

## BIOPROSPECTING OF SHELLS OF CRUSTACEANS

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## ABSTRACT

Chitin is the second most important natural biopolymer from the shells of crustaceans in the world. Development of chitosan industries on commercial scale would also generate employment opportunities which are most essential for developing countries like India. Quite a few kinds of polysaccharides occur in nature in a broad range of structures and forms, and most of them are considered to work as structural materials or suppliers of water and energy, though their functions may not have been fully comprehended. We discuss chitosan, the most important derivative of chitin, outlining the best techniques to characterize and its many applications.

**Keywords:** Crustaceans, Chitin, Crustaceans, Chitosan and Biopolymer.

## INTRODUCTION

Crustacea is a class of organisms under the phylum Arthropoda, which includes crabs, lobsters, barnacles and shrimps. All arthropods have exoskeletons and jointed appendages. The class Crustacea includes arthropods that breathe using gills or branchiae, and possess two pairs of antennae. Most marine arthropods are crustaceans. Among marine crustaceans shrimp is the single largest item landed in India. Since polysaccharides have peculiar structures and properties, quite different from those of synthetic polymers, they are considered promising biopolymers for developing desirable advanced functions. Among numerous polysaccharides, cellulose and chitin are produced in the largest amounts, estimated to be around  $10^{11}$  tons each per year, and actually, they are the most abundant organic compounds on earth. Chemically modified chitin and chitosan structures resulting in improved solubility in general organic solvents have been reported by many workers<sup>1-10</sup>. The present review is an attempt to discuss the current applications and future prospects of chitin and chitosan.

## CHITIN AND CHITOSAN

Chitin and chitosan the naturally abundant and renewable polymers have excellent properties such as, biodegradability, biocompatibility, non-toxicity, and adsorption<sup>11</sup>. The reaction of chitosan is considerably more versatile than cellulose due to the presence of  $\text{NH}_2$  groups. Various efforts have been made to prepare functional derivatives of chitosan by chemical modifications<sup>12</sup>, graft reactions, ionic interactions, and only few of them are found to dissolve in conventional organic solvents<sup>13</sup>. Chitin and chitosan are natural resources waiting for a market. They were waste products of the crabbing and shrimp canning industry. Chitin produced as processing waste from shellfish, krill, clams, oysters, squid, and fungi. Commercially chitin and chitosan are of great importance owing to their relatively high percentage of nitrogen compared to synthetically substituted cellulose.

## BIOLOGICAL APPLICATION OF CHITIN AND CHITOSAN

## Antimicrobial activity of chitosan

Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts<sup>14-17</sup>. Chitosan has wide spectrum of activity and high killing rate against Gram-positive and Gram-negative bacteria, but lower toxicity toward mammalian cells<sup>18-19</sup>. Ever since the broad-spectrum antibacterial activity of chitosan was first proposed by Allen<sup>20</sup>, along with great commercial potential, the antimicrobial property of chitosan and its derivatives have been attracting great attention from researchers. Investigation of the antimicrobial properties of chitosan has been a long journey of scientific exploration and technological development. The journey began two decades ago, with studies on the biological phenomena arising from foodborne and soilborne pathogenic fungi in the food and agriculture industries<sup>21</sup>.

## Antioxidant activity of chitosan

Chitosans showed were good in antioxidant properties, especially antioxidant activity, scavenging ability on hydroxyl radicals and chelating ability on ferrous ions. In addition, the prolonged N-deacetylation resulted in chitosan with more effective antioxidant properties. Chitosan with presumed antioxidant properties may be used as a source of antioxidants, as a possible food supplement or ingredient or in the pharmaceutical industry<sup>22</sup>. Antioxidant properties of chitosan derivatives have been studied<sup>23-25</sup>. Furthermore, antioxidant properties of fungal chitosan from *shiitake stipes* have also been studied<sup>26</sup>.

## Biosorption of heavy metal ions in aqueous solution.

Nowadays, biosorption is a strongly explored technique; it is defined as passive, not involving metabolically mediated processes, with the property to bind metals by living or dead biomass<sup>27</sup>. Considerable attention has been paid to the recovery and removal of valuable heavy metal ions from industrial and municipal wastewater by using various biosubstances or natural products, particularly because of the low cost and high availability of these materials, without needing arduous regeneration process for reuse, being capable of binding heavy metals by sorption, chelation and ion exchange processes<sup>28-29</sup>. These low-cost abundant natural materials such as chitin, chitosan, alginate, cellulose, peat and biomass require little processing and are abundant in nature, mainly when obtained as by-products and waste from industry<sup>30</sup>.

## Anticoagulant activity of chitosan

The chemical modification of the amino and hydroxyl groups can generate products for pharmaceutical applications, for example: sulfated chitosans possess a wide range of biological activities. Thus, chitosan sulfates as the nearest structural analogues of the natural blood anticoagulant heparin, demonstrate anticoagulant, antisclerotic and antiviral activities<sup>31-35</sup>.

## Wound healing property of chitosan

Chitosan, a derivative of the biopolymer chitin, has been extensively applied in biomedical and pharmaceutical research because of its low toxicity and good biocompatibility. It is able to accelerate the reepithelialization and normal skin regeneration<sup>36</sup>, and to confer considerable antibacterial activity against a broad spectrum of bacteria<sup>37-38</sup>. Chitosan (poly D-glucosamine, deacetylated derivative of chitin) and its oligomers are well known for their interesting biological properties, which have led to various applications such as drug delivery carriers, surgical thread, bone healing materials, and especially wound dressing<sup>39-40</sup>.

## Antitumour and hepatoprotective effect of chitosan

Chitoooligosaccharides (COSs), derivatives of chitosan, can be obtained by either enzymatic or acidic hydrolysis. COSs has been the

choice of interest among many researchers due to their potential biological activities such as immunity enhancing and antitumor<sup>41</sup>, antioxidant and radical scavenging activity<sup>42-44</sup> and hepatoprotective activity<sup>45</sup>.

#### Hypocholestermic and antidiabetic activity of chitosan

Chitosan, a biopolymer of glucosamine derived from chitin that is chemically similar to that of cellulose, is not digestible by mammalian digestive enzymes and acts as a dietary fiber in gastrointestinal tract<sup>46</sup>. It is well known for its cholesterol-lowering effect. However, relative less information is available about the effect of chitosan on plasma lipids and glucose control in diabetic subjects. Previous study has reported that chitosan reduced the concentration of plasma cholesterol in animals<sup>47,48,49,50</sup> and type II diabetes patients in combination with hypercholesterolemia<sup>51</sup>. Increased fecal cholesterol accompanied with or without bile acid excretion by interfering intestinal micelle formation was proposed to be the mechanisms responsible for the hypocholesterolemic properties<sup>46,49,50</sup>. One of the recently reports demonstrated that chitosan has a hypoglycemic effect in STZ-induced diabetic animals<sup>52</sup>. Other studies also found that low molecular weight chitosan (average MW about 2.0 X10<sup>4</sup> Da)<sup>53,54</sup>, as well as chitosan oligosaccharides<sup>55</sup>, can reduce plasma glucose level in diabetic animals.

#### CHITOSAN BASED MATERIALS

Chitosan is used to prepare hydrogels, films, fibers or sponges, most of the materials are used in the biomedical domain, for which biocompatibility is essential. Many systems are described in the literature, but we can cite only a few of the most promising. Chitosan is much easier to process than chitin, but the stability of chitosan materials is generally lower, owing to their more hydrophilic character and, especially, pH sensitivity. To control both their mechanical and chemical properties, various techniques are used, as mentioned previously for chitin. Often, the methods are adapted from the cellulose world.

#### ECONOMIC VALUE OF CHITOSAN

To produce 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 Kg of NaOH are required in addition to nitrogen, process water (0.5 t) and cooling water (0.9 t). Important items for estimating the production cost include transportation, which varies depending on labor and location. In India, the Central Institute of fisheries Technology, Kerala, initiated research on chitin and chitosan. From their investigation, they found that dry prawn waste contained 23% and dry squilla contained 15% chitin<sup>56</sup>. They have also reported that the chitinous solid waste fraction of the average Indian landing of shell fish ranges from 60 000 to 80 000 tonnes<sup>57,58</sup>. Chitin and chitosan are now produced commercially in India, Japan, Poland, Norway and Australia.

#### CONCLUSION

Crustacean shell waste is usually dried on the beaches. It encourages not only environmental pollution but also reduces the recoverable components from their biowaste. Solid Crustacean shell waste undergoes rapid putrefaction because of its alkaline nature (pH 7.5–8.0). Due to high perishability of Crustacean shell waste, implemented processing is needed. Improving the design and operation of biological treatment process for shrimp waste in real life application presents many challenges, including working within the following constraints: the need for robust operation, environmental parameters, and low cost operation. Therefore, extensive research should be carried out to explore bioactive compounds and their activities from Crustacean shell waste.

#### REFERENCES

1. K. Kurita, H. Yoshino, K. Yokota, M. Ando, S. Inoue, S., Ishii, S. Nishimura, Preparation of tosyl chitins as for facile chemical modification of chitin. 25, 3786 (1992).
2. K. Kurita, S. Inoue, S. Nishimura, chitin derivatives as reactive precursors for controlled modifications: tosyl- and iodo chitins, J. Polym. Sci., Part A: Polym. Chem. 29,937 (1991).

3. D.V. Luyen, V. Rossbach, Mixed esters of chitin, J. Appl. Polym. Sci. 55, 679. 2239 (1995).
4. M.T. Qurashi, H.S. Blair, S.J. Allen, Studies on modified membranes, I, J. Appl. Polym. Sci. 46, 255, (1992).
5. M.T. Qurashi, H.S. Blair, S.J. Allen, Studies on modified chitosan membranes. II Dialysis of low molecular weight metabolites, J. Appl. Polym. Sci. 46, 263, (1992).
6. N. Kubota, Permeation properties of chitosan transition metal complex membranes, J. Appl. Polym. Sci. 64, 819 (1997).
7. S. Hirano, A facile method for the preparation of novel membranes from *N*-acyl and *N*-arylidene chitosan gels, (1993) 4151. Agric. Biol. Chem. 14, 1938 (1978).
8. G.W. Urbanczyk, B. Lipp-Symonowicz, The influence of processing terms of chitosan membranes made of differently deacetylated chitosan on the crystalline structure of membranes, J. Appl. Polym. Sci. 51, 2191 (1994).
9. K. Kurita, K. Tomita, S. Ishii, S. Nishimura, K. Shimoda, b-Chitin as a convenient starting material for acetolysis for efficient preparation of *N*-acetylchitoooligosaccharides, J. Polym. Sci., Part A: Polym. Chem. 31, 2393 (1993).
10. K.R. Holme, L.D. Hall, Chitosan derivatives bearing C - 10 alkyl glycoside branches: a temperature induced gelling Polysaccharide, Macromolecules 24, 3828, (1991).
11. Hudson S M & Smith C, Polysaccharides: chitin and chitosan: chemistry and technology of their use as structural materials, Biopolymers from renewable resources, edited by D L Kaplan, (Springer-Verlag, New York), pp. 96-118, (1998).
12. Muzzarelli R A A, Some modified chitosans and their niche applications, chitin Handbook, edited by R A A Muzzarelli and M G Peter (European chitin Society, Italy), pp.47-52, (1997).
13. Li Q, Dunn E T, Grandmaison E W & Goosen m F A, Applications and properties of chitosan, Applications of chitin and chitosan, edited by MFA Goosen, (Technomic Publishing Company, Lancaster), pp.3-29, (1997)
14. Hirano, S., Nagao, N., Effects of chitosan, pectic acid, lysozyme, and chitinase on the growth of several phytopathogens. Agricultural and Biological Chemistry 53, 3065–3066, (1989).
15. Kendra, D.F., Hadwiser, L.A., Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Experimental Mycology 8, 276–281, (1984).
16. Uchida, Y., Izume, M., Ohtakara, A., In: Skjak-Braek, G., Anthonson, T., Sandford, P. (Eds.), Chitin and chitosan. Elsevier, London, UK, p. 373, (1989).
17. Ueno, K., Yamaguchi, T., Sakairi, N., Nishi, N., Tokura, S., In: Domard, A., Roberts, G.A.F., Varum, K.M. (Eds.), Advances in chitin science. Jacques Andre, Lyon, p. 156, (1997).
18. Franklin, T.J., Snow, G.A., Biochemistry of Antimicrobial Action, 3rd ed. Chapman and Hall, London, p. 175, (1981).
19. Takemono, K., Sunamoto, J., Askasi, M., Polymers and Medical Care. Mita, Tokyo; Chapter IV (1989).
20. Allan, C.R., Hardwiger, L.A., The fungicidal effect of chitosan on fungi of varying cell wall composition. Experimental Mycology 3, 285–287 (1979).
21. Rabea, E.I., Badawy, M.E.T., Stevens, C.V., Smagghe, G., Steurbaut, W., Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4, 1457–1465 (2003).
22. Ming-Tsung Yen a, Joan-Hwa Yang b, Jeng-Leun Mau, Antioxidant properties of chitosan from crab shells. Carbohydrate Polymers 74 840–844 (2008).
23. Lin, H.-Y., & Chou, C.-C. Antioxidant activities of water-soluble disaccharide chitosan derivatives. Food Research International, 37, 883–889 (2004).
24. Xie, W., Xu, P., & Liu, Q. Antioxidant activity of water-soluble chitosan derivatives. Bioorganic and Medicinal Chemistry Letters, 11, 1699–1701 (2001).
25. Xing, R., Yu, H., Liu, S., Zhang, W., Zhang, Q., & Li, Z., et al. Antioxidative activity of differently regioselective chitosan sulfates in vitro. Bioorganic and Medicinal Chemistry, 13, 1387–1392 (2005).
26. Yen, M.-T., Tseng, Y.-H., Li, R.-C., & Mau, J.-L. Antioxidant properties of fungal chitosan from shiitake stipes. LWT – Food Science and Technology, 40, 255–261 (2007).

27. N.A. Negm, H.E. Ali, Modification of heavy metal uptake efficiency by modified chitosan/anionic surfactant systems, *Eng. Life Sci.* 10, 218–224, (2010).
28. K. Kaikake, K. Hoaki, H. Sunada, R.P. Dhakal, Y. Baba, Removal characteristics of metal ions using degreased coffee beans: adsorption equilibrium of cadmium( II), *Bioresour. Technol.* 98, 2787–2791 (2007).
29. J.C.Y. Ng, W.H. Cheung, G. McKay, Equilibrium studies for the sorption of lead from effluents using chitosan, *Chemosphere* 52, 1021–1030 (2003).
30. C. Gerente, P.C. Mesnil, Y. Andres, J.F. Thibault, P.L. Cloirec, Removal of metal ions from aqueous solution on low cost natural polysaccharides Sorption mechanism approach, *React. Funct. Polym.* 46, 135–144 (2000).
31. Desai, U. R. New antithrombin-based anticoagulants. *Medical Research Review*, 24(2), 151–181 (2004).
32. Drozd, N. N., Sher, A. I., Makarov, V. A., Vichoreva, G. A., Gorbachiova, I. N., & Galbraich, L. S. Comparison of antitrombin activity of the polysulphate chitosan derivatives in vitro and in vivo system. *Thrombosis Research*, 102, 445–455 (2001).
33. Hirano, S., Tanaka, Y., Hasegawa, M., Tobetto, K., & Nishioka, A. Effect of sulfated derivatives of chitosan on some blood coagulant factors. *Carbohydrate Research*, 137, 205–215 (1985).
34. Nishimura, S., Kai, H., Shinada, K., Yoshida, T., Tokura, S., Kurita, K., et al. Regioselective syntheses of sulfated polysaccharides: Specific anti-HIV-1 activity of novel chitin sulfates. *Carbohydrate Research*, 306, 427–433 (1998).
35. Vongchan, P., Sajomsang, W., Subyen, D., & Kongtawelert, P. Anticoagulant activity of sulfated chitosan. *Carbohydrate Research*, 337, 1233–1236 (2002).
36. Azad AK, Sermsintham N, Chandkrachang S, Stevens WF. Chitosan membranes as a wound-healing dressing: characterization and clinical application. *J Biomed Mater Res Part B: Appl Biomater*;69:216–22 (2004).
37. Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN. Fabrication and characterization of sponge-like asymmetric chitosan membranes as a wound dressing. *Biomaterials*; 22:165–73 (2001).
38. Usami, Y., Okamoto, Y., Minami, S., Matsuhashi, A., Kumazawa, N.H., Tanioka, S., et al., Migration of canine neutrophils to chitin and chitosan. *J. Vet. Med. Sci.* 56 (6), 1215–1216 (1994b).
39. No, H.K., Park, N.Y., Lee, S.H., Meyers, S.P., Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Food Microbiol.* 74 (1–2), 65–72 (2002).
40. Tsai, G.J., Su, W.H., Antibacterial activity of shrimp chitosan against *Escherichia coli*. *J. Food Prot.* 62 (3), 239–243 (1999).
41. Suzuki, K., Mikami, T., Okawa, Y., Tokoro, A., Suzuki, S., & Suzuki, M. Antitumor effect of hexa-N-acetylchitohexaose and chitohexaose. *Carbohydrate Research*, 151, 403–408. (1986).
42. Castagnino, E., Ottaviani, M. F., Cangioti, M., Morelli, M., Casettari, L., & Muzzarelli, R. A. A. Radical scavenging activity of 5-methylpyrrolidinone chitosan and dibutylryl chitin. *Carbohydrate Polymers*, 74, 640–647 (2008).
43. Ngo, D. N., Kim, M. M., & Kim, S. K. Chitin oligosaccharides inhibit oxidative stress in live cells. *Carbohydrate Polymers*, 74, 228–234 (2008).
44. Park, C., So, H. S., Shin, C. H., Baek, S. H., Moon, B. S., Shin, S. H., et al. Quercetin protects the hydrogen peroxide-induced apoptosis via inhibition of mitochondrial dysfunction in H9c2 cardiomyoblast cells. *Biochemical Pharmacology*, 66, 1287–1295 (2003).
45. Mahinda Senevirathne, Chang-Bum Ahn and Jae-Young Je. Hepatoprotective effect of chitooligosaccharides against tert-butyl hydroperoxide-induced damage in Chang liver cells, *Carbohydrate Polymers* 83 995–1000 (2011).
46. Gallaher, C.M., Munion, J., Hesslink Jr., R., Wise, J., Gallaher, D.D., Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. *J. Nutr.* 130, 2753–2759 (2000).
47. Chiang, M.T., Yao, H.T., Chen, H.C., Effect of dietary chitosans with different viscosity on plasma lipids and lipid peroxidation in rats fed on a diet enriched with cholesterol. *Biosci. Biotechnol. Biochem.* 64, 965–971 (2000).
48. Yao, H.T., Chiang, M.T., Chen, H.C., Effect of chitosan on cecal bacteria metabolism and fecal b-glucuronidase activity in rats. *Taiwan. J. Agric. Chem. Food Sci.* 40, 271–279 (2002).
49. Yao, H.T. and M.T. Chiang, Chitosan shifts the fermentation site toward the distal colon and increases the fecal short chain fatty acids concentrations in rats. *Int. J. Vitam. Nutr. Res.* 76: 57–64 (2006a).
50. Yao, H.T. and M.T. Chiang, Effect of chitosan on plasma lipids, hepatic lipids, and fecal bile acid in hamsters. *J. Food Drug. Anal.*, 14: 183–189 (2006b).
51. Tai, T.S., Sheu, W.H., Lee, W.J., Yao, H.T., Chiang, M.T., Effect of chitosan on plasma lipoprotein concentrations in type 2 diabetic subjects with hypercholesterolemia. *Diabetes Care* 23, 1703–1704 (2000).
52. Yao, H.T., Hwang, S.Y., Chiang, M.T., Effect of chitosan on plasma cholesterol and glucose concentration in streptozotocin induced diabetic rats. *Taiwan. J. Agric. Chem. Food Sci.* 44, 122–132 (2006).
53. Kondo, Y., Nakatani, A., Hayash, K., Ito, M., Low molecular weight chitosan prevents the progression of low dose streptozotocin induced slowly progressive diabetes mellitus in mice. *Biol. Pharm. Bull.* 23, 1458–1464 (2000).
54. Hayashi, K., Ito, M., Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-Ay mice. *Biol. Pharm. Bull.* 25, 188–192 (2002).
55. Lee, H.W., Park, Y.S., Choi, J.W., Yi, S.Y., Shin, W.S., Antidiabetic effects of chitosan oligosaccharides in neonatal streptozotocin induced noninsulin-dependent diabetes mellitus in rats. *Biol. Pharm. Bull.* 26, 1100–1103 (2003).
56. P. Madhavan, K.G.R. Nair, Utilization of prawn waste. Isolation of chitin and its conversion to chitosan, *Fishery Tech.* 11 50, (1974).
57. P. Madhavan, K.G.R. Nair, T.K. Thankappan, P.V. Prabhu, K. Gopakumar, Production of chitin and chitosan, Project. Report, CIFT, Kochi (Priced Bulletin), (1986).
58. K. Gopakumar, P.G.V. Nair, M.K. Mukundam, J. Stephen, A.G. Radhakrishnan, K.G.R. Nair, P.D. Anthony et al., Biochemical composition of Indian food fish, CIFT Bulletin, (1997).