REVIEW: CHITIN AND CHITOSAN FROM SHELLFISH WASTES

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INTRODUCTION

Chitin, a polymer of N-acetyl-D-glucosamine residue, currently obtained from crustaceans, and with a moisture content of 5-10%, total nitrogen content of 6.9%, and average molecular weight of 1.036×10^6 daltons, has traditionally been marketed in the form of flakes, powders, beads, gels, etc.
It is widely distributed in nature and its quantitative importance in living beings is now well known [1]. It is found in marine invertebrates, insects and fungi in association with proteins, calcium deposits and pigments, where it performs a protective and supporting role [2]. Considerable amounts of chitin are present in shellfish wastes. Chitosan, the N-deacetylated form of chitin forms the body wall of most fungi, molds and yeasts [3].

Several studies have been carried out to investigate chitin chemistry, structure, modification and applicabilities and these have shown that, chitinous polymers have unusual combination of properties such as high water binding capacity [4] and good film-forming properties [5] which make them useful in the food, cosmetics, pharmaceutical, paint and textile industries, agriculture, biotechnology, and in medicine.

Both chitin and chitosan are now being produced in commercial quantities from crustacean wastes in Japan and to a relatively lesser extent in the United States [6]. Chitosan is also being produced on a laboratory scale by deacetylation and fermentation of fungal cells [7]. In Ghana, chitin is being produced on a laboratory scale [8].

The Shellfish Industry

The rapid perishability of fish and shellfish compared with meat has at all times and places made preservation against putrefaction an urgent necessity. At a very early age in history, man learned the survival value of storing day-to-day and seasonal surpluses.

In historical times, various preservation techniques were evolved, which included drying, salting and smoking of fish and shellfish, in large quantities in barrels. Canning was the result of an attempt to preserve fish satisfactorily without adversely affecting the freshness and palatability by undue desiccation, toughening, and salting. Although canning allowed large scale fishing to be done conveniently to support a growing world population, it came along with production of large quantities of shellfish processing wastes to be disposed of.

Worldwide shellfish catch per capital

The utilization pattern of shellfish is naturally determined by the size of the catch in relation to the size of the population of each fishing nation. The United States has the largest catch of crustaceans in the world [9], processing over 500,000 tons annually [10] Japan holds the second place in crustacean catching and king crab dominates the canned pack. Polish deep sea fishery has engages in harvesting marine living resources such as, the Antartic krill and squid containing chitin since 1957. Presently there is a production of about 5 tonnes per day of peeled krill meat, leaving behind a great deal of shell wastes, from which practical isolation of about 150 kg of chitin is possible [11]. Other important shellfisheries are present in India, Malaysia, Australia, South Africa, South America,

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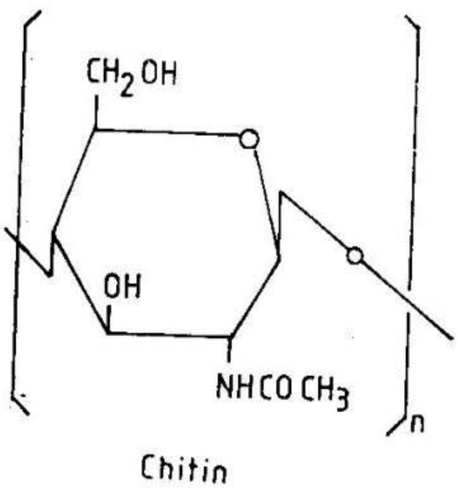
might be an attractive route to the production of chitosan. Bartnicki-Garcia [16] stated that, chitosan is made by deacetylation of chitin by the enzyme, chitin deacetylase, provided that, the deacetylation process occurs in tandem with chitin synthesis. The fungal order Mucorales is known to contain chitosan as a cell wall component [17]. Organisms of this class can be readily cultured on cheap nutrients and the cell wall material can be recovered by simple chemical procedures.

Chitosan can then be modified to produce microcrystalline chitosan, or other derivatives like carboxymethyl chitosan, which have improved solubilities compared to the native chitosan.

Physicochemical properties of chitin and chitosan

Chitin is the second most abundant polysaccharide in nature after cellulose. It is a polymer of N-acetyl-D-glucosamine in a beta-1, 4-glycosidic linkage. Chitosan, the deacetylated derivative of chitin is a polymer of D-glucosamine in a beta-1, 4-glycosidic linkage. Fig. 1 shows the structure of the repeating unit of chitin and chitosan.



Chitin

Fig.3 Structures of repeating units of chitin and chitosan

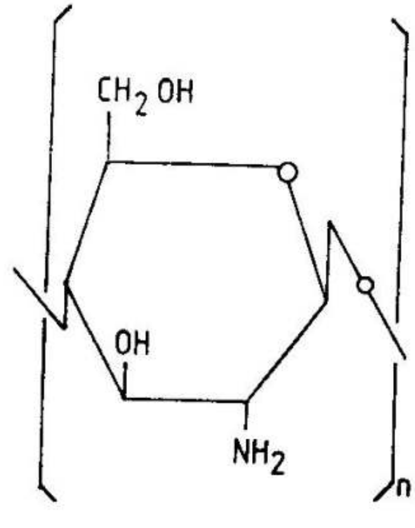
Chitin is a tasteless, odourless, non-toxic free-flowing powder. It is biodegradable and has a high molecular weight, and has less than 10% moisture content. The residual protein content based on amino acid analysis is less than 0.5%. It produces a high viscous solution of 500 - 1,000 cps. The residual metal content is less than 100 ppm per species of the following: potassium, calcium, magnesium, iro copper, zinc, aluminium, strontium, manganes barium, boron, vanadium, and less than 3 ppm p species of mercury, cadmium, lead, arseniu chromium, tin, and cobalt. [18].

Chitosan is also a free-flowing, tasteless, odourle powder, with a particle size of less than 700 um. solubilizes in 1% acetic acid: sodium acetate soluti to produce a viscous liquid of 20-3,000 cps [19].

The residual protein content by amino acid analys is less than 0.3%. Both chitin and chitosan have high water holding capacity of between 400 - 500 (w/w).

Chitinous associations

Chitin is associated with other polysaccharides su as mannans, glucans and galactans in fungal c walls while in animals, it is associated with protei [2]. The protein present in these animals are cros linked by a sclerotization process which is initiat in the cuticle by a phenoloxidase - catalyz oxidation of diphenolic substrates yielding the



Chitosan

corresponding O-quinonemethide as a reacti intermediate [20]. Chitin may also contribute to t stability of the sclerotized insect cuticle [2 Sclerotization may be accompanied by t deposition of calcium ions in the form of calciu carbonate and to a lesser extent as a calciu phosphate.

Solubility and viscosity

The choice of a solvent for chitin is always a compromise among aspects such as rate of dissolution, rate of chitin degradation, viscosity of the solvent, and viscosity of the resulting chitin solution. Austin [22] reported that, chloroalcohols in conjunction with aqueous solutions of mineral acids are effective systems for dissolving chitin in any form. An effective solvent system for chitin is N, N-dimethylacetamide or N-methylpyrolidone containing 5% dissolved LiCl, was discovered by Rutherford and Austin [23].

Film-forming properties

Chitin has a high degree of crystallinity which allows it to be cast into films or membranes. By dissolving it in a suitable solvent, the resulting solution can be coated on a suitable surface to produce a film [2]. The film-forming ability of chitosan has been the subject of many studies some of which have led to industrial uses in various fields such as photographic films [24], as reverse osmosis membranes [25] and in cosmetics [5].

Water binding properties

Chitin and chitosan are known to bind two to five times their weight of water with chitosan having a greater water binding capacity [4]. Generally, water uptake of chitinous polymers depend on factors like crystallinity, amount of salt forming groups, and amount of protein in the product. Austin et al. [26] reported that, all chitinous polymers are associated with protein residues which remain with it even after the most drastic alkali treatment.

Chelating properties

The cationic polymer has a high metal binding capacity. In solution it sequesters heavy metals and is therefore used in the treatment of waste water from chemical industries [2].

The advent of the chitin industry

In Japan, chitosan was produced industrially from chitin for the first time in the world in 1971 by Katakurachikkarin Inc. (Hokkaido) and Kyowa Reizo Inc. (Tottori) [19] from chitin. Since then, chitin and chitosan have been produced by a number of companies in Japan and by 1986, there were a total of 15 chitin producing companies. In the United States, Protan Laboratories and Kypro Company form the main chitin/chitosan industries [6]. In Poland chitin and chitosan are being produced on a laboratory scale from krill and squid pens [10]. Commercial chitin and chitosan vary in quality with each of these companies and they supply these products in the form of powders, flakes, fibres, films, beads and sheets. According to Hirano [19], the total capacity of an estimated annual production of chitin by the Japanese companies is about 2,000 tons. In 1986, these companies produced 1,270 tons of chitin. Out of this, 1,170 tons were used in the production of chitosan, 60 tons for D-glucosamine and oligosaccharide production, and 40 tons remained as excess. The yield of chitosan was 700 tons and out of this amount 500 tons was used as flocculants, with an excess of 200 tons [19]. Presently, however, there is a fairly good balance of production with utilization because of increased applications in the fields of biotechnology, medicine, foods and feeds.

Utilization of chitinous polymers

In the food, pharmaceutical and cosmetic industries, there have always been the need for inexpensive suspensions of polymers for use as thickeners, suspending, stabilizing, gelling and viscosfying agents.

Water soluble polysaccharides such as alginic acid, carboxymethyl, cellulose, guar gum, xanthan gum, starch and their derivatives have been used for these purposes. Physically modified chitinous polymers like microcrystalline chitin and chitosan are obtained via physical methods which are based on high shearing forces, centrifugation and lyophilization which result in drastic changes in the physical properties of the material. Some of the changes in properties are improved dispersibility, and unusual viscosity stability at prolonged and elevated temperatures, with the formation of powerful hydrogen bonds and development of direct film-forming properties. These properties are exploited in food systems, cosmetic manufacture, waste water treatment, and clarification processes in which viscosity control agents are required to be compatible with sterilization or other forms of heat treatment.

Chitin as dietary fiber

It has been found that additions of up to 10% of chitin in the diet of chickens results in normal growth and vigor of the animals and also increased growth of Bifidobacteria in the gut [27]. This bacterium blocks the growth of other types of micro organisms in the gut, and generates lactase, required for digestion of milk lactose. This may be exploited for use in milk products for humans with lactose intolerance. Additions of chitin above 10% in feeds depresses iron absorption [28]. When used as dietary fibre chitin exhibits a potential for reduction in cholesterol level in humans [28].

Chitin in wastewater treatment

Metals like mercury and calcium are used in metal finishing, chemical industries, and photography. Copper, nickel, zinc, chromium, and silver cause particular concern due to both public health considerations and public pressure on the environment. Their discharge in waste waters contaminate sewage sludge to an extent that, it makes it unsuitable for use on farm lands and cause harm to acquatic life. Chitosan, the cationic polymer is an excellent agent in waste water treatment because of its excellent metal binding capacity, flocculation, and coagulation abilities. Microcrystalline chitosan can remove up to 98.7% zinc in waste water at a concentration of 13 g/L at pH 7.5, and iron up to 96.8%. At concentrations of up to 12.7%, it can remove up to 100% of copper in waste water at pH 7.5 within 15 minutes. Other metals are also removed at the same rate but at relatively higher chitosan concentrations [18].

Chitin and chitosan as non-absorbable carrier of food additives and bioactive compounds

The rationale underlying the development of non-absorbable food additives stems from the concept that functional ingredients which are absorbed intact or metabolized may interact with target tissues or organs and constitute a potential risk [27]. The use of chitin/chitosan as non-absorbable food carrier for highly concentrated food ingredients was investigated in animal feeding trials with the food dye, FD & C Red No. 40. This study showed that when the dye is attached to the chitinous polymer, absorption of the dye by the animal was reduced [29]. Pesticides and fertilizers when bound to microcrystalline chitosan act as a polymeric carrier controlling the release of nutrients and thus reducing losses significantly. It also increases stability against the effect of rain water. It reduces evaporation by forming a film on the leaves, and reduces phytotoxicity of nutrients.

Use of chitosan for clarification of fruit juices

Processing of clarified fruit juices commonly involves the use of clarifying agents including gelatin, bentonite, silica sol, tannins, polyvinylpyrrolidone, or combination of these compounds. Chitosan salts, which carry a strong positive charge have proved to be equally effective in reducing juice turbidity. Soto-Peralta et al. [30] found that both acid-soluble and water-soluble and chitosan salts are effective fining agents.

Other uses of chitin and chitosan

In the field of biotechnology, chitosan has a role in plant cell permeabilization and elicitation of plant metabolities. For example, increase in product yields of existing or new phytochemicals could be made possible when chitosans are used as inducers of biosynthesis of primary metabolities for example, enzymes; or secondary metabolities for example, alkaloids, flavours, pigments and antioxidants. Osuji and Cuero [31] reported that, by the application of N-carboxymethyl chitosan by slowly pouring the solution to the soil bearing the yam tubers, the storage protein yield was enhanced.

Chitin has anti-fungal activities [32] and has been used in the treatment of tomato root rot caused by *Fusarium oxysporum* [33]. The film-forming properties of chitin and its water-soluble derivates have led to recommending chitin films as oven and other food wraps. It has also been used in storage of fruits and vegetables for delaying decay [34].

Markets

Market development holds a challenge for the future of chitin and chitosan. Though numerous applications exist, the cost effectiveness versus alternative materials is the question. The market potential is very large in water treatment, agriculture, food and textile industries. Direct incorporation into products like sutures, bandages and cosmetic products is now becoming a reality. In other areas of application the development of market has been very slow and more research is required to demonstrate the superiority of chitinous polymers over competing materials and technologies.

CONCLUSION

From the forgone discussion, it is evident that crustacean wastes need not be wasted. Efforts must be made to develop manufacturing and marketing know-how. Cooperation and coordination among all parties (crustacean farmers researchers, producers, and buyers) is required to develop a successful chitin/chitosan industry. Supply is not a problem and market development is the key to growth. Coastal dwellers can gain jobs with the development of a market place. Chitin and chitosan industry is breaking new grounds in the area of by product recovery from industrial wastes.

REFERENCES

1. **Jeuniaux, C., Voss-Foucart, M.F., Poulicek, M. and Bussers, J.C.**: Sources of chitin estimated from new data on chitin biomass and production. In: Chitin and Chitosan (Skjak-Braek, G., Anthonsen, T., and Sanford, P., Eds.). Elsevier Applied Science Pub. New York. p.3, 1988.

2. **Muzzarelli, R.A.** Chitin. Pergamon Press. Oxford N.Y. 1977

3. **Ruiz-Herera, J.** The distribution and qualitative importance of chitin in fungi. In: Proc. 1st Int. Conf Chitin/Chitosan, (Muzzarelli, R.A.A. and E.R. Pariser Eds.): MIT Sea Grant Report MITSG 78-7 p.11, 1978.

4. **Knorr, D.** Functional properties of chitin and chitosan. J. Food Sci., 47: 593, 1982.

5. **Lang, G. and Clausen, T.** The use of chitosan in cosmetics. In: Chitin and Chitosan. (Skjak-Break, G., Anthohsen, T., and Sanford, P. Eds.), Elsevier Applied Science Pub. New York. p. 139, 1988.

6. **Sanford P.** Chitosan: commercial uses and potential applications. In: Chitin and Chitosan. (Skjak-Break, G., Anthonsen, T., and Sanford, P. Eds.), Elsevier Applied Science Pub. New York. p.53, 1988

7. **White, S.A., Farina, P.R. and Fulton, I.** Production and isolation of chitosan from *Mucor rouxii*. Applied Environmental Microbiology, 38(2): 323, 1979.

8. **Tetteh, A.** Distributional review and compositional characteristics of Ghanaian shellfish and their potential utilization for chitin extraction. Journ. of Univ. of Science and Technology, Vol.15. No. 2 and 3. p19. 1995

9. **Cutting, C.L.** Historical aspects of fish. In: Fish as Food. (Borgstom, G., Ed) Acad. Press, N.Y. Vol.2: 1, 1962.

10. **CRESA** "Pollution abatement and by-product recovery in shellfish and fisheries processing" Environmental Protection Agency. Water Pollution Control Research Series, Project No. 12130FJQ, 1971.

11. **Brzeski, M.M.** 1988. Production and application of chitin and chitosan in Poland. In: Chitin and Chitosan. (Skjak-Break, G., Anthonsen, T., and Sanford, P. Eds.), Elsevier Applied Science Pub. New York. p.161, 1988.

12. **FAO**, Food and Agriculture Organisation Fisheries Statistics Yearbook, Rome p.40. 1990

13. **Revah-Moiseev, S. and Carroad, A.** Conversion of enzymatic hydrolysate of shellfish waste chitin to single-cell protein Biotech. Bioeng., 23: 1067, 1981.

14. **Kreag, R. and Smith, F.** "Seafood solid waste in Oregon: disposal or recovery?" Oregon State Univ. Extension Marine Advisory Program Report 395. Oregon State Univ., Cornallis, OR. 1975.

15. **Hanson, D.** EPA boosts fines for landfilling violations. In: Chemical and Engineering News. Nov, 5th ed. Washington. p.5. 1990

16. **Bartnicki-Garcia, S.** The biochemical cytology of chitin and chitosan synthesis in fungi. In; Chitin and Chitosan. (Skjak-Break G., Anthonsen, T., and Sanford, P. Eds.), Elsevier Applied Science Pub. New York. p.161., 1988.

17. **Shimahara, K., Takikuchi, Y., Kobayachi, O., Uda, K. and Sannan, T.** Screening of Mucorasseae strains suitable for chitosan production: Chitin and

Chitosan. (Skjak-Break, G. Anthonsen, T., and Sanford, P. Eds.) Elsevier Applied Science Pub. New York. p.171, 1988.

18. Hirano, S. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem: 385,p. 124, 1990

19. Hirano, S. Production and application of chitin and chitosan in Japan. In: Chitin and Chitosan. (Skjak-Break, G., Anthonsen, T., and Sanford, P. Eds.), Elsevier Applied Science Pub. New York. p.37, 1988

20. Hackman, R.H. and Goldberg, M. Molecular crosslinks insect cuticles,

21. Peter, M.G., Kegal, G. and Keller, R, Structural studies on sclerotized Insect Cuticle in Nature and Technology. (Muzzarelli, R.A.A., Jeuniauz, C., and Gooday; G.W. Eds.) Plenum Press, New York. p21. 1985.

22. Austin, P. R. Solvents for and purification of chitin. U.S. Patent 3, 892, 731, 1975.

23. Rutherford, F.A. and Austin, P.R. Marine chitin properties and solvents. In: Proc. 1st Inter. Conf. Chitin/Chitosan. MIT Sea Grant Report MITSG 78-7p. 182, 1978.

24. Ryan, W.H. and Yankowski, E.L. Photographic image-receiving material. Ger. 1. 116:969, 1969.

25. Yang, T. and Zall, P.R. Chitosan membranes for reverse osmosis application. J. Food Sci,. 49:91, 1984.

26. Austin, P.R., Brine, C.J. Castle, J.E. and Zikakis, J.P. Chitin: New Facets of Research Science, 212: 749, 1981.

27. Knorr, D. Use of chitinous polymers in food. Food Technol., 38(1) 85, 1984.

28. Watkins, T.R. and Knorr, D., In vivo dye binding of chitin and its effects on gerbil growth and gut function. Nutrition Reports Inter, 27: 189. 1983.

29. Soto-Peralta, N.V.; Miller, H. and Knorr, D. Effects of chitosan treatment on the clarity and colour of apple juice J. Food Sc., 54 (2); 495, 1989.

30. Osuji, G. O. and Cuero, R.G. A rapis method for enhancing the storage protein yields of yam tuber, sweet potato and maize, through N-carboxymethychitosan treatment of the crops. Med Fac. Landbouww. Rijksuniv. Gent, 56(4a). p. 1661. 1991

31. Simpson, B.K.; Tetteh, A,Y, and Oldham, J.H.
Film-forming and anti-microbial properties of chitin.
Technology and Environments Project Meeting. Jan 23-26, Dakar Senegal 1995.

32. Benhamou, N. Ultrastructural and cytochemical aspects of chitosan on ***Fusarium oxysporum*** *F.sp.radicus - lycopersici,* agent of tomato crown and root rot. **Department de** Phytologie, Faculte de Science de L'agriculture at de L'alimentation, Universite Laval, Sainte Foy, Quebec. 1989.

33. Gaouth, A.E.; Arul, J.; Ponnampalam, R. and Boulet, M. Chitosan effect on storability and quality of fresh strawberries, J. Food Science Vol.56, No.6 p.1618, 1991.