

# Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review

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**ABSTRACT:** Chitosan is a modified, natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean. Recently, chitosan has received increased attention for its commercial applications in the biomedical, food, and chemical industries. Use of chitosan in food industry is readily seen due to its several distinctive biological activities and functional properties. The antimicrobial activity and film-forming property of chitosan make it a potential source of food preservative or coating material of natural origin. This review focuses on the applications of chitosan for improvement of quality and shelf life of various foods from agriculture, poultry, and seafood origin.

**Keywords:** application, chitosan, foods, quality, shelf life

## Introduction

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin [poly- $\beta$ -(1  $\rightarrow$  4)-N-acetyl-D-glucosamine], a major component of the shells of crustacean such as crab, shrimp, and crawfish and the 2nd most abundant natural biopolymer after cellulose (No and Meyers 1995). During the past several decades, chitosan has received increased attention for its commercial applications in the biomedical, food, and chemical industries (Muzzarelli 1977; Knorr 1984; Li and others 1992). According to the "SciFinder Scholar 2001" database, 22643 references reporting chitosan and its properties and applications have been published as of June 21, 2005. Chitosan is now widely produced commercially from crab and shrimp shell wastes with different deacetylation grades and molecular weights (thus, viscosities of chitosan solutions), and, hence, different functional properties (No and Meyers 1995; Cho and others 1998b). Chitosan is water-insoluble but soluble in weak organic acid solutions. Chitosan derivatives in the form of acetate, ascorbate, lactate, and malate are water-soluble. Water-soluble chitosan can also be produced in the form of oligosaccharide by enzymatic or chemical hydrolysis (Jeon and others 2000).

To date chitosan has attracted notable interest due to its biological activities such as antimicrobial (Sudarshan and others 1992; No and others 2002), antitumor (Tokoro and others 1988), and hypcholesterolemic functions (Sugano and others 1992). The antimicrobial activity of chitosan against a range of foodborne filamentous fungi, yeast, and bacteria has attracted attention as a potential food preservative of natural origin (Sagoo and others 2002). In studies on functional properties of chitinous polymers, chitosan has been documented to possess several distinctive properties for use in water and fat uptake, emulsification (Knorr 1982), dye binding (Knorr 1983), and gelation (Vorlop and Klein 1981). Chitosan has also been documented to possess a film-forming property for use as edible films or coatings (Butler and others 1996; Jeon and others 2002; Nadarajah and others 2006). Chitosan coating can improve the storability of perishable foods by modifying the internal atmosphere as

well as decreasing the transpiration losses (El Ghaouth and others 1991; Zhang and Quantick 1997).

Chitosan is biocompatible, nonantigenic, nontoxic, and biofunctional (Hirano and others 1990; Li and others 1992). Thus, chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (Weiner 1992; FDA 1995). Biological safety of chitosan has been demonstrated by feeding trials with domestic animals (Hirano and others 1990). In 2005, shrimp-derived chitosan was submitted to the US FDA to be considered as GRAS (generally recognized as safe) based on the scientific procedures for use in foods in general, including meat and poultry, for multiple technical effects. However, according to GRAS notice nr GRN 0001-70, at the notifier's request, the US FDA ceased to evaluate the notice, effective October 31, 2005 (US FDA/CFSAN 2006).

Although the effectiveness of chitosan for its ability to enhance quality and shelf life of foods has been reported by numerous workers, no comprehensive review has been published. Therefore, in this article, we summarize the scientific publications reporting the applications of chitosan for improving quality and shelf life of foods from agriculture, poultry, and seafood origin.

## Food Applications of Chitosan

### Antimicrobial activity of chitosan

Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of foodborne filamentous fungi, yeast, and bacteria (Sagoo and others 2002). The mechanism of the antimicrobial activity of chitosan has not yet been fully elucidated, but several hypotheses have been proposed. The most feasible hypothesis is a change in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes. This interaction leads to the leakage of proteinaceous and other intracellular constituents (Young and others 1982; Leuba and Stössel 1986; Papineau and others 1991; Sudarshan and others 1992; Fang and others 1994). Other mechanisms are the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis (Hadwiger and Loschke 1981; Hadwiger and others 1986; Sudarshan and others 1992) and the chelation of metals, spore elements, and essential nutrients (Cuero and others 1991).

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Chitosan generally has a stronger antimicrobial activity against bacteria rather than against fungi (Tsai and others 2002). Recent studies on antibacterial activity of chitosan and chitosan oligomers have revealed that chitosan is more effective in inhibiting growth of bacteria than are chitosan oligomers (Uchida and others 1989; Jeon and others 2001). Furthermore, the antibacterial effects of chitosan and chitosan oligomers are reported to be dependent on its molecular weight (Uchida and others 1989; Jeon and others 2001; No and others 2002), degree of deacetylation (DD) (Tsai and others 2002), and the type of bacterium (No and others 2002). More extensive information on the antibacterial activity of chitosan is available (Sudarshan and others 1992; Roller and Covill 1999; Shahidi and others 1999; Rhoades and Roller 2000; No and others 2002).

The antimicrobial properties of chitosan have been reported widely in the literature but mainly based on the *in vitro* trials. Most foods are a mixture of different compounds (for example, carbohydrate, protein, fat, minerals, vitamins, salts, and others) and many of them may interact with chitosan and lead to loss or enhancement of antibacterial activity. Recently, Devlieghere and others (2004) extensively studied the influence of different food components (starch, protein, oil, and NaCl) on the antimicrobial effect of chitosan. For this, the media were inoculated with *Candida lambica* (2 log CFU/mL) and incubated at 7 °C with varying chitosan concentrations (43 kDa, DD = 94%; 0%, 0.005%, and 0.01%) and with the separate addition of the following food components: starch (0%, 1%, and 30% water-soluble starch), proteins (0%, 1%, and 10% whey protein isolate), oil (0%, 1%, and 10% sunflower oil) and NaCl (0%, 0.5%, and 2%). Results showed that starch, whey protein, and NaCl had a negative effect on the antimicrobial activity of chitosan. Oil conversely had no influence. A summary of microorganisms, foods, and scientific references related to the antimicrobial activity of chitosan is given in Table 1.

### Food application of chitosan

**Bread.** The shelf life of bread is generally limited due to staling and microbial growth (Leuschner and others 1999). Staling is a general term that describes the time-dependent loss in quality of flavor and texture of bread. Bread staling is a complex phenomenon in which multiple mechanisms operate (Gray and Bemiller 2003).

Applications of chitosan for extension of shelf life of bread by retarding starch retrogradation and/or by inhibiting microbial growth have been documented. Park and others (2002b) investigated the effect of chitosan (493 kDa) coating on shelf life of baguette. The surface of dough was coated with 0.5%, 1.0%, or 1.5% chitosan in 1.0% acetic acid using a brush after molding. Baguette coated with chitosan, especially with 1% chitosan, showed less weight loss, hardness, and retrogradation than the control during storage for 36 h at 25 °C. This is likely due to the moisture barrier properties of chitosan (Butler and others 1996; Nadarajah and others 2006). Chitosan coating may offer a protective barrier for moisture transfer through the bread surface, thus reducing weight loss and retarding hardness and retrogradation. By this, the shelf life (36 h) of 1% chitosan-treated baguette was extended by 24 h compared with that (12 h) of the control. In a separate experiment, Park and others (2002c) also found that the shelf life of baguette coated with 1% chitosan oligomer (2 kDa) dissolved in distilled water could be extended by 24 h compared with that (12 h) of the control, as similarly observed by Park and others (2002b) with 493-kDa chitosan.

Ahn and others (2003) reported that chitosan coating improved the shelf life and quality of bread by inhibiting microbial growth and by retarding antioxidation and retrogradation. Bread coated with 1% and 2% chitosan (120 kDa, DD = 85%) dissolved in 0.3% lactic acid showed lower total bacterial counts and thiobarbituric

acid-reactive substances (TBARS) and higher water content than those of the control after 8 d of storage at room temperature. Mold growth was detected in the control after 4 d of storage, but was not detected in bread coated with 1% and 2% chitosan throughout 8 d of storage. In other experiments, Lee and Lee (1997) reported that the shelf life of fermented pan bread containing carboxymethyl chitosan was extended by retarding retrogradation and by inhibiting growth of microorganisms. Therefore, the improved shelf life and quality of bread by chitosan coating or addition is attributed to moisture barrier property and ability to retard retrogradation and microbial growth of chitosan.

Molecular weight and concentration of chitosan may affect the shelf life of bread. Lee and others (2002a) investigated the effects of chitosans with different molecular weights (Mw = 1, 5, 30, and 120 kDa) at various concentrations on the shelf life of wheat bread. In these studies, lower Mw (1 and 5 kDa; both DD = 95%) and higher Mw (30 and 120 kDa; DD = 92% and 85%, respectively) chitosans were dissolved in 0.3% lactic acid, and added to dough at 0.01%, 0.1%, 0.3%, and 0.5% concentrations. Chitosans with higher Mw (30 and 120 kDa) were better than those with lower Mw (1 and 5 kDa) in extending the shelf life of bread. With 30 and 120 kDa chitosans, increase in chitosan concentration generally showed increased bacterial growth inhibition in bread. The bread containing 30 or 120 kDa chitosan at above 0.1% concentration showed 10<sup>1</sup> to 10<sup>3</sup> CFU/mL viable cells while the control bread containing no chitosan revealed 10<sup>6</sup> CFU/mL after storage for 8 d at room temperature.

**Egg.** Several problems are encountered during storage of eggs, such as weight loss, interior quality deterioration, and microbial contamination (Bhale and others 2003; De Reu and others 2006). The movement of carbon dioxide and moisture from the albumen through the shell governs quality changes in albumen and yolk, and weight loss of eggs (Murray and Rutherford 1963; Stadelman 1986b). To overcome these problems, considerable attention has been given to the development of coating materials from synthetic polymers (Meyer and Spencer 1973), polysaccharides (Xie and others 2002), proteins (Herald and others 1995; Cho and others 2002; Xie and others 2002; Rhim and others 2004), and oils (Knight and others 1972; Obanu and Mpieri 1984). Chitosan has been documented to possess a film-forming property for use as edible films or coatings (Butler and others 1996; Jeon and others 2002; Nadarajah and others 2006). Thus, chitosan coating may offer a protective barrier for moisture and gas transfer from the albumen through the egg shell, thus extending the shelf life of eggs (Lee and others 1996; Bhale and others 2003).

Yolk index and Haugh unit values are indices of freshness of yolk and albumen quality of egg. The Haugh unit is an expression relating egg weight and height of the thick albumen. The higher the Haugh value, the better the albumen quality of the eggs. The spherical nature of egg yolk can be expressed as a yolk index value by measuring the yolk height and width (Stadelman 1986a). A decrease in a yolk index value during storage indicates a progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by diffusion of water from the albumen (Obanu and Mpieri 1984).

Several workers (Lee and others 1996; Bhale and others 2003; Caner 2005) have reported that chitosan coating is effective in preserving the internal quality of eggs without affecting consumer acceptance. In these studies, eggs were coated with chitosan dissolved in acetic acid with (Caner 2005) and without (Lee and others 1996; Bhale and others 2003) glycerol as a plasticizer using a sponge brush (Lee and others 1996; Bhale and others 2003) or by immersing (Caner 2005). For example, Lee and others (1996) reported that chitosan coating increased shelf life of eggs. The Haugh unit value was significantly higher for 2% chitosan (dissolved in 2% acetic acid) coated eggs (45.87) than 1% chitosan (dissolved in 1% acetic acid) coated

**Table 1 – Scientific publications reporting the antimicrobial activity of chitosan**

Microorganism	Foods	References
Bacteria		
<i>Aeromonas hydrophila</i>	Sausage	Park and others (1999), Youn and others (2000)
	Seafoods	Tsai and others (2002)
<i>Bacillus cereus</i>	Fruits and vegetables	Devlieghere and others (2004)
	Meat	Rao and others (2005)
	Seafoods	Tsai and others (2002)
<i>Bacillus licheniformis</i>	Bread	Lee and others (2002b)
<i>Bacillus subtilis</i>	Bread	Lee and others (2002b)
	Meat	Darmadji and Izumimoto (1994a)
	Sausage	Park and others (1999), Youn and others (2000)
<i>Bifidobacterium bifidum</i>	Milk	Lee and Lee (2000b)
<i>Brochothrix thermosphacta</i>	Fruits and vegetables	Devlieghere and others (2004)
	Meat	Lee and others (2003)
<i>Clostridium historyticum</i>	Sausage	Youn and others (2001b)
<i>Clostridium perfringens</i>	Sausage	Youn and others (2001b)
<i>Coliform</i>	Meat	Darmadji and Izumimoto (1994b)
	Soybean sprouts	Choi and others (2000)
<i>Enterobacter aeromonas</i>	Fruits and vegetables	Devlieghere and others (2004)
<i>Enterococcus faecalis</i>	Bread	Lee and Lee (1997)
<i>Escherichia coli</i>	Bread	Lee and Lee (1997)
	Meat	Darmadji and Izumimoto (1994a), Lee and others (2003), Rao and others (2005)
	Sausage	Park and others (1999), Youn and others (2000, 2001b)
	Seafoods	Cho and others (1998a), Tsai and others (2002)
	Soybean curd	Chun and others (1997, 1999)
<i>Lactobacillus curvatus</i>	Fruits and vegetables	Devlieghere and others (2004)
	Meat	Lee and others (2003)
<i>Lactobacillus fructivorans</i>	Mayonnaise	Roller and Covill (2000)
<i>Lactobacillus plantarum</i>	Fruits and vegetables	Devlieghere and others (2004)
	Kimchi	Lee and Cho (1998), Lee and Jo (1998), Son and others (1996), Yoo and others (1998)
	Meat	Darmadji and Izumimoto (1994a), Lee and others (2003)
<i>Lactobacillus sakei</i>	Fruits and vegetables	Devlieghere and others (2004)
<i>Lactobacillus viridescens</i>	Meat	Sagoo and others (2002)
	Sausage	Youn and others (2001b)
<i>Lactobacillus</i> sp.	Kimchi	Jang and Jeong (2005)
<i>Leuconostoc mesenteroides</i>	Kimchi	Yoo and others (1998)
<i>Leuconostoc</i> sp.	Kimchi	Lee and Cho (1998), Lee and Jo (1998), Son and others (1996)
<i>Listeria innocua</i>	Meat	Lee and others (2003), Sagoo and others (2002)
	Sausage	Park and others (1999), Youn and others (2000)
<i>Listeria monocytogenes</i>	Fruits and vegetables	Devlieghere and others (2004)
	Meat	Lee and others (2003)
	Sausage	Park and others (1999), Youn and others (2000)
	Seafoods	Tsai and others (2002)
<i>Micrococci</i>	Meat	Darmadji and Izumimoto (1994b)
<i>Micrococcus varians</i>	Meat	Darmadji and Izumimoto (1994a)
<i>Pediococcus acidilactici</i>	Fruits and vegetables	Devlieghere and others (2004)
<i>Pediococcus pentosaceus</i>	Meat	Darmadji and Izumimoto (1994a), Lee and others (2003)
<i>Photobacterium phosphoreum</i>	Fruits and vegetables	Devlieghere and others (2004)
<i>Pseudomonas aeruginosa</i>	Meat	Lee and others (2003)
	Sausage	Park and others (1999), Youn and others (2000)
	Seafoods	Tsai and others (2002)
<i>Pseudomonas fluorescens</i>	Fruits and vegetables	Devlieghere and others (2004)
	Milk	Ha and Lee (2001)
<i>Pseudomonas fragi</i>	Meat	Darmadji and Izumimoto (1994a), Lee and others (2003)
<i>Pseudomonades</i>	Meat	Darmadji and Izumimoto (1994b)
<i>Pseudomonas</i> sp.	Meat	Rao and others (2005)
	Seafoods	López-Caballero and others (2005)
<i>Salmonella</i> Enteritidis	Mayonnaise	Roller and Covill (2000)
	Meat	Lee and others (2003)
	Sausage	Park and others (1999), Youn and others (2000, 2001b)
<i>Salmonella</i> Typhimurium	Bread	Lee and Lee (1997)
	Meat	Lee and others (2003)
	Sausage	Park and others (1999), Youn and others (2000, 2001b)
	Seafoods	Tsai and others (2002)
<i>Serratia liquefaciens</i>	Meat	Lee and others (2003)
<i>Serratia marcescens</i>	Bread	Lee and others (2002b)
<i>Shigella dysenteriae</i>	Seafoods	Tsai and others (2002)
<i>Staphylococcus aureus</i>	Bread	Lee and Lee (1997)
	Meat	Darmadji and Izumimoto (1994a), Rao and others (2005)
	Sausage	Park and others (1999), Youn and others (2000)
	Seafoods	Tsai and others (2002)

(continued on next page)

Table 1 – Continued.

Microorganism	Foods	References	
Yeast	<i>Staphylococci</i>	Meat	Darmadji and Izumimoto (1994b)
	<i>Vibrio cholerae</i>	Seafoods	Tsai and others (2002)
	<i>Vibrio parahaemolyticus</i>	Seafoods	Tsai and others (2002)
	<i>Candida albicans</i>	Seafoods	Tsai and others (2002)
	<i>Candida lambica</i>	Fruits and vegetables	Devlieghere and others (2004)
	<i>Cryptococcus humiculus</i>	Fruits and vegetables	Devlieghere and others (2004)
	<i>Saccharomyces cerevisiae</i>	Bread	Lee and Lee (1997), Lee and others (2002b)
		Juice	Roller and Covill (1999)
		Milk	Ha and Lee (2001)
		Juice	Roller and Covill (1999)
Mold	<i>Saccharomyces exiguus</i>	Juice	Roller and Covill (1999)
	<i>Saccharomycodes ludwigii</i>	Juice	Roller and Covill (1999)
		Meat	Sagoo and others (2002)
	<i>Schizosaccharomyces pombe</i>	Juice	Roller and Covill (1999)
	<i>Zygosaccharomyces bailii</i>	Juice	Roller and Covill (1999)
	<i>Aspergillus fumigatus</i>	Seafoods	Tsai and others (2002)
	<i>Aspergillus niger</i>	Bread	Lee and Lee (1997), Lee and others (2002b)
	<i>Aspergillus parasiticus</i>	Seafoods	Tsai and others (2002)
	<i>Botrydipodia lecanidion</i>	Fruits and vegetables	Chien and Chou (2006), Chien and others (2007a)
	<i>Botrytis cinerea</i>	Fruits and vegetables	Chien and Chou (2006), Chien and others (2007a), El Ghaouth and others (1992a)
	<i>Cladosporium</i> sp.	Fruits and vegetables	Park and others (2005)
	<i>Fusarium oxysporum</i>	Seafoods	Tsai and others (2002)
	<i>Penicillium chrysogenum</i>	Bread	Lee and Lee (1997)
	<i>Penicillium digitatum</i>	Fruits and vegetables	Chien and Chou (2006), Chien and others (2007a)
	<i>Penicillium expansum</i>	Bread	Lee and others (2002b)
	<i>Penicillium italicum</i>	Fruits and vegetables	Chien and Chou (2006), Chien and others (2007a)
	<i>Penicillium notatum</i>	Bread	Lee and Lee (1997)
<i>Rhizopus nigricans</i>	Bread	Lee and Lee (1997)	
<i>Rhizopus stolonifer</i>	Fruits and vegetables	El Ghaouth and others (1992a)	
<i>Rhizopus</i> sp.	Fruits and vegetables	Park and others (2005)	

eggs (24.28) and noncoated eggs (9.38) after 30 d of storage at 20 °C. Bhale and others (2003) evaluated internal and sensory quality of eggs coated with 1% and 2% chitosans (1100, 746, or 470 kDa) dissolved in 1% and 2% acetic acid, respectively, during a 5-wk storage at 25 °C. Use of 2% 470 kDa chitosan as a coating material was advantageous in reducing weight loss and obtaining more desirable albumen (higher Haugh unit) and yolk (higher yolk index) quality during a 5-wk storage period. The Haugh unit and yolk index values indicated that the albumen and yolk quality of chitosan-coated eggs could be preserved up to 5 wk at 25 °C, which was at least 3 wk longer than observed for the control noncoated eggs. Bhale and others (2003) investigated whether consumers have negative perception toward chitosan-coated eggs. Regarding sensory perception on external quality, these authors found that consumers could not differentiate the coated eggs from the control noncoated eggs. Overall sensory acceptability for the external quality of all chitosan-coated eggs also was not different from the control and commercial eggs. Caner (2005) also found that the shelf life of eggs coated with chitosan (3% in 1% acetic acid) could be extended by at least 2 wk longer compared with that of control at 25 °C, and that overall sensory acceptability for the external quality of chitosan-coated eggs was not different from the control eggs.

Chitosan production typically involves deproteinization (DP), demineralization (DM), decolorization, and deacetylation. Simplification of chitosan production by elimination of the DP or DM step, or reduction of the reaction time required for DP and DM, would considerably reduce production cost due to reduction in chemical usage, process time, and voluminous wastewater discharge. Chitosans prepared by the simplified process may be used for particular usages, such as coating of eggs that does not require a pure chitosan product (No and others 2005). The internal quality of eggs coated with chitosans prepared in a laboratory scale under various deproteinization (DP at 0, 5, 15 min) and demineralization (DM at 0, 10, 20, 30 min) times was evaluated by No and others (2005). Eggs

were coated with chitosan (2% in 1% acetic acid) without a plasticizer using a sponge brush. Chitosans prepared under DP 0 min/DM 30 min (viscosity of 1% chitosan in 1% acetic acid = 142.2 cP, DD = 82.3%), DP 5 min/DM 30 min (39.7 cP, DD = 84.7%), and/or DP 15 min/DM 20 min (28.2 cP, DD = 85.7%) conditions could be effectively used as an egg-coating material in preserving the internal quality of eggs compared with chitosan under the DP 15 min/DM 30 min (26.3 cP, DD = 85.0%) condition. The differences in weight (54.58 g compared with 54.59 g) and shell thickness (0.36 mm compared with 0.36 mm) of eggs before and after chitosan coating were very insignificant. The Haugh unit and yolk index values suggested that the chitosan-coated eggs could be preserved for at least 2 wk longer than the control noncoated eggs during 5 wk of storage at 25 °C.

Barrier properties of edible films may depend on their composition, such as plasticizers and solvents. In preparation of edible films, plasticizer is often incorporated into films to induce flexibility, and glycerol is one of the most widely used plasticizers (Wan and others 2005). Chitosan is insoluble in water or organic solvents, but is soluble in diluted organic acids and forms viscous solutions. The viscosity property of chitosan solution may differ with organic acid types used as a dissolving solvent, thus affecting the properties of resultant films or coatings (Kienzle-Sterzer and others 1982; Rhim and others 1998; Park and others 2002d). Most of the previous works on chitosan-coated eggs (Lee and others 1996; Bhale and others 2003; No and others 2005) have been done with chitosan dissolved in acetic acid without plasticizer. More recently, Kim and others (2006) evaluated the effects of plasticizer concentrations (0%, 0.5%, 1.0%, 1.5%, and 2.0% glycerol) and solvent types (1% acetic and 1% lactic acid) on internal quality of eggs coated with 2% chitosan (440 kDa) solution using a sponge brush. In comparison with plasticizer concentrations, eggs coated with chitosan dissolved in acetic acid containing 2% glycerol showed a significant reduction in weight loss and had at least 3 wk longer shelf life extension compared with the noncoated

eggs during a 5-wk storage at 25 °C. Use of acetic acid rather than lactic acid as a chitosan solvent was more advantageous in view of shelf life extension of eggs.

In addition to weight loss, Haugh unit, and yolk index, albumen pH also can be used as a quality index (Scott and Silversides 2000). During storage, the carbon dioxide escapes and this increases the pH up to 9.4 (Scott and Silversides 2000; Silversides and Scott 2001; Caner 2005). Egg yolks have a pH of about 6.0, which stays relatively constant during storage as long as there is no carbon dioxide loss (Caner 2005). Scott and Silversides (2000) and Silversides and Scott (2001) evaluated egg (uncoated) quality during 10-d storage at room temperature and found that with storage, albumen pH increased from 7.34 to 9.37 (Scott and Silversides 2000) and 7.43 to 9.32 (Silversides and Scott 2001), with decreasing the albumen height. Caner (2005) observed that albumen pH of chitosan (3% in 1% acetic acid) coated eggs increased from 7.49 to 8.83 while that of uncoated eggs increased from 7.48 to 9.3 after 4-wk storage at 25 °C. These results indicate that chitosan coating decreased carbon dioxide release through the shell by acting as a gas barrier. This also provides an evidence that the improved quality and extended shelf life of eggs by chitosan coating is attributed to the protective barrier properties of chitosan film for moisture and gas transfer through the egg shell.

**Fruits and vegetables.** The major postharvest losses of fruits are due to fungal infection, physiological disorders, and physical injuries (El Ghaouth and others 1991, 1992b). One of the potential approaches to extend the storability of these perishable commodities is to apply edible coatings on the surface, followed by a cold storage (Park and others 2005). Edible coatings can be used as a protective barrier to reduce respiration and transpiration rates through fruit surfaces, retard microbial growth and color changes, and improve texture quality of fruits (Kester and Fennema 1986).

Coating fruits with semipermeable film has generally been shown to retard ripening by modifying the endogenous CO<sub>2</sub>, O<sub>2</sub>, and ethylene levels of fruits (El Ghaouth and others 1991). Chitosan coating is likely to modify the internal atmosphere without causing anaerobic respiration, since chitosan films are more selectively permeable to O<sub>2</sub> than to CO<sub>2</sub> (Bai and others 1988). Therefore, chitosan coating with its ability to modify internal atmosphere in the tissue and fungistatic property has a potential to prolong storage life and control decay of fruits.

There is ample evidence that chitosan coating has the potential to prolong the storage life and control decay of fruits. Strawberry is among the most perishable fruits and is vulnerable to physical injuries and fungal infection caused by *Botrytis cinerea* and *Rhizopus* sp. (El Ghaouth and others 1991, 1992a; Park and others 2005). El Ghaouth and others (1991, 1992a) investigated the effect of chitosan coating on decay and quality of strawberries at 13 °C. Strawberry fruits were inoculated with spore suspension of *Botrytis cinerea* (El Ghaouth and others 1991, 1992a) or *Rhizopus stolonifer* (El Ghaouth and others 1992a) and subsequently dipped in chitosan solutions (1.0% and 1.5% in 0.25 N HCl). In both studies, chitosan coating significantly reduced the decay of strawberries compared to the control. However, there was no added benefit to decay control by increasing concentration of chitosan from 1.0% to 1.5%. During storage at 4 °C, chitosan-coated berries were firmer, had higher titratable acidity, and synthesized anthocyanin at a slower rate than the control and the fungicide, Rovral<sup>®</sup>-treated berries (El Ghaouth and others 1991). Chitosan coating decreased the respiration rate of strawberries with a greater effect at higher concentration (El Ghaouth and others 1991). The improved storability of fresh strawberries by chitosan-based coating also has been documented by Reddy and others (2000), Han and others (2004), Park and others

(2005), Hernández-Muñoz and others (2006), and Vargas and others (2006).

Chitosan has the ability to inhibit growth of several fungi, to induce chitinase, a defense enzyme, and to elicit phytoalexin in pea pods (El Ghaouth and others 1991, 1992b). Thus, the control of decay in strawberries could be attributed either to the fungistatic property of chitosan per se or to its ability to induce defense enzymes (that is, chitinase and  $\beta$ -1,3-glucanase) or a combination (El Ghaouth and others 1991, 1992a). In studies on antifungal activity of chitosan on 2 postharvest pathogens (*Botrytis cinerea* and *Rhizopus stolonifer*) of strawberry fruits, El Ghaouth and others (1992a) observed that coating of intact fruits with chitosan did not stimulate chitinase, chitosanase, or  $\beta$ -1,3-glucanase activities in the tissue. Stimulation of chitinase activities was observed, however, when chitosan was applied directly on freshly cut strawberries. The inability of chitosan coating to stimulate chitinase in intact fruits could quite possibly be due to the limited intimate interaction between the coating material and the tissue. Strawberry cuticle, which is nonporous, may physically separate chitosan from the tissue and consequently prevent chitosan from inducing chitinases. Based on these observations, El Ghaouth and others (1992a) concluded that mechanisms by which chitosan coating reduced the decay of the whole intact strawberries appeared to be related to its fungistatic property rather than to its ability to induce defense enzymes such as chitinase, chitosanase, and  $\beta$ -1,3-glucanase.

Edible coatings can be used as a vehicle for incorporating functional ingredients such as antioxidants, flavors, colors, antimicrobial agents, and nutraceuticals (Kester and Fennema 1986; Diab and others 2001; Han and others 2004). Several workers have endeavored to incorporate calcium (Han and others 2004; Hernández-Muñoz and others 2006), vitamin E (Han and others 2004, 2005), potassium (Park and others 2005), or oleic acid (Vargas and others 2006) into chitosan film formulation to prolong the shelf life and to enhance the nutritional value of fruits.

For example, Han and others (2004) applied 3 chitosan (DD = 89.9%, 2% in 1% acetic acid) based coatings [chitosan, chitosan containing 5% Gluconal<sup>®</sup> CAL (a mixture of calcium lactate and calcium gluconate), and chitosan containing 0.2% DL- $\alpha$ -tocopheryl acetate] to strawberries (*Fragaria ananassa*) and red raspberries (*Rubus ideaus*). These coatings proved to extend the shelf life of strawberries and red raspberries by decreasing the decay incidence and weight loss, delaying changes in color, pH and titratable acidity during storage at 2 °C. Adding calcium or vitamin E into chitosan-based coatings did not significantly alter their antifungal and moisture barrier functions. In these studies, Han and others (2004) observed no significant difference in weight loss among the 3 types of coatings. However, results on the water vapor permeability (WVP) of the films made from the same coating solutions demonstrated that the moisture barrier properties of chitosan coating containing calcium or vitamin E were significantly improved. For this, these authors explained that, by adding a high concentration of calcium salts into the film matrix, counter ion interactions increase among adjacent molecular structures with small mineral ions acting as fillers, thus resulting in decreased diffusivity of water vapor through the film matrix and a decrease in the hydrophilic tendency of chitosan films. The influence of hydrophobic additives such as vitamin E on the WVP of the films is generally expected to improve the water vapor barrier properties by providing hydrophobicity and increasing film resistance to water transmission (Amarante and Banks 2001; Han and others 2004). To this end, Vargas and others (2006) added oleic acid to chitosan (DD = 82.7%) films to improve their moisture barrier properties. Addition of oleic acid not only enhanced chitosan antimicrobial activity but also improved water vapor resistance of

chitosan (1% in 1% acetic acid) coated strawberries. However, it was recommended that oleic acid be incorporated at a ratio of less than 4:1 (oleic acid:chitosan) to avoid unpleasant changes in the sensory attributes.

In addition, Han and others (2004) observed that incorporation of calcium or vitamin E into chitosan coating significantly increased the content of these nutrients in strawberries and red raspberries; this increase was likely due to the diffusion of calcium and vitamin E during soaking. Similarly, Hernández-Muñoz and others (2006) observed that while addition of 1% calcium gluconate to the chitosan coating formulation (1.5% in 0.5% acetic acid) did not further extend the shelf life of strawberries, the amount of calcium (3079 g/kg dry matter) retained by the fruit was greater than that obtained with calcium dips alone (2340 g/kg), thus resulting in increased nutritional value of the strawberries.

Han and others (2005) evaluated sensory quality of fresh strawberries coated with three 1% chitosan (DD = 89.8%) solutions: chitosan in 0.6% acetic acid solution, in 0.6% lactic acid solution, and in 0.6% lactic acid solution plus 0.2% vitamin E. The 1% chitosan coating resulted in no perception of astringency. Lactic acid helped increase the glossiness of the coated strawberries, but the incorporation of vitamin E into the chitosan coating reduced the glossiness of coated strawberries; this could affect consumer acceptance.

Park and others (2005) evaluated the antifungal effect of 2% chitosan (DD = 89.9%; dissolved in 0.5% acetic acid) and 2% chitosan containing 0.3% potassium sorbate (PS) against *Cladosporium* sp. and *Rhizopus* sp. that were coated on fresh strawberries by dipping. Coating with chitosan containing PS did not show any advantage over the chitosan-alone coating for delay of fungal growth on fresh strawberries. However, significant synergistic inhibition activity was observed in the in vitro testing when PS was incorporated into chitosan coating. These different results may be explained by the amount of PS that spores were exposed to. In the in vitro test, spore suspensions on the surface of each agar plate were fully exposed to PS, that is, direct interactions between PS and the spores. However, in the coating application, PS was incorporated into the thin layer of the coatings and may have gradually diffused into the surface of the strawberries to interact with the spores, thus limiting antifungal action. In addition, the coating material may have interfered with the antifungal property of PS (Park and others 2005). The nonsignificant antifungal activity of chitosan with PS was also reported by Chen and others (1996), who suggested that the antimicrobial activity of chitosan films with PS could be limited by ionic interaction between PS and chitosan. Furthermore, Park and others (2005) observed that the addition of PS into chitosan coating significantly increased water vapor permeability (48.2 and 67.6 g.mm/m<sup>2</sup>.d.kPa for 2% chitosan and 2% chitosan containing 0.3% PS, respectively) of the coating without affecting thickness (44.5 and 46.2 μm, respectively) and, thus, may have compromised weight loss control.

Rapid postharvest browning of litchi fruit pericarp is the result of polyphenol oxidase activity, anthocyanin hydrolysis, and nonenzymatic polymerization of o-quinones into melanins (Caro and Joas 2005). Effects of chitosan coating on browning of litchi (*Litchi chinensis* Sonn.) fruit were investigated by several workers (Zhang and Quantick 1997; Caro and Joas 2005; Jiang and others 2005; Joas and others 2005). In these studies, coating was formed by dipping fruits in chitosan solution. Zhang and Quantick (1997) reported that chitosan coating, irrespective of concentration (1% and 2% dissolved in 2% glutamic acid), delayed changes in contents of anthocyanins, flavonoids, and total phenolics. It also delayed the increase in polyphenol oxidase (PPO) activity and partially inhibited the increase in peroxidase activity. Jiang and others (2005) also similarly

observed that chitosan (2% in 5% acetic acid) coating delayed the decrease in anthocyanin content and the increase in PPO activity. Such effects of chitosan coating were also observed with peeled litchi fruit (Dong and others 2004), longan fruit (Jiang and Li 2001), and fresh-cut Chinese water chestnut vegetable (Pen and Jiang 2003). Dependence of browning rate of chitosan-coated litchi fruit on the initial pericarp water content (Caro and Joas 2005), pericarp pH, and dehydration rate during storage (Joas and others 2005) has been reported.

Besides the above-mentioned fruits, El Ghaouth and others (1992b) reported that chitosan coating (1% and 2% in 0.25 N HCl) reduced the respiration rate and ethylene production of tomato, with a greater effect observed at 2% than 1% chitosan. Chitosan-coated tomatoes were firmer, higher in titratable acidity, and less decayed, and they exhibited less red pigmentation than the control fruit after 4 wk of storage at 20 °C. Similarly, Kim and others (1999) also observed that chitosan (318 kDa; 2% in 0.2-M acetic acid) coated tomatoes showed less weight loss and higher flesh firmness than the noncoated control during storage at room temperature for 18 d. Reduction of the respiration rate and ethylene production, control of decay, and retention of firmness as a result of chitosan coating have also been reported for apples (Hwang and others 1998; Davies and others 1989), banana (Kittur and others 2001), citrus (Chien and Chou 2006; Chien and others 2007a), mango (Kittur and others 2001; Chien and others 2007b), peach (Li and Yu 2000), carrots (Durango and others 2006), and lettuce (Devlieghere and others 2004). Apples coated with N,O-carboxymethyl chitosan films and placed in cold storage could be kept in fresh condition for more than 6 mo (Davies and others 1989). Chitosan (43 kDa, DD = 94%) coating is effective in controlling decay of strawberry and lettuce; however, its applicability for lettuce may be hampered due to a pronounced bitter taste developed after treatment (Devlieghere and others 2004).

The above documents collectively demonstrate the ability of chitosan film or coating to prolong the storage life and to better control decay of fruits and vegetables by decreasing respiration rates, inhibiting fungal development, and/or delaying of ripening by reduction of ethylene and carbon dioxide evolution. More comprehensive information on effect of chitosan on horticultural commodities is available from Bautista-Baños and others (2006).

**Juice.** Processing of clarified fruit juices commonly involves the use of clarifying aids, including gelatin, bentonite, silica sol, tannins, polyvinylpyrrolidone, or combinations of these compounds (Soto-Peralta and others 1989). Chitosan with a partial positive charge has been shown to possess acid-binding properties (Imeri and Knorr 1988) and to be effective in aiding the separation of colloidal and dispersed particles from food processing wastes (Knorr 1985; No and Meyers 2000). These properties make chitosan an attractive processing aid in fruit juice production.

Chitosan can be used as a clarifying agent in fruit juices. Clarification of fruit juices with chitosan has been attempted by Soto-Peralta and others (1989), Chatterjee and others (2004), and Rungsardthong and others (2006). Chatterjee and others (2004) applied water-soluble chitosan, hydrolyzed with 7% acetic acid, for clarification of apple, grape, lemon, and orange juices. Chitosan (2% dissolved in water) was more effective in reducing the turbidity of juices than bentonite and gelatin. The appearance and acceptability of the juices (measured on a 9-point hedonic scale), after chitosan treatment, significantly increased. Rungsardthong and others (2006) compared the effectiveness of chitosan prepared from *Abisidia glauca* var. *paradoxa* (fungal chitosan; DD = 86%) with that prepared from shrimp shells as fining agents for apple juice. They found that the fungal chitosan treatment was more effective in reducing the apple juice turbidity and yielded lighter (higher Hunter L\*

value) juices than the sample treated with shrimp chitosan. Turbidity decreased gradually with increasing chitosan (dissolved in 2% malic acid) concentration from 0.1 to 0.7 g/L of juice, but it was then increased at 1.0 g/L, which may have been due to the saturation of the active chitosan adsorption sites. Fungal chitosan treatment with approximately 0.5 to 1.0 g/L resulted in higher  $L^*$  values than the control and the juice treated with shrimp chitosan.

Chitosan may also be used to control acidity in fruit juices. Imeri and Knorr (1988) observed that chitosan (1.2% in 2% ascorbic acid) treatment had no effect on carrot and apple juice yield but significantly reduced titratable acidity due to its acid-binding properties. The effects of chitosan treatment on the reduction of titratable acidity provide a potential for acidity control in other food systems.

Enzymatic browning in apple and pear juices can be prevented by removing particulate matters by filtration or centrifugation (Sapers 1992). Sapers (1992) investigated whether the addition of chitosan (1% in 1% malic acid) to apple and pear juices would increase the effectiveness of filtration or centrifugation treatments in controlling browning. Results indicated that browning could be prevented in the McIntosh apple juice by addition of at least 200 ppm chitosan, irrespective of the type of chitosan (low or high viscosity) tested, followed by filtration with diatomaceous earth filter aid. Chitosan at 1000 ppm was required to prevent browning in juices prepared from ripen Bartlett and Bosc pears. The success of chitosan treatments in controlling enzymatic browning in filtered apple and pear juices is probably due to the ability of the positively charged polymer to coagulate suspended solids to which polyphenol oxidase (PPO) is bound (Sapers 1992).

Few attempts have been made to date to assess the antimicrobial properties of chitosan in juices (Roller and Covill 1999; Rhoades and Roller 2000). Roller and Covill (1999) reported that chitosan glutamate was an effective preservative against spoilage yeasts in apple juice. The presence of chitosan glutamate (DD = 75% to 85%) in apple juice (pH 3.4) at a level ranging from 0.1 to 5 g/L inhibited growth of all spoilage yeasts (*Zygosaccharomyces bailii* 906 and HP; *Saccharomyces cerevisiae* 3085, SD and 28; *Schizosaccharomyces pombe*; *Saccharomyces exiguus*; *Saccharomycodes ludwigii*) at 25 °C. The most sensitive strain was *Z. bailii*: it was completely inactivated by chitosan at 0.1 and 0.4 g/L for 32 d of storage at 25 °C. The most resistant strain was *S. ludwigii*, which required a level of 5 g/L of chitosan for complete inactivation and for maintaining yeast-free conditions in apple juice for 14 d at 25 °C. Rhoades and Roller (2000) also studied the antimicrobial activities of native and degraded (hydrolyzed by papaya latex) chitosan against the natural microbial flora in pasteurized apple-elderflower juice (pH 3.3) stored at 7 °C. At 0.3 g of chitosan/L addition, irrespective of chitosan types, yeasts were eliminated entirely during 13 d of storage; however, the total counts and the lactic acid bacterial counts increased at a slower rate than did in the control.

**Kimchi.** Kimchi is a traditional Korean fermented vegetable food, made mainly from Chinese cabbage with various ingredients such as garlic, red pepper powder, ginger, green onion, and fermented fish sauce (Choi and others 2006). Several workers have reported that chitosan was effective in extending the edible periods of kimchi (No and others 1995; Hur and others 1997; Lee and Jo 1998; Yoo and others 1998; Lee and Lee 2000a). The shelf life of kimchi supplemented with chitosan oligosaccharide (0.005%, 0.02%, and 0.2% in water) could be extended by 2 to 6 times compared to 2 d of the control kimchi at 20 °C (Yoo and others 1998).

In an effort to extend the shelf life of kimchi, No and others (1995) soaked Chinese cabbage in a salt solution containing various concentrations of chitosan for 24 h at room temperature. The shelf life of kimchi, prepared with Chinese cabbage soaked in 10% salt solution

containing 0.1% or 0.15% chitosan, was extended by approximately 10 d longer than that of the control kimchi. Similarly, Hur and others (1997) and Lee and Jo (1998) also observed that soaking the salted and washed Chinese cabbage in 0.5% or 1.0% chitosan solution resulted in the delay of the fermentation rate of kimchi.

Chitosan also was reported to delay softening of kimchi tissues during fermentation (Ahn and Lee 1995; Park and others 2002a) and to increase the chemopreventive effect of kimchi (Kim and others 2004). In some instances, chitosan has been applied with other preservatives, such as medicinal herb extracts (Lee and Cho 1998), Na-benzoate (Son and others 1996), and liquid calcium (Jang and Jeong 2005) for improved quality and shelf life of kimchi. For example, the color of kimchi prepared with medicinal herb extracts (3% of a 1:1 mixture of *Lithospermum erythrorhizon* and *Glycyrrhiza uralensis*) became more reddish in the presence of added chitosan (Lee and Cho 1998). The shelf life of kimchi with 0.5% chitosan-liquid calcium added was improved compared with that of kimchi with liquid calcium alone; this was due to growth inhibition of lactic acid bacteria at an early stage of fermentation (Jang and Jeong 2005).

**Mayonnaise.** Mayonnaise is an oil-in-water emulsion. A few studies have been conducted on the use of chitosan to enhance emulsification in mayonnaise preparation. Lee (1996) reported that addition of chitosan (1500 kDa, 0.1% based on egg yolk weight) increased emulsifying capacity of egg yolk by about 10% and enhanced emulsion stability of mayonnaise by 9.4% compared with those of the control. Kim and Hur (2002) also suggested the use of chitosan as an emulsion stabilizer in commercial mayonnaise preparation. Unlike other polysaccharides, chitosan possesses a positive ionic charge and has both reactive amino and hydroxyl groups, which give it the ability to chemically bond with negatively charged protein. When pH is less than 6.5, chitosan solution carries a positive charge along its backbone. Because of its polar groups, chitosan also provides additional stabilization due to hydration forces (Del Blanco and others 1999). According to Filar and Wirick (1978), chitosan functions only in acid systems to show possible utility as a thickener and stabilizer.

Del Blanco and others (1999) investigated the effect of chitosan DD (73% to 95%) on emulsification properties. Chitosan solution was prepared in 1% acetic acid at a 1% concentration. Results indicated that all DD gave stable polydispersed water/oil/water emulsions with different viscosities. Two optimum DD values were found at 81% and 88%, giving complete emulsification without residual oil or sedimentation. Chitosans with intermediate DD were less effective emulsifiers while chitosans with higher DD gave poor emulsification. Rodríguez and others (2002) also studied emulsification properties of chitosans with 7 different DD (75% to 95%). In this study, chitosan solutions (0.2%, 1.0%, and 2.0%) were prepared in 0.1 M hydrochloric acid. They found that chitosan produced stable water/oil/water (w/o/w) emulsions and that the drop size distribution was unimodal at low DD (75%) and at high DD (95%) for all chitosan concentrations. Meanwhile, drop size distribution was independent of both chitosan solution viscosity and emulsion viscosity. The emulsion viscosity and emulsion stability were proportional to chitosan concentration (Rodríguez and others 2002).

Roller and Covill (2000) investigated the effectiveness of chitosan glutamate as a preservative against both bacteria and yeast in mayonnaise. Mayonnaise containing 3 g/L of powered chitosan combined with acetic acid (0.16%) or lemon juice (1.2% and 2.6%) was inoculated with log 5 to 6 CFU/g of *Salmonella* Enteritidis, *Zygosaccharomyces bailii*, or *Lactobacillus fructivorans* and stored at 5 and 25 °C for 8 d. They found that mayonnaise samples at the same pH (4.4 to 4.5) but containing acetic acid as the acidulant were less supportive

of microbial growth and survival than those containing lemon juice. Especially in mayonnaise containing chitosan and 0.16% acetic acid, *L. fructivorans* was completely inactivated to a level below the detection limit of the plate counting method at both storage temperatures. In mayonnaise containing lemon juice at both 1.2% and 2.6%, no substantial differences were observed between the controls and the samples containing chitosan at either storage temperature.

**Meat.** Meat or meat products are highly susceptible to lipid oxidation, which leads to rapid development of rancid or warmed-over flavor. Chitosan possesses antioxidant and antibacterial capacity (Kamil and others 2002; No and others 2002), and may retard the lipid oxidation and inhibit the growth of spoilage bacteria in meat during storage.

Effectiveness of chitosan addition on storage stability of meat has been reported by several workers. For example, Darmadji and Izumimoto (1994a) observed that addition of 1.0% chitosan to beef decreased the TBA value by about 70% compared to that of the control sample after 3 d of storage at 4 °C. After 10 d of storage, the TBA value of the beef sample containing 0.5% to 1.0% chitosan was almost the same as that at day 0, whereas the TBA value of the control sample increased sharply. Chitosan had a desirable effect on the development of the red color of beef during storage. Darmadji and Izumimoto (1994b) also found that the effectiveness of chitosan addition on storage stability of minced beef was enhanced when combined with nitrite and *Lactobacillus plantarum*. The use of 3 combined treatments (0.5% chitosan, 1.0% *Lactobacillus plantarum* starter culture, and 100 ppm nitrite) inhibited the growth of bacteria by about 2-log cycle, reduced TBA value by 36%, dissipated residual nitrite by 63%, and resulted in a better color of fermented meat. Youn and others (2004) noted that the shelf life of spicy beef added with 1.0% chitosan (120 kDa, DD = 85%) dissolved in 0.3% lactic acid was remarkably improved by reducing the total bacterial cell counts and inhibiting lipid oxidation during storage for 10 d at 4 °C. Sagoo and others (2002) demonstrated that chitosan was an effective inhibitor of microbial growth in chilled comminuted pork products and that the effect of chitosan was concentration dependent. Addition of 0.3% and 0.6% chitosan glutamate to an unseasoned minced pork mixture reduced total viable counts, yeasts and molds, and lactic acid bacteria by up to 3-log CFU/g for 18 d at 4 °C compared with the untreated control. Juneja and others (2006) reported that addition of 3% chitosan glutamate (DD = 86%) to ground beef or turkey may reduce the potential risk of *Clostridium perfringens* spore germination and outgrowth during abusive cooling from 54.4 to 7.2 °C in 12, 15, or 18 h.

Lee and others (2003) investigated storage stability of pork dipped in chitosan solution. Pork was dipped for 1 min in various concentrations (0.1%, 0.5%, 1.0%) of chitosans with different molecular weights (5, 30, 120 kDa) and then stored for 8 d at 10 °C. The results indicated that the shelf life and antioxidation of pork increased by dipping with 1.0% solution of 30 and 120 kDa chitosans. The 5 kDa chitosan treatment, irrespective of its concentrations, was ineffective in extending the shelf life and preventing lipid oxidation of pork. The external redness color of pork treated with 30 and 120 kDa chitosans remained unchanged during storage. The treatment of beef loins by dipping in 1% chitosan solution, followed by 5% trisodium phosphate, effectively inhibited the growth of aerobic spoilage microorganisms during storage at 10 °C (Cheong and others 2001).

Wu and others (2000) found that wrapping with chitosan (CH, DD = 85%; 2% in 1% formic acid), wheat gluten (WG), or soy protein (SP) film was not effective in controlling lipid oxidation of precooked beef patties. These authors explained that a higher O<sub>2</sub> permeability may contribute to the higher thiobarbituric acid-reactive substances (TBARS) from these treatments. After being applied on patties and

during the 3 d of storage, the CH, WG, and SP wrappings absorbed moisture from meat samples and swelled. The plasticizing effect of water molecules may have changed the barrier properties of the edible films, and resulted in higher O<sub>2</sub> permeability or even loss of film integrity. The composition of the CH film may have also contributed to its higher O<sub>2</sub> permeability. Formic acid was used as a solvent to form the CH film in this study. Formic CH film has been reported to have higher O<sub>2</sub> permeating coefficient than lactic acid and acetic acid CH films (Caner and others 1998).

Radiation processing is an alternate technology to eliminate microbial contamination in meat and meat products; however, it accelerates lipid peroxidation (Kanatt and others 2004, 2005). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and others are being currently used to prevent oxidative changes in foods (Rao and others 2005). Kanatt and others (2004) investigated the use of irradiated chitosan as a natural antioxidant to minimize lipid peroxidation of radiation-processed lamb meat. Irradiation of chitosan at 25-kGy dose of gamma radiation resulted in a 6-fold increase in its antioxidant activity as compared to the nonirradiated chitosan, as measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. The TBA values of irradiated lamb meat containing irradiated chitosan (4 mL of 1% chitosan in 1% acetic acid/100 g meat) decreased by 88% (leg) and 54% (rib) as compared to the corresponding sample without chitosan. Further development of postprocessing rancidity was reduced by 39% (leg) and 59% (rib) in the samples treated with chitosan as compared to nontreated controls even after 1 wk of storage at 0 to 3 °C.

Rao and others (2005) also demonstrated the potential of irradiated chitosan as an antioxidant that substantially retarded lipid peroxidation and could prevent the spoilage due to microbial growth in the irradiated intermediate-moisture (IM) meat products. Chitosan (1% in 1% acetic acid) was irradiated at a 4-kGy dose in a Cobalt-60 Gamma cell-220 for the purpose of decontamination. The antioxidant activity of chitosan increased upon irradiation, as reflected by an increase in reducing power and free radical scavenging activity, without significantly affecting its antimicrobial property. The chitosan-coated irradiated (4 kGy) IM meat products, such as mutton kebabs and streaky bacon, showed lower TBARS values than the noncoated irradiated samples for up to 4 wk of storage at ambient temperature. The use of chitosan coating in place of polyethylene packaging for the preparation of safe and stable IM meat products was thus possible.

**Milk.** A few attempts have been made to evaluate the possibility of using chitosan to improve the quality and shelf life of milk. Lee and Lee (2000b) studied the effects of water-soluble chitosans with 3 different molecular weights (0.2 to 3, 3 to 10 and 10 to 30 kDa) on the physicochemical and sensory properties of milk. Consistency of chitosan-added milk increased with increasing molecular weight and concentrations (0.5%, 1.0%, and 1.5%). Milk containing 0.5% and 1.0% chitosan, irrespective of molecular weight, could be sterilized at 73 °C for 15 s without protein coagulation. Addition of 0.5% water-soluble chitosan to milk negatively affected its sensory quality of color, taste, and flavor, browning in color and chemical off-flavor with both 0.2 to 3 kDa and 3 to 10 kDa chitosan and astringent taste with 10 to 30 kDa chitosan. However, there were no differences in the sensory quality between coffee-flavored milk containing chitosan and the control; this may have been due to masking effects from coffee.

Ha and Lee (2001) investigated the effectiveness of water-soluble chitosan (0.03%) to minimize the microbial (bacterial and yeast) spoilage of processed milk. Complete inhibition of microbial growth was observed in the banana-flavored milk containing chitosan, in contrast to that observed in control milk (without chitosan), during

storage for 15 d at 4 and 10 °C. The banana-flavored milk containing chitosan also maintained relatively higher pH than that of control milk during storage for 15 d at both temperatures.

**Noodle.** Lee and No (2002) investigated the effects of addition of chitosan (Mw = 37 kDa) on shelf life and quality of wet noodle. Chitosan dissolved in 1% acetic acid was added to wheat flour at 0.0%, 0.17%, 0.35%, 0.52%, and 0.7% concentrations. During storage at 18 °C for 6 d, moisture content of wet noodle, irrespective of chitosan concentrations, slightly decreased with increasing storage periods. Increasing chitosan concentration from 0% to 0.70% decreased the numbers of viable cells during storage at 18 °C for 6 d. The shelf life of wet noodle containing 0.17%, 0.35%, 0.52%, and 0.70% chitosan was extended by 1, 2, 3, and 3 d, respectively, compared with that of the control. The wet noodle containing 0.35% chitosan had the most desirable sensory quality compared with other wet-noodle samples. In another study, Lee and others (2000) observed that the wet noodle containing chitosan (Mw = 37 kDa, 0.1% or 0.5% dissolved in 1% lactic acid) could be stored longer than 80 d compared to 7 d of the control. These studies clearly demonstrated that chitosan can be used as an effective preservative in wet noodle due to its antimicrobial activity.

**Rice cake.** Lee and others (2000) observed that chitosan treatment considerably extended the shelf life of white rice cake. These authors dipped white rice cake in either 95% alcohol, 1% lactic acid, or 1% and/or 2% chitosan (Mw = 37 kDa, dissolved in 1% lactic acid) for 10 s prior to vacuum packing. The total microbial counts for the control (no treatment), alcohol-treated, and 1% lactic acid-treated white rice cake exceeded the initial putrefactive criterion level of  $1 \times 10^6$  CFU/g, respectively, at 6, 27, and 20 d of storage at 4 °C. On the other hand, the total microbial counts ( $3.3 \times 10^5$  and  $1.4 \times 10^5$  CFU/g, respectively) of the white rice cake treated with 1% and 2% chitosan were less than the criterion level even after 76 d of storage. However, the dipping treatment, irrespective of solution types, was found to negatively affect sensory acceptability of the white rice cake. Prolonged shelf life of rice cake by addition of chitosan was also demonstrated by Park and Chong (2002). Water-soluble chitosan dissolved in water was added to rice cake at 0.0%, 0.05%, 0.1%, 0.3%, and 0.5% concentrations. During storage for 4 wk at 5 °C, the total microbial counts decreased with increasing chitosan concentrations. After 4 wk of storage, the total microbial counts of rice cake containing 0.3% and 0.5% chitosan were 2-log cycle lower than that ( $8.2 \times 10^4$  CFU/g) of the control.

Nam and Woo (2002) investigated the quality characteristics of jeung-pyun, the traditional Korean fermented rice cake leavened by yeast, as affected by the addition (0%, 2%, 4%, and 6%) of chitosan oligosaccharide. Results for sensory evaluation and rheological properties measured with rheometer indicated that addition of 2% chitosan oligosaccharide imparted positive effects on the quality of jeung-pyun.

**Sausage.** In preparation of sausage in Korea, sodium nitrite is generally used as a curing agent for color and flavor development as well as preservative effect (Park and others 1999). However, nitrite reacts with amine in meat and may produce nitrosoamine, a strong toxicant detrimental to human health. Several workers (Park and others 1999; Youn and others 1999, 2000, 2001b) have investigated the possible role of chitosan, in lieu of sodium nitrite, as curing agent in sausage, and found that addition of chitosan could reduce or replace the use of nitrite without affecting preservative effect and color development.

Park and others (1999) reported that a mixture of 0.2% chitosan (120 kDa, DD = 85%, dissolved in 0.3% lactic acid) and 0.005% sodium nitrite or 0.5% chitosan alone exhibited the same preservative effect as did 0.01% sodium nitrite alone when added in meat

sausage. Similarly, Youn and others (1999) reported that addition of 0.2% chitosan (30 kDa, DD = 92%) dissolved in 0.3% lactic acid could reduce the amount of sodium nitrite in half of the standard volume (150 ppm) without affecting quality and storage stability of sausage. In addition to the preservative effect, chitosan also notably reduced the concentration of residual nitrite in sausage (Youn and others 2001b). The concentration of residual nitrite in sausage decreased with increased molecular weights and concentrations of chitosan.

The preservative effect of chitosan in sausage may differ with its molecular weight. According to Youn and others (2000), the preservative effect of chitosan increased with increased molecular weight of chitosans (1, 5, 30, and 120 kDa). No preservative effect of chitosan with a molecular weight of 1 kDa (Youn and others 2000, 2001b) and 5 kDa (Youn and others 2000, 2001b; Jo and others 2001) added at 0.2% or 0.5% concentration in pork sausage was observed. In studies with chitosan glutamate (DD = 75% to 85%), Sagoo and others (2002) found that dipping of standard and skinless pork sausages in chitosan solution (1% dissolved in water) reduced the native microflora (total viable counts, yeasts and molds, and lactic acid bacteria) by approximately 1- to 3-log CFU/g for 18 d at 7 °C and chitosan treatment increased the shelf life of chilled skinless pork sausages from 7 to 15 d.

Lin and Chao (2001) observed that addition of 0.1% chitosan (dissolved in 1% lactic acid) with a molecular weight of 150 (DD = 85%), 600 (DD = 85%), or 1250 kDa (DD = 87%) into the reduced-fat (approximately 22%) Chinese-style sausage had no adverse effect on the textural and sensory characteristics. Jo and others (2001) also found no differences in sensory characteristics (color, flavor, texture, and overall acceptance) between sausages prepared with and without water-soluble chitosan oligomer (5 kDa, 0.2%). Compared with the control sausage, lipid oxidation was, however, lower in the sausage containing chitosan oligomer stored in an aerobic packaging after 3-wk storage at 4 °C. Chitosan may provide antioxidative effect in emulsion-type sausages. According to Youn and others (2001a), antioxidative effect of chitosan in emulsion sausage increased with increased molecular weights (1, 5, 30, 120 kDa) and concentrations (0.2%, 0.35%, and 0.5%) of chitosan.

**Seafoods and seafood products.** Seafood products are highly susceptible to quality deterioration due to lipid oxidation of unsaturated fatty acids, catalyzed by the presence of high concentrations of hemein compounds and metal ions in the fish muscle (Decker and Hultin 1992). Furthermore, seafood quality is highly influenced by autolysis, contamination by and growth of microorganisms, and loss of protein functionality (Jeon and others 2002).

Antioxidant activity of chitosans of different viscosities (360, 57, and 14 cP; corresponding molecular weight of 1800, 960, and 660 kDa) in cooked, comminuted flesh of herring (*Clupea harengus*) was investigated by Kamil and others (2002). The oxidative stability of fish flesh with added chitosans (50, 100, and 200 ppm) was compared with those added with conventional antioxidants, butylated hydroxyanisole + butylated hydroxytoluene (BHA + BHT, 200 ppm) and *tert*-butylhydroquinone (TBHQ, 200 ppm), during storage at 4 °C. Among the 3 chitosans, 14 cP chitosan was most effective in preventing lipid oxidation. The formation of TBARS in herring samples containing 200 ppm of 14 cP chitosan was reduced after 8 d of storage by 52% as compared to that of the control. At 200 ppm, 14 cP chitosan exerted an antioxidant effect similar to that of commercial antioxidants in reducing TBARS values in comminuted herring flesh. These studies indicate that antioxidant capacity of chitosan added to the fish muscle depended on the molecular weight and concentration of chitosan (Kamil and others 2002). Similarly, Kim and Thomas (2007) also observed that the antioxidative effects of chitosan in salmon depended on its molecular weight (30,

90, and 120 kDa) and concentrations (0.2%, 0.5%, and 1.0%). The 30 kDa chitosan showed the highest scavenging activity compared to 90 and 120 kDa chitosan. The scavenging activities of chitosans increased with increasing concentration, except for the 120 kDa chitosan, which showed no significant difference with concentration.

Chitosans may retard lipid oxidation by chelating ferrous ions present in the fish model system, thus eliminating prooxidant activity of ferrous ions or preventing their conversion to ferric ion. Amino groups in chitosans may participate in the chelation of metal ions (Peng and others 1998). The varying antioxidant effects of chitosans with different viscosities observed in cooked comminuted fish model systems (Kamil and others 2002) may be attributed to the molecular weight differences, which determine the extent of chelation of metal ions. In their charged state, the cationic amino groups of chitosans impart intramolecular electric repulsive forces, which increase the hydrodynamic volume by extended chain conformation. Perhaps this phenomenon may be responsible for lesser chelation by high viscosity (high molecular weight) chitosans (Kamil and others 2002). In addition, Xue and others (1998) reported that the antioxidant mechanism of chitosan could be by chelant action of ion metals and/or the combination with lipids. The protective action of chitosan is also effective when it is applied as a protective film, where it retards lipid oxidation and microbial spoilage by acting as a barrier against oxygen (Jeon and others 2002).

Jeon and others (2002) studied the effect of 3 different viscosities (360, 57, and 14 cP; corresponding molecular weight of 1800, 960, and 660 kDa) of chitosan on shelf life extension of fresh fillets of Atlantic cod (*Gadus morhua*) and herring (*Clupea harengus*) over a 12-d storage at refrigerated temperature (4 °C). Chitosan coating significantly reduced lipid oxidation, chemical spoilage (total volatile basic nitrogen, trimethylamine, and hypoxanthine), and growth of microorganisms in both fish compared to the uncoated samples. The preservative efficacy of chitosans with viscosities of 57 and 360 cP was found to be superior to that of chitosan with 14 cP viscosity. These authors concluded that chitosan as edible coating would enhance the quality of seafoods during storage. Similarly, Tsai and others (2002) also observed that the shelf life of salmon fillets dipped in chitosan (49.1 kDa) solution (1% in 0.1 N HCl) was extended from 5 to 9 d. Sathivel (2005) evaluated the effects of chitosan as edible coating on the quality of skinless pink salmon (*Oncorhynchus gorbuscha*) fillets during 3-mo frozen storage. Coating with chitosan was effective in reducing about 50% moisture loss of fillets compared to the control noncoated fillets, and in delaying lipid oxidation. There were no significant ( $P > 0.05$ ) effects of chitosan coating on  $a^*$ ,  $b^*$ , and whiteness values for cooked pink salmon fillets after 3-mo frozen storage. Chitosan coating applied on the surface of the pink salmon fillets may have acted as a barrier between the fillet and its surrounding, thus slowing down the diffusion of oxygen from the surrounding via the surface into the fillet (Sathivel 2005).

Cho and others (1998a) applied chitosan hydrolysate, hydrolyzed with chitosanase, as a natural food preservative for fish paste and found that the shelf life of the product containing 0.3% chitosan hydrolysate was extended by 6 d at 15 °C, 4 d at 20 °C, and 2 d at 30 °C. Ahn and Lee (1992) studied the preservative effect of chitosan film on quality of lightly salted and dried horse mackerel. The product was prepared by soaking the fresh horse mackerel in 15% salt solution for 30 min, coating with or without (control) chitosan, and drying for 3 h at 40 °C in a hot air dryer. During storage at 5 °C for 20 d, the chitosan-coated samples had lower volatile basic nitrogen (VBN), amino nitrogen, trimethylamine (TMA), thiobarbituric acid (TBA), and peroxide values as well as viable cell counts than those of the control. The chitosan-coated samples also obtained higher overall consumer acceptance scores than the control. As such, these au-

thors concluded that chitosan film packing was an effective method for retaining the quality of lightly salted and dried horse mackerel. Similarly, López-Caballero and others (2005) also found that coating fish (cod) patties with a chitosan-gelatin blend retarded spoilage. Addition of chitosan in a powder form into fish patties had no effect on the prevention of spoilage due to the poor insolubility of chitosan at a neutral pH and the presence of a significant proportion of uncharged amino groups.

**Soybean curd (tofu).** Freshly prepared soybean curd is quite perishable due to its rich nutrients and high moisture content, with a typical maximum shelf life of 1 to 2 d even under commercial refrigeration (Dotson and others 1977; Kovats and others 1984). This spoilage is associated with bacterial growth (Dotson and others 1977).

Several workers (Chun and others 1997, 1999) applied water-soluble chitosan as a coagulant or as an immersion solution for extension of shelf life of tofu. Addition of chitosan to a coagulant  $\text{CaCl}_2$  [chitosan: $\text{CaCl}_2$  (g/g) = 1.0:8.0, 1.5:7.5, or 2.0:7.0] extended the shelf life of tofu by more than 7 d at 4 °C compared with tofu made with  $\text{CaCl}_2$  only (Chun and others 1999). Similarly, use of 0.5% water-soluble chitosan solution as an immersion solution extended the shelf life of tofu by more than 7 d at 4 °C (Chun and others 1997). The enhanced shelf life of tofu by chitosan was due to the antimicrobial activity of chitosan.

Lee and others (2001) prepared tofu products at a commercial scale using 4 different coagulants [1:1 (v/v) mixture of 1% acetic + 1% lactic acids (solution A); 1% chitosan dissolved in A;  $\text{CaCl}_2$ ; chitosan +  $\text{CaCl}_2$ ] and compared their shelf life during storage at 10 °C for 7 d. The tofu made with 1% chitosan dissolved in solution A as a coagulant had a shelf life of 4 d compared to 3 d when made with other 3 coagulants. In the comparison of 3 different molecular-weight chitosans (746, 224, and 28 kDa) as coagulants, use of lower molecular-weight chitosan resulted in higher tofu hardness and lower turbidity in the immersion solution during storage. Addition of chitosan also has been reported to increase the yield of soybean curd by decreasing solid materials present in whey (Lee and others 2002b).

No and Meyers (2004) extensively investigated the potential of chitosan as a coagulant in tofu preparation. They used 6 different molecular weights (1106, 746, 471, 224, 28, and 7 kDa) of chitosans under various treatment conditions and established optimum processing conditions as follows: chitosan with a molecular weight of 28 kDa; chitosan concentration and solvent, 1% chitosan in a 1:1 (v/v) mixture of 1% acetic acid and 1% lactic acid; chitosan solution to soymilk ratio, 1:8; coagulation temperature, 80 °C; coagulation time, 15 min. They also reported that the chitosan tofu had a longer shelf life, about 3 d, than the tofu made with  $\text{CaCl}_2$ . Increase in gel strength and shelf life of tofu by addition of 2% chitosan was observed by Chang and others (2003). The gel strength of tofu increased with increased molecular weight (189, 720, and 2780 kDa) of chitosan while the shelf life of tofu increased with increased degree of deacetylation (DD 54%, 73%, and 91%) of chitosan. It is possible that the higher molecular weight chitosan strengthened the gel structure of tofu more than did the lower molecular weight chitosan.

**Soybean sprouts.** Several problems are encountered during cultivation of soybean sprouts, including yield reduction, quality deterioration, and rot occurrence (Lee and others 1999a, 1999b). Recent reports indicated that chitosan treatment increased the growth rate and weight (Lee and others 1999b; Choi and others 2000; Ji and others 2003; No and others 2003; Park and Kim 2003) and decreased the rot of soybean sprouts (Lee and others 1999a; Choi and others 2000).

Lee and others (1999b) reported that soaking soybeans (*Iksan* cultivar) in chitosan solution (1% in 0.25% acetic acid) for 1 h and then in water for 11 h increased the total weight of soybean sprouts and

the hypocotyl length, respectively, by 8.5% and by 25.4%, compared with the control after cultivation for 5 d at 20 °C. Choi and others (2000) observed that soaking soybeans (*Joonjul* cultivar) in chitosan solution (0.1% in 0.25% acetic acid) for 20 min increased the weight of soybean sprouts by 6.9% and the length by 9.5%, compared with the control after cultivation for 4 d at 25 °C. Lee and others (1999a) reported that soaking seeds in 1000 ppm chitosan (5 to 10 kDa) solution significantly reduced soybean sprout rot from 13.8% (of control) to 7.0%.

Recently, No and others (2003) extensively investigated the effects of molecular weights (22, 59, 224, 493, and 746 kDa), concentrations (0%, 0.01%, 0.05%, 0.10%, 0.25%, and 0.50%), and dissolving solvents (acetic and lactic acid) of chitosan, soaking times (0, 1, 3, 5, and 8 h), and soybean/chitosan solution ratios (1:4, 1:5, 1:7, and 1:10) on growth and quality of soybean sprouts. Among 5 chitosans, treatment with 493 kDa chitosan was the most effective in increasing total weight, vitamin C content, and hardness of soybean sprouts. Use of acetic acid rather than lactic acid as a chitosan solvent increased the hypocotyl weight and vitamin C content of soybean sprouts. Soaking of soybeans in chitosan solution for 8 h increased the total weight by 13% and vitamin C content by about 10% in soybean sprouts. No and others (2003) established the optimal cultivation conditions: soaking in 0.05% chitosan with 493 kDa in 0.05% acetic acid for 8 h and a soybean/chitosan solution ratio of 1:4. Under these optimal cultivation conditions established by No and others (2003), Park and Kim (2003) found that the total free amino acid content of chitosan-treated soybean sprouts was higher than that of the control after 5 d cultivation at 20 °C.

Furthermore, chitosan treatment effectively decreased the activity of lipoxygenase-2 and lipoxygenase-3, which are major causes in off-flavor formation, during cultivation of soybean sprouts (Lee and Rhee 1999). Increased shelf life of soybean sprouts treated with chitosan (200 kDa) and chitosan oligomer (20 kDa), compared with control, also was reported by Ji and others (2003).

More recently, No and others (2006) evaluated the effects of chitosans, prepared in a laboratory scale under various deproteinization (DP at 0, 5, 15 min) and demineralization (DM at 0, 10, 20, 30 min) times, on the growth of soybean sprouts in an effort to develop an ultimate economic chitosan production process for particular food applications. These authors observed that chitosan treatment increased the total weight of soybean sprouts by 10.7% to 13.8% compared with that of the control; the effectiveness of the chitosan treatment was independent of DP and DM times. The increase (12.7%) in the growth of soybean sprouts by chitosan prepared without DP and DM steps was noteworthy. Chitosan treatment may increase the production cost of soybean sprouts in commercial plants. However, the increased yield of soybean sprouts can certainly offset the increased production cost of soybean sprouts by chitosan treatment.

**Starch jelly.** The use of chitosan as an antimicrobial agent to extend the shelf life of starch jelly has been demonstrated. Moon and others (1997) studied the preservative effect of chitosan on acorn starch jelly. The shelf life of acorn starch jelly containing 0.5% chitosan (44 kDa, DD = 75.2%) dissolved in 1.0% acetic acid was extended to 6 d at room temperature, twice longer than that of the control. Addition of chitosan to the acorn starch jelly formulation also increased its hardness compared with that of the control. The observation through scanning electron microscope (SEM) revealed that acorn starch jelly containing chitosan showed a finer and more fibrous structure than that of the control without chitosan (Moon and others 1997).

Extension of shelf life of buckwheat starch jelly by chitosan addition was also observed by Lee and No (2001a). Chitosan (37 kDa) was

dissolved in 1% acetic acid and added to buckwheat starch to give a final chitosan concentration of 0%, 0.5%, 1.0%, 1.5%, and 2.0%. During storage of buckwheat starch jelly at 18 °C for 6 d, total viable counts were lower and water activity was less reduced at higher chitosan concentrations. However, increase in chitosan concentration to 1.5% and above resulted in lower overall acceptability due to noticeable astringent taste. Thus, the buckwheat starch jelly containing 1.0% chitosan had the most desirable sensory quality and its shelf life was extended by 1 to 2 d compared with that (4 d) of the control during storage at 18 °C for 6 d.

**Vinegar.** Clarification of persimmon vinegar with chitosan was studied by Lee and No (2001b). Chitosans of 150 and 37 kDa were dissolved in 1% acetic acid and applied to vinegar at concentrations of 100, 200, 300, 400, and 500 mg/L. With increasing chitosan concentrations, the coagulated solids increased, while turbidity, browning, and tannin and soluble solids contents decreased. Reduction in turbidity and soluble solids somewhat differed with molecular weight of chitosan. The most effective clarification of persimmon vinegar was achieved by treatment with 400 mg/L chitosan, irrespective of its molecular weights. Increase in chitosan (both 150 and 37 kDa) concentration to 500 mg/L resulted in lower overall acceptability due to noticeable astringent taste. The quality of persimmon vinegar clarified by the aforementioned chitosan treatment was found to be more stable than that of control (no chitosan treatment) during storage at room temperature for 6 mo (Lee and No 2001c).

### Needed Future Research

According to the SciFinder Scholar 2001 database (as of June 21, 2005), over 22600 publications related to chitin and chitosan have been published since 1907. From the perspective of food scientists, the authors would like to suggest some critically needed research related to production of chitosan, its properties, and its use in food applications.

First, from numerous research studies depicted in this review, there is no doubt that chitosan can be effectively used as food preservative or edible coating material to preserve quality and extend the shelf life of various food products. However, individual researchers used chitosans with varying physicochemical properties and perhaps from various sources. Thus, the question arises as to how to globally produce chitosans with consistent properties. Each batch of chitosan produced from the same manufacturer may differ in its quality. For proper quality control in the chitosan production, there is a critical need to establish less expensive and reliable analytical methods, especially for the evaluation of molecular weight and degree of deacetylation.

Second, the traditional chitosan production involves deproteinization, demineralization, decolorization, and deacetylation. The physicochemical characteristics of chitosan can be variously affected by production methods and crustacean species. Modification of the traditional chitosan production has been shown to affect chitosan physicochemical and functional characteristics (that is, molecular weight, viscosity, degree of deacetylation, hydrophilicity, water and fat absorption capacities, and so on), which, in turn, affect effectiveness of chitosan as food preservatives or edible coating materials. Furthermore, the amino group in chitosan is an effective functional group that can be altered chemically for production of other chitinous derivatives with specific useful characteristics. Therefore, physicochemical characteristics and functional properties of chitosan and its derivatives must be examined to effectively utilize these products for food usages and applications. Needless to say, more research in this area is needed.

Third, the typical chitosan production process is costly, thus limiting wider food applications of chitosan. Simplification of chitosan

production, for example, by elimination of deproteinization and/or demineralization, or by reduction of reaction time required for deproteinization and demineralization, would considerably reduce production cost due to reduction in chemical usage, process time, and voluminous wastewater discharge. Use of ozone treatments for decolorization and depolymerization of chitosan has been successfully attempted; the use of ozone treatment helps reduce the chemical wastes (for example, acetone and NaOCl), and the ozone treatment itself leaves no chemical waste. However, use of ozone treatment needs to be carefully examined on the possible toxic side-product due to its strong oxidative power. Recently, we (No and others 2005) prepared various chitosans by the simplified process and used them as coating materials to effectively extend the shelf life of eggs. Use of less expensive chitosan prepared from simplified production processes may increase demand of chitosan for use in food applications. More research is then needed to evaluate feasibility of using various chitosan and its oligomer products prepared from simplified production processes for specific food usage.

Fourth, functionality of chitosan as a food preservative or coating material varies with its molecular weight. Preparation of chitosan with specific molecular weight with the aid of ozone technology should be further investigated. There is a need to create a model to describe effective utilization of chitosan (in view of molecular weight and degree of deacetylation) in food applications. In other words, the model would indicate a specific chitosan(s) that is most suitable for a specific food application(s).

Fifth, to date, many attempts have been focused on applications of chitosan with relatively high molecular weight as food preservatives due to its higher antimicrobial activity compared with chitosan oligomer. However, the typical astringent/bitterness taste of chitosan currently limits its use, to some extent, as a food additive or preservative. Incorporation of L-arginine and AMP (adenosine monophosphate), both considered as GRAS in the United States, may be used to mask or minimize this effect, and should be further investigated.

Sixth, inherent antibacterial/antifungal properties and film forming ability of chitosan make it an ideal for use as biodegradable antimicrobial packaging material. One major drawback of chitosan film is its high sensitivity to humidity, and thus it may not be appropriate for use when it is in direct contact with foods and/or for direct handling. More research is needed to develop antimicrobial chitosan films that are less sensitive to humidity. In addition, effects of chitosan production protocols, film-casting solvents, and plasticizer contents on sorption behavior of chitosan films should be further investigated. Incorporation of antimicrobial and antioxidant agents, vitamins, and/or minerals in chitosan film or coating to extend shelf life of fresh-cut fruits and vegetables is of great merit for future research.

Lastly, numerous researches conducted on food applications of chitosans have been done at a small or laboratory scale. Further research on quality and shelf life of foods containing or coated with chitosan should be conducted on a scale-up or commercial trial under a large volume typical of commercial conditions. This would provide us more realistic and practical information needed for actual commercialization of food products containing or coated with chitosans.

## Conclusion

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp, and crawfish. The antimicrobial activity of chitosan against a wide range of foodborne filamentous fungi, yeast, and bacteria has made it a potential food preservative.

Chitosan also possesses film-forming and barrier properties, thus making it a potential raw material for edible films or coatings. Inherent antibacterial/antifungal properties and film forming ability of chitosan make it an ideal for use as biodegradable antimicrobial packaging material that can be used to improve the storability of perishable foods. Numerous researches have clearly demonstrated that chitosan can be used as an effective preservative or coating material for improvement of quality and shelf life of various foods. Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively. In the United States, upon receiving the US FDA approval for GRAS status, chitosan as a food additive and its applications in food systems will certainly be in more demand in the near future.

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