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NATURALLY DERIVED SUBSTANCES HAVING ANTIMICROBIAL PROPERTIES FOR EXTENDING STORAGE LIFE OF FOOD

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ABSTRACT
Chitosan is polysaccharide in nature with linearity in arrangement and composed of β-(1-4)-linked D-glucosamine (deacetylatedunit) and N-acetyl-D glucosamine (acetylated unit) which remain in a random distribution. Chitin and the derived chitosan having promising antimicrobial properties, both have potential industrial and biomedical implications for treatment of many ailments in human beings.

KEY WORDS: Biomedical, Chitosan, biopolymers, chitin

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INTRODUCTION
The shrimp waste contains biopolymers chitin, chitosan, protein with high economical values. Chitosan [ß-(1→4)-linked *N*-acetyl-D-glucosamine] which is the major structural component of the exoskeleton of crustacean is a non-toxic, biodegradable polymer of high molecular weight, and is very much similar to cellulose. In South-east Asia, the total waste produced is over 2 million metric tonnes per year. (Hossain, 2003). Production cost for 1 kg of chitosan is about US$ 15-20/kg (Stevens *et al*., 1996). 12 kg better quality chitosan is obtained from 200 kg shrimp bio waste (Stevens *et al*., 1998).

Chitosan is a new promising technology developed to kill and inactivate undesirable microorganisms in more environmental friendly way without affecting food quality. Shellfish chitosan from crab and shrimp comprises of 17-32% of the dry weight of the shell. (No and Meyers, 1992) Chitosan emerged from chitin, which is available commercially and used by a lot of people across the globe. Many studies have shown that chitosan is biodegradable polymer (Davies *et al*., 1989). Chitosan is a biomaterial with antiseptic, bioactive, and biocompatible properties (Shigemasa *et al.*, 1994). Chitosan has been approved as functional food in some south-east Asian countries during the last decade. The inclusion of chitin and chitosan was considered in 2003 by the Codex Alimentarius Commission.

Chitosan coating helps to extend shelf life of marine and seafood by reducing microbial load and respiration rate, act as antioxidant and oxygen barrier. The performance of the films will be influenced by the acetic acid (used to soluble chitosan) also. Suitability of the film will also depend on the conditions of storage, namely the temperature and humidity (Bhadra *et al.*, 2012).

Sea food industry suffers from different problems like post-processing contamination by bacteria like *Escherichia coli* and *Staphylococcus aureus* during retail display and handling, Lipid oxidation due to contact with oxygen. The above problems shows the way for future research on the use of chitosan as coating film to improve shelf life of products like PUD shrimps, fillets etc that are kept on retail display (Bhadra *et al.*, 2012).

Keeping this in view, Bhadra *et al*. (2012) performed the study to determine the proximate composition of shrimp waste, to extract chitosan from shrimp waste for determining its antimicrobial activity on *Staphylococccus aureus* and *E. coli* to assess the storage life of *P. monodon* coated with chitosan film.

Chitosan for use in industrial purpose is extracted from the chitin after deacetylation of chitin which is the major structural element of the exoskeletons of crustaceans and lobsters, prawns and cell wall of fungi.

Wide range of applications
Chitosan finds its application in water treatment plants as a part in the filtration process. It helps in the removal of hardness by the removal of sediments during salt filtration by adsorption of phosphorus, heavy minerals and oils from the water in combination with bentonite, gelatin, silica gel, isinglass or other refining agents. Chitosan also helps in clearing the turbidity in water and also used as a precipitant for casein used for manufacturing cheese from milk.

Biomedical importance
Chitosan has the property to clot blood and so also used in haemostatic agents for acceleration of wound healing property in humans. Chitosan also possess hypoallergenic and has natural antibacterial properties, which further support its use in field bandages. The flexible nature of chitin is used for making strong and tensile surgical threads having high biodegradability and wound healing property.The antimicrobial property of chitosan is attributed to its slightly acidic pH (Bhadra *et al.*, 2012).

Chotosan in ultrapure form has numerous biomedical implications. Chitosan has mucoadhesive property and so used for intradermal and sustained drug delivery (Agnihotri *et al.*, 2004), for example, in the administration of insulin in insulin dependent diabetes mellitus. Chitosan is biocompatible and biodegradable which enhances the polar drug transport across epithelial membranes and surfaces (Baldrick, 2009).

Proven research fact
Chitosan coating serve as an antioxidant and micro-diffusion barrier and prevents the loss of water, texture, odor and color thereby improving the overall accessibility of the seafood. The shelf life of *Penaeus monodon* coated with chitosan dips extends the shelf life of shrimp (Bhadra *et al.*, 2012).

Chitosan also helps in increased lipid excretion from the body system after interaction and binding with oily components in the digestive tract. Its fat binding property helps in decrease of body mass index especially in obese patients who are advised to be supplemented with the chitosan for nearly 8 weeks continuously for encouraging results. Chitosan possesses the functional antioxidant, antimicrobial and oxygen barrier properties (Fan *et al.*, 2008). No *et al.* (2002) reported that 1% chitosan concentration can retard gram negative bacteria like *E. coli*, *Vibrio*, *Salmonella* etc. The effect of chitosan may be due to the change in the outer membrane of *E. coli* cell, thereby affecting the barrier properties of bacterial cell (Helander *et al.*, 2001). Sudarshan *et al.* (1992) reported that leakage of intracellular material was one of the mechanisms of chitosan actions which mainly signify the present study. No *et al*. (2002) reported the use of chitosan is effective against Gram-positive bacteria like *Staphylococcus aureus*. Muzzarelli *et al.* (1990) reported the effect of chitosan and its derivatives in inhibiting *Staphylococcus aureus* and other gram-positive bacteria. The antibacterial mechanism of chitosan may be attributed to the interaction of charged amino group of chitosan with negatively charged microbial cell membrane leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms. (Shahidi et *al*., 1999).

Seafood provides a good niche for growth of different microorganisms. The initial total plate count of fresh *P. monodon* samples were 3.02 log CFU/g. Chitosan coatings resulted in upto 2-3 log reductions in total plate count between coated samples and control after 12 days of storage of herring and cod in refrigerated storage condition. Chitosan coating significantly lowered the TPC in fish samples ($P<0.05$) with 0.60-1.19 log CFU/g reduction being obtained in coated samples and the TPC of coated samples were below 10^7 CFU/g during first 2 weeks of cold storage. In the present study chitosan coatings resulted in 3-4.5 log CFU/g reduction in total plate count at $4^{o}\pm1^{o}C$ storage temperature as compared to uncoated one (Bhadra *et al.*, 2012).

Chitosan coating consisting of a blend of acetic acid and 1% chitosan exerts an inhibitory effect on the gram-negative flora of fish patties. Various factors affect the antimicrobial action of chitosan and its mechanism of action appears to be related to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membrane and act as a barrier against oxygen transfer. From the results of this study, it was concluded that chitosan coating has a significant ($P<0.05$) advantage in retarding the growth of bacteria. From the result of the study indicated that 1% chitosan solution coating was effective as 2% chitosan coated samples for extending the shelf life at 4º±1ºCstorage temperature, attributing to the better inhibitory effect of 1% chitosan on spoilage bacteria (Bhadra *et al.*, 2012).

Antimicrobial properties

Low molecular weight chitosan seemed to be a more effective inhibitor of microbial growth for some organisms such as *E. coli*. Although chitosan generally show stronger bactericidal effect on gram positive bacteria, in the present study it is found to be effective in inhibiting the gram negative bacteria *E .coli* at a concentration of both 1% and 2% level. The effect of chitosan may be due to the change in the outer membrane of *E. coli* cell, thereby affecting the barrier properties of bacterial cell. Leakage of intracellular material was one of the mechanisms of chitosan actions which mainly signify the present study (Bhadra *et al.*, 2012).

The use of chitosan is effective against Gram-positive bacteria like *Staphylococcus aureus*. Effect of chitosan and its derivatives is shown in inhibiting *Staphylococcus aureus* and other Gram-positive bacteria. The antibacterial mechanism of chitosan may be attributed to the interaction of charged amino group of chitosan with negatively charged microbial cell membrane leading to the leakage of proteinaceous and other intracellular constituentents of the microorganisms (Bhadra *et al.*, 2012).

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