

Flavor-Related Applications of Chitin and Chitosan in Foods: Effect of Structure and Properties on the Efficacy



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Abstract Chitin and chitosan are natural and abundant polymers with extensive structural possibilities for physical and chemical modification. The well-known inherent astringency of chitin and chitosan limits their use in the food industry. However, structural and chemical modifications may be explored for their potential in improving the flavor of foods. This chapter reviews the flavor-related applications of chitin and chitosan including enhancing saltiness perception, improving flavor

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stability, and reducing the bitter taste of food products. Also, it focuses on the effects of the physical and chemical properties on the potency of utilization.

Keywords Chitin · Chitosan · Debitterization · Flavor modulator · Flavor stability · Saltiness perception · Warmed-over flavor

1 Introduction

Chitosan is a biopolymer derived from chitin which can be found in many places in nature, including the exoskeletons of crustaceans, insects, mollusks, and fungi [1–3]. The exoskeleton of crustaceans is the major source of biomass for chitin and chitosan production in industry [4] with an estimated 1.5 billion tons available worldwide. Derived from N-deacetylation of chitin, chitosan is the most abundant cationic natural polysaccharide. It consists of β 1–4-linked D-glucosamine and N-acetyl-D-glucosamine [5] (Fig. 1). The degree of deacetylation (DD) ranges from 40 to 98%, and the molecular weight ranges from 5×10^4 to 2×10^6 Da [3, 6, 7]. Chitosan has a broad array of applications such as in agricultural, water purification, food, cosmetics, textile, and biomedical industries, owing to its biodegradable, biocompatible, biofunctional, low toxicity, gelatinous, and antimicrobial properties [1, 3, 4, 8, 9]. The DD and the molecular weight of the polymer are two important parameters dictating the use of chitosan for various applications. For example, a high DD and low molecular weight lead to a relatively high ionization

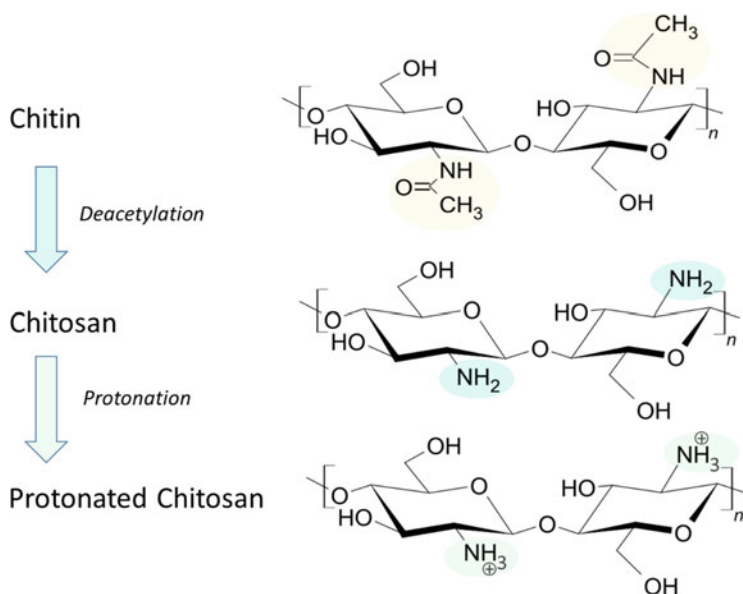


Fig. 1 Structure of chitin and chitosan

capability of chitosan, which could make it soluble in biological fluids and suitable for use in pharmaceutical fields.

Recently, the development of chitosan for use in food applications has progressed significantly and rapidly. In the food industry field, chitosan has an advantage over synthetic polymers, as it is considered to be GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA). In food-related products, it functions as a clarifying agent, preservative, pasting agent, dietary fiber, and flavor modulator. Among these applications, chitosan has received increasing attention and has been extensively explored in flavor modulation [7, 10]. The flavor of food is largely a combination of taste and odor, those are sensations that result when specialized nerve receptors in the mouth and nose detect molecules. It can be the primary quality attribute that contributes to consumer acceptance and preference. There is increasing evidence to suggest that flavor is becoming more important, perhaps because of more demanding consumers who ask for food with desirable organoleptic properties [10]. Unfortunately, some healthy food choices and ingredients with specialized biological functions are often viewed to be in conflict with enjoyable flavor. Moreover, food processing and storage are associated with losing preferable flavors and generating undesirable flavors. Therefore, it is a big challenge for academic and industrial researchers to design food products without sacrificing food flavor [11]. This review focuses on the most recent advances in the flavor-improving applications of chitosan in the food industry. This includes its use in taste masking, as a saltiness enhancer, and in preventing foods from developing off-flavors.

2 Applications of Chitin and Chitosan for Improving Saltiness Perception

2.1 Strategies for Reducing Sodium Intake

Sodium chloride (NaCl) is commonly used to season food. When dissolved in biological fluids, NaCl provides free Na^+ ions to the system which may bind to the taste bud receptor to cause a saltiness perception. As a food additive, NaCl not only provides a saltiness perception, but it also reduces the water activity of foods, which may inhibit microbial growth and therefore prolong the shelf-life of foods [12]. Moreover, it is implicated in several enzymatic reactions in food systems to modulate the texture, color, taste, and odor of food substances [13].

For the human body, sodium intake is necessary to maintain fluid balance in the body, and it is vital to muscle and nerve function. Research has indicated that 75–80% of sodium intake is from processed food products. Bakery products accounted for 13% of the sodium intake, while cereal products for 5%, processed meats for 18%, and sauces and spreads for about 11%. Spices, herbs, and salt purchased separately were responsible for 23% of the sodium intake [14]. However, excess sodium intake is associated with increased risk of non-communicable

diseases (NCDs) including stroke, hypertension, and cardiovascular disease, which are the leading causes of death globally. The World Health Organization (WHO) (2007) has recommended a daily NaCl intake (RDI) of less than 5 g. Nevertheless, the average daily NaCl intake in the USA, United Kingdom, and Asia is approximately 8.2–9.4 g, 9.4 g, and 12.0 g [15], respectively, which are much higher than the RDI suggested by WHO. Hence, techniques to reduce the salt levels in foods should be considered in order to help bring dietary sodium intake closer to those recommended for promoting public health.

Common methods for reducing the sodium level and improving the saltiness perception of foods include using substitutes such as KCl, CaCl₂, and MgSO₄ [16] as well as using flavor enhancers such as citric acid and monosodium glutamate [17]. However, the use of those metal salts and acids provides astringency and metallic flavors in food systems, thus their actual applications in food preparations are limited. Moreover, the release of sodium from the food matrix and its dissolution rate in the oral cavity have proven to be crucial for saltiness perception. Consequently, approaches such as modulating the size and shapes of salt crystals and altering the food texture have been explored recently for their potential as salt reduction substitutes [18, 19]. Saltiness is primarily perceived from free sodium ions rather than those bound with the food matrix. When consuming foods, a high proportion of sodium may be retained in the food matrix in a bound form even after being swallowed. This suggests that a significant amount of sodium might be taken into the body without being perceived [20]. Based on this principle, a higher proportion of free sodium ions released from the food matrix means that less salt addition is required in foods systems, and less sodium would be consumed [21]. Accordingly, the presence of negatively charged molecules in the food matrix (e.g., milk protein, soy protein, xanthan gum, and κ -carrageenan) would reduce saltiness perception, owing to the increased electrostatic interactions between sodium ions and the food matrix [22]. In contrast, positively charged groups interact with negatively charged groups through ionic interactions, which may therefore help release more free sodium ions and enhance saltiness perception [10].

2.2 *Chitin and Chitosan as Saltiness Enhancers*

The scientific publications reporting chitin and chitosan for saltiness enhancement applications are addressed in Table 1.

2.2.1 **Particle-Shaped Chitin and Chitosan as Saltiness Enhancers**

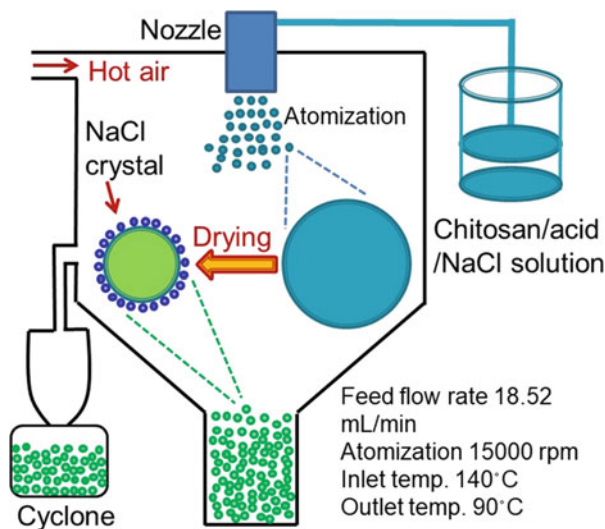
Free Na⁺ generates the salty taste in food. Negatively charged food ingredients adsorb Na⁺ through static electricity, creating bound Na⁺ and reducing the level of saltiness [22]. However, positively charged food ingredients interact with negatively charged food ingredients to decrease the amount of bound Na⁺, resulting in more

Table 1 Scientific publications reporting the saltiness enhancement applications of chitin and chitosan

Materials	Physicochemical properties	Forms	Applied foods	Outcome	Reference
Chitin	21–22% DD, 5.1–9.3 nm diameters	Nanofiber	Model solution	0.15 and 0.3 g/L of CNF showed remarkable saltiness enhancement potential (scored at 4.4 and 4.0 compared to 3.3 and 2.8 for groups without CNF, respectively)	[10]
	22.89–53.36 DD, 15.01–17.24 nm diameters	Nanocrystal, nanofiber	Model solution	The saltiness perception of CNC and DACNF suspensions performed higher than that of CNF suspension, inferring that lower aspect ratio and higher DD result in higher saltiness	[6]
	16.2% DD, 111 nm diameter	Nanofiber	Tilapia fillets	Curing solutions with 0.12–0.18 g/L of CNF are capable of providing stronger saltiness perception in the fillet than that without addition of CNF	[23]
Chitosan	32.67–50.67 particle sizes	Nanoparticles	Model solution	Compared with the control group, the saltiness intensity of 0.03% chitin nanoparticle solution treated with ultrasonication was increased by about 30%	[5]
	78.3% DD, 186.3 kDa molecular weight	Microparticles	Popcorn	The use of chitosan/NaCl microparticles provided a saltiness taste stronger than the general NaCl group. The level of saltiness can be maintained while the NaCl used can be decreased by up to 54.4%	[24]
	76.5% DD, 213.2 kDa molecular weight	Microparticles	Popcorn	Up to 45% of salt reduction can be achieved without a reduction of the saltiness perception of seasoned popcorn; the yield could be improved with addition of maltodextrin	[25]

DD degree of deacetylation, CNF chitin nanofibers, CNC chitin nanocrystals, DACNF deacetylated chitin nanofibers

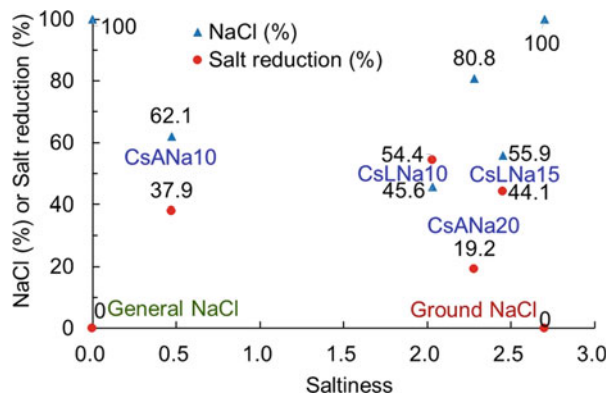
Fig. 2 The process diagram and conditions for preparing chitosan/organic acid/NaCl microparticles



free Na^+ and enhancing the level of saltiness [10]. Chitin and chitosan are nitrogen-containing linear polysaccharides composed of β -1,4 linked units of N-acetyl glucosamine, which have a positive charge under a weakly acidic environment. The positive charges from protonated amino groups may interact with the negatively charged ions in food systems, such as chloride ions from NaCl and carboxylate ions from proteins. The quantity of free sodium ions in solution may therefore be elevated, and this would be followed by an increased saltiness perception.

Recently, research has been conducted to explore the opportunities and challenges in the use of chitin and chitosan for enhancing the saltiness perception in food systems. Yi et al. [24] prepared chitosan ($78.3 \pm 2.5\%$ degree of deacetylation (DD), 186.3 ± 12.4 kDa weight average molecular weight)/acid/NaCl microparticles by the spray drying method (Fig. 2) for popcorn seasoning. It showed a promising capability for maintaining saltiness sensory perception with reduced salt addition. They dissolved chitosan and NaCl into various organic acid solutions including acetic acid, citric acid, and lactic acid. This was followed by a spray drying process to obtain the microparticles. Loss of the organic acids via evaporation occurred along with the spray drying process. The NaCl content was slightly raised from 25.0–66.7% to 30.2–80.8% in the microparticle systems. The rate of moisture absorption of the prepared microparticles was between 0.83 and 3.19 g/100 g/h, representing that these particles may rapidly dissolve in the oral cavity to provide the saltiness perception. Moreover, the prepared chitosan/acid/NaCl composites are hollow particles with relatively large sizes, and NaCl concentrations in the system ranged from 15.4 to 32.0 μm . A comparison of the saltiness in sensory evaluations of ground NaCl, chitosan/acid/NaCl microparticles, and general (ungrounded) NaCl has been carried out. Postgraduate students of the Department of Food Science at National Taiwan Ocean University (Keelung, Taiwan) were volunteers who

Fig. 3 The relationship between the saltiness score of sensory evaluations and NaCl content (%) or salt reduction (%) for chitosan/acid/NaCl microparticles. Cs Chitosan, A Acetic acid, L Lactic acid, Na10 Use 10 g of NaCl in the solution formulation



participated in a hedonic taste test. The panel consisted of 20 untrained subjects (10 females and 10 males, 23–26 years old). Twenty grams of corn kernels was popped, and 1 g of test substances was added. The mixture was stirred for 3 min to allow the seasoning agents to spread over the popcorn. The salinity of the unground NaCl group was given a score of 0 and set as the standard for comparison with the other groups. Groups with less saltiness than that of the NaCl group were given a negative score as low as -5 , whereas those with saltier taste were given a positive score as high as 5. The results shown in Fig. 3 indicated that the use of chitosan/acid/NaCl microparticles provided a saltiness taste comparable to the ground NaCl group and stronger than the general NaCl group. Because the tested microparticles had only 45.6–80.8% NaCl content, this result indicated that the level of saltiness can be maintained while the amount of NaCl used can be decreased by 19.2–54.4%. The decrease in NaCl used may be associated with the numerous small NaCl crystals on the surfaces of the chitosan/acid/NaCl. Also, the cationic effect of chitosan may play a role in increased release of free Na^+ ions. Thus, these microparticles show promise for reducing sodium addition to surface-salted foods.

Lu et al. [25] used a similar strategy and further incorporated maltodextrin into microparticles to optimize the shape and physical properties of products. Chitosan was prepared from shrimp shells and had an average DD of 76.5% and a weight average molecular weight of 213.2 ± 3.2 kDa. It was subsequently mixed with maltodextrin at weight ratios of 2:0, 1:1, and 0:2 for further use. The mixtures were then dissolved in 1% organic acid solution (acetic acid and lactic acid) at a concentration of 1%. Three percent NaCl was then dissolved in the solution as well, and the microparticles were obtained by a spray drying method. In addition, microparticles without chitosan and organic acid were prepared as the blank group. The size, density, organic acid content, NaCl content, rate of moisture absorption, and surface morphology of the particles were investigated. Along with the different acids used and the ratio of chitosan/maltodextrin, different morphological changes were observed by microscopy. Sodium chloride crystals were attached to the surface of both acetic acid and lactic acid groups, and the surface of both groups presented depressions and wrinkles, while groups without chitosan showed obvious cohesion

and could not form solid microparticles. The size of each group was between 2.3 and 9.7 μm . The average hygroscopic rates ranged from 3.4 to 5.7 g/100 g/h, and the apparent densities were between 1.7 and 1.9 g/cm³; these results were similar to Yi et al. [24]. However, with addition of maltodextrin, the yield of microparticles was greatly improved. Sensory evaluation was carried out by panelists at the laboratory level using a 5-point hedonic rating scale. The salinity of popcorn mixed with ground NaCl was given a score of 0 and set as the standard for comparison with those mixed with microparticles. As a result, chitosan/maltodextrin/NaCl particles could effectively provide salinity, and it is worth mentioning that up to 45% salt reduction could be conducted without a reduction of the saltiness perception of seasoned popcorn.

Results from Yi et al. [24] and Lu et al. [25] indicated that chitosan/NaCl and chitosan/maltodextrin/NaCl microparticles can provide a better saltiness perception than ordinary table salt. It could be concluded that the average particle size (<32 μm) is less than ordinary salt (about 500 μm), and there are a large number of micrometer-sized NaCl crystals (<4 μm) distributed on the surface of the particles. Therefore, NaCl crystals can rapidly dissolve in the oral cavity and elevate the concentration of Na⁺, which results in a strong saltiness perception. However, both studies revealed that this strategy is not suitable for use in a liquid food system such as soups and stews where the dissolved particles do not retain this dominant structure and therefore do not enhance the perception of saltiness but instead have an astringent taste owing to the chitosan in the system.

Interestingly, Somsak et al. [5] revealed that chitin nanoparticles are capable of acting as a saltiness enhancer in a solution system. They prepared α - and β -chitin from shrimp shells and squid pens, respectively. Chitins were then introduced into an ultrasonication treatment of 30, 45, and 60 min, respectively, followed by homogenization at 12,000 rpm for 20 min to produce chitin nanoparticle suspensions. Afterwards, the chitin nanoparticle suspensions were freeze-dried and ground into powder to obtain the final products. The particle sizes of the α -chitin nanoparticles were between 32.67 and 41.33 nm, while those of the β -chitin nanoparticles were between 42.60 and 50.67 nm. The particle sizes decreased with an increase in the ultrasonication treatment time. Then, the test solutions were prepared by mixing 0.03% dried nanoparticles and 0.3% NaCl in deionized water; a 0.3% NaCl solution without chitin nanoparticles was used as the control group. A panel of eight trained panelists was selected for sensory evaluation to test the ability of chitin nanocrystals to enhance the saltiness perception. The salty intensity ratings of α -chitin nanoparticles/NaCl solutions were 35.79, 43.70, and 47.33 mm on a 150-mm scale, while those of β -chitin nanoparticles/NaCl solutions were 35.89, 41.91, and 45.66 mm, respectively, for samples after 30, 45, and 60-min of ultrasonication treatment. The ratings were comparable between control groups and the 30 min-treated group, whereas there were significant increases in saltiness intensities in 45–60 min-treated groups. Compared with the control group, the saltiness intensity of 60 min-treated groups increased by about 30%. This may be related to the extremely small particle size of chitin nanoparticles by which chitin could rapidly provide amine ($-\text{NH}_3^+$) groups in the solution system to bind to Cl^- and form a Stern

layer by electrostatic interactions that caused an elevated ratio of free Na^+ in the diffuse layer and, in turn, improved saltiness. In addition, increasing ultrasonication time may improve the high specific surface area and high porosity of chitin nanoparticles, which could improve their ability to adsorb negative ions in the solution system.

2.2.2 Chitin Nanofibers/Nanocrystals for Improving Saltiness Perception

Due to the existence of numerous intramolecular and intermolecular hydrogen bonds, the chitin molecules are difficult to dissolve in water and general solvents. Chitin can only be dissolved in a few special solvents such as LiCl/dimethylacetamide [26], NaOH/urea solvent systems [27], and ionic liquids [28]. This greatly limits its application and development in biomedical fields [2]. To deal with this issue, chitin could be processed into nanomaterials, such as chitin nanofibers (CNF) and chitin nanocrystals (CNC), which can increase their dispersion in water and form a colloidal solution. Methods used for preparing chitin nanomaterials include mechanical disassembly, chemical modification, and electrospinning. The mechanical disassembly methods feature the grinding method [29, 30], ultrasonication [10, 25], the starburst system [31], dynamic high pressure homogenization [32], and micro-fluidization technology [33], whereas acid hydrolysis [34], 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO)-mediated oxidation [35], and partial deacetylation [36] could be involved in chemical modification. These prepared chitin nanomaterials are generally stable in aqueous systems and may therefore be introduced as saltiness enhancers in liquid foods.

Nanofibers are generally defined as those with a diameter less than 100 nm and an aspect ratio greater than 100. Chitin nanofiber is composed of approximately 18–25 chitin molecular chains arranged in parallel and bonded by strong hydrogen bonds (crystallization region) to form a fiber with a diameter of about 2–5 nm and a length of about 300 nm [10, 37, 38]. Due to their size and structure, chitin nanofibers not only maintain chitin properties but also have advantages such as high surface area to volume ratio, excellent mechanical properties, high flexibility, easy chemical modification, and antiviral and anti-tumor activity [34, 39]. They could potentially be used in filtration, recovery of metal ions, controlled release of drugs, tissue engineering, enzyme carriers, wound healing, cosmetics, biosensors, and medical implants [25, 37, 40, 41].

Jiang et al. [10] prepared a 0.3 g/L β -chitin aqueous suspension, and then treated it by ultrasonication at 20 kHz and 200 W for 30, 45, and 60 min. After centrifugation, the supernatant was collected as chitin nanofibers, represented as CNF30, CNF45, and CNF60, respectively. The DD were determined by FTIR at 21–22%, and the diameters of CNF30, CNF45, and CNF60 were analyzed by TEM and found to be 9.3, 5.6, and 5.1 nm, respectively. Sensory evaluation was conducted; 0.075–0.3 g/L chitin nanofiber suspensions were added with 3 g/L sodium chloride to obtain the test sample. The panel consisted of 20 untrained and volunteer subjects (10 females

and 10 males, 23–26 years of age). Every panelist was asked to drink 5 mL of 3.0 g/L NaCl as well as the CNF and CNF/NaCl solutions of varying concentrations before assessing the saltiness and astringency of the solutions which were evaluated using a 7-point scale (1 = very weak, 4 = moderate, 7 = very strong). The results showed that there was no significant effect for promoting saltiness perception observed in the 0.075 g/L CNF group, whereas the 0.15 and 0.3 g/L CNF groups showed remarkable saltiness enhancement potential (scored at 4.4 and 4.0 compared to 3.3 and 2.8 of groups without CNF), indicating that CNF had the ability to improve the salty taste. In addition, CNF60, the group with longest ultrasonication treatment time, had the best saltiness promoting effect. This was possibly because CNF can provide -NH_3^+ to adsorb anions such as Cl^- and OH^- in the Stern layer, so that the concentration of free Na^+ in the solution is higher than that of the control group. Moreover, chitin and chitosan are often hindered in the food industry by their astringency. However, with the participation of NaCl, the astringency of CNF30, CNF45, and CNF60 groups was significantly reduced compared to the group without NaCl. The astringency of chitin and chitosan could increase from the increasing in positive charges on the molecular chain [42]. When sodium chloride is added, the positive charge on chitin nanofibers will be neutralized by chlorine ions due to the interaction of charge, so its astringency will be decreased [10].

To explore the relationship between physicochemical properties and the saltiness-improving capacity of chitin nanomaterials, Tsai et al. [6] used ultrasonication treatment to prepare deacetylated chitin nanofibers (DACNF) and acid hydrolysis to prepare chitin nanocrystals (CNC) (rod-shaped in morphology with lower aspect ratios than the original nanofibers). The DDs of the original nanofibers (CNF, CNC, and DACNF) were 22.89%, 23.43%, and 53.36%, respectively. CNF, CNC, and DACNF had diameters of 17.24, 16.05, and 15.01 nm and lengths of 1.73, 0.12, and 1.81 μm , respectively. When suspended in deionized water, the overall zeta potential of chitin nanomaterials is 19.73–30.08 mV, and the concentration (0.04–0.074 mg/mL) has no obvious influence on the tested value. Sensory evaluation was also conducted using a 7-point scale to evaluate the saltiness of each group. Accordingly, the DD and the aspect ratio played roles in saltiness-improving capacity. The saltiness perception of CNC and DACNF suspensions was higher than that of the CNF suspension, inferring that lower aspect ratio and a higher DD result in higher saltiness. This may be due to the ionization of the surface amine group from deacetylated molecules under the $\text{pH} < 7$ environment. These amine groups are able to adsorb Cl^- , OH^- , and other negative ions, increasing the proportion of free Na^+ in the solution and thus improving the saltiness perception (Fig. 4). Moreover, the number of molecules and total surface area of CNC and DACNF were both larger than CNF, so more negative ions could be absorbed, resulting in more free Na^+ in the solution and a strong salty perception.

Another approach to using CNF for replacing NaCl from a curing solution of tilapia fillets was conducted by Hsueh et al. [23]. Curing solutions with CNF (with a DD of 16.2% and mean diameter of 111.6 ± 45.4 nm) at concentrations between 0.12 and 0.18 g/L were able to provide a stronger saltiness perception than those without the addition of CNF. More free Na^+ ions are assumed to be present in the

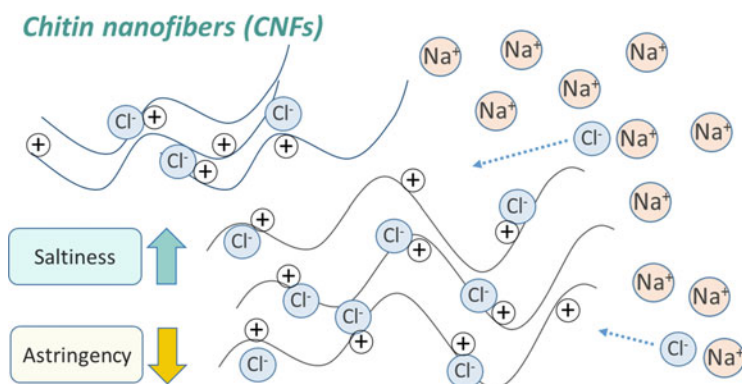


Fig. 4 The mechanism of chitin nanofibers for enhancing saltiness perception

CNF/NaCl curing solution, resulting in a higher proportion of a combination of these ions on the surface of tilapia fillets. Moreover, adding 3 g/L citric acid or 4 g/L malic acid to CNF/NaCl curing solution could effectively improve the saltiness of fish fillets without generating obvious sourness. The addition of these organic acids provided environments with a lower pH value. This may facilitate the ($-\text{NH}_3^+$) degree of protonation of amine groups in the molecular chain. As evidenced, the addition of lactic acid in CNF/NaCl curing solution elevated the zeta potential from 0.7 to 3.8 mV, which may increase the adsorption capacity of anions. It could also be concluded that the saltiness of sodium chloride at a specific concentration range may be enhanced by the addition of a small amount of acid [43, 44].

3 Chitin and Chitosan Prevent Food from Loss in the Quality of Flavor

3.1 Flavor Loss and Off-Flavor Development of Foods

The most important factor that maximizes food quality is its flavor, and there is increasing attention on the stability of flavor. The quality attribute of food flavor stability can be affected by the chemical reactivity of food flavors and the environment of food such as the availability of light and atmospheric oxygen [45]. Flavor compounds can be susceptible to chemical changes occurring from various kinds of interactions. Among the many reactions taking place in the food system, oxidation is generally considered to affect flavor stability during storage [46]. For example, meat or meat products are highly susceptible to lipid oxidation which leads to rapid generation of rancidity or a warmed-over flavor [47]. When meat is stored after cooking, lipid oxidation occurs rapidly, and this is followed by a deterioration in flavor; this phenomenon is termed warmed-over flavor (WOF). The flavor is described as “rancid,” “stale,” and like “cardboard” and even compared to “damp

dog hair” [48]. WOF was scientifically considered to be an organoleptic challenge to meat products, as cooking and subsequent refrigeration is commonly introduced when preserving foods with meat. Once cooked meat is exposed to oxygen, it could develop off-flavors in a short time. Irreversible conversion of iron from the ferrous to the ferric form of myoglobin pigments occurs during heating, and this is an important cause of rapid oxidation of lipids in meats during cooking. Concern about flavor deterioration of oxidized lipids in meats has prompted the study of antioxidants for preventing or controlling lipid oxidation [49].

Another crucial factor that causes negative effects on flavor stability is microbial spoilage. Under particular storage conditions, specific spoilage organisms (SSOs) produce metabolites responsible for off-flavors and cause organoleptic rejection of the product [50, 51]. Off-flavor production is one of the major indicators used by consumers when they evaluate the freshness of raw food materials [52]. Compounds with a specific smell such as nitrogen-related compounds, sulfuric compounds, ketones, aldehydes, and esters are mainly produced by various microorganisms during food spoilage [50, 51, 53]. One of the highly perishable foods is fish which readily undergoes autolysis followed by consumption by the microorganism. The chemical changes of flavor compound precursors, including amino acids, nucleotides, trimethylamine oxide (TMAO), and volatile organic compounds (VOC) may generate off-flavors and cause organoleptic rejection [54]. The post-mortem chemical changes related to biochemical reactions and microbial metabolism can cause deterioration of texture and flavor and finally loss of edibility.

3.2 Chitin and Chitosan for Improving the Flavor Stability of Foods

Chitosan has a remarkable antioxidant and antibacterial capacity [55, 56], and it may attenuate lipid oxidation and inhibit the growth of spoilage bacteria in food systems during storage. Since chitosan is reported to possess better antioxidation and antimicrobial capacity than chitin [57], it was preferred over chitin for use in preventing the generation of off-flavors in foods. The action of chitosan for improving the flavor stability of foods would involve antioxidant and antimicrobial pathways. Chitosan is able to adhere to the membranes of microorganisms, subsequently changing its permeability. Moreover, it can act as a trace metal chelating agent, which may inhibit the growth of microbes, or it may effectively bind to the microbial DNA and interfere with the synthesis of mRNA and proteins. Chitosan tends to adhere to or penetrate the cells depending on whether its molecular weight is high or low, respectively (Fig. 5) [58]. The addition of 0.05 and 0.1% chitosan inhibited the growth of common spoilage microorganisms, including *B. cereus*, *S. aureus*, *E. coli*, and *P. fluorescens* in vitro, while *S. typhimurium* was partially inhibited [59]. In addition, chitosan is able to chelate the free iron released from meat heme proteins, thereby inhibiting the catalytic potential of iron ions in the initial stage of lipid

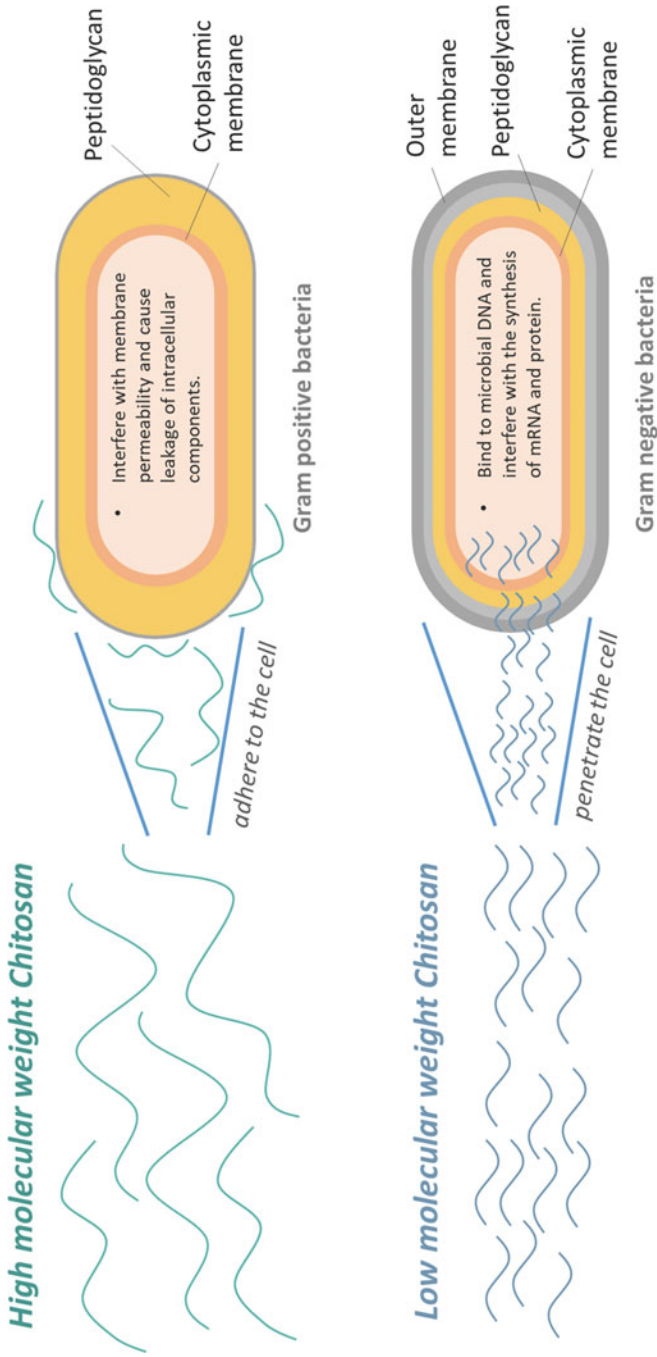


Fig. 5 The antimicrobial mechanisms of high and low molecular weight chitosan

autoxidation [60]. Xue et al. [61] reported that the protective action of chitosan toward the deterioration of foods is also effective when it is used as a protective film, where it acts as a barrier against oxygen [62]. The scientific publications reporting chitin and chitosan for improving flavor stability are addressed in Table 2.

3.2.1 Chitin and Chitosan Promote Flavor Stability in Muscle Food Products

Muscle food products are highly susceptible to off-flavor and rancidity development by the autoxidation of their unsaturated lipids. St. Angelo and Vercellotti [63] introduced chitosan at a concentration of 1% in meat and this resulted in a 70% decrease of 2-thiobarbituric acid (TBA) values, while a 93% reduction of TBA and a 99% inhibition in hexanal content were observed with N-carboxymethyl chitosan treatment after 3 days of storage at 4°C. N,O-carboxymethyl chitosan (NOCC) showed a similar effect in controlling the flavor deterioration of cooked meat within a 9-day storage period [64]. Researchers from Korea conducted a series of studies to reveal the application of chitosan as preservative agent in processed sausage, where it could potentially reduce or replace the use of nitrite [78–81]. Interestingly, they found that the preservative capacity of chitosan is increased along with increasing its molecular weight [80]. This phenomenon could be completely interpreted as chitosan with high molecular weight having bioadhesive properties such that it can attach to cells and form a covering around them. Subsequently it can alter their structure, permeability, and functioning and inhibit the survival of microorganisms. This action takes place mainly against Gram-positive bacteria because their cell wall is composed of a thick layer of peptidoglycan rather than the outer membrane [58]. The most frequent spoilage bacteria that can be identified in sausages are *Lactobacillus sakei* and *Brochothrix thermosphacta* [82], which are classified as Gram-positive bacteria. Therefore, these bacteria could be affected by high molecular weight chitosan. In addition, as a food additive, chitosan is known to form an astringent taste in a food system. However, research has indicated that addition of 0.1% chitosan with relatively high molecular weights of 150–1,250 kDa (DD = 85–87%) into reduced-fat Chinese-style sausage had no adverse effect on the sensory characteristics [66]. Also, addition of chitosan with low molecular weight (5 kDa, 0.2%) did not negatively affect the sensory characteristics of sausage, and it was certainly able to prevent the sausage from lipid oxidation within 3-week storage [66]. These results suggest a promising application of chitosan for improving flavor stability in sausage.

Serrano and Bañón [67] reported that the use of 0.02% or 0.05% chitosan (molecular weight of 340 kDa, 80% DD) can reduce the SO₂ required to preserve pork burgers and effectively enhance the flavor stability at 2°C storage for up to 21 days. The addition of either 0.02% or 0.05% chitosan was not detected by sensory analysis, but it significantly reduced the generation of undesirable odors including rancid, acidic, and putrid odors within the storage time. In the quantitative descriptive analysis, a linear intensity five-point scale was used to quantify the sensory

Table 2 Scientific publications reporting the flavor stability improving applications of chitin and chitosan

Applied methods	Materials	Physicochemical properties	Applied foods	Outcome	Reference
Immersing	NCC	Not available	Meat	93% decrease of TBA and a 99% reduction in hexanal content by treating with 3% N-carboxymethyl chitosan after 3 days of storage at 4°C	[63]
	NOCC	Not available	Cooked meat	The mean deterioration inhibitory effect of NOCC at 500–3,000 ppm was 43.4–69.9% at 4°C, for 9 days as reflected in their TBA values	[64]
	Water-soluble chitosan	80–82% DD	Oysters	Retained better freshness flavor during storage	[65]
Intact addition	Chitosan	Chitosans of different viscosity (14 cP, 57 cP, and 360 Cp)	Comminuted flesh of herring	The formation of hydroperoxides and TBARS in herring samples containing 200 ppm chitosan was reduced after 8 days of storage by 61% and 52%, respectively	[60]
	Chitosan	85–87% DD, 5 kDa molecular weight	Reduced-fat Chinese-style sausage	Addition of chitosan (0.2%) did not negatively affect the sensory characteristics of sausage, and it was certainly able to prevent the sausage from lipid oxidation within 3-week storage	[66]
	Chitosan	80% DD, 340 kDa molecular weight	Pork burger	The addition of either 0.02% or 0.05% chitosan was not detected by sensory analysis, but it significantly reduced the generation of undesirable odors including rancid, acidic, and putrid odors at 2°C storage for up to 21 days	[67]
	Chitosan nanoparticles	562 nm of average diameter	Milk	All of the sensory parameters were not significantly affected by the addition of	[68]

(continued)

Table 2 (continued)

Applied methods	Materials	Physicochemical properties	Applied foods	Outcome	Reference
Edible coating	Chitin nanofibers	50–70 nm of diameters	Raw beef	the nano-chitosan solution when stored for 15 days at 4°C The odor and overall acceptability were significantly improved by coating the gelatin-CMC films incorporated with chitin nanofiber for 12 days of storage at 4°C	[69]
	Chitosan film	Not available	Horse mackerel	The flavor could be retained well (scored at 3.5 compared to 2.2 of sample without chitosan packing, in a five-point scaled quantitative descriptive analysis) after 8 days of storage	[70]
	Chitosan film	85% DD, 400 kDa molecular weight	Grass carp fillets	Chitosan coatings resulted in significant attenuation of off-flavor compounds, such as trimethylamine, hypoxanthine, and histidine. They promoted the overall acceptance from 1.00 to up to 3.24 in a five-point scaled sensory evaluation at the 15th day of storage at 4°C	[54]
	Chitosan coating incorporated with the lactoperoxidase system	85% DD, medium molecular weight	Rainbow trout	The sensory score of odor can be improved from 2.58 and 2.53 to 6.87 and 6.70 and overall acceptability in a 9-point scaled description by this coating system	[71]
	Chitosan	85% DD, 160 kDa molecular weight	Silver carp	After 30 days of refrigerated storage, the chitosan-coated group received a better score (about 4.8) than the control group (about 3.8) in a nine-point hedonic scale	[72]

Chitosan	89% DD		Strawberries	1% of chitosan treatment showed no significant improvement in flavor stability when stored for 1 week at 2°C [73]
Chitosan	High molecular weight, 82.7% DD		Strawberries	2% of chitosan treatment could maintain edible quality of tested sample from 3 days to 21 days of storage [74]
Chitosan	95% DD		Papaya	The fruits showed acceptable quality in flavor, scored at 3.13 and 4.24 in a 5-point scale, for samples treated with 1.0 and 1.5% of chitosan solution, respectively [75]
Moringa oil/chitosan nanoparticles embedded gelatin nanofibers	85% DD		Cheshire cheddar cheese	Sensory scores for taste and overall acceptability were significantly improved with this treatment [76]
Water-soluble azido chitin derivative film	70 kDa molecular weight, degree of substitution of the azido derivative was 0.8		Ricotta Cheese	After 28 days of the storage experiment, the flavor and odor scored at 4-excellent for the 2% chitin treated sample whereas it was 2-fair for the untreated one [77]

NCC N-carboxymethyl chitosan, *NOCC* N,O-carboxymethyl chitosan, *CMC* carboxymethyl cellulose, *DD* degree of deacetylation, *TBA* thiobarbituric acid, *TBARS* thiobarbituric acid reactive substances

descriptors. In the fresh burgers preserved with low SO_2 (150 mg/kg), the sensory scores of rancid, acidic, and putrid odors developed were from 1.08, 1.08, and 1.05 to 2.14, 2.15, and 2.12, respectively, at the 21st day of storage, whereas with 0.02% and 0.05% chitosan addition, the scores of those odors were 1.76, 1.76, 1.56 and 1.59, 1.59, 1.85, respectively. Similar results were found in the storage of cooked pork burgers. The addition of chitosan could reduce the development of rancid odors, rancid flavors, putrid odors, and putrid flavors and retard the loss of meat odor and meat flavor during storage.

On the other hand, although chitin is considered to possess lower antioxidant and antimicrobial capabilities than chitosan, its nanofiber (CNF) formed materials could still be effective for improving the flavor stability of foods. Azarifa et al. [69] prepared a gelatin-CMC film incorporated with chitin nanofiber and reported that it could have a promising effect on the flavor stability of refrigerated raw beef. The odor and overall acceptability were significantly improved during 12 days of storage by coating the beef with the nanocomposite. The interaction between positively charged groups of CNF and the negative charges of the microbial membrane affected the permeability of the cell membrane, and this is considered to be the major mechanism for the antimicrobial effect of CNF.

Chitosan applications also promoted the flavor stability of aquatic products efficiently. Ahn and Lee [70] used chitosan film for packing lightly-salted horse mackerel, and they found that the flavor could be retained well (scored 3.5 compared to 2.2 for a sample without chitosan packing, in a five-point scaled quantitative descriptive analysis) after 8 days of storage. Yu et al. [54] investigated the effects of chitosan (400 kDa molecular weight, 85% DD) based coatings on flavor retention of refrigerated grass carp fillets. The results indicated that chitosan coatings resulted in a significant attenuation of off-flavor compounds such as trimethylamine, hypoxanthine, and histidine. Meanwhile, favorable flavor compounds such as inosine monophosphate and umami-associated free amino acid could be accumulated with this treatment. In sensory evaluation, chitosan coatings significantly retarded the sensory deterioration during storage, which could improve the overall acceptance from 1.00 to up to 3.24 in a five-point scaled quantitative descriptive analysis at the 15th day of storage at 4°C. Jasour et al. [71] used a chitosan (medium molecular weight, 85% DD) coating incorporated with the lactoperoxidase system to prevent rainbow trout from flavor deterioration during refrigerated storage for a period of 16 days. They evaluated the color, odor, texture, and general acceptability using a 9-point scaled description. The sensory score for odor could be improved from 2.58 and 2.53 to 6.87 and 6.70 of odor and overall acceptability by this coating system. Even coating with chitosan alone revealed a positive effect for improving the flavor stability; both the odor and acceptability were scored at 5.54. Fan et al. [72] compared the capability of chitosan (molecular weight of 160 kDa, 85% DD) and glacial acetic acid for improving the flavor stability of silver carp. After 30 days of refrigerated storage, the chitosan-coated group received a better score (about 4.8) than did the glacial acetic acid group (about 3.8) in a nine-point hedonic scale, owing to its ability to reduce the generation of TBA and total volatile basic nitrogen (TVB-N). Cao et al. [65] revealed that oysters pretreated by immersing in water-

soluble chitosan (80–82% DD) solution retained better freshness flavor during storage. This was due to the growth inhibition effect of chitosan toward microorganisms.

3.2.2 Other Foods

Due to their notable antioxidation and antimicrobial activities, chitin and chitosan can also be used to preserve the flavor of fruits, dairy, and cereal products. Strawberries could be one of the most perishable fruits, and they are characterized by a short shelf-life. Han et al. [73] developed 1% chitosan (89% DD) based solutions (containing 6% of organic acids) for coating strawberries. A trained panel developed appearance, texture, and flavor descriptors by evaluating chitosan-coated strawberries stored for 1 week at 2°C. However, the results indicated that no significant amelioration in the flavor of treated strawberries after the storage period. Another approach was conducted by Jesmin et al. [74], 2% of gamma radiation treated high molecular weight chitosan solution (82.7% DD, viscosity less than 200 mPa s and 2% acetic acid) was used to treat the test strawberries. They suggested that the treatment could remarkably prevent strawberries from weight loss (17% to about 2%) and the growth of microbes (2.00×10^5 to 1.04×10^4 CFU/g). Results from sensory evaluation showed that the treatment could maintain the edible quality of the tested samples from 3 days to 21 days of storage. From these results, it can be concluded that the concentration of chitosan used may play a crucial role in improving the flavor stability of strawberries. Han et al. [83] suggested that a 2% chitosan solution treatment could control the mold growth of strawberries, whereas the mold decay incidence of 1% chitosan treated strawberries was estimated to be over 70% after 2 weeks [73]. Cell wall degrading enzymes are suggested to be secreted onto the surface of strawberries along with the growth of microbes, and this results in the disruption of receptacles and the loss of flavor compounds. Furthermore, chitosan treatment can also help retard the flavor loss of papaya. Ali et al. [75] revealed that treatments with 1.0 and 1.5% of chitosan (95% DD) solution promoted the flavor stability of papaya. The fruits showed acceptable flavor quality; samples treated with 1.0 and 1.5% of chitosan solution scored 3.13 and 4.24 (in a 5-point scale), respectively. Interestingly, samples treated with 2.0% chitosan solution were not ripened properly during the experimental period of cold storage, and they were removed from the test due to unacceptable quality.

Dairy products are rich in nutrients and are good medium for growth of various microbes. The presence of undesirable bacteria in milk may cause a deterioration in flavor such as souring of milk, which may further lead to economic loss [84]. Several studies were conducted to use chitin and chitosan in controlling the development of undesirable flavors in dairy products. Seo et al. [68] prepared chitosan nanoparticles with an average diameter of 562 nm by a grinding method, dissolved them into a 0.3% ascorbic acid solution, and then added them into commercial milk at various concentrations. In the sensory evaluation, 8 trained sensory panelists were recruited and the rancidity, bitterness, astringency, and overall acceptability were investigated

on a 7-point scale. However, all of the sensory parameters were not significantly affected by the addition of the nano-chitosan solution when the milk was stored for 15 days at 4°C. Lin et al. [76] introduced chitosan (85% DD) moringa oil/chitosan nanoparticle embedded gelatin nanofibers as a preserving agent for reducing the flavor loss of Cheshire cheddar cheese. The cheese samples were wrapped with the nanofibers and stored at 25°C for 4 days. The sensory score for taste and overall acceptability were significantly improved with this treatment, owing to the inhibition activity toward the growth of *L. monocytogenes* and *S. aureus*. Most recently, Kritchankov et al. [77] developed water-soluble azido chitin derivatives, which possessed high antibacterial activity, to preserve Ricotta cheese. This novel material was prepared by an ultrasound-assisted treatment of chitin (70 kDa) with 1-azido-3-chloropropan-2-ol at 80°C. The prepared chitin had a degree of substitution of the azido derivative of 0.8, and it could be dissolved in water. Two percent of the azido chitin solution was used to treat the cheese sample. After 28 days of the storage experiment, the flavor and odor scored at 4-excellent for the treated sample, whereas it was 2-fair for the untreated one.

4 Chitin and Chitosan for Food Debittering

4.1 *The Bitter Taste in Food and debitterization Technologies*

Bitterness is widely distributed in foods, and basically each chemical class contains bitter molecules [85]. This sensation originates from taste receptors (TR) localized on taste buds in the oral cavity. Humans have the ability to identify a broad range of materials as bitter, which indicates that bitter molecules occur in many variations. The most important representative bitter molecules are identified as certain alkaloids (e.g., nicotine, quinine, caffeine), terpenoids (e.g., isoalpha acid, amarogentine, limonoids), and flavonoids (e.g., naringin, neohesperidin, epigallocatechin gallate) [85]. Bitter taste can be considered a major problem in the food and pharmaceutical industries, owing to its negative hedonic impact on ingestion [86, 87]. In the past, bitterness reduction technology was focused on pharmaceuticals, however, recently, most research is conducted on the reduction of bitter taste or astringency in functional food or beverage applications. These foods and beverages possess inherent off-tastes because of their fortification with healthy but poor-tasting additives. Only in limited cases will consumers accept a strong bitter taste in food and beverages (e.g., in black or green tea, black coffee, beer, grapefruit products, or bitter lemons). For most other cases, a bitter taste has to be eliminated from food or masked since it is not desirable [85].

Foods, unlike drugs, are mainly selected by the consumer based on their sensory properties. Hindering the taste of drugs should be acceptable, whereas foods should taste delicious [88]. Recently, due to the demand for healthier foods or beverages, the problem of bitter-tasting food products is surfacing again. The ingestion of these foods/beverages is not only perceived as bitter but also as astringent and/or sour.

Reduced sugar, fat, and sodium for healthy benefits can also indicate sourness, astringency, and bitterness in the base matrices. Each modality is translated by different molecular sensing mechanisms in the oral cavity, and the sensation consciously recognized is a mixture that is difficult to separate into individual sensory tastes. Therefore, the complex mixture of sensations of foods makes masking off-tastes a big challenge [85].

Methods for dealing with this issue can be varied. In general, plant breeding has selected for less-bitter varieties and processing often involves chopping and peeling to remove the bitterest parts of the plant [88]. Another example is in the juice industry. Raw orange juices are debittered by using naringinase to cleavage the bitter naringin into the less bitter naringenin or naringin-7-O-glucoside. On the other hand, most of cloudy raw apple juices are processed to remove most of the bitter-tasting or astringent polyphenols to yield clear beverages [85]. Moreover, most commonly, bitterness is masked by additives. Among the most used additives are sodium ions which antagonize the bitter taste of a broad array of compounds. However, their activity varies a great deal, depending on the chemical nature of the bitter-tasting molecule, and in some cases, they have been proven to be quite inactive. Although bitter-taste blockers have been much explored, only a few have shown a wide spectrum of activity. In fact, none of them is known to be effective against all bitter compounds. Among other bitter-taste blockers, we can mention cyclodextrins, adenosine monophosphate, and certain protein combinations such as lactoglobulin bound to phosphatidic acid [89].

4.2 Chitin and Chitosan for Debitterization Use in Foods and Herbal Extracts

Due to its natural polycationic property, chitosan is known to act as clarification agent in the juice and beverage industries [90]. It could be effective in coagulating suspended particles through ionic interaction with negatively charged molecules and thus help separate them from juice and beverages. A wide array of successful applications have been reported in the literature such as in apple, grape, lemon, passion fruit, and pomegranate juices [90–92]. Along with the clarification process, some of the major bitter-tasting molecules, such as tannins and flavonoids in juice, could be removed and give products with a lower bitterness perception.

Another debitterization technology feature in chitosan application is encapsulation, which was frequently used in masking bitter-tasting bioactive molecules in herbal extracts and functional foods. Chitosan acts as a bitter taste blocker in this technology. It enables the bitter bioactive molecules to be encapsulated into the form of free-flowing micro/nano-capsules and then reduces the solubility of such bitter bioactives by providing a physical barrier to the taste buds [87, 93–98]. The resulting microcapsules can be blended with other ingredients for food formulation and processing. The advantage of encapsulation for taste-masking of bitter bioactives

in functional foods is in the wide variety of dosage forms and product applications. In general, the coating polymer, chitosan, can be insoluble in a salivary environment at pH 6.8 and either readily dissolve in gastric fluid at pH 1.2 or it may be insoluble in gastric fluid but decomposed in the intestine fluids to release the bioactives [93]. This strategy may prevent the release of bitter-tasting bioactive molecules in the oral cavity after ingestion but allow their release where they should be absorbed. The technology can be achieved by dissolving/dispersing chitosan in a solvent or by incorporating the bitter-tasting bioactive molecule in a chitosan solution. Nowadays, most encapsulation in industry is achieved using fluidized bed processors where the coating solution is sprayed through a nozzle and dried with warm air.

Moreover, because of their high protein affinity, chitin and chitosan are promising supports for the immobilization of specific debitterization enzymes such as naringinase and exopeptidases in the food industry [99, 100]. The basic applications of chitosan for debitterization can thus be included in the aforementioned strategies (Fig. 6). The scientific publications of chitin and chitosan for Debitterization applications are addressed in Table 3.

4.2.1 Chitin and Chitosan as Carriers for Debittering Enzymes

In the food industry, immobilized naringinase is used for enzymatic debittering of several citrus juices. It can promote the hydrolysis of bitter-tasting naringin into rhamnose and prunin and further cleave prunin to glucose and naringenin [109]. In this aspect, chitin and chitosan were both used as carriers for immobilization of naringinase. Tsen and Tsai [107] used a one-step method employing glutaraldehyde and sodium borohydride to immobilize naringinase from *Penicillium* sp. on chitin. The naringin content in grapefruit juice could be reduced to about 40% within 60 min of treatment with a capacity of about 10 times the column volume. Bodakowska-Boczniewicz and Garncarek [108] obtained chitosan (medium molecular weight) microspheres by reversed-phase suspension methodology, whereby glutaraldehyde was introduced as a cross-linking reagent for the immobilization of naringinase. The immobilization yields could be up to 31.97%, and the immobilized enzyme microspheres were used for debittering grapefruit juice. Compared to the soluble form of enzyme, the K_m value of the immobilized naringinase was higher (2.56 vs. 6.59 mM), and the immobilized naringinase had good recycling stability. It retained 88.1% of its initial activity after 10 runs of the hydrolysis process from fresh grapefruit juice.

4.2.2 The Removal of Bitter-Tasting Molecules in Foods by Chitosan

Chang and Juang [104] proposed chitosan (1,850 kDa molecular weight, 97.2% DD)/activated clay composite beads for removing tannic acid, the most important bitter-tasting molecules in various juices, from a model liquid system. The beads were found to effectively adsorb tannic acid with a quantitated capacity of 1,490 g/

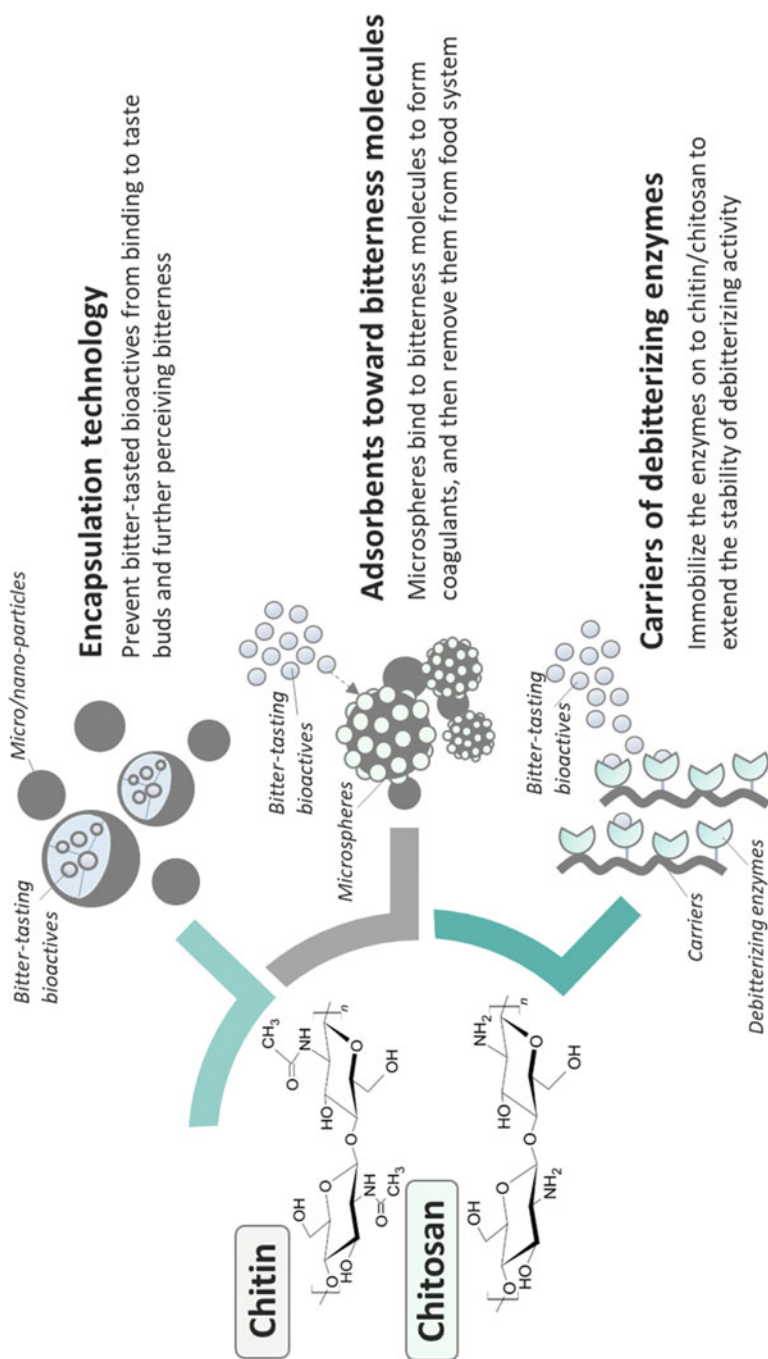


Fig. 6 The applications of chitin and chitosan for debitterization

Table 3 Scientific publications reporting the Debitterization applications of chitin and chitosan

Mechanism	Materials	Physicochemical properties	Applied foods	Outcome	Reference
Encapsulation	Chitosan-CDs adduct	70 kDa molecular weight	Caffeine and artichoke leaves, aloe and gentian extracts	Chitosan- β -CD adduct conferred the better debittering capacity than other tested groups (chitosan alone, CDs alone, and chitosan- γ -CD). It remarkably reduced the bitterness scores from 4.0 to 0.2 against caffeine, 5.0 to 0.9 against artichoke extract, 3.0 to 0.0 against gentian extract, and 3.0 to 0.0 against aloe extract in a 5-point scaled sensory evaluation	[89]
	Chitosan-coated alginate microcapsules	Medium-molecular weight, 75–85% DD	Flavonoids-rich herbal extract	Chitosan-coated alginate microcapsules had an efficiency for flavonoids up to 77.97% at optimum conditions: sodium alginate concentration 1.49%, extract concentration 1.58%, and CaCl_2 concentration 0.84%. The loaded flavonoids could be released gently in simulated gastric fluid and rapidly in simulated intestinal fluid	[101]
	Chitosan nanoparticles	75–98% DD, 10–235 kDa molecular weight	Xanthohumol and lupulone from hop extract	Nanoparticles composed of chitosan with the lowest DD and highest molecular weight can retain a small amount of bitter compounds, indicating that the encapsulation may prevent those bitter-tasting molecules from attaching to the taste buds to give a bitter perception. The release of bitter-tasting bioactives increased by increasing the molecular weight of chitosan in a pH = 7 buffer system	[102]
	Chitosan nanoparticles	Not available	Bacoside-rich <i>Bacopa monnieri</i> extract	Nanoparticles composed of 0.1% chitosan, 0.15% TPP, and 15 mg/mL of extract have an average particle size about 220 nm, and the efficiency of encapsulation was 52.0%	[103]

Adsorption	Chitosan/activated clay composite	97.2% DD, 1,850 kDa molecular weight	Model liquid system	Chitosan/activated clay composite beads were found to effectively adsorb tannic acid with a quantitated capacity of 1,490 g/kg	[104]
	Chitosan	81.23–90.54% DD	Lime juice	Chitosan with the lowest DD of 81.23% could have the highest capacity for reducing the contents of major bitterness molecules, limonin (58.56%) and naringin (22.26%)	[105]
	Carboxymethyl chitosan	85% DD, 80% degree of substitution	Yeast paste derived from beer process	2% of carboxymethyl chitosan at pH 7.5 for 58 min may greatly reduce the content of α -acid, β -acid, and iso- α -acid	[106]
Enzyme carriers	Chitin	Not available	Grapefruit juice	Naringin content could be reduced to about 40% within 60 min of treatment with a capacity of about 10 times of column volume	[107]
	Chitosan microspheres	Medium molecular weight	Grapefruit juice	Immobilized naringinase had a km value of 6.59 mM towards naringin; it retained 88.1% of its initial activity after 10 runs of hydrolysis process	[108]
	Chitin	Not available	Casein hydrolysates	The immobilized porcine pancreatic exopeptidases was effective in releasing the free amino acids from peptides, leading to the removal or a decrease in bitterness	[99]

DD degree of deacetylation, CD cyclodextrin, TPP tripolyphosphate

kg, which is much higher than those previously reported on organoclay and activated carbon, 110 and 25 g/kg, respectively [110]. Deatchewa and Buri [105] investigated the effect of DD and concentrated chitosan on the reduction of lime juice bitterness. They found that chitosan with the lowest DD of 81.23% could have the highest capacity for reducing the content of the major bitterness molecules, limonin (58.56%) and naringin (22.26%), among all tested groups. Consequently, the decrease in the DD of chitosan resulted in a decrease of bitterness scoring in sensory evaluation. However, the molecular weights of these tested chitosans were addressed in this study. In general, the deacetylation process is frequently followed by a significant decrement in the molecular weight of chitosan, hinting that the chitosan tested with relatively high DD may have lower molecular weight than others. Therefore, the molecular weight of chitosan may also play a role in this study. Jiang et al. [106] attempted to recover the yeast paste derived from beer processing and use it as a novel healthy food. Carboxymethyl chitosan (85% DD, 80% of degree of substitution) was used for debittering the paste, focusing on the removal of α -acid, β -acid, and iso- α -acid. The optimal conditions determined by response surface methodology (RSM) experiments were treatment by adding 2% of carboxymethyl chitosan at pH 7.5 for 58 min. Ye et al. [111] further described the debittering capacity of carboxymethyl chitosan in this food system. They found that a 15.19–19.49 mg/L reduction of iso- α -acid concentration could be achieved by 1 g of carboxymethyl chitosan. Li et al. [112] constructed a chitosan (550 kDa molecular weight, 85.3% DD)-Ce⁴⁺ microsphere resin system to remove the bitter-tasting molecules in processed orange juice. The adsorption system could effectively remove 43.2% of limonin and 54.86% of naringin from juice without significant nutrition loss.

Taking the aforementioned studies into consideration, chitosan was not only capable of removing bitter-tasting phenolics in juice products, but it also shows a high capacity for interacting with non-phenolic compounds such as limonin and iso- α -acid which provide significant bitterness in foods.

4.2.3 Encapsulation Technology

Chitosan itself presents strong astringency when dissolved in acidic medium, and this limits its use in oral ingestion [113]. However, when integrated with other polymers into composites or by reducing the particle size to nano-scale, it is capable of acting as an effective bitter-taste-blocker for bitterness bioactives instead of providing an astringent perception. Binello et al. [89] prepared chitosan (70 kDa molecular weight) cyclodextrin (CD) adducts using a malonyl or a succinyl bridge between these two polymers by a series of chemical syntheses, and they tested the bitter-masking potential of these materials against caffeine and artichoke leaves and aloe and gentian extracts. As a result, the chitosan- β -CD adduct conferred better debittering capacity than other tested groups (chitosan alone, CDs alone, and chitosan- γ -CD). It remarkably reduced the bitterness scores from 4.0 to 0.2 against caffeine, 5.0 to 0.9 against artichoke extract, 3.0 to 0.0 against gentian extract, and

3.0 to 0.0 against aloe extract in a 5-point scaled sensory evaluation. It is worth mentioning that chitosan alone provided a notable debittering effect as well, but it was accompanied by a strong unpleasant astringent sensation which may limit its use. Another composite proposed by Khorshidian et al. [101] also demonstrated its capability for encapsulating bitter-tasting molecules. Medium-molecular weight chitosan (75–85% DD) was introduced to prepare chitosan-coated alginate microcapsules by using the CaCl_2 method followed by filtration and centrifugation. The microcapsules were then loaded with herbal galactagogue extract (containing *Foeniculum vulgare*, *Cuminum cyminum*, *Trigonella foenum*, and *Anethum graveolens* extracts, rich in flavonoids). The encapsulation efficiency of flavonoids can be up to 77.97% at the optimum conditions: sodium alginate concentration of 1.49%, extract concentration of 1.58%, and CaCl_2 concentration of 0.84%. The loaded flavonoids could be released gently in simulated gastric fluid (SGF) and rapidly in simulated intestinal fluid (SIF); this demonstrated a desirable controlled release profile.

Leonida et al. [102] investigated the encapsulation properties of chitosan toward two specific compounds in hop extracts, xanthohumol and lupulone. These are known for their antimicrobial activity, but this is accompanied by an extremely bitter taste. Researchers prepared chitosan (varied in molecular weights and DDs) nanoparticles by ultrasonication-assisted ionic interaction with tripolyphosphate (TPP). The particle sizes ranged from 28.1 to 49.4 nm when loaded with the bioactive compounds. Notably, two different types of chitosan were used in this study, including chitosan prepared from shrimp shells (190–235 kDa molecular weight, 75% DD) and from enzymatic treatment (4, 10, and 50 kDa molecular weight, 98% DD). In the *in vitro* release study, they found that the release of bitter-tasting bioactives was increased by increasing the molecular weight of chitosan in a pH = 7 buffer system. Nanoparticles composed of shrimp shell chitin (with a relatively high molecular weight and low DD) can retain a small amount of bitter compounds. This indicated that encapsulation may prevent those bitter-tasting molecules from attaching to the taste bud to perform the bitter perception because the pH value of saliva is around 6.2–7.6, which is similar to the test condition. On the other hand, it may also hint that the release would be low in a neutral intestinal environment as well, which could limit the absorption and further bioavailability. However, the results still recommend the nanocomposites as interesting for further study in food applications as preservatives and for oral administration. Anand et al. [103] also demonstrated the potential of chitosan-TPP nanoparticles as encapsulation matrices that reduce the bitterness of bacoside-rich *Bacopa monnieri* extract (BME). They used RSM experiments to find the optimal conditions which were 0.1% chitosan, 0.15% TPP, and 15 mg/mL of BME. The nanocomposites prepared in these conditions had an average particle size of about 220 nm, and the efficiency of encapsulation was 52.0%. These technologies may facilitate the supplementation of bioactive compounds in various functional food formulations through their biochemical and physiological activity.

5 Summary

In food industry, products are mainly preferred by consumers based on their sensory qualities, and flavor is the most important among all indices. Chitin and chitosan are versatile materials with proven flavor-improving capabilities for specific food products. Physical and chemical modifications including three flavor-improving strategies using chitin and chitosan have been reviewed: (1) the particle or fibril form of materials as saltiness enhancers; (2) intact addition or edible film/coatings for improving flavor stability; (3) removing or encapsulating bitter-tasting molecules.

For use in saltiness enhancement, chitosan/NaCl and chitosan/maltodextrin/NaCl microparticles had a stronger salty perception than common market table salt. This is mainly because the average particle size ($<32\ \mu\text{m}$) is less than the general salt products (about $500\ \mu\text{m}$), and there are a large number of NaCl crystals ($<4\ \mu\text{m}$) distributed on the surface of the particle matrix. Therefore, in the oral cavity, NaCl crystal can quickly dissolve and rapidly improve the concentration of Na^+ and have a strong salty perception. This strategy belongs to the particle size and shape alteration/enhanced dissolution rate in the aspect of sodium reduction applications. It can be used as highly salty sprinkle salt with low sodium content to season the surface of dried foods such as popcorn and chips. However, it is not suitable for use in liquid foods where the dissolved particles do not retain this dominant structure and therefore do not enhance the perception of saltiness. Instead the particles produce an astringent taste due to the presence of chitosan in the system. Chitin is particularly hard to dissolve in water. By mechanical disassembly or chemical modification methods, chitin nanomaterials, including chitin nanofibers and chitin nanocrystals, can be obtained, and these are able to form a stable suspension in water. When NaCl is added to a chitin nanomaterial suspension, the amine groups on chitin are able to absorb chloride ions due to the electrostatic interaction, resulting in a decrease in the zeta potential of the suspension system, which could reduce the astringency of the suspension as well as increase the concentration of free Na^+ in the solution, resulting in an improved saltiness perception. This could be categorized into the alteration of food composition sodium reduction strategy, which can be used in soups, sauces (such as soy sauce, oyster sauce, bean paste), pickled foods, and seasoning bags of ready-to-eat food to achieve the purpose of reducing sodium.

Furthermore, chitosan possesses significant antioxidation and antimicrobial activities. It is able to improve the flavor stability of various foods by directly incorporating it into the composition or by coating it onto the surface. Chitosan with high molecular weight can inhibit the growth of Gram-positive bacteria, which would likely make it suitable for use in meat foods such as sausage. When used as a coating solution, the concentration of chitosan may significantly affect the preserving capacity. The use of chitin in this field is limited due to its lower DD than that of chitosan, resulting in relatively low antioxidation and antimicrobial activities. However, it could be applicable when it undergoes specific structural or chemical modification.

Moreover, chitosan revealed high adsorption capacity toward specific bitter-tasting molecules. Therefore, it could act as a coagulant and remove them from the

food system. Also, due to the remarkable binding capacity toward negatively charged compounds, it could effectively encapsulate the bitterness bioactives to prevent them from being perceived by bitter sensors in the oral cavity. Chitin and chitosan could also be used as carriers for Debittering enzyme immobilization in order to obtain products with desirable flavor.

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