and *B. Cerevisea* were taken as test strains and inoculated in liquid culture and incubated for 24hrs at 35°C. These were to be used as the source for the antibacterial assay. Then 200ml solid agar media was prepared and poured in three culture plates. The bacterial strains were spread over the media, one plate for each set. Two wells of 6mm diameter were dug into each plate and 12 µl of chitosan I & II dissolved in 1% acetic acid were poured in each well.

The plates were then sealed carefully using parafilm, marked accordingly and were incubated at 35°C for 24 hours. It was made sure that the plates were not being kept in an inverted position and kept erect instead. If there is any antimicrobial activity in the extracts then a zone of clearance will be formed around the corresponding well, which occurs due to diffusion of the extract through the agar.

### 2. ANTIFUNGAL ACTIVITY [14]

- Materials:

1. Compound tested: The antifungal activity of chitin and chitosan has been reported by many investigators. This study has demonstrated that chitin and chitosan from both crustacean like prawn shell wastes exhibited antifungal activity against a large number of pathogenic fungi. The tested chitin compound (chitosan I & chitosan II) has significant effect against many species.

2. Media used: For the antifungal activity, the agar media is used. The media is composed of peptone- 10gm, dextrose- 40gm, agar- 20gm for 1000ml of solution. For this study 200ml of media has been prepared, for which the amount of components are peptone 2gm, dextrose- 4gm, agar-2gm. And the pH of the media should be 5.5-6, not greater than 6.

- Method:

The antifungal activity of chitosan has been tested by normal inoculation method. For the process first a banana was taken and left for a week or more to facilitate the growth of fungi. Solid agar media was prepared and the fungi from the rotten banana was inoculated and left in normal condition for the growth of the fungi. This was to be used as the mother culture acting as the source for of fungal strain for corresponding applications. Seven sterilized culture plates were taken, among them three were taken for the chitosan I and another three for Chitosan-II and one was taken as control. In the first three culture plates equal amount of media with different concentrations of chitosan was taken - in the culture plate 1, 3ml of chitosan-I, in the culture plate 2- 5ml of chitosan-I & in the culture plate 3- 7ml of chitosan-I was poured along

with 25 ml of media. The same process was repeated with the Chitosan-II. The control plate was composed with media and no chitosan and left for solidification of media. The culture plates were then inoculated with fungal strains from the mother culture and incubated for 5 days at room temperature (32°C) and atmospheric pressure. The plates were then checked for presence of anti-fungal activity.

### 3. CHITOSAN AS FOOD PRESERVATIVE AND SHELF-LIFE ENHANCER

Use of Chitosan in food industry is readily seen due to its several distinctive biological activities and functional properties. The antimicrobial activity and film-forming property of chitosan make it a potential source of food preservative. This review [15] focuses on the applications of chitosan for improvement of quality and shelf life of various foods like some vegetables, fruits.

- Materials:

1. Fruits with thick outer layer: Cucumber, Tomato
2. Chitosan-I, Chitosan –II

- Method:

Three fresh Cucumbers & Tomatoes were taken and washed thoroughly with tap water. Two sets of experiments were devised: set-I using cucumber as test material and set II using Tomato. One cucumber and one tomato were kept as Control at room temperature (28˚C). Other two cucumbers & tomatoes were coated by Chitosan-I (1 Tomato, 1 Cucumber) & Chitosan -II (1 Tomato, 1 Cucumber) and kept at room-temperature for 2 weeks.

### 4. BLOOD-ANTI COAGULANT ACTIVITY OF CHITOSAN

Study of anti-coagulant activity showed that chitosan sulphates with lower molecular weight demonstrated a regular increase of anti-Xa activity like heparin. Sulphonated derivatives of Chitosan possess blood anti-coagulant activity [16]. It is one of the important properties of chitosan. This review focuses on the applications of Blood-Anti coagulant activity of chitosan.

- Materials:

1. Fresh chicken blood,
2. Chitosan-I, Chitosan –II.

- Method:

Seven sterile test-tubes with cotton plug were taken. Then these were divided for two sets of experiments, one for Chitosan-I & another for Chitosan-II. For set-I, three test-tubes with three different concentrations of chitosan-I (3ml, 5ml, 7ml) were taken. For set-II, similar concentrations of chitosan–II were taken. The control set didn‘t contain chitosan. Then fresh chicken blood was added to each test-tube to make the volume up to 10ml. The test-tubes were plugged properly and kept at room temperature without disturbing them for observation.

### 5. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

It is generally considered that the inhibition of lipid peroxidation by an antioxidant can be explained by various mechanisms. One is the free radical-scavenging activity where DPPH is a stable free radical with a maximum absorbance at 517 nm in ethanol. When DPPH encounters a proton-donating substance such as an antioxidant, the radical would be scavenged and the absorbance is reduced. Based on this principle, the antioxidant activity of the substance can be expressed as its ability in scavenging the DPPH radical [17]. Chitosan eliminates various free radicals by the action of nitrogen on the C-2 position of the chitosan. The scavenging activity of chitosan may be due to the reaction between the free radicals and the residual free amino group to form stable macromolecule radicals and/or the amino groups can form ammonium groups by absorbing hydrogen ions from the solution and then reacting with radicals through an additional reaction [18].

- Materials:

1. DPPH or 2, 2- Diphenyl-1-Picrylhydrazyl
2. Methanol Preparation: 2.47 mg of DDPH was measured and dissolved in 50 ml methanol to prepare the stock solution. The stock was stored in dark at room temperature.
3. Compound tested: chitosan-I and chitosan-II

- Method:

Two sets of experiments were devised and 6 test-tubes, each for two separately extracted chitosan (Process1 & Process2) and 1 for the control set was prepared. Different concentrations of DPPH (ranging from 0.25ml to 2ml) were poured in the respective test tubes and the volume was made upto 4ml by adding corresponding amount of chitosan-I and chitosan-II in the respective sets. The control/blank was prepared by adding 2ml DPPH and 2ml water. All the test tubes were completely covered to avoid exposure to light and shaken vigorously. The test-tubes were then stored in dark for about 20 minutes. A spectrophotometer was then used to measure the optical density (O.D) of the solutions. The zero measurement of the

spectrophotometer was set using distilled water (O.D=0.732 nm). The measurements obtained were compared with the O.D of the blank set and radical scavenging activity was examined.

## IV. RESULTS

*EXTRACTION OF CHITOSAN FROM PRAWN SHELL WASTES:*

1. Process I:

Initial weight of shells or chitosan produced was measured to be 0.45grams. 35 ml of 1% acetic acid was added and the mixture was shaken for 30 minutes. Final weight of shells after reaction with 1% acetic acid was measured to be 0.22 grams and hence the total dissolved weight of chitosan was calculated to be 0.23 grams.

Therefore, concentration of chitosan in 1% acetic acid: 0.0065 grams/ml.

2. Process II:

Initial weight of shells or chitosan produced was measured to be 0.45 grams. 35ml of 1% acetic acid was added and the mixture was shaken for 30 minutes. Final weight of shells after reaction with 1% acetic acid was measured to be 0.14 grams and hence total dissolved weight of Chitosan was calculated to be 0.28 grams.

Therefore, Concentration of chitosan in 1%acetic acid: 0.0080 grams/ml. It was observed that chitosan produced employing process-II or chitosan-II was more readily soluble in 1% acetic acid solution than that produced through process-I or chitosan-I.

*VARIOUS APPLICATIONS OF CHITOSAN EXTRACTED FROM PRAWN SHELL WASTES:*

1. ANTI-BACTERIAL ACTIVITY OF CHITOSAN

The plates were checked for anti-bacterial activity after 24 hours of incubation. Antibacterial activity was observed in the plates containing test strains *E. coli* and *B. subtilis* for chitosan-I and chitosan-I whereas the *B. cerevisea* showed no inhibition zone for both chitosan I & II. There was mild inhibition of *B. subtilis* by chitosan-II (inhibition zone 10mm diameter) chitosan-I showed no inhibition. Both chitosan-I (inhibition zone 13 mm diameters) and chitosan-II (inhibition zone 15 mm diameters) showed appreciable inhibition for test strain *E. coli*. It was observed that in both cases chitosan-II showed enhanced antimicrobial activity as compared to chitosan-I. The degree of deacetylation plays an important role in determining the antibacterial activity of chitosan as well. Moreover it was observed that chitosan has profound antagonistic activity against gram negative bacteria as compared to gram positive.

2. ANTI-FUNGAL ACTIVITY OF CHITOSAN

After 5 days of incubation, anti-fungal activity was observed in both experimental sets containing chitosan-I and chitosan-II as compared to the control set. In both the sets it was observed that there was an increase in the anti-fungal activity of both chitosan-I and chitosan-II with an increase in the concentration of chitosan in the media. Moreover it was observed that the anti-fungal activity of chitosan-II was more profound as compared to that of chitosan-I which might be due to the increased degree of deacetylation of chitosan-II. Both experimental sets showed a decline in fungal growth as compared to the control set without chitosan. This verifies the claim that the presence of chitosan in experiment sets I and II inhibited the growth of fungi and that the anti-fungal activity of Chitosan enhanced with the degree of deacetylation so that chitosan-II showed a better anti-fungal activity than chitosan-I.

2. CHITOSAN AS FOOD PRESERVATIVE AND SHELF-LIFE ENHANCER

- Cucumber Set:

It was observed that the control cucumber had started to show signs of spoilage by fungal attack after fifth day of storage and after 1week the control was totally rotten. The experimental sets, which were coated by chitosan I & II, however, showed no signs of spoilage after 1 week of storage were and looked as fresh. By the twelfth day, the experimental cucumbers had partially ripened but no signs of fungal or microbial attack were observed.

Table I: Observation for Cucumber Set

| Set | 5 Days | 7 Days | 12 Days |
|---|---|---|---|
| Control | Started to Spoil | Spoiled by Fungus | Totally Rotten |
| Chitosan-I | Unchanged | Unchanged | Ripe |
| Chitosan-II | Unchanged | Unchanged | Ripe |

- Tomato-Set:

The control had tomato started to ripe after second day & after five days it was totally rotten due to rodent and fungal attack. The experimental set which were coated by chitosan I & II were unchanged & looked as fresh without any sign of spoilage even after seven days.

Table II: Observation for Tomato Set

| Set | 2 Days | 7 Days |
|---|---|---|
| Control | Started to ripe | Ripe & rotten with shrink skin |
| Chitosan-I | Unchanged | No spoilage |
| Chitosan-II | Unchanged | No spoilage |

## 3. BLOOD ANTI-COAGULANT ACTIVITY OF CHITOSAN

Control test-tube‘s blood was clotted after 7mints.

- Set-I for Chitosan-I:

After 7 mints, there was no sign of formation fibrin in the test-tubes containing 3ml,5ml,7ml chitosan-I. After 30 mints, it was observed that clotting had started few fibrins started in the blood of the test tube containing 3ml chitosan-I solution, but the other two showed no such signs. After 1 hour, the test tube containing 5 ml chitosan-I started to show signs of clotting and the blood in test-tube containing 7 ml chitosan-I showed no signs of clotting even after 1 hour, 30 minutes.

Table III: Observation Set-I for Chitosan-I:

| Chitosan/ Time | 7 mins | 30 mins | 1 hour |
|---|---|---|---|
| 3ml | No clotting | Minor clot | Semi clot |
| 5ml | No clotting | No clotting | Minor clot |
| 7ml | No clotting | No clotting | No clotting |

- Set-II for Chitosan-II:

After 7 mints, there was no sign of clotting in all the experimental set test-tubes containing 3ml, 5ml, and 7ml chitosan-II solution respectively. After 40 mints, clotting just appeared to have started in that test tube containing 3ml chitosan, but there was no such sign in the other two test-tubes. Even after 1 hour, blood of the test tubes containing 5ml & 7ml chitosan-II solution showed no signs of clot formation and remained liquid.

Table IV: Observation Set-I for Chitosan-II:

| Chitosan/ Time | 7 mins | 40 mins | 1 hour |
|---|---|---|---|
| 3ml | No clotting | Minor clot | Semi clot |
| 5ml | No clotting | No clotting | No clotting |
| 7ml | No clotting | No clotting | No clotting |

## 4. DPPH RADICAL SCAVENGING ACTIVITY OF CHITOSAN

Table V: Measurement of O.D. corresponding to varying Chitosan concentrations:

| Sl. No. | Concentration | O.D. Process1 | O.D. Process2 |
|---|---|---|---|
| 1 | 0.25 | 0.678 | 0.701 |
| 2 | 0.50 | 0.658 | 0.659 |
| 3 | 0.75 | 0.648 | 0.648 |
| 4 | 1.00 | 0.646 | 0.646 |
| 5 | 1.50 | 0.645 | 0.643 |
| 6 | 2.00 | 0.643 | 0.615 |
| Control: O.D. = 0.754 | | | |

The scavenging activity of chitosan was calculated using the following formula:

*Scavenging activity (%) = (O.D. for blank – O.D. for sample / O.D. for blank)*100*

TABLE VI: Calculation of % scavenging activity:

| Sl. No. | Concentration | Scavenging activity of Process1 | Scavenging activity of Process2 |
|---|---|---|---|
| 1. | 0.25 | 10.08 | 7.03 |
| 2. | 0.50 | 12.7 | 12.6 |
| 3. | 0.75 | 14.06 | 14.06 |
| 4. | 1.00 | 14.32 | 14.32 |
| 5. | 1.50 | 14.46 | 14.72 |
| 6. | 2.00 | 14.72 | 18.43 |

It was observed that the scavenging activity increased with increase in both chitosan-I and chitosan-II concentrations but as the rate of radical scavenging is also dependent on the degree of deacetylation of chitosan, it was observed that chitosan-II showed a better activity as compared to chitosan-I.

A graph of scavenging activity vs. concentration is drawn as a comparative study between the two processes of extraction of Chitosan (Fig.1)

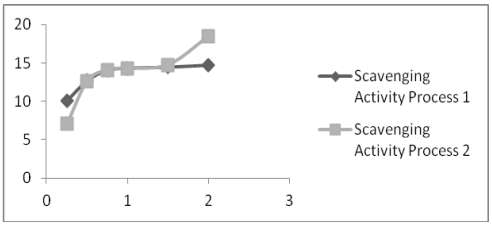


Fig.1 : Graphical representation of scavenging activities for Chitosan I & II.

## V. CONCLUSION

Prawn shell wastes from two different sources were used to obtain chitin and correspondingly for the production of chitosan by employing two different techniques. This study showed that various products of chitin and chitosan can be generated using prawn shells as starting materials. The Chitosan produced by deacetylation of chitin was observed to have many important properties like antibacterial, antifungal and radical scavenging activity to mention a few.

These important properties of chitosan are believed to have many commercial applications of high economic interests [19]. The antifungal and antibacterial activities of chitosan can be employed in production of biofertilizers and biopesticides of economical benefits [20]. Likewise the radical scavenging or the anti-oxidant activity of chitosan is of great interest in food industries and its possible use as natural additives has lead to a great interest in replacing synthetic additives [21]. The use of the antimicrobial activity of chitosan has been used for development of antimicrobial films intended for use in packaging materials for foods, medical supplies and so on, or as laminated coating on items for which surface colonization is undesirable. Chitosan used as coating on fruits and vegetables is almost as effective as the fungicide TBZ at preventing spoilage during storage at proper conditions. Chitosan activity as anti-coagulant is useful in biomedical applications [22] like wound dressing, surgical sutures and for other treatments like reducing oxidative stress in live cells [23], Antitumor activity [24][25], anti-inflammatory effect [26], HIV-1 inhibitors [27], antihypertensive [28], Hypoglycemic and hypolipidemic effect [29] etc. still research is going on. Many studies have been conducted to explore the many possibilities of utilizing the various properties of chitosan and research is still going on these aspects. Chitosan as a commercial chemical has promising range of applications.

Chitosan has shown appreciable promise as a very useful biomaterial [30] and can be obtained from a very easily available source. Despite certain difficulties and the limited means of utilization of Chitosan commercially, it is still an interesting compound with considerable potential. There have been many successful laboratory applications. There are still prospects of industrial applications and many attempts have been undertaken to improve the results of these applications. This project has been aimed at exploring the various aspects of utilizing chitosan obtained from prawn shell wastes that is hopeful to be the answer of environmental concerns as well as economic extraction of this important chemical. Furthermore, I have tried to examine the industrial viability and the claims of utilizing chitosan for diverse applications. Chitosan truly is a very useful chemical with a great scope of industrial applications in diverse fields.

## VI. REFERENCES


[1] Brine CJ, Sandford PA, Zikakis JP. Advances in chitin and chitosan. London and New York: Elsevier; 1992.

[2] Stevens WF, Rao MS, Chandrkrachang S. Chitin and chitosan—environmental friendly and versatile biomaterials. The proceedings of the second Asia Pacific Symposium, 1996.

[3] Muzzarelli RAA, Jeuniaux C, Gooday GW. Chitin in nature and technology. New York: Plenum Publishing Corporation;1986.

[4] Prashanth R, Tharanathan R. Chitin/chitosan: modifications and their unlimited application potential- an overview. Trends Food Sci Tech 2007; 18: 117-31.

[5] G. A. F. Roberts, ―Thirty Years of Progress in Chitin and Chitosan,‖ Progress on Chemistry and Application of Chitin, Vol. 13, 2008..

[6] Jiang X, Chen L, Zhong W. A new linear potentiometric titration method for the determination of deacetylation degree of chitosan. Carbohydr Polym 2003; 54: 457-63.

[7] M. N. V. R. Kumar, ―A Review of Chitin and Chitosan Applications,‖ Reactive and Functional Polymers, Vol. 46, No. 1, 2000, pp. 1-27.

[8] Thanou M, Junginger HE. Pharmaceutical applications of chitosan and derivatives. In: Dumitriu S, editor. Polysaccharides. Structural diversity and functional versatility. 2nd ed. New York: Marcel Dekker Publ.; 2005. p. 661–77.

[9] Galed G, Miralles B, Paños I, Santiago A, Heras A. N-Deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin. Carbohydr Polym 2005; 62(4):316-20

[10] Acosta N, Jimenez C, Borau V, Heras A. Extraction and characterization of chitin from crustaceans. Biomass Bioenerg 1993; 5(2): 145-53.

[11] Batista I, Roberts GAF. A novel facile technique for deacetylating chitin. Makromol Chem 1990; 191: 435-43.

[12] Sannan T, Kurita K, Iwakura Y. Studies on Chitin, 2: Effect of deacetylation on solubility. Makromol Chem 1976; 177: 3589-600.

[13] Park PJ, Je JY, Byun HG, Moon SH Kim SK. Antimicrobial Activity of Hetero-Chitosans and Their Oligosaccharides with Different Molecular Weights. J Microbiol Biotechnol 2004;14(2): 317-23.

[14] Chien P, Chou C. Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of Tankan citrus fruit (Citrus tankan Hayata). J Sci Food Agric 2006; 86: 1964-9.

[15] Oh H, Kim Y, Chang E, Kim J. Antimicrobial Characteristics of Chitosans against Food Spoilage Microorganisms in Liquid Media and Mayonnaise. Biosci Biotechnol Biochem 2001; 65(11): 2378-83.

[16] R. Jayakumar, N. Nwe, S. Tokura, H. Tamura. Sulfated chitin and chitosan as novel biomaterials. International Journal of Biological Macromolecules 2007;40(3):175-181.

[17] Yang Y, Shu R, Shao J, Xu G, Gu X. Radical scavenging activity of chitooligosaccharide with different molecular weights. Eur Food Res Technol 2006; 222(1): 36-40.

[18] Kim K, Thomas R. Antioxidative activity of chitosans with varying molecular weights. Food Chem 2007; 101: 308-13.

[19] Bade ML. Structure and isolation of native animal chitins. In: Goosen MFA, Ed. Applications of chitin and chitosan. Lancaster: Technomic Publishing 1997: 253-77.

[20] Kumar MNVR. A review of chitin and chitosan applications. React Funct Polym 2000; 46: 1-27.

[21] Shahidi, F.; Arachchi, J.K.V.; Jeon, Y.J. Food applications of chitin and chitosans. Trends Food Sci. Technol. 1999, 10, 37–51.

[22] Kim, S.K.; Nghiep, N.D.; Rajapakse, N. Therapeutic prospectives of chitin, chitosan and their derivatives. J. Chitin Chitosan 2006, 11, 1–10.

[23] Ngo, D.N.; Kim, M.M.; Kim, S.K. Chitin oligosaccharides inhibit oxidative stress in live cells. Carbohydr. Polym. 2008, 74, 228–234.

[24] Jeon, Y.J.; Kim, S.K. Potential immuno-stimulating effect of antitumoral fraction of Chitosan oligosaccharides. J. Chitin Chitosan 2001, 6, 163–167.

[25] Jeon, Y.J.; Kim, S.K. Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. J. Microbiol. Biotechnol. 2002, 12, 503–507.

[26] Yang, E.J.; Kim, J.G.; Kim, J.Y.; Kim S.C.; Lee N.H.; Hyun C.G. Anti-inflammatory effect of chitosan oligosaccharides in RAW 264.7 cells. Cent. Eur. J. Biol. 2010, 5, 95–102.

[27] Artan, M.; Karadeniz, F.; Kim, M.M.; Kim, S.K. Chitosan derivatives as HIV-1 inhibitors. J.Biotechnol. 2008, 136S, S527–S540.

[28] Hong, S.P.; Kim, M.H.; Oh, S.W.; Han, C.H.; Kim, Y.H. ACE inhibitory and antihypertensive effect of chitosan oligosaccharides in SHR. Korean J. Food Sci. Technol. 1998, 30, 1476–1479.

[29] Miura, T.; Usami, M.; Tsuura, Y.; Ishida, H.; Seino, Y. Hypoglycemic and hypolipidemic effect of chitosan in normal and neonatal streptozotocin-induced diabetic mice. Biol. Pharm. Bull. 1995,18, 1623–1625.

[30] Jayakumar, R.; Nwe, N.; Tokura, S.; Tamura, H. Sulfated chitin and chitosan as novel biomaterials. Int. J. Biol. Macromol. 2007, 40, 175–181.

◈◈◈