Recent Trends in the Use of Chitin, Chitosan and their Derivatives

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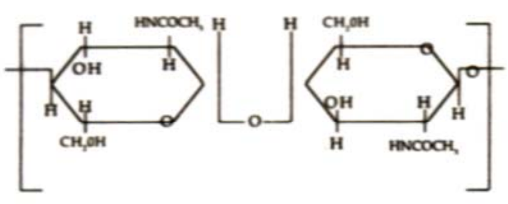
Abstract : More than 1.25 lakh tonnes of crustacean waste is available for disposal from the shell fish processing industry in India anually. Chitin, polymer of (1-4) N-acetyl D-glucosamine occurs in shell fish, clams, oysters, squid, fungi and insects. Technologies are available in India and abroad for extraction of chitin and conversion of it to chitosan and other derivatives. The current interest in chitin, chitosan and their derivatives is related to the possibilities for their biological and biotechnological applications. Chitin and chitosan have applications in textile, paper, pharmaceuticals, food and cosmetics, having potential to consume sizeable quantities of chitin and chitosan. European countries viz. Italy, Germany and France have pioneered in several valuable applications of chitin and its derivatives in pharmaceuticals and cosmetics. A review of important applications are given in this paper.

Resources

Chitin is present in a wide variety of animal species. The major sources of chitin are shell fish, krill, clams, oysters, squid, fungi and insects. Of these fungi is a very good sources of chitin. The major classes of fungi which lack chitin are the Schizomycetes, Myxomycetes and Trichomycetes. A critical report on the possible sources of chitin was presented by Allan *et al.* (1978). Antarctic krill with a resource potential of $1x10^8$ to $5x10^8$ tonnes (Naczk *et al.*, 1981) with chitin content of 0.33 to 1.74% is a very good source of chitin.

Structure

Chitin is a long chain polymer of N-ccetyl glucosamine whose units are linked by β - 1-4 bonds. It may be considered as a derivative of cellulose in which C2- hydroxyl group has been substituted by an acetylamino group.



Properties

Chitin is a white solid generally possessing some degree of crystallinity. Most commercial polysaccharides such as cellulose, dextran, pectin, alginic acid, agar, agarose, carragenans and heparin are neutral or acidic, while chitin is the only abundant basic polysaccharide. Chitin is insoluble in water, dilute acids, cold alkalis or organic solvents. Unlike cellulose chitin is insoluble in Schwertzer's (cuprammonium) reagent. It is soluble in hexaflouroisopropanol, hexaflouroacetone (Capozza, 1975), chloroalcohols in conjunction with aqueous mineral acids (Austin, 1975) and dimethyl acetamide containing 5% lithium chloride (Rutherford and Austin, 1978). On hydrolysis with concentrated acid under drastic conditions chitin gives relatively pure aminosugar, D-glucosamine. On deacetylation with strong alkali it yields the free base chitosan. Chitosan is insoluble in water but soluble in dilute acids. Pure chitosan is not hydrolysed by lysozyme while chitin or partially deacetylated chitin is hydrolysed.

Production

Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab processing waste, respectively (Ashford *et al.,* 1978). Madhavan and Nair (1978) observed that dry prawn shell waste contained 23% and dry squilla contained 15% chitin. Table 1 gives an account of yield of chitin from different sources.

Methods of chitin production from raw materials involve deproteinisation followed by demineralisation. Chitin on deacetyation with strong aqueous alkali gives chitosan (Madhavan and Nair, 1974; Anderson *et al.*, 1978; Knapczyk *et al.*, 1989). Chitin and chitosan are produced commercially in Japan, USA, India, Poland and Australia.

Application of chitin and chitosan

Chitosan is a versatile polymer and the importance of chitosan is due to large variety of useful forms that are commercially available. Several other derivatives like hydroxyethyl, carboxy methyl and ethyl chitin can be prepared from chitin by reaction with appropriate reagents. They are all known to be enzymmatically degradable by lysozyme. Chitosan nitrate and chitosan sulphate also can be prepared by nitration and sulphation of chitosan. The application of chitin and chitosan can be classified under the following heads.

1. Clarification and purification
2. Chromatography
3. Paper and textiles
4. Photography
5. Food and nutrition
6. Agriculture
7. Medical and pharmaceuticals

Clarification and purification

The ability of long chain molecules of dissolved chitosan to sediment solid particles suspended in liquids makes it suitable as a coagulant aid. It can remove transition metals (heavy metals), vegetable matter, proteins, suspended solids, etc. from vegetable processing waste, and clarify beverages. The largest commercial

Table 1. **Proximate composition of shellfish waste**

	Shrimp body peeling waste (%)	Shrimp head (%)	Crab waste (%)
A. Dry weight basis			
Chitin	27	13.5-17.5	13-15
Protein (total)	40	29.0-37.5	30-35
Ash (calcium carbonate)	33	25.0-27.0	50
B. Wet weight basis			
Water	75-80	75-80	60
Chitin	5.4-6.7	2.7-4.4	5.2-6
Protein (total)	8-10	5.8-9.4	12-14
Ash (calcium carbonate)	6.6-8.2	5.6-7.5	20

use of chitosan, at present, is in clarification of waste water. The non-food related clarification and purification applications are in the treatment of sewage effluents and gravel wash, metal finishing, electroplating waste, paper mill and radioactive wastes. The important food related applications are in purifying drinking water, recovering protein for uses in animal feeds, clarifying wastes from vegetables, poultry, egg and fish processing, clarifying fruit juices and recovery of micro algae (Standford, 1989; Bough, 1975; Bough and Landes 1976). Chitosan is reported to be a better clarifying agent than potash alum for contaminated water, because it ensures coagulation and sedimentation of bacteria along with other suspended particles.

Chromatography

Townsley (1961) da (1978) and Nageswa *et al.* (1970) used chitosan in thin layer chromatography for separation of nucleic acids. They found that ability of chitin layer to separate mixtures of either phenols, aminoacids, nucleic acids, or inorganic ions was almost equal to that of crystalline cellulose, silica gel and poly amide layers. Muzzarelli and Rocchetti (1974) proposed a method for isolation of vanadium and molybdenum in sea water using chitosan columns.

Paper and textiles

Chitosan is an ideal poymer applicable in paper finishing. Sizing with chitosan improves the wet and dry strength, printability, puncture resistance, water proofness, grease resistance etc. of paper products (Gopal *et al.*, 1981; Kobayashi *et al.*, 1982.

Photography

Chitosan is compatible with gelatin in solution; it can find application in photographic film emulsions to facilitate binding of dyes used in colour processing. Gelatin is also stiffened by this process (Kaneko *et al.*, 1991).

Food and nutrition

Chitosan is used as an ingredient for domestic animal feeds. It is a growth promoter in broiler chicks, pigs and rabbits. No abnormal symptoms were observed during the feeding trials of these animals with feeds incorporated with chitin (Hirano *et al.*, 1997, Nair *et al.*, 1993). Toxicity studies showed that chitosan has an LD-50 value greater than 6g/kg body weight and hence is as safe as salt and sugar. According to US Environmental Agency, chitosan is acceptable for potable water applications (Standford, 1989). The presence of chitin in marine invertebrate, insects, fungi, yeast and in cell walls of certain plants and of chitosan in various fungi indicates that chitin and chitosan are already part of our food supply. Chitosan finds application as thickening and stabilising agents for food (Ando and Nakanara, 1994) and enhances the storage life of meat (Wang, 1994).

Knorr (1984) reported the use of chitosan as a protein coagulant aid to recover protein from food processing waste plant. Chitosan has hypolipidemic and hypocholesterolemic activity (Nagivary *et al.*, 1979; Sugano *et al.*, 1980; Mathew *et al.*, 1994). Level of serum cholesterol, triglycerides and free fatty acids of domestic animals decreased when fed with high fat diet containing 1.2 to 1.4 g chitin/kg body weight.

Agriculture

Chitin and its derivates have potential applications in agriculture like germination and culturing to enhance self protection against pathogenic organisms in plants and supress them in soil to induce chitinase activity and protein inhibitor synthesis, for anti virus activity, in encapsulation of fertilisers, and controlled release of herbicides (Hirano *et al.*, 1984). Chitinous materials are efficient in controlling parasitic nematodes in ornamental plants, cucumber and tomato and root wilt diseases in cotton plants (Brown *et al.*, 1982). Controlled release products offer a wide range of application such as the sustained release of flavours and nutrients, as well as the controlled release of anti microbials or agricultural chemicals.

Medical and pharmaceutical uses

A wide variety of medical applications of chitin and chitosan and their derivatives have been reported over the last three decades. Chitin and its derivatives are useful as bacteristatic and haemostatic agents, drug delivery vehicle, spermicide, surgical glove powder, enzyme immobilisation, film and membrane for dialysis, contact lenses, artifical skin and sponge. Other important applications include hypocholesterolemic agent, antigastritis agent, antisore compositions, antibilirubinic agent and anti-blood coagulant. Glucosamine salts derived from chitin are widely used in medicine. It is found to be an efficient alternative to corticosteroid treatment of enteritis and colitis. Acetylated glucosamine has been found to be effective in treatment of tumour cells. Osteoarthritis can also be effectively treated with glucosamine salts (Brine, 1984). Glucosamine salts are good medicines for hypertrophy and muscular metabolic dysfunctions.

Chitin powder and chitosan containing non-woven fabrics, films and sponges can be used for wound dressings having added wound healing property. Chitosan can be used for treatment of burns. It forms a tough water absorbant and biocompatible film. Chitin fibre has all the characteristics of an ideal surgical suture such as biodegradability, resistance to alkali and digestive enzymes, softness and knot reliability, wound healing acceleration and tissue adaptation. These make it useful for digestive tract and urological surgery. Chitin has found application in the orthopaedic fields such as sutures, temporary artificial ligaments for the knee joint and materials for less rigid fixation. Sapelli *et al.* (1986) and Maida *et al.* (1984) recorded chitin braided filaments, rods and powders for their potential utility in orthopaedics. They opined that chitin promised a temporary bioengineering material pending regeneration of replacement tissues.

Enzyme immobilisation

One of the most important application of chitin is the immobilisation of enzymes and whole cells. A limited number of enzymes are of practical interest for industrial needs, and some of the enzymes that have been immobilised are given in Table 2 (Okei *et al.*, 1986, and Muzzerlli *et al.*, 1986). Mathew (1989) also reported the use of chitosan as an immobilising agent for glucose 6-phosphate dehydrogenase. Some of the advantages using immobilised enzymes include (1) possibility to control enzyme reaction (2) requirement of only small amount of enzyme (3) recovery of enzyme after use (4) absence of product inhibition (5) self digestion of the enzyme (6) possibility to use pathogenic organisms as source of enzyme and (7) convertability of batch

Table 2. **Enzymes/cells immobilised on chitin and its derivatives and proposed uses**

Enzymes/cells	Proposed use
AMP deaminase	AMP to IMP
Amylase diastase and glucoanylase	Starch and glycogen to D glucose
β.D galactosidase	Hydrolysis of lactose
D. Glucose isomerase	D glucose to D fructose and preparation of D gluconic acid
β.D. glucosidase	Hydrolysis of cellobios
Lysozyme	Preparation of pharmaceuticals
Urease	Urea to ammonia and CO_2
E. coli cells	Synthesis of L-tryptophan

process to continuous systems. The most common method of fixing enzymes to chitosan is by cross linking reagents such as dialdehydes (glyoxal and glutaraldehyde). The amino group of chitosan and the enzymes form bonds with dialdehyde and is reduced by sodium borohydride to form stable gels of active immobilised enzymes (Masri *et al.*, 1978). Chandy and Sharma (1992) also reported poly lysine - immobilised chitosan beads as adsorbents for bilirubin.

Cosmetics

Chitosan and water soluble dervatives find extensive use in cosmetic preparations. Chitosan coated liposomes are found to retain water at a greater rate than the naked liposomes (Onsoyen, 1992). A sizable quantity of chitosan is now being used in hair dressing and skin care base cosmetic products.

Conclusion

Chitinous polymers are abundant waste products with an unusual combination of properties. These products are already part of our food supply. However, there are limitations for their food applications. The application of chitin in broiler chicks as a feed additive, surgical sutures, wound healing, encapsulation of medicine, cosmetics, etc. have already reached a stage of commercial exploitation. Some other areas with good prospects are in dentistry and biodegradable films.

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