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

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Summary

Chitosan has attracted a growing attention as food preservative due to its versatility, non-toxicity, biodegradability, and biocompatibility. This review aims to provide a critical appraisal of the limitations and opportunities of the use of chitosan as food preservative. Application of chitosan as food preservatives 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 exert antimicrobial activity through perturbing the negatively charged cell envelope of microorganisms with its polycationic structure. Intrinsic characteristics, including molecular weight (MW) and degree of deacetylation (DD), and other ambient conditions, including pH, temperature, neighboring 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 of its limited antimicrobial efficacy, chitosan demonstrates both bactericidal and bacteriostatic effect 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.

Keywords: Chitosan, food preservative, mode of action, antimicrobial activity.

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 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 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 food preservative, challenge studies in food often report only a limited effect of chitosan on pathogens or spoilage organisms. This review aims to 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 food preservative.

Preparation of chitosan

Chitosan is prepared by purification, and deacetylation of chitin. Further enzymatic or chemical depolymerisation 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 demineralisation, 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 degree of deacetylation (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 DD of 87-90% and 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 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 crosslinking with polyanionic sodium triphosphate (TPP) (Chávez 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 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 microorganism (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), which is solubilized in membrane of Gram-negative bacteria only when the outer membrane is damaged (Träuble and Overath 1973; Loh et al. 1984). Chitosan at the concentration of 0.01 to 5 g L^{-1} increased in NPN fluorescence in *E. 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 and B). Quantification of the transmembrane potential with the lipophilic dye [3H] tetraphenylphosphonium bromide ([3H]TPP^+) demonstrated that addition of 10 mg L^{-1} chitosan to suspensions of *Staphylococcus simulans* reduced the membrane potential from 110 mV to 30 mV, indicating dissipation of membrane potential and perturbation of membrane integrity (Raafat et al. 2008). In addition, chitosan also initiated a progressive efflux of K+ and UV-absorbing cellular components in *S. simulans*, *S. 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 finding 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 molecular weight impact antimicrobial activity of chitosan through altering the charge density of chitosan. Chitosan with higher degree of deacetylation has a higher positive charge density, allowing for a stronger electrostatic interaction with negative 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 molecular weight of chitosan with DD of 84% for observation of antimicrobial activity was 2.3 kDa and the activity increased with increasing molecular

127 weight. With chitosan of a DD of 52%, antimicrobial activity was observed only at a
128 molecular weight of 11.9 kDa and higher (Mellegård et al. 2011). The higher antimicrobial
129 activity of chitosan with higher DD and molecular weight may be attributed to the higher
130 positive charge density and the more intensive interaction with cell envelope. In food
131 application, COS with MW of <5 kDa has no antibacterial activity while chitosan with
132 MW of >80 kDa at a concentration of 0.5 % (w/v) was bactericidal in milk and
133 bacteriostatic in cheese. Compared with chitosan, the higher reactivity and stronger
134 interaction of COS with food components, such as protein and lipid, account for the loss
135 of COS in food systems (Ausar et al. 2002; Fernandes et al. 2008).
136 The ambient conditions, including pH, temperature, divalent metal ions also affect
137 antimicrobial activity of chitosan. A low pH favors protonation of chitosan and thus
138 increases its antimicrobial activity (Tsai and Su 1999; Helander et al. 2001; Devlieghere et
139 al. 2004). Divalent metal ions, including Zn^{2+}, Ba^{2+}, Ca^{2+}, Mg^{2+}, at a concentration of 25
140 mmol L^{-1} in medium weaken the inhibitory activity of chitosan, probably through shielding
141 of negative charges on the cell envelope (Tsai and Su 1999; Chung et al. 2003). The
142 ingredients present in different food products, including NaCl and proteins, may also
143 decrease chitosan activity by shielding positive charges of chitosan (Devlieghere et al.
144 2004).
145 Antimicrobial activity of chitosan is also dependent on the target microorganisms. Since
146 media composition highly influences the *in vitro* activity of chitosan, it is not possible to
147 conclude on differences in resistance between microorganisms unless the target strains
148 were assessed in the same medium. Few studies indicated certain Gram-negative bacteria,
149 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 of cell counts of *E. coli* induced by chitosan was more than 3 log(cfu/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 teichoic acids (TA) and modification of LPS altered the susceptibility to chitosan in *S. aureus* and *S.* 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 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 g^{-1}) decrease of cell counts irrespective of the food matrix and the form of application (Table 1). A reduction of more than 5 log (cfu g^{-1}) 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

173 barrier reducing the penetration of *Salmonella* (Leleu et al. 2011). Similarly, chitosan films
174 were not bactericidal but delayed the growth of *Listeria monocytogenes* on slices of ready-
175 to-eat sausages (Moradi et al. 2011). Incorporation of chitosan powder into bread at 0.6%
176 w/w inhibited the growth of *B. cereus* and rope formation during storage at 30 °C for 3
177 days (Lafarga et al. 2013). Taken together, the disparity in lethality of chitosan shown
178 among different reports may be attributed to the variation in chitosan property, food matrix
179 and approaches of chitosan application.
180 Surface application of chitosan is the most frequent form of application (Table 1); only few
181 studies directly compared the efficacy of chitosan solutions to nanoparticles or packing
182 films. Chitosan solution exhibited stronger bactericidal activity against *L. monocytogenes*
183 on black radish when compared to a chitosan packaging film (Jovanović et al. 2016). After
184 coating of chitosan solution, samples are often drained or dried (Kanatt et al. 2013;
185 Jovanović et al. 2016). With water evaporation, chitosan becomes more concentrated than
186 the original chitosan solution, resulting in a higher local concentration of chitosan on the
187 sample surface and a more intensive interaction with target cells.
188 **Application of chitosan as food preservatives to control spoilage organisms**
189 Studies that monitored the development of the non-pathogenic microbiota of food,
190 including aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria,
191 *Brochothrix*, *Pseudomonas* spp, Enterobacteriaceae, or yeast and molds are summarized in
192 Table 2. In these cases, un-inoculated food samples were treated with chitosan solution,
193 chitosan nanoparticles, or with chitosan-based films, followed by refrigerated storage and
194 microbiological analysis during storage. Bacteriostatic effect of chitosan ranged from 1 to
195 6 log (cfu g^{-1}), depending on dosage and intrinsic characteristics of chitosan food matrix

196 and storage condition (Table 2). In addition to the enumeration of microbial populations,
197 the observation of microbial spoilage of vegetables and fruits allows assessment of the
198 effectiveness of chitosan. Coating treatment with 1% (w/v) chitosan solution reduced the
199 decay of sweet pepper by 20% after storage at 8 °C (Xing et al. 2011). Pre-harvest spray
200 with 0.1% (w/v) chitosan solution or post-harvest coating with 1% (w/v) chitosan solution
201 significantly reduced the decay index of chitosan-treated grape fruits after storage for 16 d
202 at 20 °C or 42 d at 0 °C (Meng et al. 2008). To investigate the mechanisms of chitosan-
203 mediated reduction of spoilage of fruits and vegetables, artificially wounded fruits were
204 first coated with chitosan solution then inoculated with indicator fungal strains (Chien et
205 al. 2007), or artificially wounded samples, inoculated, and then coated with chitosan (Shao
206 et al. 2015). Independent of the sequence of inoculation with fungi and chitosan
207 application, chitosan treated samples reduced the decay incidence when compared to
208 controls (Chien et al. 2007; Shao et al. 2015). Chitosan also inhibited spore germination,
209 germ tube elongation and mycelial growth of many phytopathogens (Ben-Shalom et al.
210 2003; Liu et al. 2007). The antifungal activity of chitosan in combination with the
211 mechanical barrier provided by a chitosan coating probably contribute to the decreased
212 decay incidence through inhibiting growth of indigenous microorganisms and protecting
213 samples from exogenous infection.

214 **Use of chitosan to enhance the efficacy of other antimicrobial hurdles.**

215 Chitosan potentiates the efficacy of commercial intervention technologies, such as heat and
216 high hydrostatic pressure. Chitosan is generally applied as dilute solution in acetic acid.
217 Those studies that used a solvent control demonstrated, however, that the carry-over of
218 acetic acid or acetate, 1 – 20 mg kg-1 , does not impact the antimicrobial activity of chitosan

(Table 1 and 2). Addition of chitosan to 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 g^{-1}) (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 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 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 color 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. 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). The ability of chitosan to control lipid oxidation relate to scavenging of reactive radicals (Kim and Thomas 2007; Wan et al. 2013), forming stable complex with volatile aldehydes derived from decomposition of lipid (Shahidi et al. 1999), and to providing a barrier to oxygen diffusion (Sathivel et al. 2007). The color of specific foods strongly affects purchasing decisions of consumers (Gao et al. 2013). Chitosan treatments in different forms retarded the color 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 color retention caused

241 by chitosan was achieved through decreasing MetMb concentration, and may also relate to
242 the anti-oxidative activity of chitosan (Qin et al. 2013).
243 Melanosis is a type of spoilage specific for crustaceans. During post-mortem storage of
244 crustaceans, microbial compounds, including peptidoglycan binging protein (PGBP)
245 produced by Gram positive bacteria, lipopolysacharide and β-(1→3)-glucan binding
246 protein (LGBP) produced by Gram negative bacteria, and β-(1→3)-glucan binding protein
247 (BGBP) produced by fungi, accumulate and activate polyphenoloxidase (PPO). PPO
248 oxidizes monophenols, particularly tyrosine, into quinones, followed by non-enzymatic
249 polymerization of quinones to form dark pigments called melanin. The accumulation of
250 melanin incurs the formation of black spots on carapace, namely, melanosis, thus
251 substantially decreasing the commercial value of crustacean products (Garcia-Molina et al.
252 2005; Amparyup et al. 2013; Gonçalves et al. 2016). Coating shrimps with 1-1.5% chitosan
253 solution significantly retarded melanosis in shrimps (Huang et al. 2012; Yuan et al. 2016),
254 and the protective effect against melanosis likely relates to its anti-oxidative activity and
255 antimicrobial activity (Huang et al. 2012).
256 The texture profiles is a widely used freshness indicator for seafood products (Cheng et al.
257 2014). Myofibrillar and connective tissue proteins are the major elements maintaining the
258 textural properties of shrimps and fish. Microbial and endogenous proteases lead to
259 softening of the texture during storage (Hultmann and Rustad 2004; Yuan et al. 2016). In
260 some cases, surface application of chitosan solution retarded the softening during storage
261 of fish, presumably through inhibition of microbial spoilage or interactions with
262 myofibrillar proteins to form the compact structure (Huang et al. 2012; Yang et al. 2015;
263 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 eggshell surface may offer all these benefits through decreasing transfer of carbon dioxide and water vapor through the eggshell pores, eventually enhancing 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 continual decline in fruit firmness (Lazan and Ali 1993; 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 favoured by the presence of O_2 (Ayranci and Tunc 2004) and coating of fruits with chitosan solution significantly reduced O_2 diffusion into 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 direct protective effect, 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 protection of vegetables and fruits through acting as a defensive-enzyme enhancer (Xing et al. 2011; Hong et al. 2012).

Concluding remarks

Chitosan has antimicrobial activity only if it is in the polycationic form at pH values below the pKa. 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 positive charge of chitosan is neutralized. Therefore, inactivation of pathogens by chitosan on food is typically limited to a decrease of 1 - 2 log (cfu g^{-1}), which provides a significant challenge to the application of chitosan as general food preservative. In specific applications, however, provide opportunities for the use of chitosan as effective preservative. First, 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, e.g. 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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571

572 **Figure legends**

573 **Fig 1.** Mode of action of chitosan against Gram negative bacteria **(Panel A)** and Gram positive
574 bacteria **(Panel B)**: When the ambient pH is lower than pKa of chitosan, chitosan is polycationic
575 chitosan molecules, which enables electrostatic interactions with negatively charged structures of
576 the cell envelope, including the lipopolysaccharide (LPS) in the outer membrane of Gram negative
577 bacteria (A), lipoteichoic acid and wall teichoic acids of Gram positive bacteria (B), and the
578 cytoplasmic membrane. These electrostatic interactions can disrupt the integrity of cell envelope,
579 subsequently cause dissipation of membrane potential, leakage of cells, leading to cell death
580 (Helander et al. 2001; Liu et al. 2004; Raafat et al. 2008; Mellegård et al. 2011).

581

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[1]
	2% w/v	2 (*S. aureus*) 2.5 for *B. cereus*; 1 for *E. coli*; 0.5 for *P. fluorescens*	Chicken or mutton seekh kabab[2]
	2% w/v; 340 kDa	2 (*E. coli* O157:H7); 1 (*Salmonella*)	Fresh turkey meat[3]
Packaging film	0.389 mg chitosan/cm^2; 150 kDa	0.8 (*Listeria innocua*)	Ready-to-eat turkey meat[4]
	150 mg chitosan/g starch; 190–310 kDa	1 (spoilage bacteria cocktail of *Brochothrix thermosphacta*, *Carnobacterium maltaromaticum*, *Leuconostoc gelidum* and *Lactobacillus sakei*)	Ham[5]
Seafood			
Microoparticles (CM)	Surface application of 0.5% w/v CMs solution from chitosan with 50-190 kDa	1.9-3.9 (*V. vulnificus*); 1.9-2.6 (*V. parahaemolyticus*)	Live oysters[6]
Vegetables and fruits			
Nanoparticles (CN)	Washing samples with 800 mg/L CNs solution, which was produced from chitosan with 30 or 2100 kDa	1 (*E. coli*) 1 (*S.* Typhimurium)	Fresh vegetables[7]
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 (*L. monocytogenes*)	Zucchini, corn and radishes; mixed salad, carrots and zucchini; apples and grapes[8]
	2% w/v; 150 kDa;	1 (*Salmonella*)	Cantaloupe[9]

	1% w/v; 1600 kDa	0.5 (*L. monocytogenes*)	Broccoli florets[10]
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[11]

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

[1] Menconi et al. 2013 [2] Kanatt et al. 2013 [3] Vardaka et al. 2016 [4] Guo et al. 2014 [5] Zhao et al. 2018 [6] Fang et al. 2015 [7] Paomephan et al. 2018 [8] Anacarso et al. 2011 [9] Chen et al. 2012 [10] Severino et al. 2014 [11] Jovanović et al. 2016

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 °C.	Chicken skin[1]
	1.0% w/v	Cell counts of mesophilic and psychrotrophic bacteria, lactic acid bacteria, and yeast and mold were lower than controls after storage at 4 °C for 60 d by 3 – 6 log (cfu/g).	Sausage[2]
	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-moulds, were lower than controls by 1-2 log (cfu/g) after storage at 4 °C for 12 days, extending the microbial shelf-life by more than 9 days.	Chicken breast meat[3] Turkey meat[4] Ready to cook chicken product [5]
	1 % w/v; 800 kDa	Cell counts of pseudomonads, lactic acid bacteria, and coliforms were lower than controls after 6 d of storage at 4 °C by 3.9-4.9 log (cfu/g).	Chicken breast fillets[6]
	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[7]
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/g) after storage of minced pork at 5 °C for 8 days	Minced Pork[8]
	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/g) after storage at 4 °C for 28 days.	Fresh pork sausages[9]
Packaging film	Prepared from 2% w/v chitosan (100 kDa)	Total viable cell counts, cell counts of lactic acid bacteria, and yeasts and molds were lower than controls by 1.5-5 log (cfu/g) after storage at 4 °C for 20 days.	Cooked pork sausages[10]

	Prepared from 2% w/v chitosan	Total viable cell counts were lower than controls by 1 log (cfu/g) after storage at 4 °C for 12 days.	Pork meat patties[11]
		Seafood	
Surface application	1% w/v; 320 kDa	Inhibition of H_2S-producing organisms during storage at 4 °C.	Shrimp[12]
	1% w/v; 25 kDa	Total aerobic plate counts were lower than controls by 2 log (cfu/g) after 10 days of iced storage.	Pacific white shrimp[13]
	2% w/v; 450 kDa	Total viable counts and psychrotrophic counts were lower than controls by 1-3 log (cfu/g) after storage at 4 °C for 16 days.	Rainbow trout[14]
	3% w/v;	Total viable cells and cell counts of psychrotrophic bacteria were lower than controls by 1 log (cfu/g) after storage at 4 °C for 12 days.	Ready-to-eat peeled Shrimps[15]
	3% w/v; 149 kDa	Total plate counts were lower than controls by 4 log (cfu/g) after vacuum or modified atmosphere packaging storage at 2 °C for 14 days.	Lingcod (*Ophiodon elongates*) fillets[16]
Incorporation	1.0% w/v; 1800, 960 or 660 kDa	Total viable counts were lower than controls by 2 log (cfu/g) after storage for 12 days at 4 ± 1 °C.	Herring and Atlantic cod[17]
	Chitosan (10 kDa) insurimi at 2% w/w.	Aerobic plate counts were lower than controls by 1 log (cfu/g) after storage at 4 °C for 12 days.	Surimi gel made from African catfish (Clarias gariepinus)[18]
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/g) after storage at 4 °C for 24 days. Conventional solution was more bacteriostatic than nanoparticles solution.	Shrimp Muscle[19]
		Vegetables, fruits and juice	
Surface application	1.5%w/v;	Total viable counts and cell counts of yeast and mold were lower than controls by 0.5-1 log (cfu/g) after storage at 4 °C for 7 days.	Pears[20]
	1% w/v; 190 to 310 kDa;	Cell counts of mesophilic aerobic bacteria, yeast and molds were lower than controls by 1 log (cfu/g) after storage at 10 °C for 7 days.	Fresh Blueberries[21]
	1.0% w/v	Lower decay incidence by 20% after at 8 °C for 35 days.	Sweet pepper (*Capsicum annuum L.*)[22]

	Pre-harvest 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[23]
Incorporation	Solution **(**0.4% w/v; 1674 kDa) in apple juice at 2 g/L.	Total bacterial counts, cell counts of psychrotrophic bacteria, yeast and mould were lower than controls by 0.5-3.0 log (cfu g^{-1}) after storage at 5 °C for 15 days.	Apple juice[24]
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 g^{-1}) after storage at 5 °C for 10 days. Solution and nanoparticles exhibited comparable bacteriostatic effect.	Fresh-cut apples[25]
		Bakery products	
Incorporation	Chitin(124±10 kDa; DD 19%) in bread at 1%.	Delay of mold growth in bread during storage of 3 days at 30 °C.	Bread[26]
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/g) after storage for 8 days at room temperature (about 25 °C).	Butter cake [27]
		Eggs	
Surface application	1 % w/v	Total aerobic cell counts chitosan-coated eggs were under detection limit while those of non-coated eggs increased to 20 cfu/ml after 5-weeks of storage at 22±1 or 32±1 °C.	Eggs[28]

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

[1] Menconi et al. 2013 [2] Bostan and Mahan 2011 [3] Petrou et al. 2012 [4] Vasilatos and Savvaidis 2013 [5] Giatrakou et al. 2010 [6] Latou et al. 2014 [7] Lekjing 2016 [8] Malinowska-Pańczyk et al. 2009 [9] Soultos et al. 2008 [10] Siripatrawan and Noipha. 2012 [11] Qin et al. 2013 [12] Arancibia et al. 2015 [13] Yuan et al. 2016 [14] Ojagh et al. 2010 [15] Carrión-Granda et al. 2016 [16] Duan et al. 2010 [17] Jeon et al. 2002 [18] Amiza and Kang 2013 [19] Chouljenko et al. 2017 [20] Cé et al. 2012 [21] Sun et al. 2014 [22] Xing et al. 2011 [23] Meng et al. 2008 [24] Malinowska-Pańczyk et al. 2009 [25] Pilon et al. 2015 [26] Lafarga et al. 2013 [27] Sangsuwan et al. 2015 [28] Suresh et al. 2015

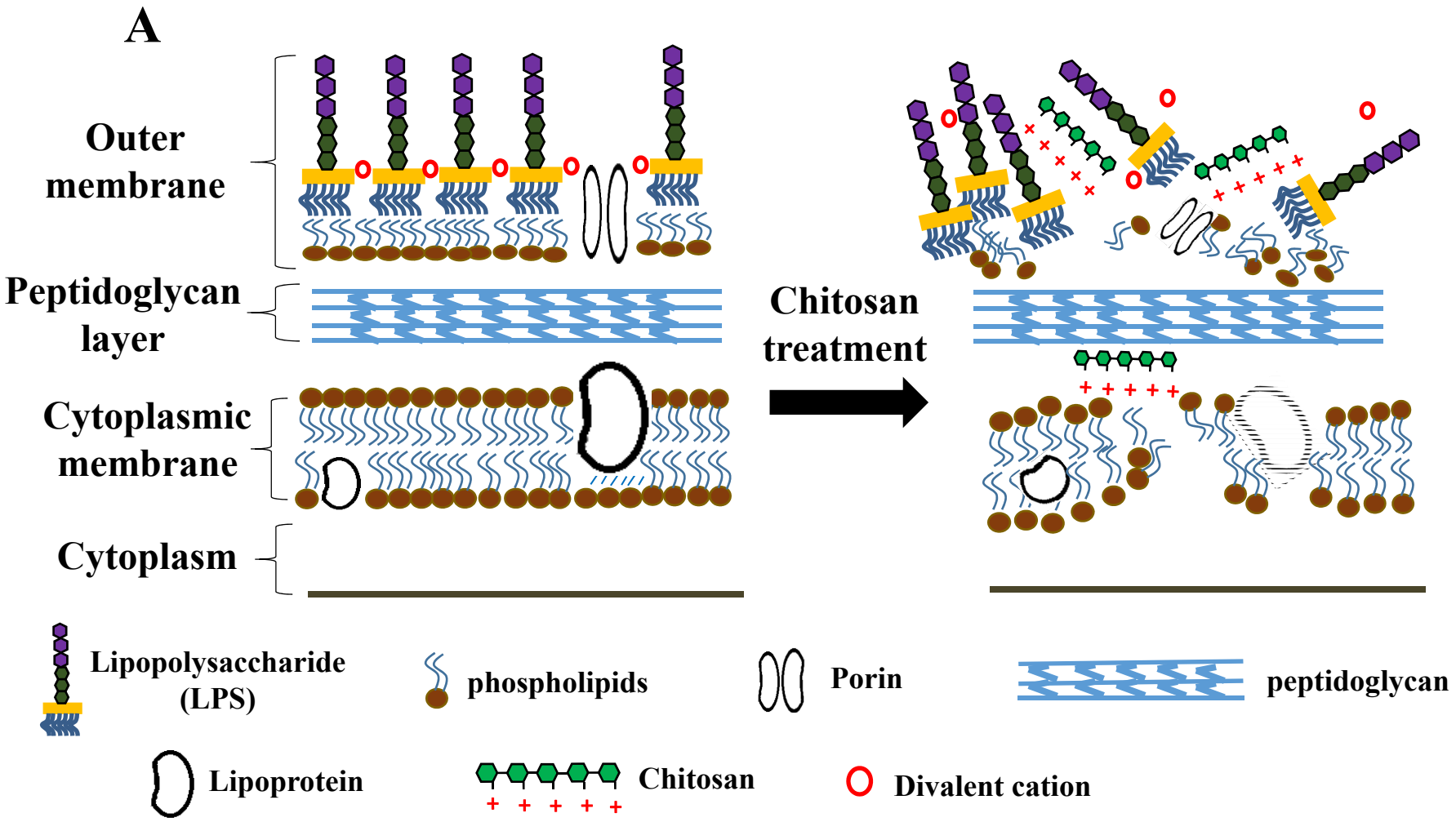
Table 3. Effect of chitosan on food quality

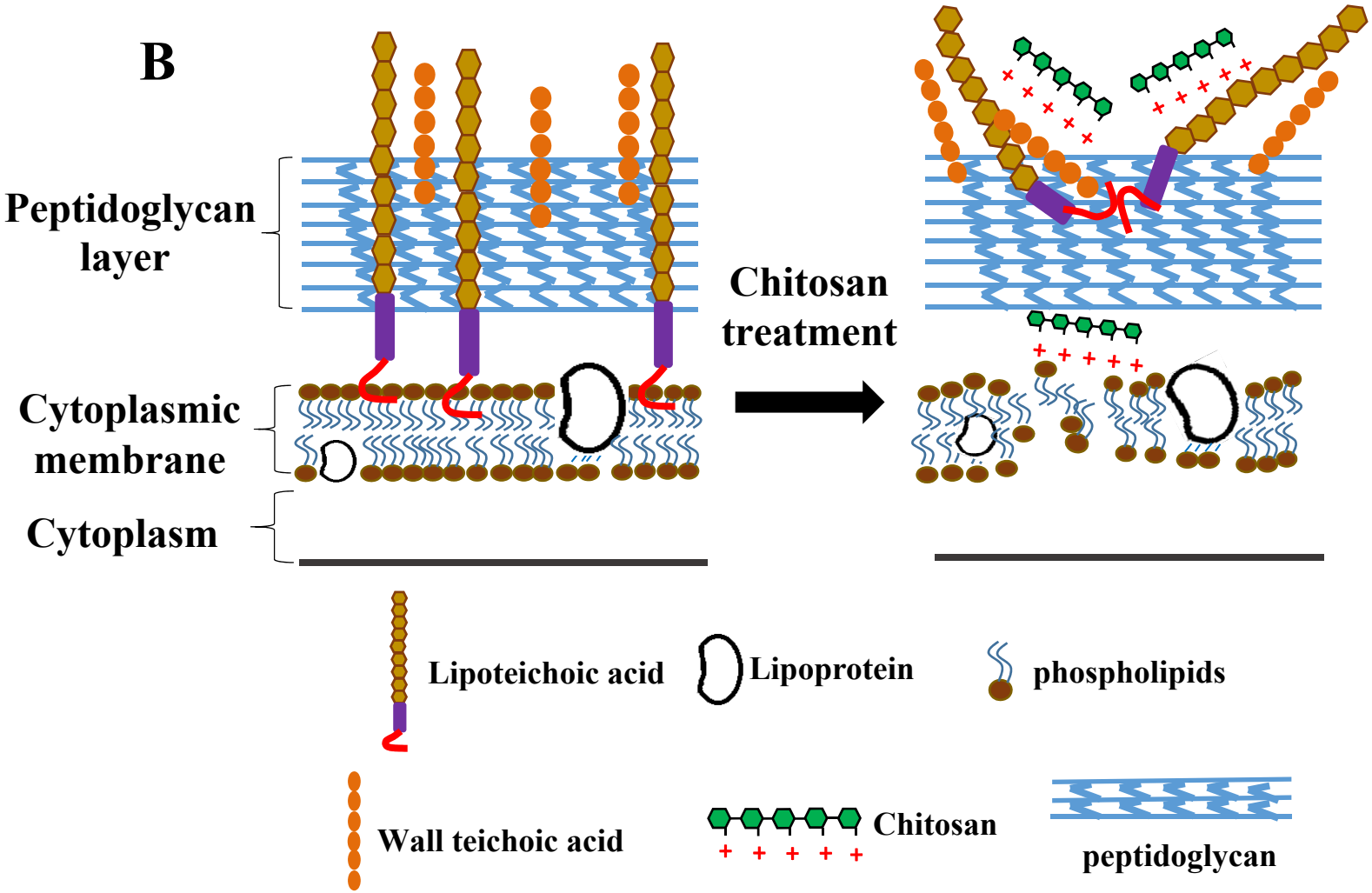
Chitosan preparation and application		Effect of chitosan	Products (reference)
		Meat products	
Surface application	1.0% w/v	Brighter and more attractive color.	Sausage[1]
	1.5% w/v; 340 kDa	Improvement in sensory attributes.	Chicken breast meat[2] Turkey meat [3] Chicken product[4]
	1 % w/v; 800 kDa	Retardation of decline in odor and taste scores.	Chicken breast fillets[5]
	2% w/v; MW: 897 kDa	Retardation of lipid oxidation, change in color and sensory attributes.	Cooked pork sausages[6]
Packaging film:	Prepared from 2% w/v chitosan (100 kDa) solution.	Retardation of lipid oxidation, changes in color, texture, and sensory characteristics.	Cooked pork sausages[7]
	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[8]
Incorporation	Chitosan **(**490 kDa**)** in sausages at 1% w/w	Retardation of lipid oxidation	Fresh pork sausages[9]
		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[10]
	2% w/v; 450 kDa	Retardation of increase in peroxide value and total volatile base nitrogen.	Rainbow trout[11]
	2% w/v;	Retardation of lipid oxidation and improvement in sensory attributes.	Fresh *Channa Argus*[12]
	3% w/v; 149 kDa	Retardation of lipid oxidation under vacuum or modified atmosphere packaging.	Lingcod (Ophiodon elongates) fillets[13]

	1.5% w/w	Retardation of increase in melanosis and loss in freshness and sensory quality.	Whiteleg shrimp (*Litopenaeus vannamei*) [14]
	1.0% w/v of chitosan with 1800, 960, or 660 kDa	Retardation of lipid oxidation.	Herring and Atlantic cod[15]
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)[16]
		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.)[17]
	1% w/v	Retardation of loss in weight	Grape fruits[18]
	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.)[19]
	1.0%, 1.5% or 2.0% (w/v)	Retardation of loss in weight, firmness and changes in the peel colour.	Papaya[20]
		Sauce	
Incorporation	Chitosan (310 or 123 kDa) in mayonnaise at 100 mg kg^{-1}.	Improvement in odor and taste attributes, and retardation of lipid oxidation.	Mayonnaise[21]
		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[22]
	3% w/v	Retardation of loss in weight, decline in Haugh unit and yolk index.	Eggs[23]
	1% w/v; 1110 kDa.	Retardation of loss in weight and decline in Haugh unit.	Eggs[24]

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

[1] Bostan and Mahan 2011 [2] Petrou et al. 2012 [3] Vasilatos and Savvaidis 2013 [4] Giatrakou et al. 2010 [5] Latou et al. 2014 [6] Lekjing 2016 [7] Siripatrawan and Noipha 2012 [8] Qin et al. 2013 [9] Soultos et al. 2008 [10] Yuan et al. 2016 [11] Ojagh et al. 2010 [12] Yang et al. 2015 [13] Duan et al. 2010 [14] Huang et al. 2012 [15] Jeon et al. 2002 [16] Amiza and Kang 2013 [17] Xing et al. 2011 [18] Meng et al, 2008 [19] Hong et al. 2012 [20] Ali et al. 2011 [21] García et al. 2014 [22] Suresh et al. 2015 [23] Caner and Cansiz 2007 [24] Wardy et al. 2014


A
Outer membrane
Peptidoglycan layer
Cytoplasmic membrane
Cytoplasm
Chitosan treatment
Lipopolysaccharide (LPS)
phospholipids
Porin
peptidoglycan
Lipoprotein
Chitosan
Divalent cation


B
Peptidoglycan layer
Cytoplasmic membrane
Cytoplasm
Chitosan treatment
Lipoteichoic acid
Lipoprotein
phospholipids
Wall teichoic acid
Chitosan
peptidoglycan