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A Review on Application of Chitosan as a Natural Antimicrobial

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***Abstract*—**In recent years application of natural antimicrobials instead of conventional ones, due to their hazardous effects on health, has got serious attentions. On the basis of the results of different studies, chitosan, a natural bio-degradable and non-toxic biopolysaccharide derived from chitin, has potential to be used as a natural antimicrobial. Chitosan has exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms, including fungi, and Gram-positive and Gram-negative bacteria. The antimicrobial action is influenced by intrinsic factors such as the type of chitosan, the degree of chitosan polymerization and extrinsic factors such as the microbial organism, the environmental conditions and presence of the other components. The use of chitosan in food systems should be based on sufficient knowledge of the complex mechanisms of its antimicrobial mode of action. In this article we review a number of studies on the investigation of chitosan antimicrobial properties and application of them in culture and food mediums.

***Keywords*—**Antimicrobial**,** Chitosan, Preservative

I. Introduction

CHITIN, after cellulose, is the most abundant polysaccharide in nature, and is primarily present in the exoskeletons of crustaceans (such as crab, shrimp, lobster etc.) and also in various insects, worms, fungi and mushrooms in varying amount. In addition, recent advances in fermentation technology suggest that the cultivation of fungi can provide an alternative source of chitosan. Chitin makes up to 45% of the cell wall of *Aspergillus niger* and *Mucor rouxii* and 20% of the cell wall of *Penicillium notatum* [1]. Fungal culture media and fermentation condition can be manipulated to provide chitosan of more consistent physico-chemical properties compared to that derived chemically from chitin [2]. For example, Chatterjee et al. (2005) isolated chitosan from *Mucor rouxii* cultured in molasses salt medium.

Today, preference of consumers for foods without chemical preservatives has led to the discovery of new natural antimicrobial agents. The antimicrobial and antifungal activities of chitosan and its degradation products such as, chitooligomers and low molecular weight chitosans have been studied by several researchers, with particular emphasis on their ability as a food preservative [3].

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The present review will summarize critical properties of chitosan affect its antibacterial ability, and a number of new efforts on usage of chitosan as additive or antibacterial packaging.

II. General Properties

The chemical structure of chitin [poly-β-(1 → 4)-N-acetyl-D-glucosamine] is similar to cellulose, with this difference that in chitosan one hydroxyl group on each monomer has substituted with an acetylamine group. Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin (Fig. 1) with the average molecular weight 1.0 to 5×10^5 Da [4]. Deacetylation step often performed using concentrated alkaline solution at elevated temperature for defined periods. Two the most important physicochemical characteristics of chitosan are its degree of deacetylation (DDA) and the molecular weight. The DDA has influence on all the physicochemical properties such as molecular weight, viscosity, solubility, etc. This parameter can also influence the solubility of the polymer in organic or aqueous solvents. By increasing the DDA, the solubility increases. Molecular weight affects antibacterial properties [5],[6].

Chitosan is insoluble in most organic solvents and in water at neutral pH, but dissolves in dilute solutions of organic acids such as acetic, formic, tartaric, valeric, lactic, glycolic, and citric acids and also dissolves in dilute inorganic acids such as hydrochloric and sulfuric acids. Water-insolubility of chitosan is disadvantageous for its wide application as an antibacterial agent [7]. In the recent decades, extensive investigations have been carried out to prepare functional chitosan and to increase its solubility in water in order to broaden its application. One way is modification of production process of chitosan. Qin et al. (2006) found that optimum chitosan as food preservative should be water-insoluble chitosan from mild depolymerization of native chitosan. Another way is modification of chitosan with derivatioztion which explain more below.

III. Antimicrobial Activity

Researchs on the possibility of developing chitosan as a natural antimicrobial have increased especially in tow decades. Chitosan can be applied to extend the storage life of different categories of food materials. Martín-Diana et al. (2009) incorporated chitosan in unpasteurised orange juice and evaluated of quality and nutritional markers. Their results recommend the use of chitosan at concentrations up to 1 g L^{-1} to extend quality and preserve ascorbic acid and carotenoids during storage time of fresh orange juice, thus avoiding the

use of standard thermal treatments which produces a negative impact on the nutritional value. Coma et al. (2003) suggested the application of chitosan as preservative in dairy products. Devlieghere et al. (2004) investigated the influence of food component on chitosan activity and concluded that the effect of chitosan as an antimicrobial preservative for food will be limited to food products with low protein and NaCl content. Table 1 summarizes the results of experiments that have used chitosan as antibacterial and antifungal additive.

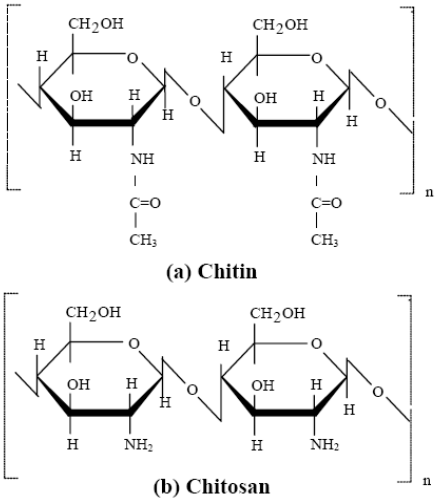

Fig. 1 Structure of chitin and chitosan

A number of researchers have focused on the antibacterial action modes of chitosan and its derivatives. The tow major suggested mechanism are: (1) The binding of cationic chitosan to sialic acid in phospholipids, and consequently restraining the movement of microbiological substances, and (2) penetration of oligomeric chitosan into the cells of microorganisms and prevention the growth of cells by preventing the transformation of DNA into RNA [7]. Chung et al. (2004) demonstrated the relationship between the antibacterial activity of chitosan and the surface characteristics of the cell wall of Gram-positive and Gram-negative bacteria. They saw that more adsorbed chitosan, due to more negatively charged cell surfaces, would result in greater changes in the structure of the cell wall and in the permeability of the cell membrane. As a result, Gram-negative bacteria due to their more negatively charged cell surfaces are more susceptible to chitosan, and sensitivity of the Gram-positive bacteria was highly variable. Latter, Raafa et al. (2008) demonstrated that the mode of action of chitosan is probably more complex than simple interaction of chitosan with cell membrane, and it involves a number of events, which may ultimately lead to a killing process. Analysis of transcriptional response data in their study revealed that chitosan treatment leads to multiple changes in the expression profiles of *Staphylococcus aureus* SG511 genes involved in the regulation of stress and autolysis, as well as gene associated with energy metabolism.

Important parameters affecting antimicrobial activity of chitosan are the molecular weight (MW) and concentration of chitosan, and type of the microorganism. Jeon and kim (2000) reported the MW of chitooligosaccharides is critical for microorganism inhibition and required to be higher than 10 kDa. No et al. (2002) found chitosan has higher antimicrobial activity than chitosan oligomers at a 0.1% concentration. In a study, Liu et al. (2006) evaluated the antibacterial properties of chitosans with different MW (55 to 155 kDa) but with same degree of deacetylation (80% ± 0.29), against *E. coli* with different concentrations. According to their result, all chitosans had antibacterial avtivity at concentrations over 200 ppm, thought the antibacterial activity of low MW chitosan was higher than that of the high MW samples. Omura et al. (2002) measured the antimicrobial activity of chitosan and chitooligosaccharides with different MW without acetylated groups. They observed high MW chitosans showed strong antimicrobial activity against Gram-positive bacteria, whereas chitosans of 11 kDa and 20-30 kDa molecular weights were most effective against Gram-negative bacteria. Zheng and Zhu (2003) reported that the effect of chitosan with MW below 300 kDa on *Staphylococcus aureus* was strengthened as the MW increased, whereas the antimicrobial effect on *E. coli* increased as the MW was decreased.

IV. Derivatization Of Chitosan

Chitosan possesses three types of reactive functional groups: an amino group at the C-2 position of each deacetylated unit, as well as primary and secondary hydroxyl groups at the C-6 and C-3 positions, respectively, of each repeat unit (Fig. 1). These reactive groups are readily subjected to chemical derivatization under mild conditions for improving compatibility and mechanical and physicochemical properties of chitosan [17]. Water soluble derivatives, which can be attained by chemical introduction of CH_3 in the main chain, enhancing the chitosan applicability in a large pH range and also improve the antimicrobial activity [18]. The method and ingredients using for derivatization extensively depend to the application of derivatized chitosan. For example, Cyclodextrin linked chitosan is interesting for the viewpoint of pharmaceutics, including drug delivery, cosmetics, and analytical chemistry and Quaternized chitosan has potential as an absorption enhancer across the intestinal epithelium due to its mucoadhesive and permeability enhancing properties [7].

N-alkylation reaction of chitosan at the C-2 position with disaccharides including lactose, maltose and cellobiose effectively enhanced the solubility of chitosan [19]. Yang et al. (2005) investigated water-soluble N-alkylated disaccharide chitosan derivatives against *Escherichia coli* and *S. aureus.* They showed that *E. coli* and *S. aureus* were most susceptible to cellobiose chitosan derivative DS 30–40% and maltose chitosan derivative DS 30–40%, respectively. Antibacterial activity of the chitosan derivatives (DS 30–40%) against *E. coli* increased as the pH increased from 5.0 and reached a maximum around the pH of 7.0–7.5. Furthermore, the N-alkylated disaccharide chitosan derivatives tested showed a higher antibacterial activity than the native chitosan at pH 7.0. Li et al. (2011) used xylan for doing N-alkylation reaction and reported the antimicrobial activity of the xylan–chitosan conjugate was higher than chitosan.

Methylation is the other mechanism for functionalizing of chitosan. Rúnarsson et al. (2007) synthesized series of methylated chitosaccharide derivatives that had antibacterial effects at pH 7.2, whereas they did not contribute to the antibacterial activity under acidic conditions. Vallapa et al. (2011) using a tow step process (reductive alkylation and methylation) introduced quaternary ammonium groups to the chitosan surface and could significantly improve the antibacterial activity of the chitosan, especially in a neutral environment.

Liu et al. (2000) prepared a kind of derivatized chitosan, *N,O*-carboxymethylated chitosan, that had higher antibacterial activity against *E. coli*. than underivatized chitosan. Recently, other derivatized chitosans such as salicyloyl chitosan [25], O-fumaryl-chitosan [26], arginine-functionalized chitosan [27] and guanidinylated chitosan derivatives [28] have been reported to have more ability to inhibition of bacterial growth and higher solubility than underivatized chitosan.

TABLE 1
THE POTENTIAL OF CHITOSAN AS ANTIBACTERIAL AND ANTIFUNGAL ADDITIVE

Activity	Microorganism	Product	Result	Ref.
Antibacterial				
	L. monocytogenes	ready-to-eat roast beef	2–3 log CFU/g reduction after 14 days	[29]
	Pseudomonas and *Vibrionaceae*	Refrigerated Pacific oysters	Increasing the shelf-life from 8–9 days to 14–15 days.	[30]
	L. monocytogenes, *Escherichia coli* 0157:H7 and *S. typhimurium*	Agar containing culture medium (diffusion assay)	Chitosan biopolymer films were more effective at inhibition of *L. monocytogenes* than *S. typhimurium* and *E. Coli O157:H7*	[31]
	Staphylococcus aureus, salmonella enterica serovar typhimurium, and *vibiro vulnificus*	Raw oysters	S. aureus growth was strongly inhibited in raw oysters coated with 2% chitosan	[32]
	Vibrio parahaemolyticus	Shrimp	Chitosan has the ability to replace chlorine for decontamination of seafoods	[33]
	Aeromonas hydrophila		Growth and haemolysin production were clearly suppressed at 0.04% of chitosan. Ions reduced chitosan's activity	[34]
	Bacillus cereus	cooked rice	Low-molecular-weight chitosan effectively inhibited growth of *B. cereus* in cooked rice.	[35]
	Listeria monocytogenes, Salmonella spp. and *Staphylococcus aureus*	Laboratory media and fish soup	The application of chitosonium acetate as an internal coating of the packaging material could be a very suitable means to assure safety of liquid food products such as fish soup	[36]
	L. monocytogenes	ham steaks	Chitosan-coated plastic films containing 0.001 g/cm^2 of sodium lactate have a potential to be used on ham steaks to control *L. monocytogenes.*	[37]
Antifungal				
	Candida lambica and *Cryptococcus humicolus*	Culture medium	Chitosan activity depend to NaCl and protein content	[11]
	Aspergillus flavus, Cladosporium cladosporioides, Mucor racemosus, Penicillium aurantiogriseum, Byssochlamys spp., *Saccharomyces* spp. and *Zygosaccharomyces bailii*	Culture medium and apple juice	Growth inhibition and inactivation was concentration-, pH- and temperature-dependent. *Zygosaccharomyces bailii* is the most sensitive and *Saccharomycodes ludwigii* is the most resisitance starin	[38]
	Alternaria sp, *Penicillium* sp, and *Cladosporium* sp	as an edible film and as a dough ingredient in precooked pizza	Use of chitosan as edible coating delayed the fungal growth but was not effective into the dough	[39]

V. Chitosan As Antibacterial Film And Blending It With Other Packaging And Coating Materials

A promising form of active packaging for extending the shelf life of foods is antimicrobial packaging. Chitosan not only has been applied for preparation of films itself, but also it has been incorporated into other packaging materials and used for preserving and extending the shelf life of foods. Kim et al. (2011) used chitosan alone and prepared chitosan biopolymer films. They showed the oxygen permeability of the films increased with increasing viscosity of chitosans, but water vapor transmission rate was not similarly affected. The combination of chitosan with other polymers invstigates below.

A. Synthetic polymers

Park et al. (2010) incorporated chitosan as an antimicrobial additive into low density polyethylene (LDPE) with different concentrations. These chitosan incorporated films were applied on red meat surfaces to determine the microbial growth inhibition, and unexpectedly saw that microorganisms on the meat surface were not inhibited. They suggested that

interactions between chitosan and meat components may reduce the amount of chitosan interacting with microorganisms on meat surface. Ethylene copolymer film was coated with chitosan by attachment of the polymer to the corona-treated surface of the film, and the composite film was analyzed for antimicrobial activity [41]. The film was active against bacteria in 0.625 mM phosphate buffer and reduced colony counts of *Escherichia coli* 25922 and of *Listeria monocytogenes* Scott A by 5 and 2–3 $\log_{10}$, respectively, after 24 h exposure. Fernandez-Saiz et al. (2010) investigated the morphology, water barrier and the antimicrobial activity of high and low molecular weight chitosonium-acetate based solvent-cast blends with ethylene–vinyl alcohol (EVOH) copolymers. They reported that the blends based on the low molecular weight chitosan grade have enhanced phase morphology, transparency, enhanced water barrier properties, up to 86% water permeability reduction compared to pure chitosonium-acetate films, as well as excellent antimicrobial activity. Poly(vinyl alcohol) (PVA) a biodegradable, synthetic polymer, innocuous, non-carcinogenic and have good biocompatible properties. Tripathi et al. (2009) developed a novel antimicrobial coating based on chitosan and PVA and evaluated its effect on minimally processed tomato. The results indicated the film may be a promising material for food packaging applications.

B. Natural polymers

Modification and enhancement of natural and environmental friendly polymers for replacing synthetic polymers is very interested for many scientists. Sebastien et al. (2006) prepared composite films from chitosan and poly(lactic acid) (PLA) by solution mixing and a film casting procedure. This first study showed that the PEG improved the mechanical properties of the chitosan and PLA films by increasing the flexibility of materials but decreases their water vapor barrier properties. Also, taking into account the interesting mechanical and barrier properties of PLA films, due to the relatively hydrophobic nature of the polymer, PLA would be a good candidate to associate with the chitosan. Liu et al. (2009) produced antibacterial membranes from a mixture of hydrolyzed starch and chitosan. The elongation-at-break and water vapor transmission rate of starch/chitosan blending membranes were largely improved compared with each single component due to the interaction formed between the hydroxyl groups of starch and the amino ones of chitosan. Shih et al. (2009) investigated cellulose/chitosan blend films preparation and examined the mechanical and antimicrobial properties of them. Chitosan-gelatin copolymer has been also used for coating of chilled cod patties [47] and meat products [48].

VI. CONCLUSION

Chitosan antibacterial and antifungal potency in culture and food systems has been affirmed by several studies. Its unique preiperties, antimicrobial ability and good film-forming properties, separated it from other degradable polymers.

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