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REVIEW ARTICLE

Challenges and opportunities related to the use of chitosan as a food preservative

Ziyi Hu[1] and Michael G. Gänzle[1,2]

1 Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
2 College of Bioengineering and Food Science, Hubei University of Technology, Wuhan, Hubei, China

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Correspondence
Michael Gänzle, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB Canada, T6G 2P5.
E-mail: mgaenzle@ualberta.ca

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Summary

Chitosan has attracted a growing attention as a food preservative due to its versatility, nontoxicity, biodegradability and biocompatibility. This review aims to provide a critical appraisal of the limitations and opportunities of the use of chitosan as a food preservative. The application of chitosan as a food preservative necessitates insights into mechanisms of chitosan-mediated cell death and injury, factors affecting chitosan activity and effects of chitosan on food safety and quality. Chitosan exerts antimicrobial activity by perturbing the negatively charged cell envelope of micro-organisms with its polycationic structure. Intrinsic characteristics, including molecular weight and degree of deacetylation (DD), and other ambient conditions, including pH, temperature and neighbouring components, affect chitosan activity. Because the antimicrobial activity of chitosan is mainly based on ionic interactions with negatively charged components of the bacterial cell envelope, the food matrix can strongly interfere with the antimicrobial activity of chitosan. Despite its limited antimicrobial efficacy, chitosan demonstrates both bactericidal and bacteriostatic effects in specific food products. Moreover, chitosan can also enhance the efficacy of commercial intervention technologies, such as heat and pressure treatment, and aid the preservation of food quality, including retardation of lipid oxidation, weight loss and deterioration in sensory attributes.

Introduction

Food safety and quality are fundamental concerns for consumers and the food industry. Current intervention and preservation technologies, however, do not prevent outbreaks of foodborne bacterial disease, or food spoilage and food waste (Hussain 2013). Moreover, the negative public perception of commercial preservatives prompts an increasing preference of consumers for the replacement of chemical preservatives by 'natural' alternatives that are derived from biological systems (Amit *et al.* 2017; Román *et al.* 2017). To meet the consumers' demand for 'natural preservatives' including essential oils extracted from plants (Sanchez-Maldonado *et al.* 2015; Pandey *et al.* 2017), bacteriocins from lactic acid bacteria (LAB), such as nisin or pediocin PAß1-AcH, and bacteriocin-producing protective cultures, such as *Carnobacterium maltaromaticum* UAL307 (Micocin®; Liu 2014; Barbosa *et al.* 2017), are used commercially as food preservatives. Further improvement of food safety and quality, however, necessitate the development of other antimicrobials from natural resources.

Chitosan is a linear polysaccharide consisting of β-(1→4)-linked glucosamine and N-acetyl-D-glucosamine that has been proposed for use as a food preservative. Chitosan is prepared by deacetylation of chitin, which is present in the exoskeleton of crustaceans and insects and in the cell walls of most fungi and some algae (Ma *et al.* 2017; Muxika *et al.* 2017). When the proportion of glucosamine exceeds the proportion of N-acetyl glucosamine, corresponding to a degree of deacetylation (DD) of more than 50%, the polymer is termed chitosan (Khor and Lim 2003; Ramírez *et al.* 2010). Owing to its positive charge and unique functional groups, including the amino/acetamido groups at the C-2 position, and hydroxyl groups at the C-3 and C-6 positions, chitosan is a versatile biopolymer with applications in the biomedical field, in wastewater

treatment, agriculture, food protection, cosmetics, papermaking and the textile industry (Ma *et al.* 2017; Muxika *et al.* 2017). While several reviews indicate the potential applications of chitosan as a food preservative, challenge studies in food often report only a limited effect of chitosan on pathogens or spoilage organisms. This review aims to not only provide a critical appraisal of the challenges to food applications of chitosan that are imposed by the molecular structure of chitosan and its interactions with the food matrix but also outline opportunities of the use of chitosan as a food preservative.

Preparation of chitosan

Chitosan is prepared by purification, and deacetylation of chitin. Further enzymatic or chemical depolymerization of chitosan yields water-soluble chitosan oligosaccharides (COS). To purify chitin from the shells of crustaceans, the shells are ground (Abdou *et al.* 2008), processed with HCl to achieve demineralization and boiled in dilute NaOH to remove proteins (Puvvada *et al.* 2012; Arbia *et al.* 2013; Kumari *et al.* 2015). Deacetylation of chitin is achieved through alkaline treatment at more than 80°C. The DD is dependent on the reaction conditions (Teng 2011; Yuan *et al.* 2011). Treatment with 12·5 mol l^{-1} NaOH at 95–100°C deacetylates chitin within 2 h, yielding chitosan with a DD of 87–90% and an average MW of 160–1600 kDa (Puvvada *et al.* 2012).

Generally, chitosan is acid soluble and has antimicrobial activity only when the ambient pH is lower than its pKa, which ranges from 6·2 to 7·0 (Tsai and Su 1999; Helander *et al.* 2001; Devlieghere *et al.* 2004). For food applications, chitosan is either dissolved in acetic acid to a concentration of 1–2%, or applied as a chitosan-based packaging film (Jovanović *et al.* 2016; Muxika *et al.* 2017; Zhao *et al.* 2018). Chitosan has also been converted to chitosan nanoparticles or microparticles (CN/CM) through ionic cross-linking with polyanionic sodium triphosphate (TPP) (Chávez de Paz *et al.* 2011; Zhao *et al.* 2011). CN/CM were reported to be effective food preservatives (Fang *et al.* 2015; Pilon *et al.* 2015; Chouljenko *et al.* 2017; Paomephan *et al.* 2018), however, there is no evidence that CN/CM have superior antimicrobial activity when compared to chitosan solutions. Chitosan can also be depolymerized by chitosanases and chitinases (Aam *et al.* 2010). COS have higher solubility and lower antimicrobial activity when compared to high molecular weight (MW) chitosan (Fernandes *et al.* 2008; Mellegård *et al.* 2011).

Mode of action and factors affecting the antimicrobial activity of chitosan

Chitosan exhibits bacteriostatic or bactericidal effects against a wide range of micro-organisms (Devlieghere *et al.* 2004). The mode of action of chitosan relates to alterations of the cell envelope and a compromised integrity of the cytoplasmic membrane. The mode of action of chitosan against Gram-negative and Gram-positive bacteria is depicted in Figure 1 and described in more detail below.

Polycationic chitosan disrupts the integrity of the Gram-negative outer membrane (Fig. 1a). Outer membrane damage caused by chitosan was demonstrated through use of the fluorescent dye N-phenyl-1-naphthylamine (NPN). NPN is solubilized in the membrane of Gram-negative bacteria only when the outer membrane is damaged; its fluorescence intensity is increased in the hydrophobic interior of the membrane (Träuble and Overath 1973; Loh *et al.* 1984). Chitosan at the concentration of 0·01 to 5 g l^{-1} increased the NPN fluorescence in *Escherichia coli*, indicating permeabilization of the outer membrane (Liu *et al.* 2004; Mellegård *et al.* 2011). Similar chitosan-induced permeabilization of the outer membrane was also observed in *Salmonella* (Helander *et al.* 2001).

Chitosan also permeabilizes cytoplasmic membrane (Fig. 1a,b). Quantification of the transmembrane potential with the lipophilic dye [3H] tetraphenylphosphonium bromide ([3H]TPP^+) demonstrated that the addition of 10 mg l^{-1} chitosan to suspensions of *Staphylococcus simulans* reduced the membrane potential from 110 to 30 mV, indicating dissipation of membrane potential and perturbation of membrane integrity (Raafat *et al.* 2008). In addition, chitosan initiated a progressive efflux of K+ and UV-absorbing cellular components in *S. simulans*, *Staphylococcus aureus*, *E. coli* and *Bacillus cereus*, further supporting an increased permeability of cytoplasmic membrane (Helander *et al.* 2001; Liu *et al.* 2004; Raafat *et al.* 2008; Mellegård *et al.* 2011).

A *pmrA*-negative mutant of *Salmonella* Typhimurium with a more positively charged lipopolysaccharide (LPS) was more resistant to chitosan than its parent strain (Helander *et al.* 2001), and *S. aureus* mutants lacking teichoic acids (TA) or lipoteichoic acid (LTA) were also more resistant to chitosan than wild-type *S. aureus* (Raafat *et al.* 2008). These findings suggest that the electrostatic interactions between positively charged chitosan and negatively charged LPS (Fig. 1a), TA or LTA (Fig. 1b) contribute considerably to the chitosan-mediated cell death and injury.

The degree of acetylation and the MW impact antimicrobial activity of chitosan by altering the charge density of chitosan. Chitosan with higher DD has a higher positive charge density, allowing for a stronger electrostatic interaction with the negatively charged cell surface and leading to an enhanced antimicrobial activity (Chung *et al.* 2004; Mellegård *et al.* 2011; Younes *et al.* 2014; Chien *et al.* 2016). The minimum MW of chitosan with DD of 84% for observation of antimicrobial activity was 2·3 kDa and the

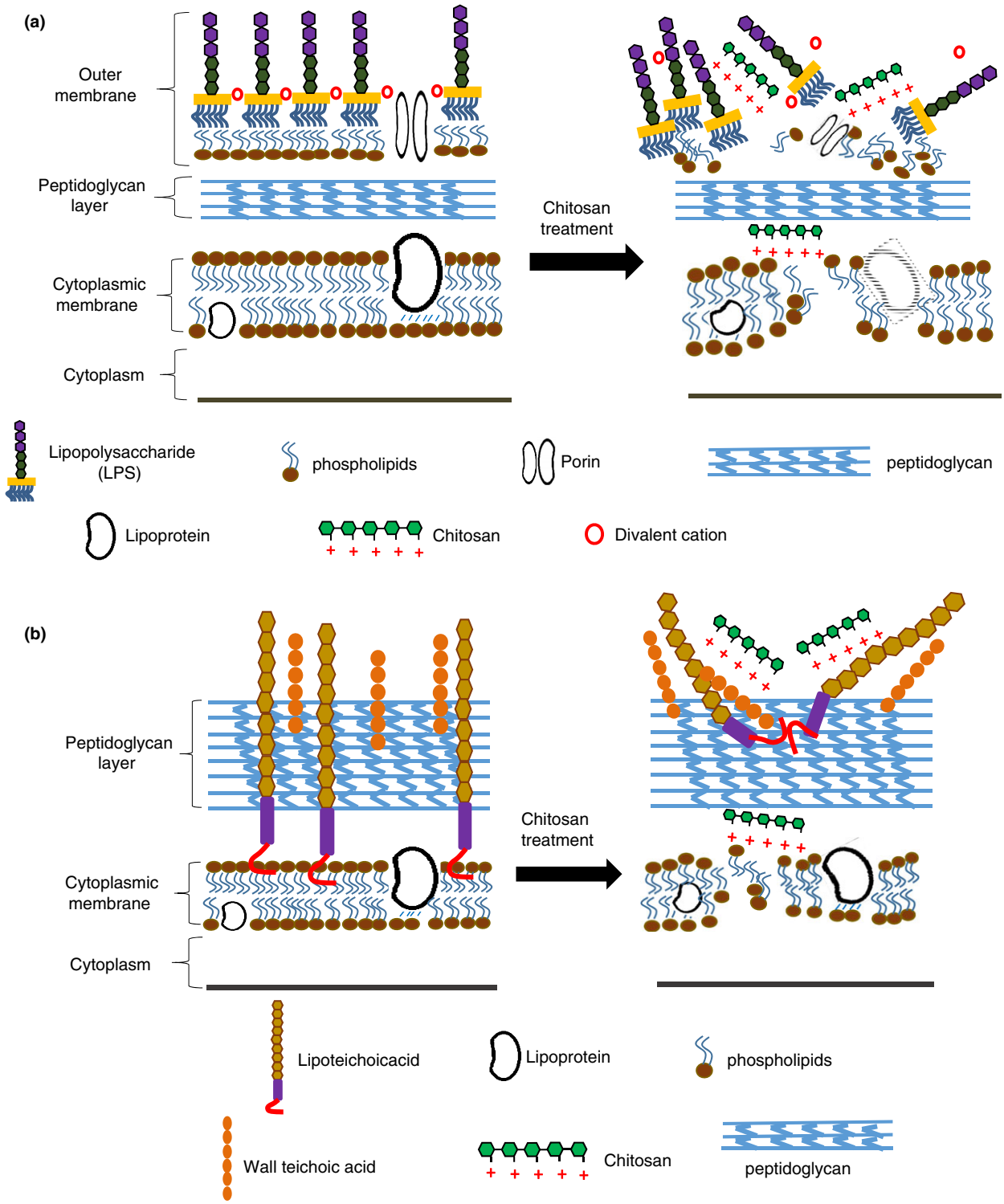

Figure 1 Mode of action of chitosan against Gram-negative bacteria (a) and Gram-positive bacteria (b): When the ambient pH is lower than the pKa of chitosan, chitosan is polycationic, which enables electrostatic interactions with negatively charged structures of the cell envelope, including the lipopolysaccharide in the outer membrane of Gram-negative bacteria (a), lipoteichoic acid and wall teichoic acids of Gram-positive bacteria (b) and the cytoplasmic membrane. These electrostatic interactions can disrupt the integrity of cell envelope, subsequently cause dissipation of membrane potential and leakage of cells, leading to cell death (Helander *et al.* 2001; Liu *et al.* 2004; Raafat *et al.* 2008; Mellegård *et al.* 2011). [Colour figure can be viewed at wileyonlinelibrary.com]

activity increased with increasing MW. With a DD of 52%, the antimicrobial activity of chitosan was observed only at a MW of 11·9 kDa and higher (Mellegård *et al.* 2011). The higher antimicrobial activity of chitosan with higher DD and MW may be attributed to the higher positive charge density and the more intensive interaction with the cell envelope. In food application, COS with MW of <5 kDa has no antibacterial activity while chitosan with a MW of >80 kDa at a concentration of 0·5% (w/v) was bactericidal in milk and bacteriostatic in cheese. Compared with chitosan, the higher reactivity and stronger interaction of COS with food components, such as protein and lipid, account for the loss of COS in food systems (Ausar *et al.* 2002; Fernandes *et al.* 2008).

The ambient conditions, including pH, temperature and divalent metal ions also affect the antimicrobial activity of chitosan. A low pH favours protonation of chitosan and thus increases its antimicrobial activity (Tsai and Su 1999; Helander *et al.* 2001; Devlieghere *et al.* 2004). Divalent metal ions, including Zn^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, at a concentration of 25 mmol l^{-1} in medium weaken the inhibitory activity of chitosan, probably through shielding of negative charges on the cell envelope (Tsai and Su 1999; Chung *et al.* 2003). The ingredients present in different food products, including NaCl and proteins, may also decrease chitosan activity by shielding positive charges of chitosan (Devlieghere *et al.* 2004).

The antimicrobial activity of chitosan is also dependent on the target micro-organisms. Since media composition highly influences the *in vitro* activity of chitosan, it is not possible to conclude on the differences in resistance between micro-organisms unless the target strains were assessed in the same medium. Few studies indicated certain Gram-negative bacteria, including *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella* Typhi, were more susceptible to chitosan than certain Gram-positive bacteria, including *S. aureus*, *B. cereus*, *Enterococcus faecalis* and *Micrococcus luteus* (Younes *et al.* 2014). Similarly, chitosan also exhibited a higher activity against *E. coli* when compared to *B. cereus* (Mellegård *et al.* 2011). When cells were suspended in buffer containing 0·5% chitosan at pH 5·4, the decrease in cell counts of *E. coli* induced by chitosan was more than 3 log(CFU per ml) higher when compared to *S. aureus* (Liu *et al.* 2004). The reasons for these species-specific differences in resistance to chitosan are still unclear. The loss of TA and modification of LPS altered the susceptibility to chitosan in *S. aureus* and *Salm.* Typhimurium respectively (Helander *et al.* 2001; Raafat *et al.* 2008; Mellegård *et al.* 2011). These studies highlight that the difference in charge distribution on the cell surface may account for the species- and strain-specific differences in resistance to chitosan.

Challenge studies with pathogens to evaluate the use of chitosan as a food preservative

A summary of challenge studies with chitosan, chitosan nanoparticles or chitosan-based films in food is provided in Table 1. In most cases, the lethality of chitosan is limited to a 2·5 log (CFU per g) decrease in cell counts irrespective of the food matrix and the form of application (Table 1). A reduction of more than 5 log (CFU per g) of *Listeria monocytogenes* was observed on apples and grapes coated with 2% (w/v) chitosan solution (Anacarso *et al.* 2011). This high antilisterial activity may be attributed to the smooth surface of apples and grapes, resulting in a high local concentration of chitosan and an intense interaction of bacterial cells with chitosan. Other studies observed bacteriostatic rather than bactericidal effects of chitosan in artificially contaminated food. Coating eggs with 2% chitosan solution was not lethal to *Salmonella* Enteritidis when chitosan solution was applied on egg shells and dried prior to the inoculation of bacterial cells, but offered a protective barrier reducing the penetration of *Salmonella* (Leleu *et al.* 2011). Similarly, chitosan films were not bactericidal but delayed the growth of *L. monocytogenes* on slices of ready-to-eat sausages (Moradi *et al.* 2011). Incorporation of chitosan powder into bread at 0·6% (w/w) inhibited the growth of *B. cereus* and rope formation during storage at 30°C for 3 days (Lafarga *et al.* 2013). Taken together, the disparity in lethality of chitosan shown among different reports may be attributed to the variation in chitosan property, food matrix and approaches of chitosan application.

Surface application of chitosan is the most frequent form of application (Table 1); only few studies directly compared the efficacy of chitosan solutions to nanoparticles or packing films. Chitosan solution exhibited stronger bactericidal activity against *L. monocytogenes* on black radish when compared to a chitosan packaging film (Jovanović *et al.* 2016). After coating of the chitosan solution, samples are often drained or dried (Kanatt *et al.* 2013; Jovanović *et al.* 2016). With water evaporation, chitosan becomes more concentrated than the original chitosan solution, resulting in a higher local concentration of chitosan on the sample surface and a more intensive interaction with target cells.

Application of chitosan as a food preservative to control spoilage organisms

Studies that monitored the development of the non-pathogenic microbiota of food, including aerobic mesophilic bacteria, psychrotrophic bacteria, LAB, *Brochothrix*, *Pseudomonas* spp, Enterobacteriaceae or yeast and

Table 1 Bactericidal effect of different forms of chitosan on artificially contaminated foods

Chitosan preparation and application		Lethality (log N_0/N)	Product (reference)
Meat products			
Surface application	0·5% (w/v); 350 kDa	0·5 (*S.* Typhimurium)	Chicken skin (Menconi *et al.* 2013)
	2% (w/v)	2 (*Staphylococcus aureus*) 2·5 for *Bacillus cereus* 1 for *Escherichia coli* 0·5 for *Pseudomonas fluorescens*	Chicken or mutton seekh kebab (Kanatt *et al.* 2013)
	2% (w/v); 340 kDa	2 (*E. coli* O157:H7); 1 (*Salmonella*)	Fresh turkey meat (Vardaka *et al.* 2016)
Packaging film	0·389 mg chitosan cm^{-2}; 150 kDa	0·8 (*Listeria innocua*)	Ready-to-eat turkey meat (Guo *et al.* 2014)
	150 mg chitosan g^{-1} starch; 190–310 kDa	1 (spoilage bacteria cocktail of *Brochothrix thermosphacta*, *Carnobacterium maltaromaticum*, *Leuconostoc gelidum* and *Lactobacillus sakei*)	Ham (Zhao *et al.* 2018)
Seafood			
Microparticles (CM)	Surface application of 0·5% (w/v) CM solution from chitosan with 50–190 kDa	1·9-3·9 (*Vibrio vulnificus*) 1·9-2·6 (*Vibrio parahaemolyticus*)	Live oysters (Fang *et al.* 2015)
Vegetables and fruits			
Nanoparticles (CN)	Washing samples with 800 mg l^{-} CN solution, which was produced from chitosan with 30 or 2100 kDa	1 (*E. coli*) 1 (*S.* Typhimurium)	Fresh vegetables (Paomephan *et al.* 2018)
Solution	2% (w/v); 150 kDa	1·5 on zucchini, corn and radishes 2 on mixed salad, carrots and zucchini >5 on apples and grapes (*Listeria monocytogenes*)	Zucchini, corn and radishes Mixed salad, carrots and zucchini Apples and grapes (Anacarso *et al.* 2011)
	2% (w/v); 150 kDa	1 (*Salmonella*)	Cantaloupe (Chen *et al.* 2012)
	1% (w/v); 1600 kDa	0·5 (*L. monocytogenes*)	Broccoli florets (Severino *et al.* 2014)
Solution coating or packaging film	Solution: 1% (w/v) Film: 0·5% (w/w) 190–310 kDa	2·5 (*L. monocytogenes*) with solution 1·0 (*L. monocytogenes*) with packaging film	Black radish (Jovanović *et al.* 2016)

The degree of deacetylation was >75% for all studies included in this table.
Lethality: Reduction of log (CFU per g) or log (CFU per ml); MW, Molecular weight.

moulds, are summarized in Table 2. In these cases, uninoculated food samples were treated with chitosan solution, chitosan nanoparticles or with chitosan-based films, followed by refrigerated storage and microbiological analysis during storage. The bacteriostatic effect of chitosan ranged from 1 to 6 log (CFU per g), depending on dosage and intrinsic characteristics of chitosan food matrix and storage condition (Table 2). In addition to the enumeration of microbial populations, the observation of microbial spoilage of vegetables and fruits allows assessment of the effectiveness of chitosan. Coating treatment with 1% (w/v) chitosan solution reduced the decay of sweet pepper by 20% after storage at 8°C (Xing *et al.* 2011). Preharvest spray with 0·1% (w/v) chitosan solution or postharvest coating with 1% (w/v) chitosan solution significantly reduced the decay index of chitosan-treated grape fruits after storage for 16 days at 20°C or 42 days at 0°C (Meng *et al.* 2008). To investigate the mechanisms of chitosan-mediated reduction of spoilage of fruits and vegetables, artificially wounded fruits were first coated with chitosan solution and then inoculated with indicator fungal strains (Chien *et al.* 2007), or artificially wounded samples were inoculated and then coated with chitosan (Shao *et al.* 2015). Independent of the sequence of inoculation with fungi and chitosan application, chitosan-treated samples reduced the decay incidence when compared to controls (Chien *et al.* 2007; Shao *et al.* 2015). Chitosan also inhibited spore germination, germ tube elongation and mycelial growth of many phytopathogens (Ben-Shalom *et al.* 2003; Liu *et al.* 2007). The antifungal activity of chitosan in combination with the mechanical barrier provided by a chitosan coating probably contributes to the decreased decay incidence by inhibiting the growth of indigenous micro-organisms and protecting samples from exogenous infection.

Table 2 Effect of chitosan on the microbial quality of food

Chitosan preparation and application		Effect of chitosan	Products (reference)
Meat products			
Surface application	0·5% (w/v); 350 kDa	Psychrotrophic spoilage bacteria in samples treated with chitosan remained below detectable levels during storage at 4°.	Chicken skin (Menconi *et al.* 2013)
	1·0% (w/v)	Cell counts of mesophilic and psychrotrophic bacteria, lactic acid bacteria, and yeast and mould were lower than controls after storage at 4°C for 60 days by 3–6 log (CFU per g)	Sausage (Bostan and Mahan 2011)
	1·5% (w/v); 340 kDa	Total plate counts and cell counts of spoilage organisms including *Pseudomonas* spp., lactic acid bacteria, *Brochothrix thermosphacta*, coliforms, and yeasts and moulds, were lower than controls by 1–2 log (CFU per g) after storage at 4°C for 12 days, extending the microbial shelf life by more than 9 days	Chicken breast meat (Petrou *et al.* 2012) Turkey meat (Vasilatos and Savvaidis 2013) Ready-to-cook chicken product (Giatrakou *et al.* 2010)
	1% (w/v); 800 kDa	Cell counts of pseudomonads, lactic acid bacteria and coliforms were lower than controls after 6 days of storage at 4°C by 3·9–4·9 log (CFU per g)	Chicken breast fillets (Latou *et al.* 2014)
	2% (w/v); 897 kDa	Total viable count and cell counts of psychrotrophic bacteria were lower than controls by 1 log after storage at 4°C for 25 days	Cooked pork sausages (Lekjing 2016)
Integration of chitosan to product formula	Chitosan (1674 kDa) at 2 mg g^{-1} in minced pork	Total bacterial count and psychrotrophic counts were lower than controls by 1 log (CFU per g) after storage of minced pork at 5°C for 8 days	Minced Pork (Malinowska-Pańczyk *et al.* 2009)
	Chitosan (490 kDa) at 1% (w/w) in pork sausage	Total viable counts, and cell counts of lactic acid bacteria, *Pseudomonas* spp., *Brochothrix thermosphacta*, Enterobacteriaceae, yeasts and moulds were lower than controls by 0·5–1 log (CFU per g) after storage at 4°C for 28 days	Fresh pork sausages (Soultos *et al.* 2008)
Packaging film	Prepared from 2% (w/v) chitosan (100 kDa)	Total viable cell counts, cell counts of lactic acid bacteria, and yeasts and moulds were lower than controls by 1·5–5 log (CFU per g) after storage at 4°C for 20 days	Cooked pork sausages (Siripatrawan and Noipha 2012)
	Prepared from 2% (w/v) chitosan	Total viable cell counts were lower than controls by 1 log (CFU per g) after storage at 4°C for 12 days	Pork meat patties (Qin *et al.* 2013)
Seafood			
Surface application	1% (w/v); 320 kDa	Inhibition of H_2S-producing organisms during storage at 4°C	Shrimp (Arancibia *et al.* 2015)
	1% (w/v); 25 kDa	Total aerobic plate counts were lower than controls by 2 log (CFU per g) after 10 days of iced storage	Pacific white shrimp (Yuan *et al.* 2016)
	2% (w/v); 450 kDa	Total viable counts and psychrotrophic counts were lower than controls by 1–3 log (CFU per g) after storage at 4°C for 16 days	Rainbow trout (Ojagh *et al.* 2010)
	3% (w/v)	Total viable cells and cell counts of psychrotrophic bacteria were lower than controls by 1 log (CFU per g) after storage at 4°C for 12 days	Ready-to-eat peeled Shrimps (Carrión-Granda *et al.* 2016)
	3% (w/v); 149 kDa	Total plate counts were lower than controls by 4 log (CFU per g) after vacuum or modified atmosphere packaging storage at 2°C for 14 days	Lingcod (*Ophiodon elongates*) fillets (Duan *et al.* 2010)
	1·0% (w/v); 1800, 960 or 660 kDa	Total viable counts were lower than controls by 2 log (CFU per g) after storage for 12 days at 4 ± 1°C	Herring and Atlantic cod (Jeon *et al.* 2002)
Incorporation	Chitosan (10 kDa) in surimi at 2% (w/w)	Aerobic plate counts were lower than controls by 1 log (CFU per g) after storage at 4°C for 12 days	Surimi gel made from African catfish (*Clarias gariepinus*) (Amiza and Kang 2013)

(Continued)

Table 2 (*Continued*)

Chitosan preparation and application		Effect of chitosan	Products (reference)
Coating with solution or nanoparticles	Solution: 1% (w/v); 300 kDa; DD 65%; Nanoparticles: 1% (w/v); DD 20%	Cell counts of aerobic bacteria were lower than controls by more than 1 log (CFU per g) after storage at 4°C for 24 days. Conventional solution was more bacteriostatic than nanoparticles solution	Shrimp muscle (Chouljenko *et al.* 2017)
Vegetables, fruits and juice			
Surface application	1·5% (w/v)	Total viable counts and cell counts of yeast and mould were lower than controls by 0·5–1 log (CFU per g) after storage at 4°C for 7 days	Pears (Cé *et al.* 2012)
	1% (w/v); 190–310 kDa	Cell counts of mesophilic aerobic bacteria, yeast and moulds were lower than controls by 1 log (CFU per g) after storage at 10°C for 7 days	Fresh Blueberries (Sun *et al.* 2014)
	1·0% (w/v)	Lower decay incidence by 20% after at 8°C for 35 days	Sweet pepper (*Capsicum annuum L.*) (Xing *et al.* 2011)
	Preharvest spray with 0·1% (w/v) or coating with 1% (w/v) solution	Lower decay index after storage for 16 days at 20°C or 42 days at 0°C	Grape fruit (Meng *et al.* 2008)
Incorporation	Solution (0·4% (w/v); 1674 kDa) in apple juice at 2 g l^{-1}	Total bacterial counts, cell counts of psychrotrophic bacteria, yeast and mould were lower than controls by 0·5–3·0 log (CFU per g) after storage at 5°C for 15 days	Apple juice (Malinowska-Pańczyk *et al.* 2009)
Coating with solution or nanoparticles	0·2% (w/v); 71 kDa	Cell counts of mesophilic and psychrotrophic bacteria were lower than controls by 3 log (CFU per g) after storage at 5°C for 10 days. Solution and nanoparticles exhibited comparable bacteriostatic effect	Fresh-cut apples (Pilon *et al.* 2015)
Bakery products			
Incorporation	Chitin (124 ± 10 kDa; DD 19%) in bread at 1%	Delay of mould growth in bread during storage of 3 days at 30°C	Bread (Lafarga *et al.* 2013)
Packaging film:	Prepared form 1·5% (w/v) chitosan	Delay of time to visible mould growth by 3 days and cell counts of mould were lower than controls by 2 log (CFU per g) after storage for 8 days at room temperature (about 25°C)	Butter cake (Sangsuwan *et al.* 2015)
Eggs			
Surface application	1% (w/v)	Total aerobic cell counts chitosan-coated eggs were under detection limit while those of noncoated eggs increased to 20 CFU per ml after 5 weeks of storage at 22 ± 1 or 32 ± 1°C	Eggs (Suresh *et al.* 2015)

The degree of deacetylation of chitosan was higher than 75% unless otherwise noted.

Use of chitosan to enhance the efficacy of other antimicrobial hurdles

Chitosan potentiates the efficacy of commercial intervention technologies, such as heat and high hydrostatic pressure. Chitosan is generally applied as a dilute solution in acetic acid. Those studies that used a solvent control demonstrated, however, that the carry-over of acetic acid or acetate, 1–20 mg kg^{-1}, does not impact the antimicrobial activity of chitosan (Tables 1 and 2). The addition of chitosan at a concentration of 0·01% (w/w) enhanced the thermal inactivation of *E. coli* O157:H7 (EHEC) in ground beef by 1·5 log (CFU per g) (Surendran Nair *et al.* 2016). Chitosan at a concentration of 0·1% (w/v) acted synergistically with pressure treatment of apple juice to inactivate *E. coli* (Kumar *et al.* 2009). The combined application of chitosan and pressure demonstrated synergistic effects in the elimination of *S. aureus* and *E. coli* in buffer, and in controlling bacterial growth in apple juice and minced pork during refrigerated storage (Malinowska-Pańczyk *et al.* 2009).

Application of chitosan to improve the quality of food products

Chitosan also exerts other beneficial effects on food quality that are independent of its antimicrobial activity and include retardation of lipid oxidation, retention of colour

and nutrients, maintaining freshness and sensory attributes. The effects on food quality are dependent on the food matrix and are summarized in Table 3.

Meat and seafoods

The application of chitosan significantly reduced the rate of lipid oxidation, which is usually indicated by thiobarbituric acid-reactive substances and peroxide value on meat and seafood (Table 3). Chitosan controls lipid oxidation by the scavenging of reactive radicals (Kim and Thomas 2007; Wan *et al.* 2013), forming a stable complex with volatile aldehydes derived from the decomposition of lipids (Shahidi *et al.* 1999) and by providing a barrier to oxygen diffusion (Sathivel *et al.* 2007).

The colour of specific foods strongly affects the purchasing decisions of consumers (Gao *et al.* 2013). Chitosan treatments in different forms retarded the colour alteration in sausage, pork meat patties and pacific white shrimp (Table 3). Metmyoglobin (MetMb) is the major factor causing the browning of fresh meat (Bekhit *et al.* 2007). The colour retention caused by chitosan was achieved by decreasing the MetMb concentration, and may also relate to the anti-oxidative activity of chitosan (Qin *et al.* 2013).

Melanosis is a type of spoilage specific for crustaceans. During the postmortem storage of crustaceans, microbial compounds, including the peptidoglycan-binding protein produced by Gram-positive bacteria, the lipopolysaccharide and β-(1→3)-glucan-binding protein (LGBP) produced by Gram-negative bacteria and the β-(1→3)-glucan-binding protein (BGBP) produced by fungi, accumulate and activate polyphenoloxidase (PPO). PPO oxidizes monophenols, particularly tyrosine, into quinones, followed by nonenzymatic polymerization of quinones to form dark pigments called melanin. The accumulation of melanin incurs the formation of black spots on carapace, namely, melanosis, thus substantially decreasing the commercial value of crustacean products (Garcia-Molina *et al.* 2005; Amparyup *et al.* 2013; Gonçalves *et al.* 2016). Coating shrimps with 1–1·5% chitosan solution significantly retarded melanosis in shrimps (Huang *et al.* 2012; Yuan *et al.* 2016), and the protective effect against melanosis likely relates to its anti-oxidative activity and antimicrobial activity (Huang *et al.* 2012).

The texture profile is a widely used freshness indicator for seafood products (Cheng *et al.* 2014). Myofibrillar and connective tissue proteins are the major elements maintaining the textural properties of shrimps and fish. Microbial and endogenous proteases lead to the softening of the texture during storage (Hultmann and Rustad 2004; Yuan *et al.* 2016). In some cases, the surface application of chitosan solution retarded the softening during the storage of fish, presumably through the inhibition of microbial spoilage or interactions with myofibrillar proteins to form the compact structure (Huang *et al.* 2012; Yang *et al.* 2015; Yuan *et al.* 2016).

Eggs

Coating treatment with chitosan solutions also preserved the freshness and enhanced the commercial value of eggs (Table 3). The protective barrier formed by chitosan coating on the eggshell surface may offer all these benefits through a decreasing transfer of carbon dioxide and water vapour through the eggshell pores, eventually enhancing the storability of eggs (Robinson 1987; Williams 1992; Wardy *et al.* 2014; Suresh *et al.* 2015).

Vegetables and fruits

During the storage of vegetables and fruits, metabolism and respiration of plant tissue leads to weight loss, oxidation of vitamin C and a decline in fruit firmness (Zhu *et al.* 2008; Ali *et al.* 2011; Xing *et al.* 2011; Hong *et al.* 2012; Han 2014). Coating with chitosan solution significantly reduced the rate of vitamin C loss in Guava and sweet pepper (Xing *et al.* 2011; Hong *et al.* 2012). Vitamin C loss is attributed to the presence of O_2 (Ayranci and Tunc 2004) and coating of fruits with chitosan solution significantly reduced O_2 diffusion into the plant tissue (Ali *et al.* 2011). Chitosan coatings delayed the ripening process and tissue softening of guava (Hong *et al.* 2012), litchi fruit (Dong *et al.* 2004), papaya (Ali *et al.* 2011) and grapes (Meng *et al.* 2008).

In addition to performing a direct protective effect, the coating treatment with chitosan solution also enhanced the activities of peroxidase (POD) and superoxide dismutase (SOD), plant-defensive enzymes that aid self-detoxification under stress (Jahnke *et al.* 1991; Meng *et al.* 2008; Xu *et al.* 2009), in sweet pepper and guava fruits, concomitantly resulting in a decreased membrane injury (Xing *et al.* 2011; Hong *et al.* 2012). These findings suggest that chitosan can also promote the protection of vegetables and fruits by acting as a defensive enzyme enhancer (Xing *et al.* 2011; Hong *et al.* 2012).

Concluding remarks

Chitosan has antimicrobial activities only if it is in the polycationic form at pH values below the pKa. The antimicrobial activity of chitosan depends on the electrostatic interactions between polycationic chitosan molecules and negatively charged cell envelopes. Food components, including NaCl, proteins and starch, adversely affect chitosan activity if the positive charge of

Table 3 Effect of chitosan on food quality

Chitosan preparation and application		Effect of chitosan	Products (reference)
Meat products			
Surface application	1·0% (w/v)	Brighter and more attractive colour	Sausage (Bostan and Mahan 2011)
	1·5% (w/v); 340 kDa	Improvement in sensory attributes	Chicken breast meat (Petrou *et al.* 2012) Turkey meat (Vasilatos and Savvaidis 2013) Chicken product (Giatrakou *et al.* 2010)
	1% (w/v); 800 kDa	Retardation of decline in odour and taste scores	Chicken breast fillets (Latou *et al.* 2014)
	2% (w/v); MW: 897 kDa	Retardation of lipid oxidation, change in colour and sensory attributes	Cooked pork sausages (Lekjing 2016)
Packaging film:	Prepared from 2% (w/v) chitosan (100 kDa) solution	Retardation of lipid oxidation, changes in colour, texture and sensory characteristics	Cooked pork sausages (Siripatrawan and Noipha 2012)
	Prepared from 2% (w/v) chitosan solution	Retardation of lipid oxidation and increase in MetMb content, as well as improvement in sensory attributes	Pork meat patties (Qin *et al.* 2013)
Incorporation	Chitosan (490 kDa) in sausages at 1% w/w	Retardation of lipid oxidation	Fresh pork sausages (Soultos *et al.* 2008)
Seafood			
Surface application	1% (w/v); 25 kDa	Retardation of increase in melanosis and improvement in the texture parameters and sensory attributes	Pacific white shrimp (Yuan *et al.* 2016)
	2% (w/v); 450 kDa	Retardation of increase in peroxide value and total volatile base nitrogen	Rainbow trout (Ojagh *et al.* 2010)
	2% (w/v)	Retardation of lipid oxidation and improvement in sensory attributes	Fresh *Channa Argus* (Yang *et al.* 2015)
	3% (w/v); 149 kDa	Retardation of lipid oxidation under vacuum or modified atmosphere packaging	Lingcod (Ophiodon elongates) fillets (Duan *et al.* 2010)
	1·5% (w/w)	Retardation of increase in melanosis and loss in freshness and sensory quality	Whiteleg shrimp (*Litopenaeus vannamei*) (Huang *et al.* 2012)
	1·0% (w/v) of chitosan with 1800, 960, or 660 kDa	Retardation of lipid oxidation	Herring and Atlantic cod (Jeon *et al.* 2002)
Incorporation	Chitosan (10 kDa) in surimi at 2% (w/w)	Retardation of lipid oxidation, extension of shelf life by 4 days	Surimi gel made from African catfish (*Clarias gariepinus*) (Amiza and Kang 2013)
Vegetables and fruits			
Surface application	1·0% (w/v)	Reduction of cell injury in plant tissue, retention of vitamin C content and enhancement of self-defence system	Sweet pepper (*Capsicum annuum* L.) (Xing *et al.* 2011)
	1% (w/v)	Retardation of loss in weight	Grape fruits (Meng *et al.* 2008)
	0·5, 1·0 or 2·0% (w/v); 50–190 kDa	Retardation of loss in firmness, weight, chlorophyll and vitamin C, as well as reduction of cell injury in plant tissue and enhancement of self-defence system	Guava (*Psidium guajava* L.) (Hong *et al.* 2012)
	1·0, 1·5 or 2·0% (w/v)	Retardation of loss in weight, firmness and changes in the peel colour	Papaya (Ali *et al.* 2011)
Sauce			
Incorporation	Chitosan (310 or 123 kDa) in mayonnaise at 100 mg kg^{-1}	Improvement in odour and taste attributes, and retardation of lipid oxidation	Mayonnaise (García *et al.* 2014)
Eggs			
Surface application	1% (w/v)	Retardation of loss in weight, increase in air space and decline in Haugh Unit value, yolk index, shell strength and quality grade	Eggs (Suresh *et al.* 2015)
	3% (w/v)	Retardation of loss in weight, decline in Haugh unit and yolk index	Eggs (Caner and Cansiz 2007)
	1% (w/v) 1110 kDa	Retardation of loss in weight and decline in Haugh unit	Eggs (Wardy *et al.* 2014)

The degree of deacetylation of chitosan was higher than 75% unless otherwise noted.

chitosan is neutralized. Therefore, inactivation of pathogens by chitosan in food is typically limited to a decrease of 1–2 log (CFU per g), which provides a significant challenge to the application of chitosan as a general food preservative. Specific applications, however, provide opportunities for the use of chitosan as an effective preservative. First, the surface application of chitosan on smooth fruits and vegetables concentrates chitosan and allows effective microbiocidal activity. Second, chitosan can potentiate the efficacy of other intervention technologies, including heat and pressure treatments, to become part of an effective hurdle concept. Third, chitosan improves food quality independent of its antimicrobial activity in some cases, for example, by retardation of lipid oxidation, plant metabolism or melanosis, which may favour chitosan applications even if the antimicrobial effect is limited. Chitosan is thus a promising food preservative in specific applications.

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Conflict of Interest

The authors declare no conflict of interest.

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