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Review

Review of Antimicrobial and Antioxidative Activities of Chitosans in Food

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ABSTRACT

Interest in chitosan, a biodegradable, nontoxic, non-antigenic, and biocompatible biopolymer isolated from shellfish, arises from the fact that chitosans are reported to exhibit numerous health-related beneficial effects, including strong antimicrobial and antioxidative activities in foods. The extraordinary interest in the chemistry and application in agriculture, horticulture, environmental science, industry, microbiology, and medicine is attested by about 17,000 citations on this subject in the Scopus database. A special need exists to develop a better understanding of the role of chitosans in ameliorating foodborne illness. To contribute to this effort, this overview surveys and interprets our present knowledge of the chemistry and antimicrobial activities of chitosan in solution, as powders, and in edible films and coating against foodborne pathogens, spoilage bacteria, and pathogenic viruses and fungi in several food categories. These include produce, fruit juices, eggs and dairy, cereal, meat, and seafood products. Also covered are antimicrobial activities of chemically modified and nanochitosans, therapeutic properties, and possible mechanisms of the antimicrobial, antioxidative, and metal chelating effects. Further research is suggested in each of these categories. The widely scattered data on the multifaceted aspects of chitosan microbiology, summarized in the text and in 10 tables and 8 representative figures, suggest that low-molecular-weight chitosans at a pH below 6.0 presents optimal conditions for achieving desirable antimicrobial and antioxidative-preservative effects in liquid and solid foods. We are very hopeful that the described findings will be a valuable record and resource for further progress to improve microbial food safety and food quality.

Chitin, a component of the exoskeletons of insects and *Crustacea* including crab and shrimp, consists of *N*-acetylglucosamine residues joined by β-1,4-glycosidic links. It is the second most abundant biopolymer in the world after cellulose. Structurally, chitin (poly-*N*-acetylglucosamine) resembles cellulose, except that the substituent at the C-2 atom is an acetylated amino (–NH–CO–CH_3) instead of a hydroxyl (–OH) group. Deacetylation is achieved by exposing chitin to strong NaOH solutions or to the enzyme chitinase (Fig. 1). Interest in chitin resides in the fact that its deacetylated product called chitosan exhibits desirable functional and biological traits, including antimicrobial and antioxidative properties, and that there is the possibility of its addition to the generally recognized as safe (GRAS) list in the United States *(249)*.

Chitosan exhibits strong antimicrobial effects against a variety of pathogenic and spoilage organisms, reviewed in *(67, 168, 189, 210, 227)*. Numerous studies have been carried out on the antimicrobial, antifungal, and antiviral effects of chitosan and chitosan derivatives *(11, 14, 105, 116, 121, 122, 145, 147, 148, 156, 176, 186, 238, 268, 272, 273)* as well as chitosan coatings and films *(67, 136, 160, 174, 178, 181–183, 252)*. The molecular size of chitosan, which may range from about 2,000 to more than 100,000 Da, as well as particle size *(35, 185)* and pH, also influences biological activity *(128, 173, 205, 222, 248, 277)*.

The objective of this overview is largely limited to outlining and summarizing reported studies on the beneficial effects of chitosan on microbial safety and quality of cereal and dairy products, fruits, fruit juices, vegetables, meat and poultry products including eggs, and seafood. Also covered are antimicrobial nanochitosans, metallochitosans, chitosan-treated antimicrobial fabrics, potential therapeutic uses for infectious and other diseases, possible mechanisms of beneficial antimicrobial and antioxidative effects, and food-related research needs. What follows is a discussion of antimicrobial effects of chitosans in laboratory media and on or in different food categories in alphabetical order.

ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITIES OF CHITOSANS IN LABORATORY MEDIA

Chitosan films and coatings. Because some of the cited observations may stimulate interest in food applications, we first briefly mention reported studies on antimicrobial effects of chitosans in the absence of food. Chitosan films and coating with and without antimicrobial additives have been extensively evaluated for their ability to inhibit pathogenic organisms. Detailed discussion of the factors that influence

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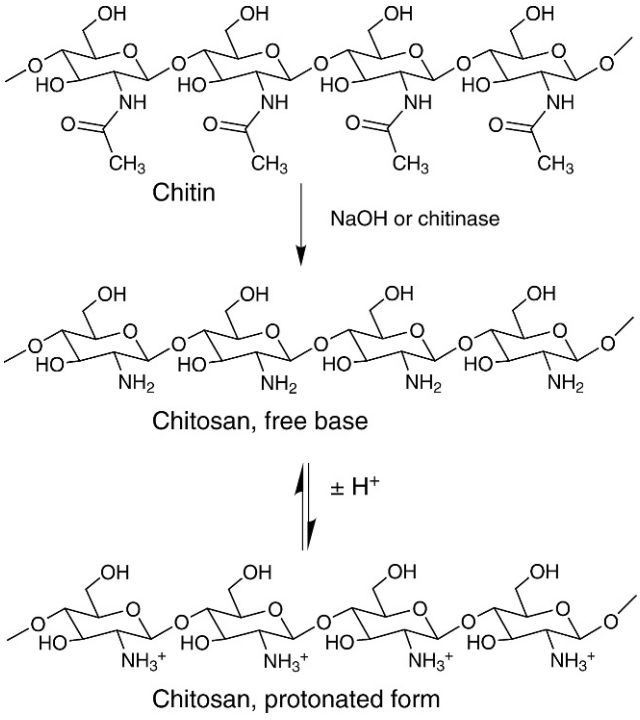

FIGURE 1. *Deacetylation of chitin to chitosan and acid-base equilibrium of chitosan. The antimicrobial effect of the free base is postulated to involve chelating to trace elements and metalloenzymes, and of the protonated form to disruption of cell membranes.*

antimicrobial activities of the films in laboratory media is beyond the scope of this review. For convenience, we have summarized the salient aspects in Table 1. This and the other tables list the following information: nature of the chitosan formulation, the antimicrobial assay used, the pathogens that were inhibited, the possible mechanism of the observed effects, observed outcomes, and the relevant references. These references provide an entry into the widely scattered literature on the potential beneficial role of chitosan in the diet.

Nanochitosans. Because nanosized composites (nanoparticles, nanomaterials) are expected to be more effective in penetrating and disrupting bacterial cell membranes, they may have great potential for use in the ongoing battle against pathogenic bacteria *(262).* However, except for water, no information is available on the use of chitosan nanoparticles as antimicrobial agents in foods. Table 2 shows that nanochitosans are effective against a range of foodborne and spoilage organisms, and that added silver salts or other functional antimicrobial agents to chitosan nanoparticle composites enhance antimicrobial activity. These pioneering observations suggest the potential for use of nanochitosans against pathogens on or in contaminated foods. This aspect awaits future studies.

A word of caution is in order. As noted by Das et al. *(52),* because nanoparticles may possess new chemical and physical properties as compared with normal macroparticles of the same composition, they may interact with and penetrate living tissues and fluids differently, possibly causing toxicity. Studies designed to ascertain the value of nanochitosans as antimicrobial agents in or on contaminated foods should therefore include a risk assessment.

ANTIMICROBIAL AND ANTIOXIDATIVE ACTIVITIES OF CHITOSANS IN FOOD PRODUCTS

Cereals, legumes, and eggs. Grains and legumes are occasionally contaminated with two major environmental hazards: toxic weed seeds *(48, 65, 74, 79–81, 85),* and foodborne pathogens and spoilage organisms *(239).* Below we mention efforts to overcome microbial contaminants with the aid of chitosan in cereal and legume products. The experimental aspects are summarized in Table 3.

(i) Bread. Feeding of bread coated with chitosan to diabetic subjects improved the high-density-lipid to low-density-lipid cholesterol profiles *(13, 123).*

(ii) Noodles. Addition of 0.05 g of chitosan per 100 ml of acetic acid to fresh noodles resulted in extension of shelf life for 6 more days during storage at 4°C *(102).* The chitosan treatment also inhibited the growth of bacilli.

(iii) Pasta. The combined effect of chitosan and modified atmosphere packaging improved the microbiological safety of amaranth-based homemade fresh pasta *(53, 54).* The treatment inhibited the growth of mesophilic bacteria, *Staphylococcus* spp., yeasts, molds, and total coliforms during storage at 4°C for 2 months. The cited effects and the compatible sensorial properties of the chitosan-added pasta suggest the potential of the nonthermal preservation for large-scale use.

(iv) Rice. Treatment prior to vacuum packaging with 1 and 2% solutions of 37-kDa chitosan extended the shelf life of rice cakes and rice noodles *(142),* reviewed in *(168).* Black rice cultivars contain strong antioxidative compounds, which merit study for potential antimicrobial properties in food *(164–166).*

(v) Chickpeas. Chitosan at low pH inhibited the growth of spoilage organisms (*Candida* sp., *Zygosaccharomyces bailii*) in a chickpea (hummus) dip *(205).*

(vi) Eggs. Table 3 summarizes the available information on eggs. Hard-boiled eggs coated with a chitosan–lysozyme composite coating inhibited the growth of injected *Listeria monocytogenes* and *Salmonella enterica* as well as multiplication of coliforms, molds, and yeasts *(126, 131).* The treatment also retarded moisture loss and pH changes during storage at 10°C. The authors conclude that chitosan–lysozyme-based coatings can enhance microbial safety and extend shelf life of hard-boiled eggs by controlling postprocessing contamination and delaying undesirable changes in egg quality. A related study *(131)* showed that chitosan coating extended the shelf life and quality of eggs. The total amino acid content of albumen and fatty acid composition of noncoated and chitosan-coated eggs was the same after storage for 5 weeks. Similar observations are reported by Su et al. *(240).* Added chitosan inhibited the growth of spoilage organisms *Serratia liquefaciens* and *Z. bailii* in egg-containing mayonnaise stored at 25°C *(169).*

Dairy products. Here, we briefly outline reported studies on the application of chitosan-based films and coatings, and free chitosan to improve the shelf life, quality,

TABLE 1. *Antimicrobial and antioxidative activities of chitosan films against foodborne pathogens in the absence of food*

Target	Chitosan type used	Application/assay	Microorganism(s) inhibited	Antimicrobial mechanism	Outcome/significance	Reference
Bacteria	Chitosan-lysozyme composite films	Petri dish assay	*E. coli, S. faecalis*	Release of lysozyme	Lysozyme enhanced chitosan activity	*64*
Bacteria	Chitosan–hydroxypropyl methyl cellulose films	Solid medium enumeration	*L. monocytogenes*	Only thermally treated films were effective	FTIR and NMR were used to study chitosan chemistry[a]	*160*
Bacteria	Chitosan films with garlic oil, potassium sorbate, and nisin	Agar diffusion method	*B. cereus, E. coli, L. monocytogenes, S. aureus, Salmonella* Typhimurium	Inhibition zone surrounding film strips	Added garlic oil enhanced activity	*182*
Bacteria	Glucomannan films plus chitosan and nisin	Agar diffusion method	*B. cereus, E. coli, L. monocytogenes, S. aureus*	Chitosan improved antimicrobial effects	Films had good physical properties	*143*
Bacteria	Chitosan–cassava starch–oregano oil	Disk inhibition zone assay	*B. cereus, E. coli, S. aureus, Salmonella* Enteritidis	Oregano oil improved antimicrobial effect	Good film physical properties	*178*
Bacteria	Chitosan–tapioca starch–potassium sorbate films	Agar well diffusion assay	Lactobacilli, *Z. bailii*	Not effective against lactobacilli	Useful barrier against *Z. bailii* contamination	*252*
Bacteria	Quaternized chitosan	Petri dish assay	*L. monocytogenes, Salmonella* Typhimurium	Positively charged side chains enhance activity/solubility	Quaternization produced water-soluble agent	*19*
Bacteria	Chitosan-lactoferrin film	Agar diffusion assay	*E. coli* O57:H7, *L. monocytogenes*	Added lactoferrin and lysozyme inhibited growth	Apparent synergism of antimicrobials in chitosan films	*23*
Bacteria	Chitosan-divergicin film	Agar diffusion–critical microdilution	*L. monocytogenes*	Additive effect chitosan and divergicin	Activity a function of molecular weights	*20*
Bacteria	Chitosonium acetate	Macrodilution method	*Salmonella* spp., *S. aureus*	Release of positive glucosamine fractions	Method permits standardization of antimicrobial assays	*70*
Bacteria	Chitosans of different molecular weight	Cell fluorescence assay membrane disruption	*Campylobacter* serotypes	Disruption of cell membranes	MIC values ranged from 0.005 to 0.05%	*89*
Bacteria	Chitosan-silver films	Agar diffusion methods; electron microscopy	*S. aureus*	Films caused elongation and disruption of cells	Images show chitosan antimicrobial effects	*59*
Bacteria	Chitosan in sweet potato starch films	Inhibitory zone method	*E. coli, S. aureus*	Added chitosan inhibited pathogens	Chitosan enhanced film tensile strength	*229*
Bacteria	Chitosan–vinylsulfonic acid–sodium salt	Agar dilution method	*Micrococcus luteus, Achromobacter xylosoxidans*	Good antimicrobial and metal chelating properties	Water-soluble chitosans	*33*
Bacteria	Chitosan-flavonoid derivatives	AATCC antimicrobial test 100-2004[b]	*B. subtilis, K. pneumonia, P. aeruginosa, S. aureus*	Flavonoids increased antioxidative and antimicrobial effects	More effective against gram-positive than against gram-negative bacteria	*238*
Bacteria, fungi	Chitosan–tree tea composite film	Petri dish assay	*L. monocytogenes, Penicillium italicum*	Significant antimicrobial and limited antifungal activities	Tea tree oil improved mechanical properties of films	*216*
Fungi	Chitosan coating	Spore assay in liquid medium	*Aspergillus niger*	Both lag and stationary phases were inhibited	High inhibitory activity at low concentrations	*225*

[a] FTIR, Fourier transform infrared spectroscopy; NMR, nuclear magnetic resonance.
[b] AATCC, American Association of Textile Chemists and Colorists.

TABLE 2. *Antimicrobial activities of nanoscale chitosans (nanochitosans)*

Target	Chitosan type used	Application/assay	Microorganism(s) inhibited	Antimicrobial mechanism(s)	Outcome/significance	Reference
Bacteria	Nanosilver-chitosan films	Inhibition zone method	*S. aureus, E. coli, L. monocytogenes, Salmonella* Typhimurium	Activity depended on nanoparticle type	Prepared by solvent-casting method	*204*
Bacteria	Chitosan-silver nanoparticles	Green fluorescent protein	*E. coli*	Attached bacteria fragmented rapidly	Growth of bacteria stopped after exposure to chitosan	*219*
Bacteria	Chitosan-silver porous nanoparticle films	Disk diffusion and viable cell count method	*Bacilli* sp., *E. coli, K. pneumoniae*	Porosity of films enhanced antimicrobial effects	Superior antimicrobial properties	*253*
Bacteria	Chitosan–polyvinyl alcohol nanofibers	Shake flask method	*S. aureus, E. coli*	$AgNO_3$-TiO_2 enhance activity	Total inhibition of bacteria	*234*
Bacteria	Chitosan magnetic nanoparticles	Bioluminescence	*E. coli, Vibrio* strains	Inhibition of bacterial signaling	Enhanced activity via magnetic effect	*69*
Contaminated water	Chitosan nanoparticles	Flocculation	Multiple organisms	Trace element chelation; membrane damage	Limitation of antimicrobial effect due to aggregation	*144*

TABLE 3. *Antimicrobial and antioxidative activities of chitosans in and/or on cereal and legume products and eggs*

Target	Chitosan type used	Application(s)/assay(s)	Microorganisms inhibited	Antimicrobial mechanism	Outcome/significance	Reference
Noodles, fresh	0.5% chitosan and chitosan-xylose suspensions	Agar dilution method, MIC	*B. cereus, E. coli, P. fluorescens, S. aureus, Salmonella* Typhimurium, *Vibrio parahaemolyticus*	Growth inhibition	Extended shelf life of noodles up to 14 days	*102*
Pasta, amaranth, fresh	Chitosan and modified atmosphere packaging	Chitosan mixed with pasta dough; plate count agar method	*Staphylococcus* spp., *Mesophilic* bacteria	Growth inhibition	Microbial quality controlled for 2 mo; gluten-free cereal	*54*
Rice cake	Chitosan solution	None	None	None	Extended shelf life	*142*
Chickpea dip, hummus	Chitosan, 5g/kg, mixed with food	Agar plate assay and absorbance at 620 nm	*Bacillus* sp., *Candida* sp., *P. fragi, S. ludwigii, Z. bailii*	Growth inhibition during storage at 7°C for 7 days	Reduced bacterial but no yeast count	*205*
Eggs, hard boiled	Chitosan-lysozyme coating	Eggs inoculated with bacteria; total plate count assay	*L. monocytogenes, S. enterica,* molds, yeasts	Growth inhibition	4-log reduction of *S. enterica* after 4-wk storage; reduced weight loss	*126*
Eggs	Chitosan-glycerol, propylene glycol, sorbitol coatings	Brushing, dipping, spraying of chitosan on eggs	None	None	Shelf life of eggs extended by ~3 wk	*130*

and microbial safety of cheese, milk, and yogurt (Table 4). Selected studies in this area include the following findings.

(i) Cheese. Chitosan-based edible coating adjusted to pH 5.0 inhibited the growth of gram-positive *L. monocytogenes* and *S. aureus,* but not gram-negative *Pseudomonas aeruginosa,* on a cheese food product *(45).* Because chitosan increased the microbial lag phase and decreased microbial density, the chitosan films and coatings have the potential to be used to preserve dairy products. Chitosan-lysozyme films and coatings inhibited the growth of *Escherichia coli, L. monocytogenes, Pseudomonas fluorescens,* as well as molds and yeast in mozzarella cheese *(64),* and prolonged the shelf life of mozzarella cheese *(4).* Films could be used as cheese packaging to control postprocessing microbial contaminants.

A combination chitosan coating and modified atmosphere packaging inhibited the growth of coliform and *Pseudomonas* spp. bacteria, resulting in improved microbial and sensory quality, as well as longer shelf life of stored Fior di latte cheese *(55).* Addition of chitosan increased the encapsulation efficiency of the enzyme flavourzyme used to control cheese ripening *(9).*

(ii) Milk. Chitosan-containing antimicrobial paper board suppressed the growth of aerobic bacteria in milk and yeast in orange juice at 3 and 10°C, but not at 20°C *(140).*

(iii) Yogurt. Addition of probiotics encapsulated in chitosan-coated chitosan beads increased survival of the probiotic bacteria (*Lactobacillus acidophilus*) in yogurt during storage *(133).* Addition of chitosan to yogurt reduced the in vitro availability of nutrients such as glucose and calcium, suggesting that added chitosan behaved as a dietary fiber *(208).*

Fruits, fruit juices, and vegetables. Protecting fruit, fruit juices, and vegetables against pathogenic and spoilage organisms and bacterial toxins with the aid of natural antimicrobials is a challenging problem *(82, 153, 197, 203).* The experimental data in Table 5 and the following observations suggest that the use of chitosan in various forms may help ameliorate this problem.

(i) Cantaloupes and pineapples. Studies with cantaloupe and pineapple showed that (i) coating of fresh-cut cantaloupes with chitosan–methyl cellulose films reduced the growth of mesophilic aerobes, psychrotrophs, lactic acid bacteria, yeasts, and molds and prevented the multiplication of *E. coli* and *Salmonella* organisms *(134)*; and (ii) chitosan–methyl cellulose–vanillin films inhibited the growth of *E. coli* and *Saccharomyces cerevisiae* yeast on fresh-cut cantaloupe and pineapple, while maintaining quality attributes of the fruit *(217, 218).*

(ii) Citrus. Chitosan and chitosan coatings increased postharvest quality, reduced postharvest rotting, and extended shelf life of citrus (*Murcott tangor*) fruit *(27, 39).*

(iii) Grapes. Studies with grapes showed that (i) chitosan and grapefruit seed extract appear to act synergistically in reducing postharvest fungal rot of table grapes caused by *Botrytis cinerea (260)*; and (ii) chitosan acetate

TABLE 4. *Antimicrobial and antioxidative activities of chitosans in or on dairy products listed alphabetically, and on stainless steel surface*

Target	Chitosan type used	Application/assay	Microorganisms inhibited	Antimicrobial mechanisms	Outcome/significance	Reference
Cheese, Emmental	Chitosan coating	Agar plate method	*S. aureus, L. monocytogenes, P. aeruginosa*	Antimicrobial activity	Increase in microbial lag phase	*45*
Cheese, Mozzarella	Chitosan-lysozyme composite	Antimicrobial	*E. coli, L. monocytogenes,* molds, *P. fluorescens*	Activity enhanced by lysozyme	Inhibited growth of bacteria and molds	*64*
Cheese, Fior di latte	Chitosan coating with lysozyme and EDTA	Shelf life	None	Synergism between chitosan and added antimicrobials	Shelf life increased up to 5 days	*56*
Milk	Paper board coated with chitosan or nisin	Antimicrobial effect	Aerobic bacteria and yeast	Baranyi's growth model[a]	Antimicrobial effect most effective at 10°C	*140*
Milk and yogurt	Alginate beads coated with chitosan	Inhibition zone method	*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum*	Encapsulation	Survival of probiotic bacteria was higher by ~1 log cycle	*133*
Yogurt	Chitosan plus apple, bamboo, and wheat dietary fibers	Digestive model for nutrients	None	Yogurt-fortified chitosan and plant fibers	Different fibers decreased both Ca^{2+} and glucose availability	*208*
Stainless steel surface	Chitosan-glutamate suspension	Suspension and surface test procedures	*L. monocytogenes, S. aureus, S. enterica, Saccharomyces cerevisiae*	Activity of chitosan enhanced by carvacrol	Effective against pathogens adhered to steel surfaces	*132*

[a] The growth rates of the bacteria were interpreted with the aid of the Baranyi mathematical model, which correlates the impact of food ingredients and environmental factors on inhibition of growth *(18, 163).*

TABLE 5. *Antioxidative and antimicrobial activities of chitosans in fruit juices and on produce*

Target	Chitosan type used	Application(s)/assay(s)	Microorganism(s) inhibited	Mechanism	Outcome/significance	Reference(s)
Fruit juices						
Apple/orange juices	Chitosan solutions	Antioxidative activity	None	Antioxidant and metal chelation	Low-molecular-weight chitosan most active	*38, 153, 203*
Apple juice	Chitosan plus moderate pressure	Homogenization with chitosan	*E. coli* K-12	Antimicrobial effect	Pathogens were inactivated	*137, 152*
Apple-elderflower juice	Chitosan, 0.3 g/liter	Antimicrobial activity; shelf life	Lactic acid bacteria, yeast	Growth inhibition	Yeast eliminated during 13 days of storage at 7°C	*205*
Orange juice	Paper board coated with chitosan or nisin	Antimicrobial effect	Aerobic bacteria and yeast	Baranyi's growth model	Antimicrobial effect most effective at 10°C; alternative to pasteurization	*140*
Fruit						
Cantaloupe and pineapple, fresh cut	Chitosan–methyl cellulose–vanillin films	Coated slices	*E. coli, S. cerevisiae*	Microbial control of fruit quality	Vanillin reduced vitamin C during storage to 10% of original	*218*
Garlic cloves	Agar-agar coatings incorporated with chitosan	Counts of filamentous fungi and yeasts	Filamentous fungi and yeast	Coatings reduced respiration rate and water loss	Improvement in garlic quality during 6-day storage	*92*
Grapes, table	Chitosans dissolved in acids	Immersion of berries	None	Reduction of gray mold (decay) during storage	Chitosan acetate was most effective	*157, 213*
Litchi peeled pulp	Chitosan coatings	Immersion in water solutions and coatings	None	Antioxidative effect	Enhanced quality and shelf life	*53, 60, 109*
Mango slices	Treated with 0.5–2% chitosan solutions	Pour-plate microbial assays	Spoilage organisms	Inhibited growth of microorganisms	Improved quality attributes and extended shelf life	*40*
Melons, whole	Chitosan-natamycin coatings	Potato dextrose agar plate assay	*Alternaria alternata, Fusarium semitectum*	Inhibited growth of fungi	Reduced postharvest spoilage	*46*
Mushrooms, fresh-cut	Chitosan solutions, 0.5–2%	Microbial plate counts; oxidative enzyme assays	Total bacterial growth and yeast counts	Inhibited microbial growth and discoloration	Extended quality and shelf life	*68*
Oranges	Chitosan coatings	Antimicrobial assay	*Guignardia citricarpa*	Reduction of infection	Extended shelf life	*27, 39*
Squash slices	Chitosan coatings	Antimicrobial plate assay	*Mesophilic* aerobic microorganisms	Inhibition of spoilage organisms	Best coating was chitosan dried for 30 min at 50°C	*161*
Strawberries, freshly cut	Chitosan coatings	Growth of microorganisms	*Enterobacteriaceae,* lactic acid bacteria, molds, yeasts	Microbial control	Prolonged shelf life and quality of freshly cut strawberries	*26*
Strawberries, whole	Chitosan–acetic acid coating solutions	Consumer testing	None	Sensory attributes unaffected	Coatings with vitamin E had waxy appearance	*97*
Vegetables						
Carrots, sliced	Samples immersed in 0.5–1.5% chitosan in yam starch coating	Growth inhibition	*S. aureus, E. coli,* lactic acid bacteria, molds, yeasts	Up to 2.5 log inhibition during storage for 15 days	Viable alternative for shelf life extension of carrots	*66*

TABLE 5. *Continued*

Target	Chitosan type used	Application(s)/assay(s)	Microorganism(s) inhibited	Mechanism	Outcome/significance	Reference(s)
Tomato fruit	2% Chitosan solution in 1% HCl	Petri dish plating	*Botrytis cinerea, Penicillium expansum*	Inhibited growth of fungi	Induced resistance against blue and gray mold on tomato fruit	*146*
Tomatoes and grapes	1–2.5% chitosan aqueous solutions	Petri dish assay	*Colletotrichum* sp., *Dracaena sanderiana*	Strong antifungal effect	Chitosan is a safe alternative to synthetic fungicides	*162*
Vegetables, fermented	Chitosan-lactate polymers, partly hydrolyzed	Antimicrobial assay on methylene blue agar	Lactic acid bacteria, *Saccharomyces unisporus*	Inhibited growth of spoilage organisms, but also fermenting bacteria	Useful preservative in acidic foods after fermentation	*222*

effectively controlled postharvest gray mold of table grapes at cold and ambient storage temperatures, without any apparent injury to the grapes *(157, 213)*.

(iv) Litchi. Several studies showed that (i) application of chitosan coatings maintained quality parameters and extended the shelf life of peeled litchi (*Litchi chinensis*) fruit *(60)*; (ii) chitosan coatings enhanced the microbial safety of cold-stored litchi fruit *(109)*; and (iii) chitosan improved the quality of litchi cultivars *(56)*.

(v) Mangoes. Chitosan and chitosan coatings delayed the ripening, improved the quality, reduced decay, and extended the shelf life of sliced mango (*Mangifera indica*) fruit *(40, 110, 256, 276)*.

(vi) Strawberries and raspberries. Studies with berries showed that (i) a chitosan coating significantly reduced the decay of fresh strawberries and raspberries, and had beneficial effects on firmness and anthocyanin and vitamin C content of the berries *(270)*; (ii) a chitosan–lactic acid–sodium lactate dip solution inhibited the growth of pathogenic and spoilage organisms on strawberries (*Fragaria* × *ananassa*) and lettuce *(57)*; (iii) chitosan-based coating exhibited antifungal properties against *Cladosporium* spp. and *Rhizopus* spp. on strawberries. The treatment also reduced total aerobic count, coliforms, and weight loss of the strawberries during storage *(177)*; (iv) chitosan coatings did not affect consumer acceptability of flavor, sweetness, or firmness of strawberries *(97)*; and (v) treatment with 1% chitosan solutions reduced mesophilic and psychrotrophic microflora, improved the quality, and prolonged the shelf of fresh-cut strawberries *(26)*.

(vii) Apple juice. Studies with apple juice indicated that (i) chitosan exhibited antifungal properties in juice *(211)*; (ii) Figure 2 shows that low-molecular-weight chitosan exhibited higher antioxidative and free radical scavenging effects than did high-molecular-weight chitosans *(38)*; and (iii) chitosan and pressure at 193 MPa at 20°C exerted synergistic antimicrobial effects against *E. coli, S. aureus,* psychrophiles, psychrotrophs, and yeast during storage of the juice *(152)*.

(viii) Orange juice. Added chitosan extended the shelf life of fresh orange juice. The preservative action of chitosan merits further study as a replacement of heat pasteurization *(153)*. Possible beneficial effects of chitosan in tomato and other vegetable juices have apparently not been studied.

(ix) Carrots. Dipping (submerging) of carrot slices in coatings prepared from yam starch containing 1.5% chitosan, followed by storage for 15 days at 10°C inhibited the growth of coliforms and spoilage organisms *(66)*. The treatment significantly extended the shelf life of the carrot slices.

(x) Tomatoes. Chitosan inhibits the late-blight-causing fungus *Phytophthora infestans* as well as *Colletotrichum* in tomatoes and grapes *(12, 162)*.

(xi) Squash. Chitosan-coated squash slices showed significant log reductions of mesophilic aerobic microor-

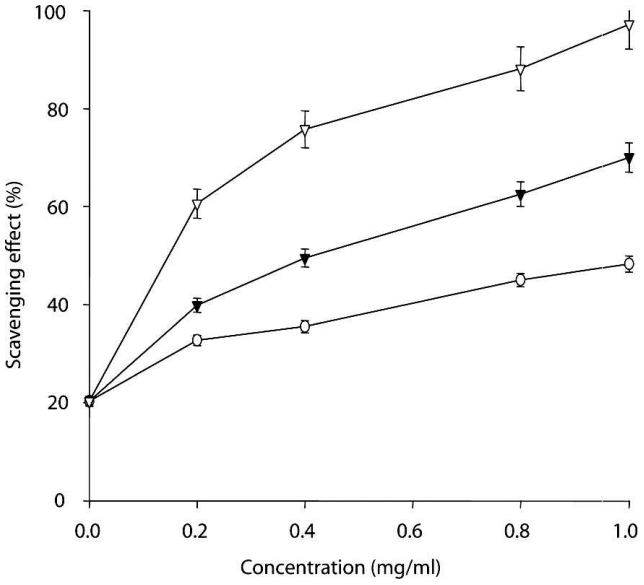

FIGURE 2. *Low-molecular-weight chitosan (12 kDa, open triangles) is more effective in scavenging peroxy radicals in apple juice than are intermediate-molecular-weight (95 kDa, closed triangles) or high-molecular-weight (318 kDa, open circles) molecules. Conditions: To 45 ml of apple juice was added 5-ml chitosan (1 mg/ml), pH 5, solutions; the reaction mixture was incubated for 30 min at room temperature, and the radical scavenging effect was then determined by four different assays. Adapted from (38).*

ganisms *(161, 180)*. Those dried at 50°C for 30 min showed the highest reductions (5.02 log CFU/g).

(xii) Sweet potatoes. Chitosan enhanced the quality characteristics of sweet potatoes during 17-day refrigerated storage *(254)*.

Meat products. Chitosan has been extensively evaluated for its antimicrobial and antioxidative (preservative) properties in a variety of meat products (Table 6). Here, we briefly review selected reported observations for different meat categories in alphabetical order.

(i) Bacon. Low-molecular-weight, irradiated chitosan exhibited enhanced antioxidative activity, without affecting antimicrobial microflora of bacon and mutton seekh kababs *(192)*.

(ii) Beef. Edible chitosan films dissolved in acetic or lactic acids reduced *L. monocytogenes* pathogens on the surface of ready-to-eat roast beef by 2 to 3 log on day 14 *(21)*. The acetic acid–chitosan coatings were more effective in controlling the pathogens than were the lactic acid coatings.

Chitosan alone and in combination with either rosemary extract or α-tocopherol had better antioxidative properties during frozen storage of beef burgers than either rosemary or α-tocopherol had alone *(91)*. The best antioxidative effects were observed with the combination of chitosan and rosemary extract.

Figure 3 shows that the shelf life of minced meat containing irradiated chitosan plus lysozyme was extended up to 15 days at chilled temperatures *(193)*. This beneficial effect, associated with the synergistic action of chitosan and lysozyme, was accompanied by complete elimination of *Bacillus cereus, E. coli,* and *P. fluorescens,* and inhibited *S. aureus* cells, as well as enhanced resistance to oxidative spoilage.

(iii) Bologna. Application of chitosan films enriched with oregano essential oil on bologna, followed by sensory evaluation showed that 45 ppm oregano oil in bologna that diffused from the films would be acceptable to consumers *(37)*. Related studies on the effectiveness of antimicrobial chitosan films are described in *(174, 278)*.

(iv) Ham. Antimicrobial films prepared from chitosan inhibited *L. monocytogenes* and surface spoilage bacteria in processed hams *(174, 265)*.

(v) Lamb. Irradiated chitosan reduced the rancidity of radiation-processed lamb meat *(119)*. Chito-oligosaccharides produced by γ-irradiation of chitosan and lysozyme exhibited a synergistic effect (they were more effective than the individual compounds were) against pathogens on meat *(193)*. The combined treatment induced complete elimination of *B. cereus, E. coli,* and *P. fluorescens*. The shelf life of treated lamb meat was extended up to 15 days at chilled temperatures.

(vi) Pork. Sagoo et al. *(214)* found that (i) chitosan at 0.05% in 0.9% saline at pH 6.2 inhibited the growth of the spoilage microorganisms *Saccharomycodes ludwigii*; (ii) higher concentration (0.25 to 0.5%) inactivated *Lactobacillus viridescens* and *Listeria innocua*; and (iii) dipping of skinless pork sausages in chitosan solutions (1.0%) reduced the native microflora by 1 to 3 log during 18 days at 7°C and increased shelf life from 7 to 15 days (Fig. 4). The combination of chitosan and a rosemary extract showed intense antioxidative and antimicrobial effects in fresh pork sausages stored at 4°C, similar to those mentioned above for beef burgers *(90)*.

Because meat is susceptible to both microbial and oxidative spoilage, Kannat et al. *(120)* evaluated the combination of chitosan and an antioxidant mint plant extract as a preservative for meat products. The shelf life of pork cocktail salami was enhanced after exposure to the combination. The combination also induced reduction in the following pathogenic and spoilage organisms: *B. cereus, E. coli, Salmonella* Typhimurium, and *P. fluorescens*. These observations suggest that combinations of chitosan mint mixture are a potent antimicrobial and antioxidative agent that can be used for preservation and shelf life extension of meats and meat products.

The combination of chitosan (0.5 and 1%) with nitrites (150 ppm) reduced the viable counts of lactic acid bacteria, *Pseudomonas* spp., *Brochothrix thermosphacta, Enterobacteriaceae,* yeast, and molds in fresh pork sausages *(236)*. The rate of lipid oxidation was also significantly decreased. The cited data indicate that chitosan can be used to extend shelf life of stored pork products.

Poultry products. Here, we outline the use of chitosan to reduce pathogens in chicken and turkey products (Tables 6 and 7).

(i) Chicken. Chitosan coatings reduced *Salmonella* levels in modified atmosphere packaged fresh chicken

TABLE 6. *Antimicrobial and antioxidative activities of chitosans in and/or on meat products*

Target	Chitosan type used	Application(s)/assay(s)	Microorganism(s) inhibited	Antimicrobial mechanism	Outcome/significance	Reference
Beef and turkey, ground	0.5–3% chitosan mixed with ground beef	Antimicrobial assay	*C. perfringens*	Inhibited spore germination and outgrowth	Reduced potential risk of *C. perfringens* spores	*115*
Beef, roasted	Chitosan films	Spread plating	*L. monocytogenes*	Acetic acid–chitosan coating was most effective	2.5-log reduction of pathogens	*21*
Beef burgers	Chitosan plus rosemary extract and tocopherol	Lipid oxidation	None	Antioxidative effect	Chitosan plus rosemary extract provided best antioxidative effect	*91*
Bologna, cooked ham, pastrami	Chitosan films plus antimicrobials	Circular slab assay	*Enterobacteriaceae,* lactobacilli, *Serratia liquefaciens*	Activity enhanced by cinnamaldehyde	*Enterobacteriaceae* and *S. liquefaciens* completely inhibited	*174*
Bologna	Chitosan film with oregano oil	Consumer acceptability	None	None	Addition of 45 ppm of oregano oil to bologna acceptable to consumers	*37*
Chicken, ready to cook	Chitosan with thyme oil	Chitosan solutions sprayed into pouch	*Enterobacteriaceae* and spoilage organisms	Growth inhibition of microbes	Shelf life extended up to 6 days	*93*
Ham steaks	Chitosan-coated plastic films	Shake-flask assay	Five-strain cocktail of *L. monocytogenes*	Activity enhanced by sodium lactate	Effective during 12-wk storage	*265*
Pork sausage	Chitosan plus rosemary extract and tocopherol	Antimicrobial assay; lipid oxidation	*Enterobacteriaceae, Pseudomonas* spp., lactic acid bacteria, molds, yeasts	Rosemary extract enhanced chitosan effect	Shelf life of pork sausage was doubled by chitosan plus rosemary extract	*90*
Pork sausage, Greek style	Chitosan plus nitrites	Antimicrobial assays	*Enterobacteriaceae,* lactic acid bacteria, yeast, molds, *Brochothrix thermosphacta*	Inhibition of microbial growth; decreased rate of lipid oxidation	Chitosan plus nitrite samples were most effective	*236*
Pork cocktail salami	Chitosan plus mint extract	Antimicrobial and antioxidative assays	*B. cereus, E. coli, S. aureus, Salmonella* Typhimurium, *P. fluorescens*	Effective antimicrobial–antioxidant blend	Good meat preservative for extended shelf life	*120*

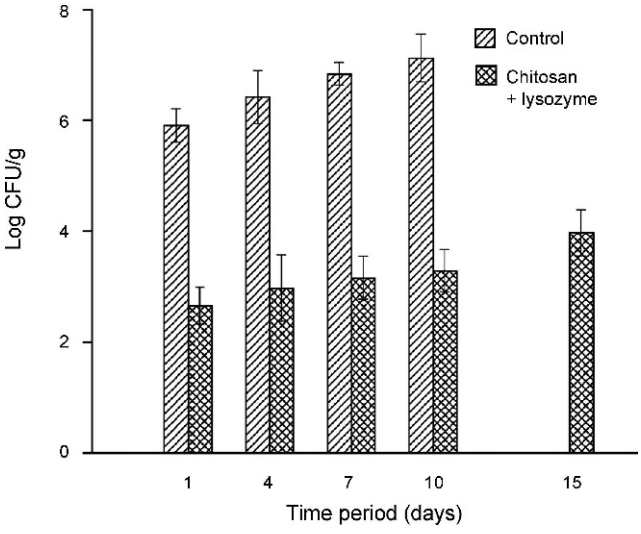

FIGURE 3. *Reduction (organisms recovered) of log CFU from control (*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescens*) by a chitosan-lysozyme mixture in minced meat in chilled storage for 15 days. Addition of chitosan (8.3 kDa, 200 μg/ml)–lysozyme (2 mg/ml) to minced meat resulted in a decrease in microbial populations of 3 log CFU. Control meat samples spoiled within 4 days of storage. By contrast, minced meat containing the chitosan-lysozyme mixture had a shelf life of 15 days during storage at 4°C. Adapted from (193).*

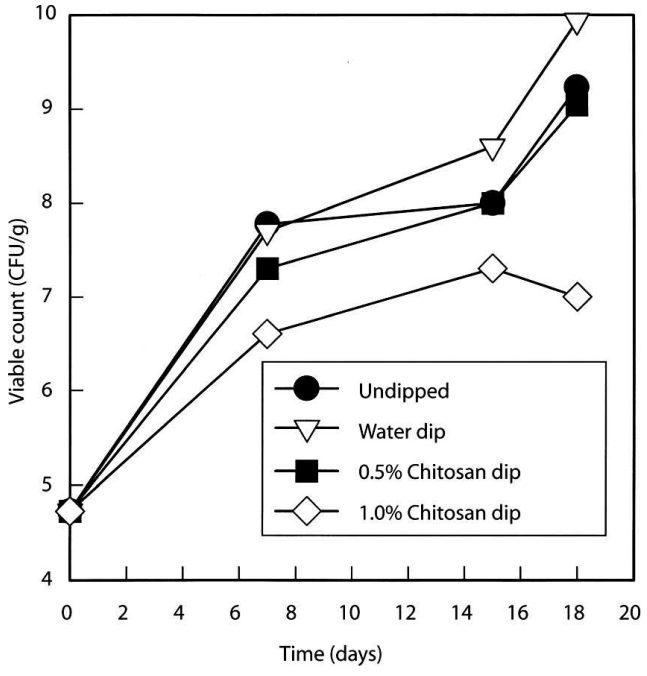

FIGURE 4. *Skinless pork sausage was dipped in chitosan solutions and stored at 7°C for 18 days. Chitosan treatment increased shelf life of chilled skinless sausages from 7 to 15 days. Addition of 0.3 to 0.6% chitosan to a minced pork mixture reduced total viable counts, yeasts, and molds, and lactic acid bacteria by up to 3 log CFU/g for 18 days at 4°C. Adapted from (214).*

breasts *(47)*. Combinations of chitosan (1.5%) and thyme oil (0.2%) inhibited the growth of spoilage lactic acid bacteria, *Pseudomonas,* and *B. thermosphacta,* as well as *Enterobacteriaceae* in ready-to-cook chicken–pepper kebab stored under anaerobic condition at 4°C for 12 days *(93)*. The treatment extended the shelf life of the organoleptically acceptable products by 4 to 6 days.

(ii) Turkey and ground beef. We investigated the inhibition of *Clostridium perfringens* spore germination and outgrowth by the biopolymer chitosan during abusive chilling of cooked ground beef and turkey obtained from a retail store *(115)*. Table 7 shows that chilling of ground beef resulted in germination and outgrowth of *C. perfringens* spores, and that added chitosan reduced the outgrowth of the pathogen. These data show that in the control samples without chitosan, cooling from 54.4 to 7.2°C in 12, 15, 18, or 21 h, resulted in 3.10, 4.51, 5.03, and 4.70 log CFU/g increases, respectively, in *C. perfringens* populations of the ground meat. The corresponding increases for ground turkey are 5.27, 4.52, 5.11, and 5.38 log CFU/g. The results suggest that incorporation of 3% chitosan into ground beef or turkey may reduce the potential risk of *C. perfringens* spore germination and outgrowth during abusive cooling from 54.4 to 7.2°C in 12, 15, or 18 h. A chitosan-based film induced 1.7-log reductions of *L. monocytogenes* on turkey breast after 10 days and 1.2-log reductions after 15 days of storage at 4°C *(111)*.

Seafood. Although chitosan alone possesses antimicrobial properties, the use of chitosan in combination with other antimicrobials enhances its activity in seafood. (See Table 8 for experimental details.) The following examples illustrate the use of chitosan formulations to improve shelf life and the microbial safety of seafood.

(i) Cod. Low levels (50 to 200 ppm) of chitosan prepared from snow crab with different molecular weights and viscosities were effective in controlling the oxidation of lipids in comminuted cod (*Gadus morhua*) during cooking *(228)*. The inhibition of lipid oxidation was concentration dependent. The mechanism of the protective effect may involve formation of chitosan-iron complexes, thus reducing or eliminating the pro-oxidant effect of this metal ion in the cod. A related study further demonstrated the ability of chitosan to act as a preservative in cod *(107)*.

Chitosan fish oil coatings increased total lipid and omega-3 fatty acid content threefold, reduced lipid oxidation, and inhibited growth of total and psychotropic microorganisms in fresh lingcod (*Ophidian elongates*). The coatings did not affect the color of the fish fillets, but lowered the pH and moisture content of the samples. These observations suggest that chitosan coatings containing fish oils have the potential to be applied in fish packaging to increase omega-3 fatty acid content and extend shelf life of seafood *(63)*.

(ii) Fish fillets. Pretreatment of fish fillets (*Oncorhynchus nerka*) with a 1% chitosan solution for 3 h retarded the increase in volatile basic nitrogen content as well as the growth of coliforms, *Vibrio* spp., *Aeromonas* spp., mesophiles, and psychrotrophs, and extended the shelf life from 5 to 9 days *(247)*.

(iii) Herring. Chitosan effectively protected cooked comminuted herring (*Clupea harengus*) samples against

TABLE 7. *Populations of* Clostridium perfringens *in cooked ground beef and turkey containing chitosan, immediately after heat treatment and after cooling exponentially in 12, 15, 18, or 21 h (adapted from (115))*

	Cooling time[a]:							
	12 h		15 h		18 h		21 h	
Chitosan (%) in:	Cook[b]	Chill[c]	Cook	Chill	Cook	Chill	Cook	Chill
Ground beef								
0 (control)	2.14	6.35	3.29	7.80	3.23	8.26	2.86	7.56
0.5	2.23	4.31	3.27	7.77	3.18	7.81	3.05	6.85
1	2.43	4.37	3.47	7.10	3.20	7.87	2.82	7.21
2	2.72	3.44	3.50	5.59	3.27	6.20	3.06	6.02
3	2.96	3.22	3.61	3.60	3.47	3.61	3.31	5.59
Ground turkey								
0 (control)	2.42	7.69	3.25	7.77	3.01	8.12	2.33	7.71
0.5	2.34	3.27	2.93	7.55	2.80	7.80	2.49	6.46
1	2.47	3.22	3.25	6.39	2.96	7.64	3.06	6.59
2	2.74	2.96	3.15	3.65	3.20	5.08	3.11	6.29
3	2.49	2.91	3.47	3.41	3.41	3.26	3.49	5.14

[a] Populations are expressed as mean log CFU per gram.
[b] Cook, heat treatment process was at 60°C for 1 h.
[c] Chill, chilling of meat from 54.4 to 7.2°C.

oxidation *(107, 118, 228)*. The formation of hydroperoxides and 2-thiobarbituric acid reactive substances were reduced in herring after 8 days of storage by 61 and 52%, respectively. Because chitosan has the potential to prevent lipid oxidation, the growth of spoilage bacteria, and moisture loss, the authors suggest that chitosan extracted from crab processing wastes appears to be a natural antioxidant and antimicrobial for stabilizing lipid-containing foods.

(iv) Oysters. Oysters are highly susceptible to contamination by pathogens *(202)*. A water-soluble sulfobenzoyl chitosan derivative inhibited the growth of *Aeromonas hydrophila, B. cereus, Salmonella* Typhimurium, and *Shigella dysenteriae (34)*. Solutions containing 1,000 and 2,000 ppm of the chitosan derivative also retarded the growth of coliforms, *Aeromonas, Pseudomonas,* and *Vibrio* spp. and extended the shelf life of oysters. Chitosan inhibited the growth of the virulent *S. enterica*, *S. aureus*, and *Vibrio vulnificus* in raw oysters *(36)*. Figure 5 shows that treatment with a chitosan solution (5 g/liter) changed the freshness score of Pacific oysters (*Crassostrea gigas*) stored at 5°C from 8 to 9, to 14 to 15 days *(28)*.

Evaluation of water-soluble chitosan oligosaccharides in vitro and in vivo against the gram-negative pathogen *V. vulnificus* that contaminates oysters and other shellfish showed that oligosaccharide with a molecular weight of 10,000 at concentrations of 0.5 to 10 mg/ml suppressed the growth of the pathogen.

(v) Salmon. Chitosan coatings were effective against *Lactobacillus* spp. and *Zygosaccharomyces bailii* on salmon slices *(252)*. Chitosan-coated plastic films incorporating the antimicrobials nisin, sodium lactate, sodium benzoate, sodium diacetate, and potassium sorbate inhibited the growth of a five-strain cocktail of *L. monocytogenes* on cold-smoked salmon samples *(266)*. The film incorporating nisin plus sodium lactate completely inhibited the growth of the bacteria during 10 days of storage. Storage of the test samples in a refrigerator continued to inhibit the bacteria up to 6 weeks. Chitosan coatings also improved the quality and shelf life of pink salmon *(220, 221)*. These observations suggest the potential of use of chitosan containing synthetic plasticizers and plastics in conjunction with contemporary packaging polymer film materials that would be removed before food preparation *(130, 168, 265, 266)*.

(vi) Shrimp. Chitosan coatings (9 mg/g of shrimp) inhibited the growth of spoilage flora in raw shrimp from 8 log CFU in the controls to 4 log CFU during 4 weeks *(212)*. Additional studies indicate that chitosan can also be used for the preservation of shrimp salad and other seafood *(231, 247)*. These results suggest chitosan has the potential for fish and shellfish preservation.

(vii) Trout. Immersion of contaminated rainbow trout fingerlings in a chitosan solution of 125 μg/ml caused 100% mortality of *Saprolegnia parasitica* zoospores *(269)*. A chitosan–cinnamon oil coating improved the quality of refrigerated rainbow trout *(171)*.

ANTIMICROBIAL CHITOSAN-TREATED TEXTILES

Chitosan-treated fabrics could potentially be used to protect food against pathogens, similar to proposed uses of other wraps. Here, we briefly mention reported studies with cotton, jute, and wool.

Cotton. Cellulose fibers cross-linked to chitosan can be used to prepare antimicrobial cotton fabrics *(2)*. These fabrics inhibited the growth of *E. coli* and *Penicillium chrysogenum*. Another study showed that chitosan is irreversibly bound to the cotton fabric *(25)*.

Jute. Treatment of scoured jute with 1% chitosan in acetic acid, followed by fixation at higher temperatures

TABLE 8. *Antioxidative and antimicrobial activities of chitosans in and/or on seafood*

Target	Chitosan type used	Application/assay	Microorganisms inhibited	Mechanism	Outcome/significance	Reference(s)
Cod	Chitosan of different molecular weights	Mixing with chitosan	None	Inhibition of lipid oxidation	Prevents off-flavors	*228*
Lingcod	Chitosan–fish oil coatings	Storage of coated lingcod for 3 wk	Psychrotrophic organisms	Antioxidative effect	Extended shelf life, omega-3 fatty acids have added benefit	*63*
Herring	Chitosan coatings	Coating of fish flesh	None	Inhibition of oxidation	Hydroperoxides were reduced during storage	*118*
Herring/Atlantic cod	Chitosan coatings	Coating of seafood	None	Antioxidative effect	Enhanced seafood quality during storage	*107*
Oysters	*N*-Sulfonated and *N*-sulfobenzoyl chitosan solutions	Antimicrobial plate assay	*Salmonella* Typhimurium, *Shigella dysenteriae*, *Aeromonas hydrophila*	Enhanced antimicrobial effect	Shelf life of oysters extended up to 7 days	*34*
Trout	2% chitosan plus 1.5% cinnamon oil coating solution	Pour plate method using plate count agar	Total viable count; psychrotrophic count	Growth inhibition of spoilage organisms	Improved shelf life and sensory properties	*170, 171*
Shrimp salads, mayonnaise based	Chitosan-glutamate coating	Antimicrobial plate assay	*Salmonella* Enteritidis, *Lactobacillus fructivorans*, *Z. bailii*	Inhibition of pathogenic and spoilage organisms	Chitosan with acetic acid is a good preservative	*212*
Fish soup	Chitosonium-acetate films	Addition of films to liquid soup	*L. monocytogenes*, *S. aureus*, *Salmonella*	Reduction in bacterial growth	Sensory properties were not affected	*71*

FIGURE 5. *Immersion of oysters in chitosan solutions in sterile water (5.0 g/liter) for 10 min improved the freshness of oysters determined by sensory analysis during refrigerated storage. Adapted from (28).*

resulted in an antimicrobial fabric that inhibited the growth of *Candida albicans* and *S. aureus (100)*. Additional studies showed that treatment of chitosan with metal complexes of silver, zinc, and zirconium produced fabrics that had better antimicrobial properties than those treated with either chitosan or metal salts alone. The zinc-containing jute had the highest antimicrobial properties.

Wool. Wool cross-linked by chitosan biguanidine exhibited antimicrobial properties, which were retained after washing *(271)*. These observations suggest that other positively charged protonated pyridinium side chains in wool *(73, 86)* may also exhibit antimicrobial properties *(7, 101)*.

ANTIVIRAL ACTIVITIES OF CHITOSANS

Because pathogenic viruses can also contaminate food, we briefly summarize the following observed antiviral activities of chitosans. Treatment of tobacco plants with 0.1% chitosan solutions suppressed the growth of the tobacco necrosis necrovirus *(104)*. Although the monomeric chitosan molecules—glucosamine and *N*-acetylglucosamine—exhibited no antiviral activity, low-molecular-weight chitosans at concentrations of 10 or 100 μg/ml prevented infection of beans (*Phaseolus vulgaris*) by a mosaic virus *(135)*. Sulfated chitosan at a concentration as low as 0.29 μg/ml completely inhibited the infection of the AIDS virus (human immunodeficiency virus I) in blood cells (T lymphocytes) *(167)*. Chitosan facilitates the absorption and biological utilization of the antiviral drug acyclovir *(58, 226)* and of peptide antimicrobials *(251)*. Possible mechanisms of viral inhibition by polycationic chitosan and derivatives are described in *(30, 41, 189)*.

The observed cytotoxicity of chitosan against viruses suggests that chitosans can be used to manage viral-induced plant and human diseases. There is a need to find out whether the biopolymer will inhibit foodborne viruses such as the

hepatitis A virus on spinach *(230)* and noroviruses in other foods *(159, 215)*. See also ''Vaccine adjuvants,'' below.

MECHANISMS OF BENEFICIAL EFFECTS

Understanding the biochemical basis of antimicrobial and antioxidant effects of chitosans should minimize adverse microbial and maximize beneficial sensory, nutritional, and health effects of treated foods in the diet. Such efforts should lead to better and safer foods and improved human health.

Antimicrobial mechanisms. The main mechanism that appears to govern the bacteriostatic and bactericidal effects of chitosan appears to involve binding of its positively charged amino ($-NH_3^+$) groups to negatively charged carboxylate ($-COO^-$) groups located on the surface of the bacterial cell membranes *(189)*. Such electrochemical binding can alter the distribution of negative and positive charges on the surfaces of the cell membranes, leading to weakening and/or disruption of the membranes, followed by leakage of cell components. This mechanism is supported by electron microscopy studies that showed that the polymer binds to and weakens the outer membrane of bacteria *(99)*, as well as by atomic force microscopy studies that indicate that chitosan nanoparticles induced disruption of cell membranes and leakage of cytoplasm of *Salmonella choleraesuis* organisms *(185)*.

The pH of the microenvironment in which chitosan operates determines the relative concentrations (ratios) of unprotonated and protonated amino groups which are governed by the equilibrium

$$\text{chitosan} - NH_2(\text{unprotonated}) + H^+ \leftrightarrow \text{chitosan} - NH_3^+(\text{protonated}); \quad \text{pKa} = \sim 6.5 \quad (1)$$

At a pH = pKa, 50% of amino group are protonated. At pH 5.5, the positively charged amino group contribute 90%, and at pH 4.5, 99% to the equilibrium shown in equation 1.

The antimicrobial effectiveness of chitosan appears to be highest below pH 6.0, where the protonated form predominates and where chitosan is most soluble (Fig. 6). By contrast, only the unprotonated form can chelate essential metal ions. These considerations suggest that depending on pH, different mechanisms may operate in different food categories and that lowering the internal pH of meat may enhance the antimicrobial activity of chitosan. The internal pH values of the ground meat and turkey used in our studies *(115)* were 6.25 and 6.46, respectively.

The following experimental findings contribute to our understanding of possible mechanisms of bactericidal and antifungal effects of chitosans and chitosan metallocomplexes. These effects appear to be largely due to their specific perturbations of the ordered structure of phosphatidylcholine and phosphatidylethanolamine bilayers constituting bacterial cell wall membranes via electrochemical and hydrogen bond interactions, as described in detail elsewhere for tea catechins *(76, 233)*.

Based on the following (and additional) reported MICs (in parts per million) gram-negative bacteria appear more

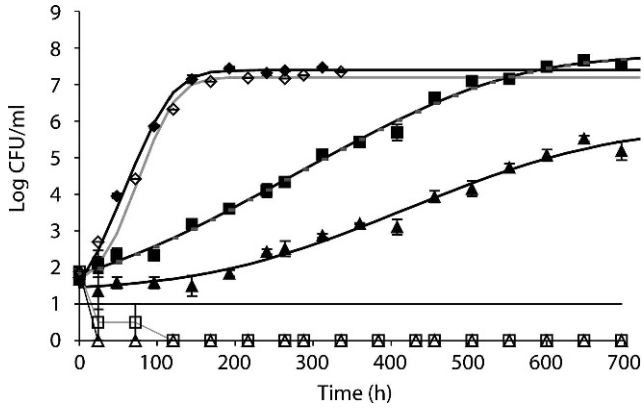

FIGURE 6. *pH dependence and concentration dependence of antifungal activity of 43-kDa chitosan at 7°C. Diamond, control without chitosan; square, 0.005% chitosan; triangle, 0.01% chitosan. Open symbols, pH 4.0; closed symbols, pH 6. Dipping in chitosan solutions (40 to 750 μg/ml) inactivated spoilage organisms on whole strawberries and on lettuce. Adapted from (57).*

susceptible to chitosan, while relative sensitivities of gram-positive bacteria are less clear cut: *E. coli,* 20; *S. aureus,* 20; *Klebsiella pneumonia,* 700; *P. fluorescens,* 500; and *B. cereus,* 1,000 *(57)*. Another study reported that among a number of pathogens tested, *Campylobacter* spp. were most susceptible to inactivation by chitosan, with MICs ranging from 0.005 to 0.05% *(89)*. All the gram-negative bacteria (three *Campylobacter jejuni* strains, *Campylobacter coli* LP2, *E. coli* ATCC 25922, and *P. aeruginosa* ATTCC 27853) were more sensitive to inhibition by high-molecular-weight chitosan than were three gram-positive bacteria (*S. aureus* ATCC 25923, *Lactobacillus salivarius* IFa, and *Lactobacillus casei* CECT 475). Inhibition of *Campylobacter* was accompanied by loss of membrane integrity. There was a change in cell membrane resistance toward a loss of integrity as the cells entered the stationary phases. The finding with *Campylobacter* confirms our previous observations that *Campylobacter* strains are also much more susceptible to inactivation by plant essential oils and phenolic compounds than *E. coli* O157:H7, *L. monocytogenes,* and *S. enterica* organisms *(83, 84)*.

The molecular mechanism of antibacterial action of chitosan may involve cross-linking or association between positively charged amino and negatively charged anions on the bacterial surface. This results in changes in the membrane permeability, leakage of cell components, followed by cell death *(107)*. Chitosan (800 ppm, 98% deacetylated) at pH 6.0 caused leakage of glucose and lactate dehydrogenase from *E. coli* cells *(246)*. Lower concentrations (0.1 mg/ml) of chitosan induced greater leakage of intracellular components from *E. coli* than did higher concentrations (2.0 and 5.0 mg/ml) *(189, 241)*. Based on the observed relative antibacterial lethality rates induced by chitosan powder against *E. coli, Enterococcus faecalis, P. aeruginosa,* and *Staphylococcus saprophyticus,* Andres et al. *(8)* suggest that the antibacterial mechanism involves cell wall disruption induced by chitosan amino groups.

However, the cited mechanistic aspects may not be the only ones that govern the mode of antimicrobial action of

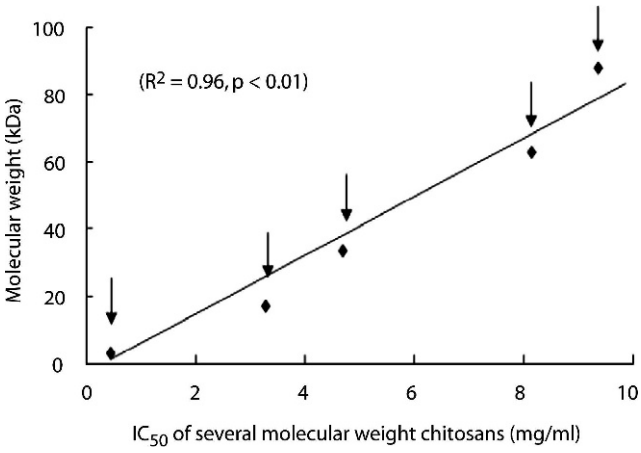

FIGURE 7. *Protective effect of chitosan (2.5 mg/ml) solutions against peroxy radical formation in human plasma proteins is linearly related to the molecular weights of chitosans in the range from ~2,000 to ~100,000 kDa. Radical scavenging activities by three methods were measured in a pH 5.5 ethanol–2-(*N*-morpholino)ethanesulfonic (MES) buffer (1:1, vol/vol). Half maximal inhibitory concentration values are concentrations of chitosan (in milligrams per milliliter) that inhibited 50% of the peroxy radicals; the lower the value the greater the inhibition. Adapted from (244).*

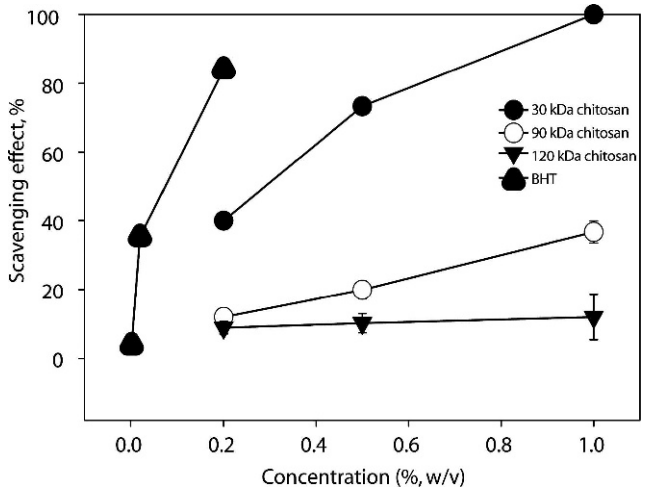

FIGURE 8. *Comparison of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity by butylated hydroxytoluene and three 0.2 to 1% chitosan solutions at 4°C. Activity increases with the molecular weights of the chitosan molecules. The antiradical effect of the 30-kDa chitosan was comparable to the activity of butylated hydroxytoluene. Adapted from (127).*

chitosan. Based on data derived from killing kinetics of *S. aureus* SG511 and membrane potential, cellular leakage, electron microscopy, and transcriptional response measurements, Rafaat et al. *(188)* suggest that binding of chitosan to teichoic acids accompanied by extraction of membrane lipids also results in a sequence of events leading to bacterial death. The authors conclude that chitosan's mode of action is not confined to a single target. Cell death may result from a sequence of the cited molecular events occurring simultaneously or successively.

The following MICs (in parts per million) of chitosan against fungi show a 500-fold difference in resistance to inhibition among seven fungi that contaminate food plants and food: *Botrytis cinerea, Drechslera sorokiniana, Micronectriella nivalis* (10, least resistant to inhibition), *Fusarium oxysporum* (100), *Rhizoctonia solani* (1,000), *Trichophyton equinum* (2,500), and *Pyricularia oryzae* (5,000, most resistant) *(189)*.

It is also important to note that differences in membrane composition, structure, thickness, and electrochemistry (distribution of ionic charges) may be largely responsible for variations in sensitivities of different classes of microorganisms to inactivation by chitosan. We do not know the molecular basis for the apparent large differences in susceptibilities among gram-positive and gram-negative bacteria, as well as fungi, to chitosan *(77, 102, 143)*. This aspect for both susceptible and antibiotic-resistant microorganisms merits further study.

Antioxidative mechanisms. Because the antioxidative effect of chitosan also contributes to preservation and shelf life extension of foods, there is a need to better understand the molecular basis of the reported antioxidative free radical scavenging activity. An antioxidative effect can, in principle, occur during mixing of chitosan with foods containing peroxide free radicals, which results in transfer of the free electron from the peroxide to the electron sink of chitosan. It is not, however, immediately apparent why chitosan behaves as an antioxidant in foods because it does not contain carbonyl or phenolic groups, which are reported to stabilize the free electron abstracted from other food ingredients, as discussed in detail elsewhere *(43, 44, 72, 166)*. The following observations suggest that chitosan does exhibit antioxidative effects in foods and in vivo.

Added chitosans of different molecular weights (30, 89, and 120 kDa) at 0.2 and 0.5% (wt/vol) exhibited antioxidative abilities in salmon *(252, 266)*. Scavenging of free radicals increased with concentration and decreased with increased molecular weights of chitosans. Antioxidative activities may be due to bonding of chitosan amino groups to iron in ferritin, hemoglobin, and myoglobin present in salmon. The iron in these metalloproteins is known to be released during storage. The released ferrous ions can then activate oxygen and initiate lipid oxidation *(51, 118, 261)*.

Grafted eugenol and carvacrol (16 mg/ml in soy broth medium) enhanced both antimicrobial and antioxidative activities of chitosan nanoparticles *(35)*. The antimicrobial activity of the grafted eugenol and carvacrol against *S. aureus* and *E. coli* was better than that observed with the unmodified chitosan nanoparticles. Modification with flavonoids also increased both antimicrobial and antioxidative effects of *(238)*. We do not know whether the chitosan backbone, the attached antimicrobials, or the newly created chitosan derivatives govern the observed antimicrobial-antioxidative effects. Both chitosan and grafted moieties probably contribute to the beneficial effects.

Tomida et al. *(244)* measured the ability of several chitosans (2.5 mg/ml, pH 5.5 solutions) with a range of molecular weights to protect plasma proteins of human volunteers against oxidation by peroxyl radicals. They

observed a linear correlation between antioxidant activity and the molecular weights of the chitosans in vitro (Fig. 7). Low-molecular-weight chitosans (20 to 30 kDa) were most effective in protecting the proteins against formation of carbonyl oxidation products. The authors suggest that differences in intramolecular hydrogen bonding between amino and hydroxyl group may govern the observed relative antioxidative effects. Figure 8 shows that a 1% chitosan solution exhibited higher radical-scavenging activity than did the known food preservative butylated hydroxytoluene *(127)*.

Metal ion chelating mechanisms. Friedman and colleagues *(87, 155)* found that chitosan and other natural biopolymers have strong affinity for toxic (cadmium, cobalt, copper, gold, iron, lead, and mercury) and for nutritionally essential (copper, iron, manganese, and zinc) metal ions. Based on this observation, they suggested that the natural biopolymers may be useful for the removal of toxic metal salts from contaminated water supplies. The validity of this suggestion was later realized by numerous studies designed to demonstrate this possibility *(106, 150, 158, 179, 275)*.

Table 9 summarizes experimental data on the antimicrobial/antioxidative effects chitosan metallocomplexes. The inhibitory effects of chitosan-metal complexes with Cu (II), Zn (II), and Fe (II) ions against two gram-positive bacteria (*S. aureus* and *Staphylococcus epidermidis*), two gram-negative bacteria (*E. coli* and *P. aeruginosa*), and two fungi (*C. albicans* and *Candida parapsilosis*) was dependent on the metal ion, the molecular weight, the degree of deacetylation of chitosan, and the pH of the environment *(257)*. Electron microscopy studies indicated that the antimicrobial action of the chitosan–Cu (II) complex against *S. aureus* resulted in the disruption of the bacterial cell envelope *(257)*. Binding of bacterial trace metals by chitosan inhibited both microbial growth and production of bacterial toxins *(132)*. It is also likely that binding of chitosan to trace elements, such as ferric and zinc ions that the bacteria need for growth, may contribute to its antimicrobial action *(189)*. The use of chitosan to eliminate toxic metal ions from contaminated liquid foods (milk, fruit, and vegetable juices, etc.) merits study.

TABLE 9. *Antimicrobial–antioxidative activities of chitosans–metal complexes*

Target	Chitosan type used	Application(s)/assay(s)	Microorganism(s) inhibited	Antimicrobial mechanism	Outcome/significance	Reference
Bacteria and fungi	Chitosan-metal complexes	Agar dilution method; electromicroscopy	*C. albicans*, *C. parapsilosis*, *S. aureus*, *S. epidermidis*	Structure-inhibition relationships	Complexes better inhibitors than metal salts are	*257*
Bacteria	Chitosan-maltose soluble derivative	Agar plate assay	*E. coli* O157:H7	Stationary-phase more resistant than late-phase cells were	Growth inhibition enhanced with added metal salts	*264*
Bacteria	1% chitosan treated jute fabrics	Petri dish assay	*C. albicans*, *S. aureus*	Growth inhibition	Antimicrobial food wrap	*100*
Fish feed with cadmium	Chitosan	Cadmium assay	None	None	Cadmium level reduced up to 24%	*108*
Ochratoxin A	Chitosan-polyaniline in pH 6.4 buffer	Immunosensor assay	None	Assay for mycotoxin in food	Highly sensitive assay	*125*

THERAPEUTIC PROPERTIES OF CHITOSANS

In addition to described antimicrobial effects in food, chitosans are reported to also exhibit numerous therapeutic properties, reviewed in *(6, 267)*. These include anticarcinogenic, anticholesterol, anti-obesity, antihypertensive, controlled drug delivery, hemostatic, immunoprotective, and neuroprotective properties summarized in Table 10. Here, we briefly describe therapeutic properties associated with infectious diseases.

Antibiofilm properties. Studies by Carlson et al. *(31)* revealed that surface coating with chitosan resisted biofilm formation by bacteria and yeast. Reduction in biofilm viable cell populations ranged from 95 to 99.99% for *S. epidermidis, S. aureus, K. pneumoniae, P. aeruginosa,* and *C. albicans*. Fluorescence microscopy studies showed permeabilization of cells as they alighted on chitosan-coated

TABLE 10. *Medical applications of chitosans*

Target(s)	Chitosan used	Application(s)/assay(s)	Pharmacology/medicine	Mechanism(s)	Outcome/significance	Reference(s)
Cancer genes	Nanochitosan, 15.5 kDa complexed with alginate	Gene transfection of cancer cells	Integrity of plasmid DNA	Expression of transgene products	Promising method for safe and effective gene therapy	*263*
Cancer cells	*N*-Succinyl chitosan, 3,000-kDa nanoparticles	Inhibition of human cancer cells	Changes in morphology and cell properties	Induction of apoptosis and necrosis	Inhibition of cells with low IC_{50} of 14.3 μg/ml[a]	*149*
Cholesterol/blood lipids	Chitosan	Effect on human serum lipids; meta analysis	Decrease in serum lipids	Chitosan amino groups bind to negatively charged lipids	Significant decrease of total but not of HDL and LDL cholesterols	*15, 259*
Drug delivery, amoxicillin antibiotic	Chitosan–glutamic acid nanoparticle hydrogels	Treatment of *H. pylori* infection	Carrier for amoxicillin to digestive tract	Protection from action of gastric juice	Potential improved treatment of human ulcers	*16, 32*
Drug delivery, ellagic acid cancer drug	Chitosan-glycerophosphate gel	MTT assay for release to brain cancer cells	Increased release rate of ellagic acid in the presence of lysozyme	Inhibition of cancer cell growth	Promising therapy for brain cancers via local delivery of drug	*129*
Drug delivery, fluorouracil cancer drug	Chitosan–chondroitin sulfate microcapsules	Release of drug via UV-VIS spectroscopy	Release of drug influence by structure of chitosan and pH	Controlled release of drug to cancer sites	Promising treatment for cancer via local delivery	*103*
Drug delivery, insulin for diabetes	Chitosan–polyglutamic acid nanoparticles	Oral delivery of insulin to rats	Dissolved in small intestine and absorbed into the circulation	Protection of insulin degradation in the stomach	Prolonged reduction in blood glucose levels	*6, 235*
Drug delivery, nimodipine blood pressure drug	Chitosan lactate–alginate buccal adhesive tablets	Release of drug in the buccal cavity	Treatment of hypertension	Effective controlled release pattern of drug	Promising treatment of blood pressure via local delivery of drug	*98*
Drug delivery, timolol for glaucoma	Chitosan-gellan gum gel	Enhanced retention of drug in eye	Glaucoma therapy	Controlled release of drug to diseased tissues in eyes	Improved alternative to eye drops for treatment of ocular diseases	*96*
Fungal human infections	Chitosan, high molecular weight	Broth microdilution	*Candida* spp. clinical strains	Inhibited at 1.25 mg/ml, pH 4; inhibits biofilms	Could be used in vulvovaginal candidiasis	*154, 243*
Hemostasis, rabbit and pig cardiac surgery	Carboxymethyl chitosan film	Reduced postsurgical adhesions	Acts as a biophysical barrier that prevents adhesions in tissues	Reduced severity and area of adhesions	Improved outcome of cardiac surgery	*274*
Hemostasis, rat and pig hepatic hemorrhage	Chitosan sponge and patch	Absorbable surgical hemostatic agent	Reduced bleeding during liver surgery	Improved control of lethal organ bleeding	Promising agent for postoperative hemostasis	*22, 95*
Hemostasis, human sinus surgery	Chitosan-dextran gel; chitosan-based dressing	Hemostatic agent	Control of postoperative bleeding and adhesion	The gel was rapidly hemostatic and prevented adhesion	Improved wound healing following nasal surgery	*50, 250*
Immunity	Chitosan mouse vaccine adjuvant	Nasal vaccination of mice	Protected mice against multiple subtypes of influenza A virus	Improved efficacy of vaccine	Candidate for influenza virus vaccine	*242*
Immunity	0.5% chitosan poultry vaccine adjuvant	Oculonasal delivery of live vaccine	Protected poultry against viral Newcastle disease	Stimulation of immune system	Promising safe live poultry vaccine	*199*
Immunity	Chitosan pentamers and hexamers	Gene transcription	Promoted expression of interleukins and cytokines in vivo	Immunomodulation	Improves immune system	*258*

TABLE 10. *Continued*

Target(s)	Chitosan used	Application(s)/assay(s)	Pharmacology/medicine	Mechanism(s)	Outcome/significance	Reference(s)
Obesity, humans	Chitosan, 6 500-mg capsules/day	Weight loss by obese human subjects	Depletion of excess body fat with minimal loss of lean body mass	Greater body composition improvement	Potential value in treatment of obesity	*117*
Obesity, rats	300 mg of chitosan per kg of rat	Administered orally by gavage	Weight loss; enhanced antioxidant defense system	Weight loss; hypolipidemic and antioxidative effect	Potential value in treatment of obesity and hypolipidemia	*124*
Obesity, mice	Chitosan oligosaccharides, 200 mg/kg per mouse	Proteomic analysis of plasma	Intraperitoneal administration	Decreased body weight gain and improved glucose and lipid profiles	Potential treatment for obesity and diabetes	*138*
Spinal cord injury	Chitosan	Topical application; stem cell assay	Restored conduction of nerve impulses	Suppressed peroxidation of lipid membranes	Treatment of spinal cord and brain injury; repair of neurons by stem cells	*42, 15, 223*

[a] IC_{50}, half maximal inhibitory concentration values are concentrations of chitosan (in milligrams per milliliter) that inhibited 50% of cell growth.

surfaces, suggesting that chitosan disrupts cell membranes as microbes settle on the surface. Related observations suggest that chitosan should be considered for the prevention or treatment of microbial biofilms on central venous catheters and other medical devices *(29, 154, 245).*

Antifungal effects against clinical *Candida* strains. High-molecular-weight chitosan inhibited the growth of clinical strains of *Candida* spp., suggesting that these compounds could potentially be used in vulvovaginal candidiasis *(243).*

Antimicrobial dressing for infected burns. An antimicrobial chitosan acetate bandage effectively controlled the growth of *P. aeruginosa* and *Pseudomonas mirabilis* on third-degree burns in mice and prevented systemic sepsis *(49).* The survival of mice treated for 21 days was 73.3%, compared with 13.3% for untreated mice. The authors suggest that chitosan acetate bandage has the potential to prevent fatal burn infections.

Controlled delivery of antibiotics to target tissues. Chitosan-containing adhesives, capsules, and gels facilitate controlled delivery of several medicines, including the antibiotic amoxicillin used to treat *Helicobacter pylori* infections of the digestive tract *(6, 32, 149, 175, 190).*

Protection against infections. Mice pretreated by intraperitoneal injection of chitin and chitosan showed resistance to intraperitoneal infections by *L. monocytogenes* and *P. aeruginosa (172),* suggesting that the antimicrobial action may also occur in humans. Lee et al. *(139)* evaluated the in vivo antibacterial activity of two water-soluble chitosan oligosaccharides (molecular weights of 1,000-A and 10,000-B) against *V. vulnificus.* Oligosaccharide A, but not B, inhibited both the growth of the pathogens and cytotoxicity in human intestinal epithelial INT-407 cells. Orally administered B as water solutions at 0.1 to 0.5 mg per mouse increased the survival of infected mice *(139).* The number of viable pathogens in the blood, liver, small intestine, and spleen was significantly lower in the treated mice as compared with controls. These results suggest that chitosan has the potential to prevent and treat humans infected with clinical and foodborne pathogens.

Vaccine adjuvants. Chitosans enhanced the protective immunity effect of vaccines against the avian influenza A virus *(242)* and hepatitis B infection *(184).* They were also found to be a promising adjuvant for the delivery of live vaccine against Newcastle viral poultry disease *(199).* Enhancement of the antigen-specific cell-mediated immune response in the spleen appears to govern the mechanism of immune stimulation.

The cited observations indicate that antibiotic and immune-stimulating effects of chitosans in vivo complement antimicrobial activities in food. We do not know whether chitosan-treated food will exhibit similar beneficial properties in vivo.

CONCLUSIONS AND OUTLOOK

We can only surmise as to why nature created the cellulose derivative chitin, the precursor of chitosan. Chitin undoubtedly serves as a barrier that protects tissues of some sea animals against damage, analogous to that of the human skin. Since the discovery that chitosan (deacetylated chitin) is safe to consume and exhibits antimicrobial and antioxidative effects in vitro, extensive efforts have been made to explore and exploit the potential value of chitosans of different degrees of acetylation and of different molecular weights as well as of chitosan derivatives and of nano- and metallochitosans for their ability to enhance antimicrobial and preservative properties in laboratory media and on and in food.

Because chitosan is regarded as safe, with no apparent undesirable sensory properties, it can be added to liquid and solid foods or applied as an antimicrobial film and coating. However, we do not know whether chitosans with added antibiotics such as lysozyme and nisin or chemically modified chitosans containing synthetic plasticizers and plastics as well as grafted side chains designed to enhance antimicrobial properties are safe to consume. This aspect merits study.

To cross-fertilize information among several disciplines and to enhance their utility, this review attempts to integrate and correlate the widely scattered literature on antimicrobial–antioxidative–preservative effects of different chitosan formulations in wide range of widely consumed food. The range of topics covered includes a variety of specific and general interest. Because of the multidimensional nature of chitosan chemistry and microbiology, the widest possible interchange of ideas, viewpoints, and expertise is needed to transcend present limitations on efforts designed to define and enhance the value of chitosans for microbial safety and shelf life of food. There is a need to define a relative antimicrobial/antioxidative potency scale of different chitosan formulations against pathogenic and spoilage organisms described in this overview. The most active formulations could then be recommended for use with food.

Our assessment also shows the need for additional research designed to mitigate with the aid of chitosans adverse consequences of consuming contaminated food. To further enhance the potential of chitosans to help assure food quality and safety, future studies need to address the following additional food-related aspects of this widely studied natural biopolymer:

1. Determine whether the potent antimicrobial-preservative effects of chitosan in vitro can be duplicated in vivo, especially in humans. Are inhibitory effects by chitosan clinically significant, i.e., would human consumption of chitosan-containing food be expected to reduce both the incidence and severity of infectious diseases?
2. Determine whether animal and human consumption of chitosan-treated food will contribute to reported beneficial effects of chitosan against cancers *(52, 141),* cholesterol *(3, 15, 17),* obesity *(112, 209),* and wound-healing *(3, 206).*
3. Compare effectiveness of chitosan against susceptible and antimicrobial-resistant foodborne pathogen serotypes *(75, 78, 200)* and determine whether chitosan added to animal feed can replace standard antimicrobials, whose use is being discontinued *(1, 10).* Will orally fed chitosan reduce the *E. coli* O157:H7 populations in the guts of cattle and pigs?
4. Define additive and/or synergistic activities of mixtures of chitosans with other plant-derived antimicrobials such as oregano oil, sodium lactate, and polyphenol-rich apple, grape, olive, and tea extracts *(113, 114),* as well as with medical antibiotics such as vancomycin. Combinations of natural antimicrobials that act synergistically will lessen amounts needed to design effective antimicrobial food formulations. They will be safer and will affect flavor and will taste better compared with the use of individual compounds.
5. Because we previously found that added carvacrol concurrently facilitated heat-induced reduction of *E. coli* O157:H7 populations and inhibited the formation of potentially carcinogenic heterocyclic amines in grilled beef patties *(88),* it would be of interest to find out whether chitosan will also simultaneously inhibit both pathogens and heterocyclic amines during baking and grilling of meat, poultry, and seafood products.
6. Determine whether chitosan continues inhibiting the growth of bacteria during post-thermal processing and storage of ground beef and poultry products and produce *(114).*
7. Because, as discussed in detail elsewhere *(61, 62, 201),* edible films prepared from apples and tomatoes may have advantages over nonedible films, determine whether adding chitosan to apple and tomato purees used to prepare the films can provide novel edible antimicrobial coatings for contaminated food. Determine long-term stabilities of added natural antimicrobials in chitosan films, coatings, and microspheres *(5, 201).*
8. Determine organoleptic properties of food exposed to chitosan. It is worth noting that chitosan coatings did not change consumer acceptability of flavor, sweetness, or firmness of whole strawberries *(97).*
9. Determine whether molecular modeling of chitosan structure–cell membrane interactions can be used to predict antimicrobial activities of chitosan and derivatives, as appears to be the case for antimicrobial tea catechins *(232, 233).*
10. Determine whether chitosan can concurrently inactivate pathogenic bacteria such as *C. botulinum, E. coli, S. aureus,* and the production of botulinum, Shiga, and *Staphylococcus* toxins produced by these bacteria *(76, 187, 194–196, 198).*
11. Because the mode of action of chitosan against phytopathogenic bacteria is not known, determine whether the mechanisms of protection against foodborne pathogens are similar to those that may govern the inhibition of phytopathogenic bacteria, including strains of *Agrobacterium, Clavibacter, Pseudomonas, Erwinia,* and *Xanthomonas* that contaminate cabbage, eggplants, grapes, lettuce, onions, potatoes, and tomatoes *(207).*
12. Determine whether chitosan can be used to both inactivate pathogenic organisms and sequester toxic

and radioactive metals (cadmium, lead, mercury, uranium) from liquid and solid foods *(158, 191, 255).*

13. Determine whether added chitosans will inactivate the hepatitis A virus and the norovirus in food *(26, 94, 224).*
14. Determine whether chitosan-treated antimicrobial fabrics (cotton, wool, jute) can be used as antimicrobial food wraps.
15. Determine whether chitosan solutions could be used as sanitizers for cutting boards.

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