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Chitosan and Derivatives: Bioactivities and Application in Foods

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Abstract

Chitosan is a biodegradable, biocompatible, and nontoxic aminopolysaccharide. This review summarizes and discusses the structural modifications, including substitution, grafting copolymerization, cross-linking, and hydrolysis, utilized to improve the physicochemical properties and enhance the bioactivity and functionality of chitosan and related materials. This manuscript also reviews the current progress and potential of chitosan and its derivatives in body-weight management and antihyperlipidemic, antihyperglycemic, antihypertensive, antimicrobial antioxidant, anti-inflammatory, and immunostimulatory activities as well as their ability to interact with gut microbiota. In addition, the potential of chitosan and its derivatives as functional ingredients in food systems, such as film and coating materials, and delivery systems is discussed. This manuscript aims to provide up-to-date information to stimulate future discussion and research to promote the value-added utilization of chitosan in improving the safety, quality, nutritional value and health benefits, and sustainability of our food system while reducing the environmental hazards.

INTRODUCTION

Chitosan, the deacetylated derivative of chitin, is a cationic linear polysaccharide composed of β-(1,4)-linked glucosamine and *N*-acetyl-D-glucosamine (Hu & Gänzle 2019). Chitin is found naturally in crustaceans, mollusks, fungi, and insects, and the shells of crustaceans, including shrimps, crabs, and lobsters, are the primary commercial source (Yadav et al. 2019). In some Asian countries, people consume snack foods rich in chitin (e.g., baby shrimp with shells or insects) (de Gier & Verhoeckx 2018, Gao et al. 2018). Fungi also contain chitin in their cell walls/mycelium and are another popular food form.

Depending on its source, chitin in nature is present in three different types of crystalline forms: α, β, and γ. The α-chitin is the most stable and abundant polymorph; it is usually isolated from the exoskeleton of crustaceans, yeast cell walls, and the arthropod cuticle in general. The β-chitin can be extracted from squid pens and the tubes are synthesized by vestimentiferan and pogonophoran worms and are easily converted to the α form via alkaline treatment. The γ-chitin is a combination of α and β forms (El Knidri et al. 2018). Chemical methods, microbial fermentation, and enzymatic hydrolysis have been used to produce chitosan through deacetylation of chitin, with alkaline deacetylation the most commonly used procedure (Kumari et al. 2015, Puvvada et al. 2012).

Chitosan is biodegradable, biocompatible, and nontoxic. Chitosan prepared from different chitin sources using different methods may differ in molecular weight (MW) and degree of deacetylation (DDA) as well as bioactivities and functional properties in food systems. Studies have demonstrated the potential body-weight management and antihyperlipidemic, antihyperglycemic, antihypertensive, antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory activities of chitosan and its derivatives as well as their ability to interact with gut microbiota in vivo and in vitro (Riaz Rajoka et al. 2019). Chitosans have been widely used in films and packages, delivery systems, and functional ingredients in food systems (**Figure 1**). In addition to the food industry, chitosan and its derivatives have been widely used for water and waste treatment and in the cosmetic, agricultural, and medical fields (Muzzarelli 2009, Yadav et al. 2019). This review aims to summarize the recent progress in structural modifications and biological activities of chitosan and its derivatives as well as their functionalities and applications in food systems.

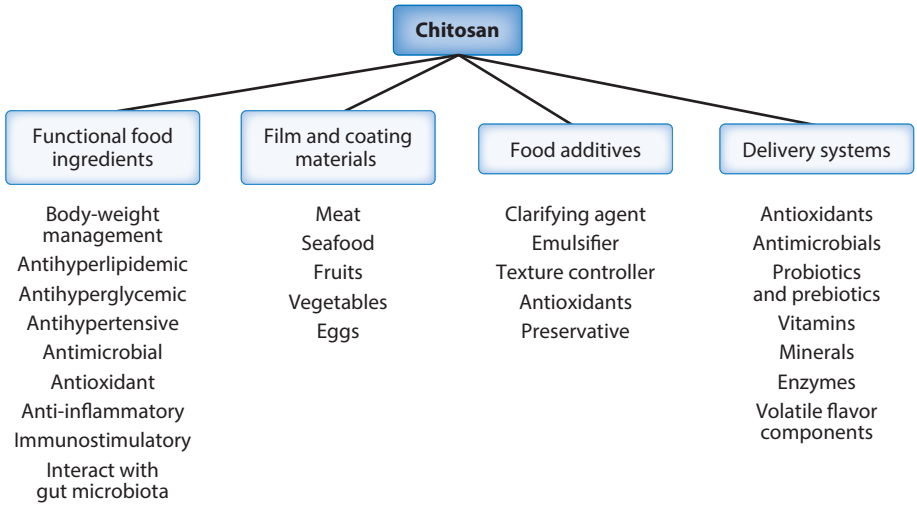

Figure 1

The applications of chitosan in food systems as functional ingredients, film and coating materials, food additives, and delivery systems.

BIOLOGICAL ACTIVITIES

Body-Weight Management

Chitosan and its derivatives have been shown to possess antiobesity properties in several human and animal studies. In 2019, a meta-analysis by Huang et al. (2019) using the data from 11 randomized control trials (RCTs) with 768 obese adults revealed that oral administration of chitosan at 1.0–4.5 g/day for 4–24 weeks or a β-glucan-chitin-chitosan fraction at 3.0 g/day for 12 weeks significantly reduced body weight without altering food intake. The findings agreed with an earlier study in healthy adults that showed that chitosan intervention reduced body weight (Moraru et al. 2018). Together, these findings indicated the potential of chitosan and its derivatives to reduce body weight in obese adults.

Animal studies have also shown the antiobesity properties of chitosan (**Table 1**). An animal study by Pan et al. (2018) showed that oral administration of chitosan with MW $\leq$ 1 kDa and a DDA = 95.6% (0.15, 0.30, 0.60 g/kg of body weight/day) by gastric gavage to the high-fat-diet-induced obese Sprague-Dawley (SD) rats (5-week-old males) for 8 weeks reduced body-weight gain without significant change in food intake. The chitosan promoted the release of leptin and downregulated the expression of acetyl-CoA carboxylase, fatty acid synthase, peroxisome proliferator-activated receptor gamma (PPAR-γ), and sterol regulatory element-binding protein-1c (SREBP-1c). These earlier findings indicated that chitosan oligosaccharides (COSs) may reduce appetite and inhibit triglyceride (TG) synthesis and adipocyte differentiation (Pan et al. 2018). Intraperitoneal injection of COS (MW and DDA, not available; 0.2 g/kg of body weight/day) to male C57BL/6J *ob/ob* mice for 28 days also downregulated PPAR-γ and SREBP-1c and inhibited adipocyte differentiation (Rahman et al. 2010). The COSs reduced intracellular glycerol level by upregulating aquaporin-7, which facilitates permeation of glycerol out of the cell, and inhibited TG synthesis. Another study by Wang et al. (2019a) reported that oral administration of chitosan (DDA $\geq$ 85%) or COS (DDA $\geq$ 90%) (0.6 g/kg of body weight/day) by gastric gavage to high-fat-diet-induced obese SD rats (8-week-old males) for 8 weeks reduced body-weight gain. Chitosan and COS promoted the expression of fat browning genes and proteins, such as PPAR-γ coactivator-1α (PGC-1α) and PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM-16), that induce the conversion of fat-storing white adipose tissue into energy-consuming brown adipose tissue (BAT) as well as the expression of mitochondrial uncoupling protein-1, which facilitates thermogenesis in BAT. Egan et al. (2015) reported that oral administration of basal diet (with no limit) mixed with shrimp shell chitosan (MW = 124,000 g/mol and DDA = 15%; 1 g/kg of body weight/day) by gastric gavage to healthy females pigs (large white × land-race genetic lines) for 63 days lowered dietary intake, body-weight gain, and final body weight compared with the pigs on the basal diet alone. Chitosan also downregulated the expression of fatty acid binding protein 2, which facilitates long-chain fatty acid uptake by cells, and reduced fatty acid utilization and TG synthesis (Egan et al. 2015).

The antiobesity effects of chitosan and its derivatives generally depend on MW. Huang et al. (2015b) reported that oral administration of COS with an average MW $\leq$ 1 kDa (0.25, 0.50, and 1.00 g/kg of body weight/day) by gastric gavage to high-fat-diet-induced obese SD rats (4-week-old males) for 5 weeks resulted in lower body weight compared with the rats given COS with an average MW $\leq$ 3 kDa and control rats. This might be due to higher absorption of COS with lower MW under gastric acidic conditions (Huang et al. 2015b).

To conclude, chitosan and COS may be used for weight loss and body-weight management. Further research is needed to assess the molecular and cellular mechanisms via which chitosan

Table 1 An overview of antiobesity properties of chitosan and its derivatives

Animal model	Treatment		Findings	Reference
Large white × land-race pigs	Chitosan, MW 124,000 g/mol, DDA = 5%	1 g/kg diet, 9 weeks, ad libitum feeding	Chitosan inhibited body-weight gain, reduced final body weight and fat content	Egan et al. 2015
Sprague-Dawley rats; obesity model induced by high-fat diet	Chitosan oligosaccharide (COS)-1, MW ≤ 1 kDa, DDA = unknown COS-2, MW ≤ 3 kDa, DDA = unknown	250, 500, 1,000 mg/(kg·day), 5 weeks, orally by gavage	COS-1 and COS-2 inhibited weight gain in rats 1,000 mg/(kg·day) of COS-1 was more effective than 1,000 mg/(kg·day) of COS-2, and this effect was dose-dependent 1,000 mg/(kg·day) COS-2 reduced the body fat and body fat ratio in obese rats; COS-1 also reduced rat body fat, but this reduction was not significant	Huang et al. 2015b
Sprague-Dawley rats; obesity model induced by high-fat diet	COS, MW ≤ 1 kDa, DDA = 95.6%	150, 300, 600 mg/(kg·day), 8 weeks, orally by gavage	All dose groups of chitosan inhibited body-weight gain and reduced fat pad and body fat ratio, and these effects were dose-dependent	Pan et al. 2018
C57BL/6J *ob/ob* mice; spontaneous obesity model	COS, MW = unmentioned, DDA = not available	200 mg/(kg·day), 4 weeks, intraperitoneal injection	COS inhibited body-weight gain and reduced adipose tissue mass	Rahman et al. 2010
Sprague-Dawley rats; obesity model induced by high-fat diet	COS-T, MW ≤ 1 kDa, DDA ≥ 90% COS-M, MW ≤ 3 kDa, DDA ≥ 90% CTS, MW = not available, DDA ≥ 85%	600 mg/(kg·day), 8 weeks, orally by gavage	All treatment groups inhibited body-weight gain and the inhibition degree of weight gain in the COS-M group was greater than that in the COS-T and chitosan groups All treatment groups reduced fat content, and the fat content in the COS-T and chitosan groups was lower than that in the COS-M group	Wang et al. 2019a

Abbreviations: CTS, chitosan; COS, chitosan oligosaccharide; DDA, degree of deacetylation; MW, molecular weight.

and COS manage body weight. The role of other factors such as polymerization, DDA, and substitution on overall body-weight reduction also needs to be assessed.

Antihyperlipidemic Activity

A meta-analysis by Moraru et al. (2018) using the data from a total of 8 RCTs with 756 obese adults with a body mass index greater than 23.6 indicated that chitosan supplementation at 0.34–3.10 g/day for 56–365 days by capsules and tablets significantly reduced total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and TG.

Oral administration of chitosan (MW = 250 kDa and 38 kDa; 0.45 g/kg of body weight/day) by gastric gavage to male SD rats fed a high-fat diet for 30 days reduced blood TG, TC, and LDL-C,

whereas it increased HDL-C and fecal fat and cholesterol excretion (Wang et al. 2019a). These results were consistent with a previous study on male SD rats fed a high-fat diet supplemented with 5% COS [degree of polymerization (DP) of approximately 5] from *Clanis bilineata* (Lepidoptera) (Xia et al. 2013). These findings together indicated that chitosan and COS may bind and trap fat and cholesterol in the gastrointestinal (GI) tract, which may enhance fecal excretion and reduce serum cholesterol and TG concentrations.

In a recent study by Chiu et al. (2020), 6-week-old male SD rats were randomly assigned to different treatment groups given a high-fat diet supplemented with 5% high-MW chitosan (HMWC; MW = 740 kDa, DDA = 91%; crustacean shell), 5% low-MW chitosan (LMWC; MW = 80 kDa, DDA = 83.9%; crustacean shell), 5% COS (MW = 0.719 kDa, DDA = 100%; crustacean shell), or 5% cellulose for 8 weeks; HMWC, LMWC, and COS supplementations significantly reduced plasma TC, LDL-C+VLDL-C, and the TC:HDL-C ratio compared with the 5% cellulose group; however, only HMWC and LMWC downregulated the acyl-CoA cholesterol acyltransferase 2 level and upregulated LDL receptor (LDLR), cholesterol 7α-hydroxylase (CYP7A1), adenosine monophosphate-activated protein kinase-α, and PPARα expression levels (Chiu et al. 2020). In contrast, the COS supplement had no detectable effect on these lipid-metabolism-related proteins. Interestingly, in another study, oral administration of COS (MW < 1 kDa, DDA = 95.6%; 0.15, 0.30, and 0.60 g/kg of body weight/day) by gastric gavage to high-fat-diet-induced high-cholesterol male SD rats for 6 weeks upregulated LDLR, PPARα, and CYP7A1 (Jiang et al. 2018). Notably, the COS diet downregulated 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which catalyzes the rate-limiting step of endogenous cholesterol synthesis (Jiang et al. 2018). Together, these studies indicated that chitosan and COS may have potential antihyperlipidemic effects by modulating TG and cholesterol biosynthesis and metabolism as well as liver LDL-C reuptake; however, the molecular mechanisms behind the hypolipidemic effects and the contribution of the molecular and chemical structure of chitosan and COS to the antihyperlipidemic activities remain unclear.

Zhang et al. (2013) reported that a high-fat diet with a 3% chitosan (MW = 326 kDa and 56 kDa) or 3% chitosan nanopowder (MW = 315 kDa and 51 kDa) supplement in male SD rats for 6 weeks had the antihyperlipidemic effects with lower serum TG, TC, and LDL-C and higher HDL-C compared with the control rats. Interestingly, the chitosan nanopowder intervention groups had lower serum TG, TC, and LDL-C, with a higher HDL-C compared with the rats fed chitosan with a similar MW, indicating that the antihyperlipidemic activity of chitosan nanopowder was better than the counterpart chitosan (Zhang et al. 2013). This finding also indicated that stronger hyperlipidemic activity of chitosan may be associated with lower particle size. Additionally, chitosan with a MW of 56 kDa was more effective in lowering blood lipid levels than that with a MW of 326 kDa (Zhang et al. 2013). These results indicated that decreases in particle size and/or molecular size increased the antihyperlipidemic activity of chitosan and its potential impact on health. In contrast, Yao et al. (2008) observed that feeding of a basal diet including 5% shrimp shell chitosan with high MW (1,000 kDa) to streptozotocin-induced diabetic SD rats (6-week-old males) for 4 weeks significantly lowered the plasma TC, TG, and TC:HDL-C ratio, whereas chitosan with a lower MW (14 kDa) had no significant effect.

A recent study by Wang et al. (2019b) suggested the possible influence of substitution groups on the antihyperlipidemic effect of chitosan. Wang et al. (2019b) synthesized chitosan quaternary ammonium salts (MW = 240 kDa and 25 kDa, DDA of 92%) by combining chitosan with glycidyl trimethyl ammonium chloride at 75°C under alkaline conditions and compared the lipid-lowering effect with that of chitosan alone. The results showed that oral administration of chitosan quaternary ammonium salt suspension (0.45 g/kg of body weight/day) by gastric gavage for 30 days was more effective in reducing blood TG, TC, and LDL-C levels and in increasing

HDL-C levels than the chitosan counterpart (MW = 250 kDa and 38 kDa, DDA = 92%) at the same dose and for the same treatment period. Wang et al. (2019b) concluded that quarternary ammonium substituents in chitosan molecules may elevate positive charge density, which improves chitosan's capacity to bind with negatively charged lipids and bile acids in the intestine and consequently reduce lipid absorption.

In conclusion, the antihyperlipidemic properties of chitosan and COS may be due to the reduction in lipid absorption in the GI tract, modulation of cholesterol and TG biosynthesis and metabolism in the liver, and absorption of circulating LDL-C into hepatocytes. Molecular size, particle size, and positive charge density are critical for the overall antihyperlipidemic effect of chitosan and COS. These properties may alter their charge along the GI tract and consequently alter the viscosity of GI contents that help bind and trap lipids for excretion. However, the mechanisms via which chitosan compounds may modulate liver lipid biosynthesis and metabolism are unknown. The positive charge is unique to chitosan and COS as compared to other digestion-resistant polysaccharides with antihyperlipidemic properties.

Antihyperglycemic Activity

In 2019, a randomized, double-blind, controlled crossover trial by Jeong et al. (2019) showed that COS (MW < 1 kDa) administration at 0.25 g/day, 15 min before sucrose consumption, for 7 days in impaired fasting glucose participants and healthy participants (20–75 years old) reduced blood glucose levels within 2 h post-treatment compared to the placebo control group. Jo et al. (2014) also reported that oral intake of 0.5 g COS (MW < 1 kDa) 20 min before sugar administration in healthy participants (mean age of 28.9 ± 7.2 years) lowered blood glucose levels within 2 h post-treatment compared with the control group. Taken together, these findings revealed that COS intake before a meal may suppress blood glucose elevation in healthy adults and individuals with impaired fasting glucose.

Several animal studies have also investigated the antihyperglycemic properties of chitosan and COS. Feeding of a basal diet supplemented with 5% or 7% chitosan (MW = 830 kDa, DDA = 94%; shrimp shell) to nicotinamide- and streptozotocin (STZ)-induced diabetic SD rats (6-week-old males) for 10 weeks significantly decreased the levels of plasma glucose and inflammatory factors, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and thus ameliorated insulin resistance (Hsieh et al. 2012). Liu et al. (2010) reported that feeding a 5% chitosan (MW = 860 kDa and 17.7 kDa; shrimp chitin)–supplemented diet to STZ-induced diabetic SD rats (7-week-old males) for 4 weeks effectively reduced the blood glucose levels; a low-MW chitosan was more effective than a high-MW chitosan. Furthermore, feeding a 5% chitosan–supplemented diet downregulated the expression of phosphoenolpyruvate carboxykinase and upregulated the expression and translocation of glucose transporter-4, which is necessary for gluconeogenesis and glucose uptake into skeletal muscle and adipose tissues, respectively (Liu et al. 2010). In another study, feeding a basal diet supplemented with 5% chitosan from shrimp shell (MW = 1,000 kDa, DDA = 80%) to STZ-induced diabetic SD rats (6-week-old males) for 4 weeks was significantly more effective in reducing plasma glucose concentration than a diet containing 5% chitosan with an MW of 14 kDa and DDA of 91% (Yao et al. 2008). This study also showed that sucrase, lactase, and maltase concentrations were more effectively reduced by the high-MW chitosan than by the low-MW chitosan (Yao et al. 2008). In contrast, oral administration of sucrose (2.0 g/kg of body weight/day) mixed with COS (0.1 g/kg of body weight/day) by gastric gavage to SD rats (5-week-old males) significantly reduced the postprandial blood glucose levels within 1 h post-treatment compared to control (without COS) (Jo et al. 2013), although the COS with a low MW (<1 kDa) demonstrated maximum reduction in blood glucose (Jo et al. 2013).

Several human and animal studies have so far suggested the antihyperglycemic properties of chitosan and COS, which may act via suppression of inflammatory cytokines (reduce insulin resistance), regulation of glycometabolism in the liver, and reduction of glucose absorption in the GI tract (Hsieh et al. 2012, Liu et al. 2010, Yao et al. 2008). The MW and DDA play significant roles in determining their overall antihyperglycemic properties; however, the exact mechanism of chitosan's antihyperglycemic property remains unclear.

Antihypertensive Activity

A meta-analysis by Huang et al. (2017) using the data from a total of eight RCTs with 617 adults with systolic blood pressure (SBP) of 120–139-mm Hg and/or diastolic blood pressure (DBP) of 80–89-mm Hg found that the supplementation of chitosan at a dose of more than 2.4 g/day for a short period (<12 weeks) significantly reduced DBP. Similar findings that indicated the potential of chitosan to lower human blood pressure were reported by Moraru et al. (2018) via meta-analysis.

Several animal studies have also demonstrated the antihypertensive properties of chitosan and COS. Oral supplementation of 5% chitosan (DDA = 81%) in the diet for 40 days significantly decreased SBP caused by a high-salt diet (3% NaCl) in the stroke-prone spontaneously hypertensive rats and in the age-matched Wistar rats (normotensive rats); increased chloride (Cl^-) excretion, which consequently reduced renal afferent artery resistance; and promoted renal blood flow and glomerular filtration rate (Kato et al. 1994). Furthermore, chitosan supplementation significantly reduced the activity of serum angiotensin I converting enzyme (ACE) activity, which converts angiotensin I into angiotensin II (a powerful vasoconstrictor) and inactivates bradykinin (capable of relaxing blood vessels and lowering blood pressure) (Kato et al. 1994). This observation indicated that chitosan might lower blood pressure by modulating the renin-angiotensin system (RAS) (Kato et al. 1994). Another animal study by Park et al. (2009) showed that including 3% chitosan (MW = 100 kDa; crab chitin) in the basal diet for two months decreased SBP and angiotensin II concentration in spontaneously hypertensive rats (SHRs) possibly via RAS modulation. In 1998, Hong et al. (1998) also reported that oral supplementation of COS at 2.14 mg/kg of body weight/day reduced the blood pressure of SHRs; chitosan trimer was the most effective in decreasing blood pressure among all the tested oligomers with a DP of 1–10.

A few in vitro studies indicated that ACE and renin inhibitory activities of chitosan may be influenced by MW, DDA, and the substitution groups. COS with a medium MW (MMW; 1–5 KDa) exhibited the strongest ACE and renin inhibitory activities among the tested COSs (MW < 1, 1–5, and 5–10 KDa) (Park et al. 2003, 2008). Meanwhile, COS with a DDA of 50% demonstrated stronger ACE inhibition and weaker renin inhibition compared to the one with 90% DDA and the same MW. In addition, carboxymethylation (Huang et al. 2005), aminoethyl grafting (Ngo et al. 2008), and sulfation (Qian et al. 2010) enhanced the ACE inhibitory activity of COS. These studies proposed possible approaches to further improve the antihypertensive activity of COS.

In conclusion, MW, DDA, and substitution play important roles in determining the antihypertensive properties of chitosan and COS; however, the relationship between their structures and antihypertensive properties needs to be investigated further. An in-depth investigation to clarify the underlying molecular mechanisms of the antihypertensive activity, especially the interaction with RAS and ACE, is needed to recommend them for commercial use.

Antimicrobial Activity

Chitosan and its derivatives with quaternary ammoniumyl, carboxyalkyl, hydroxyalkyl, guanidinyl, and thiol-containing groups have shown antibacterial and antifungal activities in cultured

microorganisms, food systems, and packaging materials (Elkholy et al. 2014, Ignatova et al. 2006, Oh et al. 2019, Orellano et al. 2019, Sahariah & Masson 2017). Chitosan and its derivatives can also reduce the pathogenic microorganisms in animals. Feeding a chitosan-supplemented diet (4 g/kg of diet dry matter) to dairy cows for 23 days altered ruminal fermentation and decreased *Butyrivibrio* population and microbial protein synthesis (Zanferari et al. 2018). In another study, Jeong et al. (2011) reported that supplementation of 8 g/day chitosan microparticles in the diet for 6 days decreased *Escherichia coli* O157:H7 (a major human pathogen that resides in healthy cattle) in the rectal swabs of calves (steers).

MW, DDA, and substitution type and degree are the major structural factors that determine the antimicrobial effects of chitosan. To date, no conclusion has been made on how MW may alter the antimicrobial activities of chitosan (Liu et al. 2006, Zheng & Zhu 2003). A greater DDA may be associated with a stronger antimicrobial activity of chitosan (Taşkın et al. 2014). An increased substitution degree of hydrophobic groups via alkylation, acylation, quaternization, and metalization enhanced the antimicrobial activity of chitosan (Ignatova et al. 2006, Kong et al. 2010, Peng et al. 2010). Environmental conditions, including pH and cationic charge density, can alter the antimicrobial activity of chitosan. Chitosan is polycationic at a pH < pKa; it easily interacts with negatively charged residues of carbohydrates, lipids, and proteins located on the surface of microorganisms. Thus, the electrostatic interaction may cause changes in cell membrane structure and permeability and nutrient uptake, thereby inhibiting microbial survival and growth (Kong et al. 2008, 2010). Chitosan with a MW of less than 5,000 KDa may penetrate multiple layers of the bacterial cell wall, bind to cellular DNA to suppress DNA transcription and translation, and finally kill the bacteria (Choi et al. 2014, Goy et al. 2009). Chitosan can also selectively chelate metal ions and suppress microbial growth and toxin production (Sahariah & Masson 2017, Zhang et al. 2016). Moreover, chitosan adsorbed to the surface of microbial cells can form a high-MW polymer film, which prevents nutrients from being transported into the cells or acts as an oxygen barrier, and inhibit microbial growth (Devlieghere et al. 2004).

Owing to antimicrobial activity, chitosan has been mainly explored as an alternative natural antimicrobial agent in foodstuffs to improve storage stability. Chitosan can effectively inhibit the growth of spoilage microorganisms in meat and meat products, wheat products, and beverages (**Table 2**) (Huang et al. 2007, Lee et al. 2002, Malinowska-Pańczyk et al. 2009, Petrova et al. 2016, Roller & Covill 2000). Malinowska-Pańczyk et al. (2009) reported that the total bacterial (psychrophilic and psychrotrophic) count was lower in the minced pork treated with a 2 mg/g chitosan solution compared to the control samples. Lee et al. (2002) reported that chitosan (30 kDa and 120 kDa; 1%) addition inhibited bacterial growth on wheat bread and improved shelf life. Huang et al. (2007) reported that the addition of chitosan (0.05 g/100 mL) to a fresh noodle

Table 2 Antimicrobial effects of chitosan in food systems

Food systems	Chitosan dosage	Effects	Reference
Minced pork	2 mg/g	Lowered the total bacterial (psychrophilic and psychrotrophic) count	Malinowska-Pańczyk et al. 2009
Wheat bread	1%, m/v	Inhibited bacterial growth Improved shelf life	Lee et al. 2002
Noodle	0.05 g/100 mL	Extended shelf life by at least 6 days	Huang et al. 2007
Red wine	4, 8, and 12 g/hL	Reduced *Brettanomyces bruxellensis*	Petrova et al. 2016
Mayonnaise	3 g/L	Inactivated *Lactobacillus fructivorans*	Roller & Covill 2000
Chinese cabbage in kimchi	0.1% and 0.15%, m/v	Extended shelf life by about 10 days	No et al. 1995

formulation extended its shelf life by at least 6 days during storage at 4°C. Petrova et al. (2016) proved the antimicrobial effect of chitosan against *Brettanomyces bruxellensis* in red wine. Roller & Covill (2000) reported that chitosan at 3 g/L concentration in mayonnaise inactivated the inoculated *Lactobacillus fructivorans* during chill storage for 8 days. No et al. (1995) reported that the shelf life of kimchi prepared using Chinese cabbage immersed in 0.1% or 0.15% chitosan solutions extended shelf life by approximately 10 days compared to the control kimchi.

Collectively, chitosan and its derivatives with appropriate MW, DDA, and substitutions may be used as antimicrobial agents in food systems, such as food packages, and other agricultural systems, including animal feeds, to improve food quality and safety.

Interaction with Gut Microbiota

The role of chitosan and its derivatives in regulating the gut microbiota has been shown in various in vivo studies. A human study performed by Mrázek et al. (2010) reported that chitosan supplementation (3 g/day) via capsules for 28 days significantly affected fecal microbiota with high complexity and individuality for each subject; higher fecal levels of *Bacteroides* were found with chitosan supplementation than the control. Animal studies on mice, rats, and pigs have also shown that chitosan and its derivatives alter gut microbiota (Koppová et al. 2012, Yu et al. 2017, Zhang et al. 2018a). Zhang et al. (2018a) reported a lower level of the probiotic genera *Lactobacillus* and *Bifidobacterium* and harmful genus *Desulfovibrio*, and a higher level of the genus *Akkermansia* in C57BL/6J mice after being fed a COS (0.2 g/kg of diet weight)-supplemented diet for five months. Moreover, the effect of COS was opposite to that of chitosan. Yu et al. (2017) reported that feeding a low-MW chitosan (50 mg/kg of diet weight)–supplemented diet for 28 days increased the relative abundance of *Bacteroidetes*, *Prevotella*, *Succinivibrio*, and *Anaerovibrio* and decreased the count of *Firmicutes* and the ratio of *Lactobacillus* in piglets. These earlier studies demonstrated the prebiotic potential of chitosan and its derivatives. Moreover, recent studies have positively correlated the alteration of gut microbiota by chitosan and its derivatives with improvement in diabetes, hyperglycemia, hyperlipidemia, inflammation, obesity, and immune regulation (Shang et al. 2018, Udayangani et al. 2017, Xiao et al. 2016, Zheng et al. 2018). However, there is also a report that found chitosan had an adverse effect on gut microbiota. Koppová et al. (2012) reported that feeding a 1% chitosan and COS-supplemented diet for 3 weeks increased the population of the *Bacteroides–Prevotella* group and decreased that of *Enterobacteriaceae* in the GI tract of healthy rats.

Complex polysaccharides and nondigestible carbohydrates, which are not digested by humans, are processed by gut microbiota in the intestine with different hydrolytic enzymes. Different types of oligosaccharides may also be fermented by the gut microbiota. The efficient utilization of polysaccharides in the gut occurs via a series of fermentation reactions in which the metabolite of one microorganism is used as the substrate of the subsequent microorganisms (Yadav et al. 2018). Similarly, digestive enzymes of the host may not be able to digest chitosan and its derivatives (Ringø et al. 2012, Xiao et al. 2016). Therefore, it may be metabolized by the gut microbiota. Previous studies have demonstrated that the effects of interaction between gut microbiota and chitosan and its derivatives depend on MW and DP. The degradation kinetics of chitosan and its derivatives by gut microbiota are different. COS with a lower DP showed a faster degradation, whereas those with higher DPs showed slower degradations (Zhang et al. 2018a). Low-MW chitosan is degraded by the gut microbiota at a faster rate (Vernazza et al. 2005). To summarize, chitosan and its derivatives have the ability to regulate the gut microbiota. However, the impact of chitosan on gut microbiota and the mechanisms underlying this interaction need to be further investigated.

Antioxidant Activity

Chitosan and its several derivatives have antioxidant properties. Oral supplementation of chitosan (Abdel-Wahhab et al. 2016, Toz & Deger 2017, Wu et al. 2017, Xu et al. 2016), COS (Kong et al. 2018, Lan et al. 2019, Tao et al. 2019), and a chitosan–caffeic acid conjugate (Park et al. 2017) elevated the activities of antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), and reduced the concentration of malondialdehyde (MDA) in D-galactose-induced aging mice, high-fat-diet-fed mice, ethanol-treated mice, heat-stressed rats, lead toxicity–induced rats, and ochratoxin-A-induced oxidative stress and renal genotoxicity rats. An experiment on the human neuroblastoma cell line (SH-SY-5Y) showed that a low-MW sulfated chitosan pretreatment suppressed the rotenone-induced oxidative injury in the cells in a dose-dependent manner with reduced levels of lipid peroxidative markers such as thiobarbituric acid reactive components and MDA and increased levels of the cellular antioxidatives SOD, CAT, GPx, and glutathione (Manigandan et al. 2018). Furthermore, chitosan and its derivatives, such as gallic acid–grafted chitosan, suppressed the oxidative spoilage of white wine (Marín et al. 2019), bulk oil (Gim et al. 2017), and cupcake (Allam & Dolgnova 2017). Chitosan has also been used in food packaging materials to protect lamb meat (Sharafati-Chaleshtori & Sharafati-Chaleshtori 2017), sliced dry-cured ham (Yan et al. 2019), strawberries (Resende et al. 2018), guava (Silva et al. 2017), nectarine (Zhang et al. 2019), and sweet pepper (Xing et al. 2011) from oxidative deterioration.

Chemical mechanisms involved in the antioxidant activities of chitosan and its derivatives include scavenging free radicals and chelating transition metal ions (Yang et al. 2016). The number and relative position of hydroxyl and amino groups in the molecules determine their interactions with radicals and chelating metal ions (Luan et al. 2018). Generally, chitosan with a lower MW and a higher DDA is favorable for free-radical scavenging activity (Kim 2018). In addition, approaches to improve water solubility or grafting with phenolic groups may enhance the antioxidant properties of chitosan compounds (Cho et al. 2011, Luan et al. 2018, Zhang et al. 2018b). In a biological system, chitosan/derivative intake may neutralize intracellular reactive oxygen species and elevate antioxidant enzymes such as SOD, GPx, CAT, glutathione-S-transferases, uridine diphosphate-glucuronosyl transferases, and nicotinamide adenine dinucleotide phosphate quinone oxidoreductase (Hunyadi 2019).

Chitosan has been explored as an alternative natural antioxidant agent in foodstuffs to improve storage stability and effectively delay food oxidation during storage and thereby maintain food quality with an extended shelf life. Kanatt et al. (2004) found a significant decrease in thiobarbituric acid values in radiation-processed lamb meat treated with an irradiated chitosan. Youn et al. (2004) reported that chitosan (120 kDa; 1.0%) addition to spicy beef remarkably inhibited lipid oxidation during storage at 4°C and improved the shelf life. Jo et al. (2001) reported that the addition of a chitosan oligomer (5 kDa; 0.2%) to sausage effectively inhibited lipid oxidation during storage for 3 weeks at 4°C.

Anti-Inflammatory Activity

COS has shown anti-inflammatory activities in several cell and animal studies. Oral administration of a mixture of glucosamine (80.00%) and COS (9.80%; MW = 20–30 KDa, DDA = 85%–95%) at doses of 0.04, 0.08, and 0.16 g/kg body weight/day by gastric gavage for 15 days significantly reduced the serum levels of TNF-α, IL-6, and IL-1β and alleviated knee joint swelling and damage in osteoarthritic mice (Li et al. 2018). Oral administration of a low-MW COS consisting of glucosamine (GlcN)n (n = 3–5, MW ≤ 1 kDa, DDA = 100%) at 0.016 g/kg of body weight/day

for 29 days to an ovalbumin-induced asthma mouse model significantly reduced both mRNA and protein levels of proinflammatory cytokines such as IL-4, IL-5, IL-13, and TNF-α in the lung tissue and bronchoalveolar lavage fluid, which indicated the effectiveness in alleviating allergic inflammation (Chung et al. 2012). A basal diet supplemented with COS (300 μg) for 21 days significantly reduced TNF-α, IL-6, and IL-8 serum concentrations, mononuclear cell infiltration, histological injury, and epithelial erosion in lipopolysaccharide-induced intestinal inflammation piglet models (Huang et al. 2015a). These findings indicated the potential anti-inflammatory role of COS.

Several studies have investigated the in vitro anti-inflammatory activities of COS, gallic acid-grafted COS, and 4-hydroxybenzyl-chitooligomer (Lee et al. 2009, Pangestuti et al. 2011, Trinh et al. 2014, Vo et al. 2017). Pangestuti et al. (2011) reported that COS of four different MWs (<1, 1–3, 3–5, and 5–10 kDa) reduced the production of proinflammatory cytokines by inhibiting mitogen-activated protein kinase (MAPK) in the murine BV2 microglia, and a lower MW was associated with a stronger anti-inflammatory activity. This observation contrasted with that reported in RAW264.7 macrophage cells where COS with a greater MW and higher DDA demonstrated stronger anti-inflammatory activity (Lee et al. 2009). Interestingly, the gallic acid–grafted COS was stronger than the COS in suppressing the prostaglandin E_2 production from arachidonic acid and inhibiting cyclooxygenase-2 in human lung epithelial A549 cells (Vo et al. 2017). In addition, 4-hydroxybenzyl-chitooligomer was more effective than the COS in inhibiting nuclear factor-κB and MAPK and reducing proinflammatory cytokine levels in human Chang liver cells (Trinh et al. 2014). Taken together, these results suggested that the introduction of a simple phenolic moiety into COS may enhance its anti-inflammatory effect.

In summary, COS might be used in modulating inflammatory disorders in different human organs and tissues. However, the molecular and cellular mechanisms underlying the anti-inflammatory effect of COS and the possible structure–activity relationship need to be investigated. Meanwhile, further confirmation of their efficacy in vivo is also needed.

Immunostimulatory Activity

Chitosan and COS have shown immunostimulatory activities in several cell and animal studies. Intraperitoneal administration of COS (MW = 1,000 Da, DDA = 95%) at doses 0.02, 0.04, and 0.08 g/kg of body weight/day for 10 days significantly increased spleen and thymus indices in a dose-dependent manner, enhanced NK cell cytotoxicity and macrophage phagocytic activity, and improved T- and B-cell proliferation in cyclophosphamide-induced immunosuppressed mice (Zhai et al. 2018). Another study by Zhang et al. (2014) showed that oral administration of COS containing 2–8 glucosamines at doses 0.10, 0.25, and 0.50 g/kg of body weight/day by gastric gavage for 20 days increased the relative organ weight of the spleen and thymus and enhanced immunoglobin G content in the serum of ICR mice. They also observed that the COS promoted the proliferation and phagocytic functions of RAW264.7 macrophage cells. An in vitro study showed that COS upregulated nitric oxide and TNF-α secretion, indicating that the immunostimulatory effect of COS may be induced via the increase in immunological cell proliferation and function as well as secretion of proinflammatory mediators (Zhai et al. 2018). Together, these findings suggested the potential immunostimulatory properties of COS.

Several in vitro studies have reported the immunostimulatory effects of chitosan and its derivatives, such as C2,3,6-sulfated chitosan and 2-hydroxypropyltrimethyl ammonium chloride chitosan (Wu et al. 2015; Yang et al. 2017, 2018). Generally, chitosan with relatively low MW demonstrated a stronger immunostimulatory activity (Wu et al. 2015).

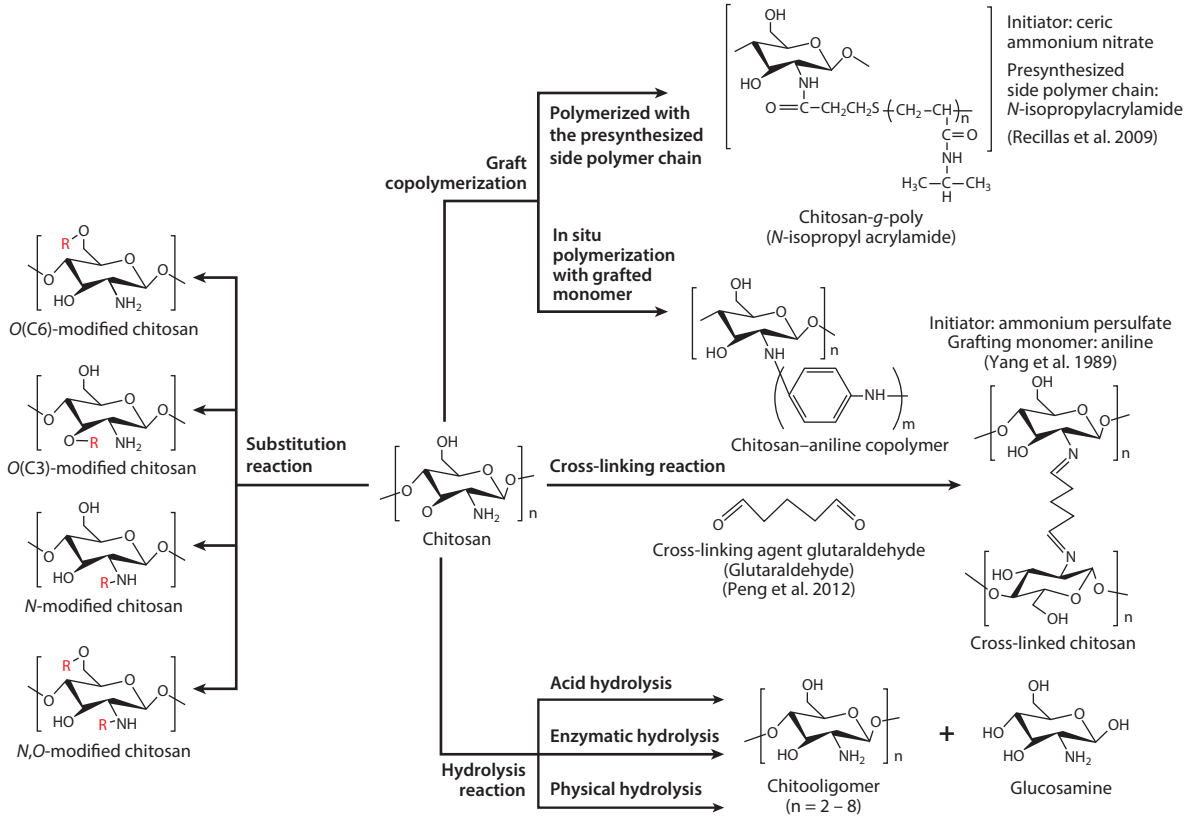

Figure 2

Substitution, graft copolymerization, cross-linking, and hydrolysis reactions of chitosan.

In summary, chitosan and COS might be used as potential immunostimulatory agents. However, no studies have so far investigated the difference in immunostimulatory effects of chitosan and COS with differences in structure.

DERIVATIZATION OF CHITOSAN

According to the current food- and drug-related standards of various countries, the consumption of chitosan is restricted to its original form. However, in view of many current research findings, the biological activities of chitosan can be improved through derivatization. Therefore, chitosan derivatization products have the potential to improve their application value. Chemical, physical, and biological methods have been used to modify the structure of chitosan preparations with different DDAs and MWs to improve their physicochemical properties, as well as to enhance their bioactivities and functionalities. The commonly used reactions in derivatization and modification are substitution, grafting copolymerization, cross-linking, and hydrolysis (**Figure 2**). The amino group ($-NH_2$) on C2 and the hydroxyl group ($-OH$) on C6 and C2 of chitosan can be modified to obtain the derivatives. The physicochemical properties of chitosan such as structure, MW, solubility, charge density, lipophilicity, and hydrophilicity can be altered by derivatization to improve the biocompatibility, bioadhesion, coagulation, film-forming, coating, swelling, binding, and

trapping capacities and various bioactivities and thus broaden the scope of chitosan (Argüelles-Monal et al. 2018).

Substitution

Substitution is a chemical reaction during which hydrogen atoms in organic compounds are replaced by other atoms or atomic groups. This reaction is widely used in the derivatization of chitosan. The hydroxyl groups at C3 and C6 and the amino group at C2 of the chitosan monomer are the main reactive groups substituted to form *O*-modified, *N*-modified, and *N*,*O*-modified chitosan derivatives under different conditions (**Figure 2**). Substitution can be achieved by acylating with organic acids and their anhydrides and with acid chlorides, esterifing with oxygen-containing inorganic acids (sulfuric and phosphoric acids), and alkylating with alkanes or aromatic hydrocarbons and their derivatives (alcohol, carboxylic acid, aldehyde, and ketone). The amino group at C2 is the most reactive group for substitution followed by the hydroxyl group at C6; the hydroxyl group at C3 is less reactive because of the steric hindrance (Motiei et al. 2017).

Substitution can be achieved by a one-step or multistep reaction. One-step substitution can be either nonselective or selective, whereas a multistep reaction is mainly selective. In a one-step nonselective substitution, the substitution group(s) can be introduced onto any reactive groups of chitosan; however, the substitution rate of the reactive groups at different C positions varies depending on the relative reactivity (Costa et al. 2017, Sun et al. 2007). One-step selective substitution can be achieved to a certain degree by controlling the reaction conditions. For example, during chitosan acylation, acylation with *N*-hexanoic anhydride occurs selectively only on the C2 amino group in methanol/ethanol and water systems to form *N*-acyl products because of the competitive effect of the hydroxyl group in the alcohol (Xiang 2009), whereas chitosan reacts with acid chloride to form *N*,*O*-acyl products with a higher degree of substitution in pyridine/chloroform systems (Zong et al. 2000).

Substitution via multistep reactions often protects the amino group at the C2 position first to achieve selective substitution on the C3 and C6 hydroxyl groups; this procedure involves three steps: protection of the amino group at the C2 position, substitution of the hydroxyl groups at the C6 and/or C3 positions, and removal of the protective group. The *N*-phthaloyl group (Kurita et al. 2007), Schiff base (Piisko et al. 1977), and methanesulfonic acid salts (Tong et al. 2005) have been used to protect the amino group at C2. The phthaloyl group can be removed using hydrazine or a water-mixed solvent (Kurita et al. 2007), and Schiff base or methanesulfonic acid can be removed in an acidic or alcoholic system (Sashiwa et al. 2002). Several reactions can be used to selectively remove the substituents on C6 or C3 hydroxyl groups to produce the selective *O*-modified chitosan. For example, to achieve sulfation on the hydroxyl group at the C3 position of chitosan, phthalic anhydride was used to protect the amino group at C2 and a sulfur trioxide (SO_3)-amine complex was used to sulfate the hydroxyl groups at C3 and C6. The *N*-phthaloyl protective group at C2 was removed using hydrazine/water and the sulfate group at C6 was removed using *N*-methylpyrrolidinone-water to obtain a (C3) sulfated chitosan derivative (Baumann et al. 1998, Nishimura et al. 1998).

Substitution introduces new groups into chitosan without changing the skeletal structure. This improves the functional and biological attributes of chitosan. For example, the introduction of hydrophilic groups increases water solubility (Uragami et al. 1997), of long-chain hydrophobic groups enhances lipophilicity and self-assembly (Tong et al. 2005), of charged functional groups modulates charge characteristics and behavior in solution (Je & Kim 2006), and of antioxidative groups improves antioxidant activity (Amato et al. 2018).

Graft Copolymerization

Graft copolymerization is an approach in which one or more side polymer chains can be grafted onto the hydroxyl and amino groups of chitosan to form a copolymer. During graft copolymerization, chitosan is either polymerized with the terminal functional group of a presynthesized side polymer chain or directly polymerized in situ with monomers successively to form the side polymer chain (**Figure 1**) (Argüelles-Monal et al. 2018). A presynthesized side polymer can be designed according to the needs, and the commonly used grafting monomers may include acrylic monomers, ε-caprolactone, styrene, oligoethylene glycol methacrylate, 2-pyrrolidone *N*-sulfate, lactide, carbamate, naphthoquinone, and aniline (García-Valdez et al. 2015).

Graft copolymerization of chitosan has been commonly carried out by chemical and radiation methods. In a chemical method, the commonly used polymerization reaction is free-radical polymerization, which requires free-radical initiators such as ammonium persulfate, potassium persulfate, and cerium (IV) to initiate the free-radical chain reaction (Kurita et al. 1991). In contrast, a radiation method uses gamma rays or ultraviolet rays to initiate graft polymerization of monomers (such as acrylic acid) onto chitosan through a radical chain reaction (Wang et al. 2007). This method is advantageous because of a low reaction temperature, high reaction rate, and short polymerization time.

The obtained chitosan copolymer may have a network structure that expands the scope of application (Pérez-Calixto et al. 2016). Moreover, graft copolymerization of chitosan was able to improve the biocompatibility, bioadhesion, antibacterial, and antioxidant activities (Argüelles-Monal et al. 2018, Božič et al. 2012).

Cross-Linking

Chitosan modification via cross-linking results in the cross-linked chitosan derivatives with improved stability in water and dilute acids. This method uses cross-linking agents such as bifunctional aldehyde, anhydride, epoxy compound, and cyanide (Jabeen et al. 2016, Tong et al. 2005). For example, the aldehyde cross-linking agents such as glutaraldehyde, glyoxal, and other bifunctional aldehydes may react with the amino groups of the two chitosan molecules and form Schiff bases to cross-link the two molecules (**Figure 1**). Under most circumstances, cross-linking of two amino groups in the same chitosan molecule may occur and often needs to be avoided. The cross-linked chitosan is often used in food packaging materials, hemostatic agents, medical dressings, and nanomaterials because of the network structure with unique mechanical properties and stability (Wahba 2020).

Hydrolysis

Chitosan can be hydrolyzed into lower-MW chitosan preparations, oligosaccharides, or monomers via a chemical, physical, or biological method (**Figure 2**) (Poshina et al. 2018). Acid hydrolysis is the most common chemical method used to break down the glycosidic bond of chitosan. Nitrous and hydrochloric acids are commonly used; the number of glycosidic bonds that break down during nitrous acid hydrolysis of chitosan depends on the amount of acid used as well as the reaction time and solvent (Allan & Peyron 1995). Although acid hydrolysis is a common and rapid method, it is disadvantageous because of difficulty in controlling hydrolysis degree, poor selectivity, difficult separation and purification, high residual acidity, and low yield.

The main biological method used for chitosan hydrolysis is either enzymatic degradation using chitinase, chitosanase, and glucanase or fermentation with microorganisms such as *Bacillus licheniformis* (Affes et al. 2020, Jung et al. 2014). One important advantage of the biological method is

its selectivity due to the different types of enzymes used that can break different glycosidic bonds. Therefore, the biological method can achieve selective depolymerization of chitosan under mild conditions; however, it is time-consuming and expensive.

Physical methods include irradiation, ultraviolet or gamma rays, and thermal depolymerization (Feng et al. 2008, Holme et al. 2008). Chitosan is usually irradiated in an ionic solution. Different low-MW COSs can be obtained by controlling the temperature and irradiation time. Physical methods are simple with no reaction waste, low cost, less time, and high product purity; however, lack of selectivity is its disadvantage.

A COS with low MW generally exhibits high solubility (neutral media such as water), low viscosity, easier absorption, and high bioavailability (Lee et al. 2013, Mei et al. 2013). COS can also be modified for their molecular and chemical structures using the protocols summarized in **Figure 2**.

FUNCTIONALITIES IN FOOD SYSTEMS

Film and Coating Materials

Chitosan has good film-forming properties owing to cross-linking among the molecules via intermolecular hydrogen bonds and the formation of crystalline phase regions in the network structure (Hosseini et al. 2013). Chitosan can be used as a food packaging material in the form of films and coatings that may help extend food stability and shelf life with the advantages of biodegradability and nontoxicity. Chitosan films are dry, prefabricated, thin structures used to wrap foods or make pouches and bags. Chitosan coatings are thin layers on the surface of food products. These coatings are applied in the form of a liquid with certain viscosity by spraying, dipping, or brushing, and followed by drying to get on the food surface (Pascall & Lin 2013). Moreover, edible chitosan films and coatings do not adversely affect the sensory properties of the packed food products, such as meat, seafood, fruits, vegetables, and eggs (Bostan & Mahan 2011, Hong et al. 2012, Izci et al. 2018, Suresh et al. 2015).

Chitosan films and coatings possess good mechanical and barrier properties, such as high tensile strength, appropriate viscosity, and good swelling ability as well as a selective permeability (water vapor, CO_2, and O_2) (Hosseini et al. 2013, Zargar et al. 2015). These properties can be altered by manipulating the structure, preparation condition, and additives. The DDA, relative MW, and molecular cross-linking degree of chitosan are major structural factors affecting its film properties. Generally, a higher DDA is associated with higher tensile strength or water vapor permeability but lower swelling degree or water absorbability (Chen 1996, Moura et al. 2011). Meanwhile, a higher MW is often associated with higher tensile strength and lower permeability (Chen 1996, Garcia et al. 2000). As the degree of cross-linking among the chitosan molecules increases, the tensile strength of the film increases while the water and gas permeability and swellability decrease (Garavand et al. 2017, Yang et al. 2005). Substitution groups can also improve the film properties of chitosan (Zargar et al. 2015). Alkylation with a shorter alkyl chain and a higher substitution degree resulted in an improved barrier property of the chitosan film (Nicu et al. 2013). Preparation conditions, including drying temperature and humidity, can also affect the film properties of chitosan. Lower drying temperature may increase the tensile strength while decreasing the water vapor and gas permeability (weaker barrier properties) (Fernández-Pan et al. 2010, Liu et al. 2019). A lower relative humidity with a longer drying time can increase the tensile strength (Mayachiew & Devahastin 2008). In addition, plasticizers, such as glycerol, sorbitol, sucrose, and polyethylene glycol, may improve the mechanical properties and increase the water vapor permeability of the chitosan film (Srinivasa et al. 2007, Ziani et al. 2008).

Studies have reported that chitosan films and coatings improved the nutritional and sensory qualities as well as shelf life of food products, such as meat, seafood, fruits, vegetables, and eggs (Bostan & Mahan 2011, Hong et al. 2012, Izci et al. 2018, Suresh et al. 2015). Bostan & Mahan (2011) reported that coating sausages with chitosan (DDA of greater than or equal to 85%) solutions (0.25%, 0.5%, and 1% w/v) effectively reduced microorganisms and prolonged the shelf life; the coatings also improved the sensory properties of sausages by increasing the brightness. Izci et al. (2018) reported that chitosan films inhibited microbiological spoilage as well as reduced total volatile basic nitrogen, thiobarbituric acid reactive substances, and trimethylamine nitrogen and thereby prolonged the shelf life of sea bream fillet. Hong et al. (2012) reported that coating guava with 0.5, 1.0, and 2.0% w/v chitosan (MW = 50–190 kDa, DDA ≥ 75%) solutions reduced weight loss and firmness, delayed the ripening process by reducing respiration, and suppressed vitamin C oxidation during storage. Suresh et al. (2015) reported that chitosan coatings prevented weight loss and microbial contamination by acting as a barrier for moisture and gas transfer from the albumen through the eggshell, thus extending the shelf life of eggs.

Chitosan derivatives are specifically prepared to improve the film and coating performance in food systems (Britto & Assis 2012, Kumar et al. 2018, Tripathi et al. 2009). Britto & Assis (2012) reported that 2 g/L chitosan quaternary salt (including *N*,*N*,*N*-trimethylchitosan, *N*-butyl-*N*,*N*-dimethylchitosan, *N*-octyl-*N*,*N*-dimethylchitosan, and *N*-dodecyl-*N*,*N*-dimethylchitosan) coatings restrained the fungus *Penicillium expansum* growth on sliced apple by up to 30% and reduced the browning effect on cut-apple surface during storage. Similarly, Kumar et al. (2018) reported that the film of a binary grafted chitosan prepared with acrylamide and acrylonitrile via microwave-initiated graft copolymerization prevented microbial infection and extended the shelf life of apple and guava. Tripathi et al. (2009) reported that cross-linked chitosan-poly(vinyl alcohol) (PVA) film prepared by blending chitosan and PVA with glutaraldehyde as the cross-linker inhibited microbial growth on and extended the shelf life of processed tomato.

Active chitosan films and coatings were also prepared to improve their performance in food systems. Tolouie et al. (2013) described that coating with a 2% w/v chitosan solution incorporated with 0.4% w/v α-tocopherol effectively reduced lipid oxidation in farmed trout with a lower peroxide value, anisidine value, and free fatty acid content compared to the control chitosan group during refrigeration (28 days). In another study, chitosan films with different concentrations of anise essential oil (0, 0.5, 1, 1.5, and 2%, v/v of chitosan solution) delayed lipid oxidation in a chicken burger and reduced pathogenic bacterial population on it, thus improving the shelf life (Mahdavi et al. 2017). Chitosan film containing divergicin M35 effectively inhibited the growth of *Listeria monocytogenes* in cold-smoked wild salmon and maintained the acceptable color and texture of the product during 3 weeks of refrigeration (Benabbou et al. 2018).

In summary, chitosan and its derivatives serve as excellent candidates for incorporation into edible films and coatings in food systems because of their excellent safety and mechanical properties. Chitosan-derived materials may become more important as consumers and food manufacturers are more interested in eco-friendly green biodegradable packaging options. Further research is needed to better understand the contribution of molecular and chemical structures to different characteristics of chitosan film, coating, and food packing materials.

Delivery System

Chitosan is a promising polymer for delivery systems because of its cationic, gelling, mucoadhesive, and permeation-enhancing properties as well as its nontoxicity, biodegradability, and biocompatibility (Reza et al. 2019). Chitosan-based delivery systems have been widely used in different food active ingredients and have been reported to improve stability, increase bioavailability, and enhance sensory quality, and are also used for target delivery and controlled release.

Chitosan-based delivery systems can improve the stability of food active ingredients because chitosan acts as a physical barrier for oxygen and other molecules and possesses antioxidant and antibacterial activities. Lekshmi et al. (2019) reported that squalene encapsulated in chitosan–whey protein showed better oxidative stability, solubility, and flow properties and increased thermal stability in baked products. Souza et al. (2014) reported improved antioxidant properties (1,1-diphenyl-2-picryl-hydrazyl scavenging capacity, ferric reducing antioxidant power, and antilipid peroxidation assay) of quercetin encapsulated in the lecithin/chitosan nanoparticles, which were stable within a wide range of temperatures (5–70°C) and pH (3.3–5.0). Ricardi et al. (2018) reported that β-D-galactosidase immobilized on a silica–chitosan composite improved the operational stability of a fixed bed reactor during lactose hydrolysis (200 hours of continuous use). Therefore, it could be used as an excellent alternative to reduce lactose concentration.

Active ingredients with low absorption and bioavailability normally have poor stability, poor passive diffusion, and active efflux in the GI tract (Lekshmi et al. 2019). Chitosan-based delivery systems can enhance the bioavailability of such food active ingredients. Liang et al. (2017) reported that a chitosan-based nanoparticle improved the stability of tea polyphenols, protected them from oxidative degradation, and promoted their absorption in the GI tract. In 2015, Chatterjee & Judeh (2015) reported that fish oil microencapsulated in *N*-lauroyl chitosan showed high stability with no sign of undesired flocculi formation or disintegration during digestion, which is beneficial for its delivery, stability, and bioavailability in oral administration. Lee et al. (2004) also reported that alginate microparticles with high-MW chitosan coating enhanced the bioavailability of *Lactobacillus bulgaricus* KFRI 673 by an effective colon-specific delivery and maintained stability during storage.

Chitosan-based delivery systems can also control the release of active ingredients. Lee et al. (2017) demonstrated less release of iron under simulated gastric conditions and more release under simulated intestinal conditions through in vitro release tests using chitosan-coated nanostructure lipid carriers (CH-NLCs). Iron release from CH-NLCs was slower compared to that from NLCs because of the swollen outer layer of their chitosan coating. Similarly, Teng et al. (2013) reported a controlled release of vitamin D3 encapsulated in carboxymethyl chitosan and soy protein complex nanoparticles in the simulated gastric fluid but not under the simulated intestinal conditions. A chitosan-based delivery system has been used for colon-targeted delivery. This type of delivery system can also protect the entrapped active ingredients from absorption and degradation by the upper digestive tract and facilitate delivery to the colon. Iyer et al. (2010) investigated the release characteristics of *Lactobacillus casei* strain Shirota from chitosan-coated alginate-starch (CCAS) capsules in different regions of the ex vivo porcine GI tract. CCAS capsules effectively protected probiotic bacteria from adverse gastric conditions and released viable probiotic cells to the porcine ileal and colon contents.

A chitosan-based delivery system for active food factors can also enhance the sensory qualities by masking unpleasant flavors and stabilizing pigments. Fathi & Varshosaz (2013) reported that hesperetin-loaded nanostructure lipid carriers coated with chitosan could be used in milk fortification to mask bitterness, inhibit color change, and enhance its solubility. Wani et al. (2015) reported that fish liver oil encapsulated in chitosan/calcium alginate capsules had a less unpleasant taste. Estevinho et al. (2013) successfully encapsulated flavoring substances in water-soluble chitosan through a spray-drying process, which could be utilized in the food industry. Chitosan may be used to deliver flavoring substances to improve food sensory quality.

Functional Ingredients in Food Systems

Chitosan has been used in functional foods in several countries such as China, Japan, and South Korea (China State Mark. Superv. Adm. 2020, Inmaculada et al. 2009). For example, chitosan has been used as a functional ingredient of snacks, wheat products, and vinegar products in Japan

(Shahidi et al. 1999). Chitosan has been added to food systems directly to clarify and deacidify beverages, stabilize emulsion, and improve texture and mouthfeel as well as improve food storage stability. Abdelmalek et al. (2017) reported that 0.2–1.0 g/L of chitosan effectively reduced the turbidity and improved the appearance, acceptance, and stability of sugar and ascorbic acid of apple juice. The clarification effect of chitosan is related to the cationic flocculating property, which binds negatively charged pectin, soluble starch, protein, and microparticles through charge attraction to form floc precipitation. Klinkesorn (2013) confirmed that polycationic chitosan could stabilize emulsion by being adsorbed at the oil–water interface, enhancing the viscosity of the continuous phase, and interacting with surface-active agents, including surfactants, proteins, and polysaccharides. Therefore, chitosan could be utilized in food emulsions such as mayonnaise and tofu (Kim & Hur 2002, Chang et al. 2003). Chitosan can also be used to improve food storage stability. For example, coating fresh pork sausages with a 1% chitosan solution effectively inhibited microbial growth, decreased lipid oxidation rate, and improved flavor during storage (28 days) at 4°C, thus extending the shelf life of pork sausages (Soultos et al. 2008). China, Japan, and South Korea have already approved chitosan as a food additive (Jpn. Food Chem. Res. Found. 2014, Minist. Food Drug Saf. 2019, NHFPC 2014).

FUTURE PROSPECTS

Chitosan is a nonexpensive, nontoxic, and environmentally friendly polysaccharide and the only polysaccharide able to carry a positive charge. Developing the utilization of chitosan and its derivatives may enhance the quality, safety, health benefits and nutritional value, and sustainability of food systems while reducing the environmental hazards. Additional research is needed to further understand why and how their molecular and chemical structures may contribute to their functionalities and applications in food packaging, delivery systems, and functional ingredients. Additional research is also needed to investigate their possible adverse effects and safety problems, including their effects on nutrient bioavailability, although chitosan preparations have been accepted as food additives and functional food ingredients in a few countries, including China, Japan, and Korea. However, there are still several limitations for the extensive application of chitosan and its derivatives. In addition, there are only limited data about the in vivo process, including about absorption, distribution, metabolism, and excretion of chitosan and its derivatives (especially for COS) after oral administration. This information is needed to understand the molecular and cellular mechanisms underlying the bioactive activities of chitosan and its derivatives. More chitosan-derived materials for food application should be designed, prepared, and evaluated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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