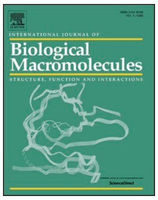
International Journal of Biological Macromolecules 235 (2023) 123716

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac



Review

Application of functionalized chitosan in food: A review

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ARTICLE INFO

Keywords:
Chitosan
Modifying strategy
Functionality
Food applications

ABSTRACT

Environmental and sustainability issues have received increasing attention in recent years. As a natural biopolymer, chitosan has been developed as a sustainable alternative to traditional chemicals such as food preservation, food processing, food packaging, and food additives due to its abundant functional groups and excellent biological functions. This review analyzes and summarizes the unique properties of chitosan, with a particular focus on the mechanism of action for its antibacterial and antioxidant properties. This provides a lot of information for the preparation and application of chitosan-based antibacterial and antioxidant composites. In addition, chitosan is modified by physical, chemical and biological modifications to obtain a variety of functionalized chitosan-based materials. The modification not only improves the physicochemical properties of chitosan, but also enables it to have different functions and effects, showing promising applications in multi-functional fields such as food processing, food packaging, and food ingredients. In the current review, applications, challenges, and future perspectives of functionalized chitosan in food will be discussed.

1. Introduction

Chitosan (CS) is the second most abundant biopolymer on Earth after cellulose [1,2]. CS is a polycationic polysaccharide derived from chitin that is composed of *N*-acetyl-d-glucosamine units linked by β-(1,4)-glycosidic bonds [3], which is soluble in aqueous solutions such as acetic acid and lactic acid, and its solubility depends on the degree of deacetylation (DD) and molecular weight [4]. CS is a special biological material because of its biodegradability, non-toxicity, biocompatibility, and good film-forming properties, it is highly valued by academia and industry in food applications [5,6]. At present, a variety of functionalized chitosan derivatives have been developed in many fields (such as food preservation [7–9], food packaging [10–14] food processing [15,16]) to meet the needs of modern society.

However, CS also suffers from inherent disadvantages, including low mechanical properties, thermal stability and high sensitivity to humidity, which make it more limited in industrial applications. To overcome these shortcomings, researchers have attempted to functionalize CS using physical, chemical and biological modification methods. At present, some articles have comprehensively discussed the specific application of CS or a specific type of functionalized chitosan material containing chitosan-based encapsulants [17], chitosan nanomaterials [18] and chitosan-based copolymers [19]. In addition, several specific application areas have also been reviewed, such as food packaging [20], food coatings [13], food processing [21], and food production [22]. The rational modifications of CS materials are important prerequisites for their successful application in the food field, and this is also a major challenge. However, a comprehensive review of the properties and modifications of chitosan for food applications has not yet been reported. In recent years, the review of chitosan in food is mainly focused on its application in a certain direction of food, such as food packaging and food preservatives, and the review of functional chitosan is only focused on its single form. At present, there is still a lack of comprehensive description of different forms of functional chitosan in the application of food. The purpose of this paper is to provide a comprehensive literature review of the potential applications of different functionalized chitosan in food over the past five years. In addition, this paper also aims to highlight some possible trends and future research on

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https://doi.org/10.1016/j.ijbiomac.2023.123716
Received 11 November 2022; Received in revised form 5 February 2023; Accepted 12 February 2023
Available online 16 February 2023
0141-8130/© 2023 Published by Elsevier B.V.

functionalized CS in the food field and strengthen the summary and discussion of its safety evaluation.

2. The structure of CS

Fig. 1 shows a model of the chitosan molecule. CS is rich in -OH, —O— and -NH_2 groups, among which the amino group content is related to the viscosity, ion exchange capacity, flocculation capacity and N-selective acylation capacity of chitosan products [23]. In addition, there are many highly extended hydrogen-bonded semi-crystalline structures in the structure of CS, so it is only soluble in weakly acidic solutions. CS also contains a large number of primary amines and is one of the rare polycationic polymers in nature, which sets it apart from other polysaccharides. The properties of CS are also related to many parameters, such as molecular weight, degree of deacetylation (DD) (in the range of 50–100 %), sequence of amino and acetamido groups, and product grade [24].

3. Properties and mechanism of action of chitosan

CS provides many excellent physical and chemical properties, such as antibacterial properties, antioxidant properties, adsorption properties, good biocompatibility, non-toxicity and film-forming properties. Among them, the excellent antibacterial and antioxidant properties of chitosan make it widely used in food packaging, food preservation, food additives and other fields. In the field of food packaging, the functionalized chitosan packaging film has good mechanical properties and selective permeability to O_2 and CO_2 [25]. Therefore, it plays an important role in keeping food quality by avoiding chemical and microbial influences on food during transportation, storage and distribution [26]. The macromolecular chain of chitosan contains a large number of amino, hydroxyl and ether bonds, which can be used as a matrix material for acylation, esterification, carboxylation, etherification, oxidation, Schiff alkalization and other reactions [27], which makes chitosan more widespread application in food. The mechanisms of action related to the antibacterial and antioxidant properties of chitosan were listed in the following.

3.1. Antibacterial properties of CS

Previous studies have found that the antibacterial activity of chitosan is determined by many factors. Currently, the existing study and put forward the antibacterial mechanism of chitosan and its derivatives because the chitosan molecular chain has positively charged amino groups that interact with the microbial cell membrane, prevent the cells inside and outside the transport of molecules, and the microorganisms cannot maintain basic metabolism, which will also lead to the leakage of microbial proteins and other cellular components, thereby inhibiting the normal growth of microorganisms [28–31]. In addition, amino groups on the surface of chitosan can also be modified by different antibacterial agents to enhance the inhibitory effect on microbial cell growth [32]. However, the inhibitory mechanism of chitosan for bacteria, molds, and viruses is also different.

3.1.1. Inhibitory mechanism against bacteria

The mechanism of antimicrobial activity of CS is different for gram-positive and gram-negative bacteria. The unique outer membrane (OM) structure of gram-negative bacteria is located on the outside of the cell wall peptidoglycan and is composed of lipopolysaccharides, lipid bilayers (phospholipids) and lipoproteins (LPS). The presence of divalent cations in OM plays an important role in the stabilization of the core anionic charge of LPS molecules. CS is assumed to replace the binding site of divalent cations and reduce the interaction between LPS molecules. The loss of LPS may lead to the appearance of phospholipids in the outer OM lobules and form channels where hydrophobic molecules can diffuse [33,34]. Bacterial membranes are susceptible to attack by CS molecules and pore formation, leading to membrane disruption and cell lysis (Fig. 2). Unlike gram-negative bacteria, gram-positive bacteria do not have an OM. CS, as a polycationic long-chain molecule, can better adhere to negatively charged Gram-positive bacteria [35]. Therefore, the inhibitory effect of CS is more effective against gram-positive bacteria than gram-negative bacteria [32,36,37].

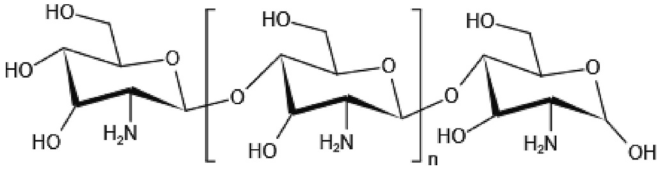

Fig. 1. The molecular formula of chitosan.

3.1.2. Inhibitory mechanism against fungal

CS is a deacetylated derivative of chitin, a component of many fungal cell walls. It has been reported [38] that chitosan is a compound synthesized by fungal cells, which can not only induce the synthesis of vicilin, but also inhibit the germination and growth of fungi. Dutta et al. [39] believed that when chitosan is released from the cell wall of fungal pathogens, it penetrates the fungal nucleus and interferes with RNA and protein synthesis. The research of GHAOYTH and his colleagues [40] confirmed the strong inhibitory activity of chitosan against fungi. Compared with *N*, *O*-carboxymethyl chitosan, polygalacturonic acid or D-glucosamine, chitosan inhibited *Alternaria alternate*, *Botrytis cinerea*, *Colletosporum anthracis* and *Stolon* better, and its inhibitory activity increased with the degree of deacetylation.

3.1.3. Inhibitory mechanism against virus

Chitosan and its derivatives have been recognized as antiviral materials in the past and recent years. Previously, Kochkina [41] proposed that chitosan can inhibit phage replication through several mechanisms: (a) reducing the viability of cultured bacterial cells; (b) neutralizing the infectivity of mature phage particles in inoculum and progeny phage particles; (c) Blocking the replication of virulent phages. Chirkov [42] cited several findings to support the antiviral mechanism of chitosan and its various derivatives. He pointed out that chitosan can promote the expression of ribonuclease (RNAse), which is associated with the induction of resistance to potato virus X (PVX) infection, thereby affecting viral replication. This was later verified by Iriti et al. [43]. Recently, the novel coronavirus pneumonia (Corona Virus Disease 2019, COVID-19) caused by *SARS-CoV-2* infection has caused outbreaks and epidemics in many countries and regions around the world, so the suppression of *SARS-CoV-2* virus has triggered a research trend. Sharma et al. [44] discussed the external and internal factors of the antiviral efficacy of chitosan, and pointed out the effect of chitosan on different SARS virus strains, and chitosan targeting CD147 receptor, which is a new way for *SARS-CoV-2* to invade host cells. It provides a theoretical basis for the potential application of chitosan as an anti-*SARS-CoV-2* virus. Tan et al. [45] also gave this result.

3.2. Antioxidant properties of CS

Chitosan has shown substantial scavenging ability against various free radical compounds, and its antioxidant effects are similar to those known to be antioxidants [46]. The sulfation products of CS exhibit excellent scavenging activity against peroxide radicals, but CS with the lowest MW exhibits stronger ferrous (Fe^{2+}) ion chelation. Notably, chitosan's ability to chelate metal ions highlights its potential role as a natural antioxidant for stabilizing lipids in foods to extend their shelf life. CS may inhibit lipid peroxidation by coordinating Fe^{2+} found in the surrounding environment, thereby inhibiting its pro-oxidative activity or conversion to Fe^{3+} [47].

Wei et al. [48] proposed that the scavenging ability of chitosan and

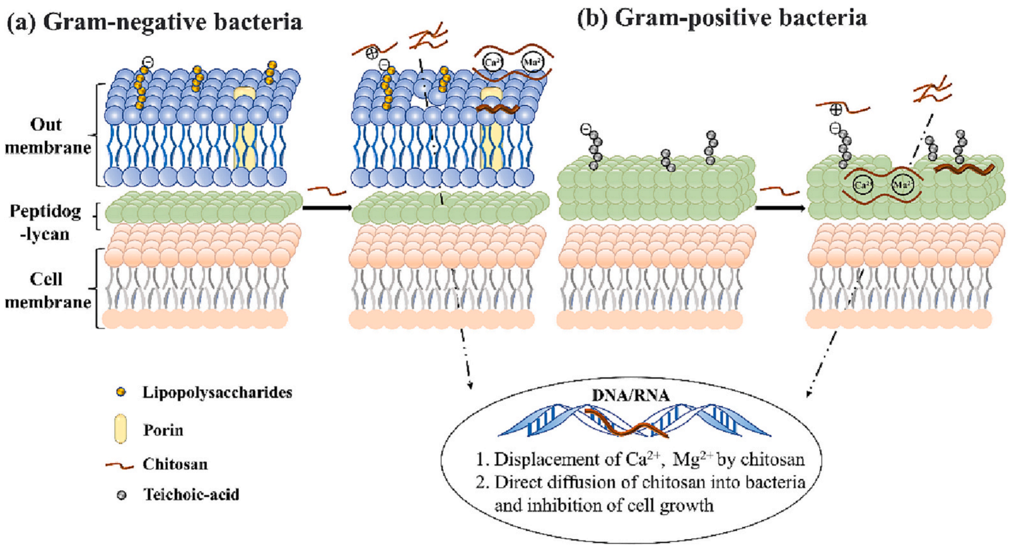

Fig. 2. Antibacterial mechanism of chitosan against gram-negative bacteria (a) and gram-positive bacteria (b) [34].

its derivatives against 2,2-diphenyl-1-picrohydrazine (DPPH) radicals was attributed to the presence of functional amino and hydroxyl groups on its backbone. The ability of chitosan to exert its antioxidant activity by scavenging free radicals makes it widely used in various fields. Ol. et al. [49] pointed out that chitosan can scavenge free radicals or chelate metal ions by donating hydrogen or lone pair electrons. The interaction between metal ions and chitosan occurs through various mechanisms, such as ion exchange, chelation, electrostatic attraction and adsorption. Under acidic conditions with pH less than 6, the amino groups of chitosan are fully protonated and adsorb other anions strongly through electrostatic interactions. Furthermore, the antioxidant and scavenging activities of chitosan were related to its molecular weight. The scavenging activity of low molecular weight chitosan on DPPH free radicals is stronger than that of high molecular weight chitosan. Low molecular weight chitosan can not only scavenge DPPH free radicals, but also inactivate peroxy radicals [50]. Many studies have demonstrated the role of chitosan as a reactive oxygen species (ROS) scavenger to prevent lipid oxidation in food and biological systems [51–53]. However, due to the semi-crystalline structure of chitosan with strong hydrogen bonds [54], the chemistry with amino and hydroxyl groups as reaction sites makes its accessibility to biological macromolecules extremely challenging.

4. Functionalization of chitosan groups

Despite the many advantages of CS, pure chitosan still does not meet the needs of most applications. The low thermal and mechanical stability of CS is a major disadvantage of its application as a biodegradable material in the food industry scale [55,56]. An effective combination can not only improve the stability of chitosan, but also fully combine the advantages of both. Several studies have confirmed that chitosan combined with other bio-based materials enhances the physical, thermal and mechanical properties of chitosan composites while improving their antibacterial and antioxidant properties [13,57,58]. Therefore, the preparation of these chitosan composites effectively expands the application of chitosan in the food industry. Among the modification methods for functionalized chitosan, physical, chemical and biological modification methods are the most common means of application.

4.1. Physical modification of CS

Physical modification is an attractive process because it does not involve the use of toxic and harmful chemicals, especially when modified chitosan is used in food contact, which is relatively inexpensive and simple compared to other technologies. Using physical interactions (such as hydrogen bonding, electrostatic interactions and hydrophobic interactions) to introduce the advantages of other components further enhances their application value [59]. For example, composite films that physically modify chitosan with gelatin, collagen, casein, whey protein, zein, and essential oils (EO) have been shown to have excellent physical properties to protect food, facilitate transportation, and extend the shelf life of perishable items, especially those susceptible to pressure, air, light, and even temperature [60–63].

Modification of chitosan by physical crosslinking mainly depends on two key conditions: i) The interchain interactions of the polymer must be strong enough to form semi-permanent connection points in the molecular network, and ii) The polymer network must be able to satisfy the exchange and diffusion of water molecules [64]. Fig. 3(a) shows the main interactions (ionic, polyelectrolyte, interpolymer complex, and hydrophobic associations) of physically modified chitosan. And these interactions can be easily tuned according to parameters including i) the charge density and size of the anionic agent, ii) the degree and concentration of deacetylation of the chitosan polymer, iii) the method of cross-linking, iv) the solubilization (aqueous or alcoholic solution) [65].

4.1.1. Ionic complexes

Fig. 3(b) shows the ionic crosslinking between chitosan polycations and negatively charged particles. Chitosan molecular chains have positively charged amino groups, and ionic interaction may occur between chitosan and negatively charged molecules and anions, thus forming ion complexation of a mixed charge system [66]. The interaction is easily affected by environmental pH value and pKa of the substance itself [67]. In acidic solutions, the solubility of chitosan is determined by the balance of its repulsive forces (positively charged amino groups) and the propensity of their polymer chains to self-associate through hydrogen bonding and hydrophobic interactions [68]. When the pH value is lower than its pKa ($pH = 6.3–7$), the chitosan molecule is water-soluble and the amino groups are positively charged, inducing electrostatic repulsion between polymer chains [69]. Adding bicarbonate or NaOH to the chitosan solution increases its pH, and when the pH is above 6.5, chitosan undergoes a sol-gel transition to form a hydrated gel-like precipitate [68].

Nie et al. [70] prepared CS hydrogels in alkaline and acidic solvent systems by physical cross-linking. In the acidic system, the gelation process of C (OH-) has the characteristic of layer by layer due to the equipotential surface of C (OH-). Significantly different from the acidic

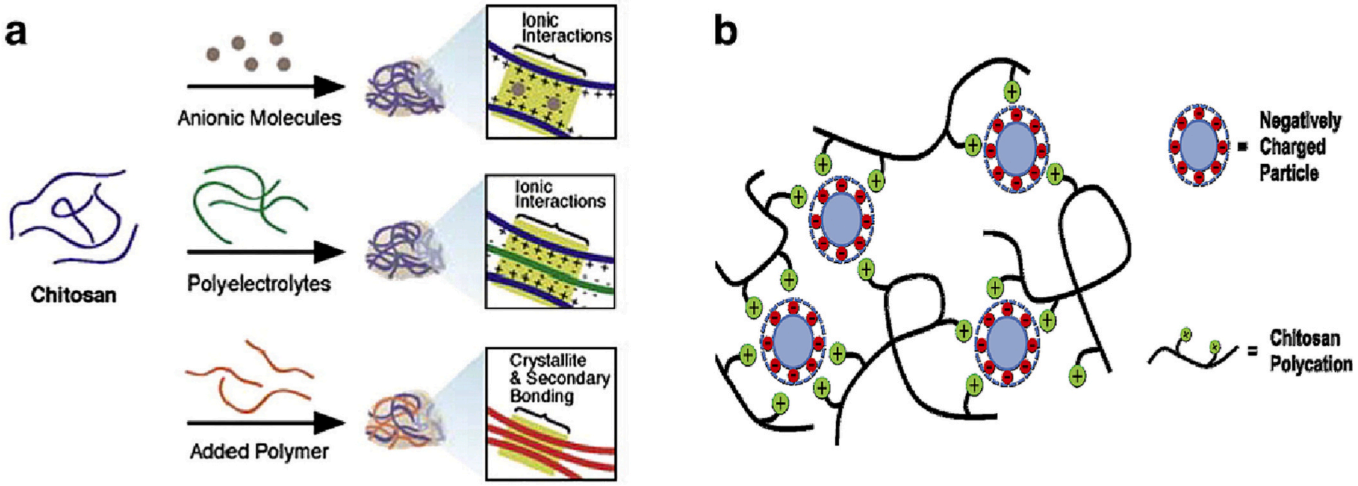

Fig. 3. Schematic diagram of different physical modification methods of chitosan groups: (a) networks of chitosan formed with ionic molecules, polyelectrolyte polymer and neutral polymers [64]; (b) schematic diagram of ionic crosslinking between chitosan polycations and negatively charged particles [74].

system, in the basic system, CS forms intermolecular/intramolecular H bonds with each other rather than forming effective interactions with OH-. The effect of pH value on chitosan-based hydrogel material was confirmed. Csaba et al. [71] used ion gelation technology to prepare CS/ Tripolyphosphate (TPP) carriers with high-concentration and low-concentration chitosan solution, respectively. Studies show that high concentration of CS/ TPP nanoparticles has a high positive Zeta potential. The interaction with the negatively charged cell membrane was strong, resulting in a slight decrease in cell viability. Therefore, anionic molecules that maintain a high charge density are chosen to ensure strong ionic interactions and have a suitably small MW for free diffusion and rapid electrostatic bond formation throughout the polymer matrix. Although, the ionic crosslinking of chitosan does not use any toxic precursors, catalysts or reducing agents, which makes the system biocompatible and ideal for food applications [72,73]. However, ionically prepared chitosan-based materials have poor mechanical stability and are highly sensitive to environmental changes (such as pH, temperature, and ionic strength). Therefore, there are limitations in their application.

4.1.2. Polyelectrolyte complexes (PECs)

Different from low molecular weight ionic crosslinking, chitosan can cross-link with macromolecular polyelectrolytes (carboxymethyl cellulose (CMC), sodium alginate, polyacrylic acid (PAA), pectin, gelatin, xanthan gum) by electrostatic interaction [74,75] (Fig. 4). The crosslinking density and the physicochemical properties of the resulting polymer (such as mechanical stability, swelling behavior, and dissolution curves) can be adjusted by changing the net charge ratio and molecular weight of the polyelectrolyte [76]. Yu et al. [77] used LBL (layer by layer) multilayer assembly technology to deposit CS and sodium alginate (ALG) on the surface of polypropylene (PP) - PAA. The multi electrolytic complexation between CS and ALG forms the biobased active layer. With the increase in the number of active layers, the reaction equilibrium time and chelation ability increased, and the PP-PAA/(CS/ALG) $_n$ film was confirmed to have the potential as antioxidant food packaging. Furthermore, Maciel et al. [78] developed a PECs matrix formed between chitosan and pectin as a pH indicator device to detect pH changes in food, and pointed out that the resulting charge of the system formed between chitosan and pectin was pH dependent. Under extremely acidic conditions of pH 1.5, PECs could not be promoted between pectin and chitosan. When the pH value (3.8–5.4) increases, the optimal ratio of pectin to chitosan constantly changes with the pH change [79]. The maximum was achieved when the ratio of chitosan to pectin of PECs is 1:1 and 3:7. at pH 5.0 [80].

The association between chitosan and polyelectrolytes is stronger than other secondary binding interactions, such as hydrogen bonds or van der Waals interactions [64]. The advantages of this type of complex are significant. Moreover, since PECs is composed only of chitosan and polyelectrolyte, their complexation is direct and reversible. Although the selection of anionic molecules used for PECs formation is highly

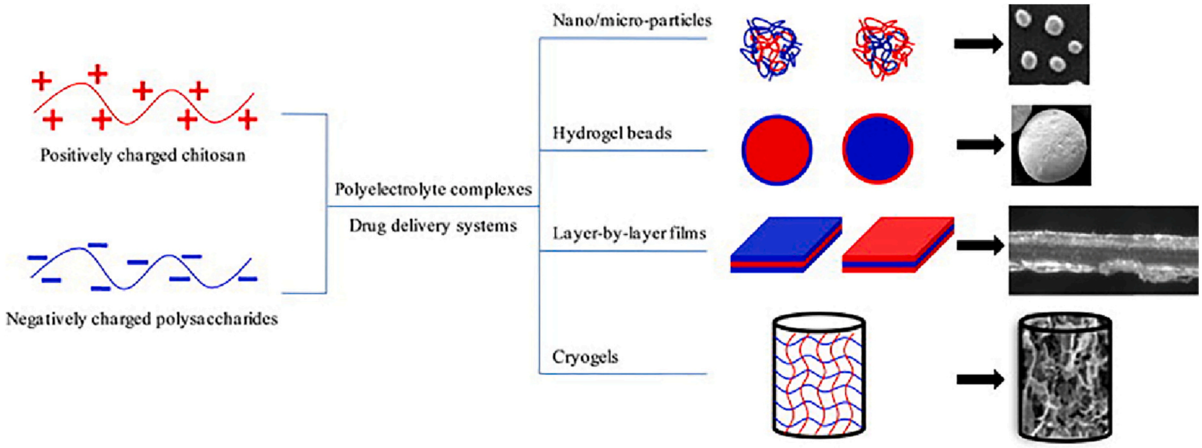

Fig. 4. Different types of structures formed by the multi-electrolytic complexation between chitosan and polyanions [75].

dependent on their charge under physiological conditions, pH can modulate ionic interactions and subsequently alter PECs properties. However, if the electrostatic interactions of the polymers are strong enough, the physical association of the polymers at physiological pH can be maintained.

4.2. Chemical modification of CS

Chitosan has abundant functional groups, which allow it to undergo various chemical reactions, such as acylation, esterification, etherification and other covalent grafting reactions [81]. These reactions may target –NH_2, –OH, glycosidic bonds, or their combinations, to form multifunctional chitosan derivatives. The formation of these derivatives not only achieved better solubility, electrostatic charge, porosity, and mechanical strength, but also maintain the main unique properties of chitosan. In recent years, many types of chemical modifications of chitosan have been reported, including: EDC-mediated modifications, radical-induced modifications, bromide-mediated modifications, and methods for the direct formation of Schiff bases. In this section, the mechanisms and characteristics of EDC/NHS coupling and bromide-mediated modification are discussed separately (Fig. 5).

4.2.1. Modification of EDC/NHS coupling

Chemical coupling reagents can promote the covalent attachment of carboxyl groups to chitosan amino groups, thereby improving the grafting efficiency. Among them, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) is the most commonly used coupling reagent [82]. As shown in Fig. 5(a), the coupling reaction of EDC/NHS between carboxyl-containing substances and chitosan. The reaction of EDC with carboxyl groups yields unstable O-acylisourea intermediates, which react with NHS to give semi-stable amine-reactive NHS-esters. The amines of chitosan react with NHS ester intermediates, resulting in stable amide linkages. Excess EDC can be easily removed during this reaction. Recently, EDC/NHS coupling has been widely used in the synthesis of chitosan derivatives. However, studies have shown that the chitosan modification of the EDC/NHS coupling is sensitive to pH control. Xu et al. [83] controlled the grafting rate by controlling pH in EDC/NHS-mediated synthesis of protocatechuate-chitosan derivatives, and the maximum grafting rate was achieved at pH 6.0. Zhang et al. [84] developed a non-migratory active packaging material by immobilizing CS covalently on the surface of polylactic acid (PLA) film through EDC/Sulfo-NHS coupling, and the results showed that it exhibited good antibacterial properties at pH 5.5, which effectively prolonged the shelf life of grass carp. Madison et al. [85] pointed out that for one-step amidation, the chosen pH value is slightly higher, usually pH 5.5–8.

Although EDC/NHS-mediated modification has a higher grafting rate, excessive addition of EDC and NHS may lead to the formation of insoluble complexes [86]. In addition, adding a large amount of chemical cross-linking agents (such as EDC, NHS and HOBt) is not only expensive but also pollutes the environment. Therefore, the reaction time as well as the reactants and pH must be strictly controlled during the reaction.

4.2.2. Bromide-mediated modification

Part of the halide can react with the hydroxyl group of the alcohol, so that the hydroxyl group is replaced by a halogen atom. This reaction has been widely used in organic synthesis. Lin et al. [87] synthesized geraniol chitosan derivatives (COS-O-Ger) by grafting chitosan oligosaccharide (COS) with geraniol (Ger) through substitution reaction and deprotection reaction. As shown in Fig. 5(b), the hydroxyl group on COS was replaced by geranyl bromide, and the benzaldehyde in the molecule was removed under the combined action of HCl and EtOH to realize the synthesis of COS-O-Ger. Mao et al. [88] proposed a bromide-mediated synthesis of chitosan derivatives, chemical modification of COS with citronellol (Cit). During the grafting process, the degree of bromination of Cit in anhydrous ether system reached 76.75 % at −5 °C. In the *N*, *N*-

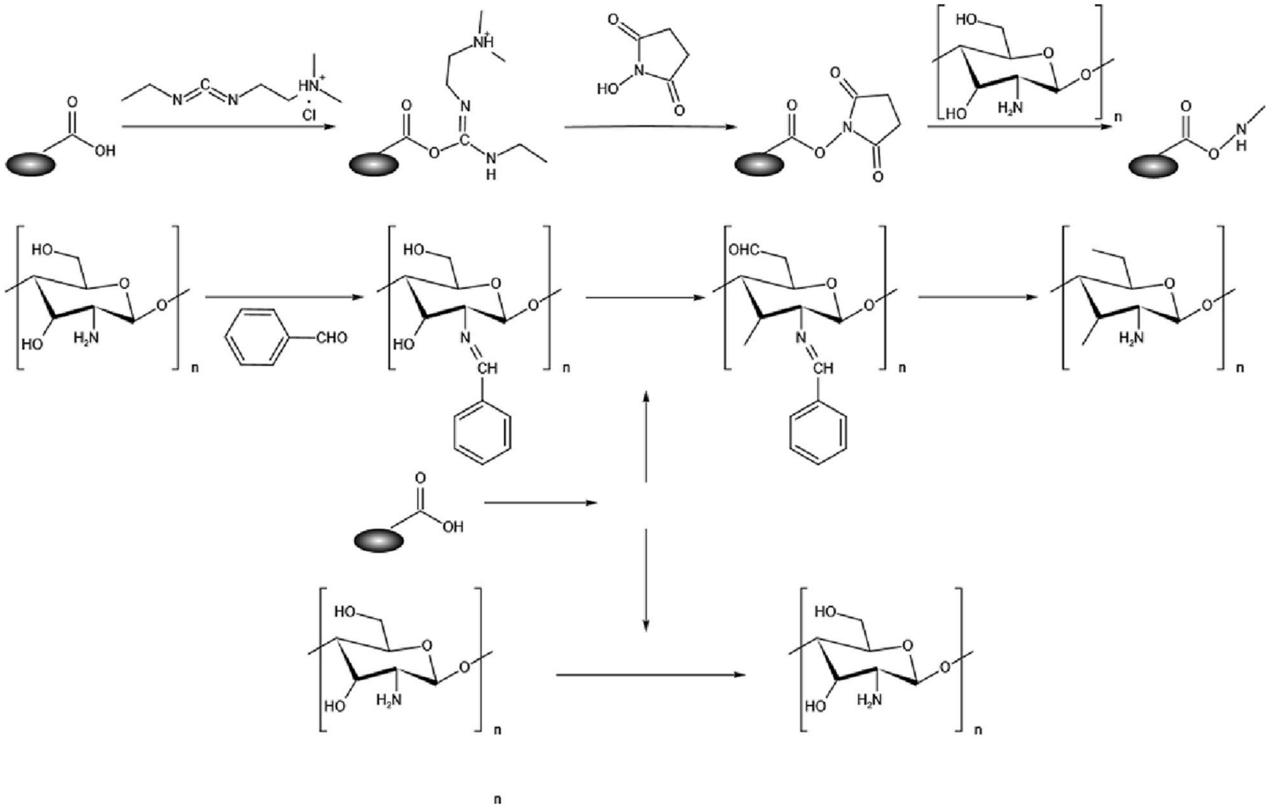

Fig. 5. Chemical modification of chitosan by different methods: (a) EDC/NHS-mediated process of grafting chitosan. (b) Bromide-mediated modification of chitosan: grafting on the hydroxyl and amino groups of chitosan, respectively.

dimethylformamide (DMF) system, the interaction of Cit-Br with -NH was successfully grafted onto COS to realize the synthesis of COS-g-Ger (Fig. 5(b)). Compared with COS-O-Ger, the synthesis of COS-g-Ger does not require the introduction and removal of protecting groups. When triethylamine was used as a catalyst, geraniol could form COS-N-Ger on the amino group directly grafted to chitosan (Fig. 5(b)). Furthermore, COS-N-Ger showed better antibacterial activity [89].

These bromide-mediated methods chemically modify chitosan to form chitosan derivatives with excellent antibacterial activity, anti-inflammatory activity, and biocompatibility, which have potential applications in the food industry. However, bromides are inherently toxic and the use of halogen atoms is not environmentally friendly [90]. The practical application value of this method in production needs to be further evaluated.

4.3. Biomodification of CS

In addition to physical and chemical modification, the application of biomodification in chitosan modification shows the advantages of selectivity, high efficiency and environmental friendliness [91]. Enzymatic synthesis and modification of polymers can eliminate harmful substances associated with reactive monomers, and this is very beneficial to human health and safety. Furthermore, the specificity of the enzyme can offer the possibility to accurately modify the polymer structure without losing any desirable polymer functionality. However, different enzymes are subjected to different immobilization methods, such as adsorption, covalent attachment, microencapsulation and affinity [92]. Among these methods, affinity immobilization techniques have shown advantages by exploiting the high affinity between ligands and enzymes. The successful immobilization of α-galactosidase on Concanavalin A-functionalized chitosan by using sugar-lectin affinity interactions have been reported, and it is proved to be effective for raffinose hydrolysis in a batch model system, which has been reported in the food industry [93]. Facin et al. [94] confirmed that the chitosan-grafted hydrogel technology containing immobilized enzymes can be used to produce lactose-free food and high-performance controlled enzyme release.

5. Application of functionalized chitosan in food

5.1. Food processing

In recent years, the demand for the safety and nutrition value of food products has grown greatly, and new materials or techniques are needed to meet the demand. Accordingly, functionalized chitosan has been widely used as a clarifying additive in food and beverages, an antibacterial agent and flavor enhancer in food, and a stabilizer for food texture [21].

The production of beverages such as wine, beer, tea and juices requires a clarifying step to remove their suspended solids. The application of functionalized chitosan to clarify food and beverage is listed in Table 1. The clarifying mechanism is that chitosan exhibits polycationic properties in acidic media and can interact with anionic compounds through electrostatic interactions, so chitosan can be used as a clarifying agent for food and beverages [9,10]. Chitosan can also interact with polyphenols, proteins, polysaccharide metal ions via hydrogen bonds and van der Waals forces to reduce suspended solids in food and beverages, thereby reducing turbidity [95]. In addition, the use of nanotechnology to encapsulate the magnetic nanoparticles with chitosan improves the aggregation of the magnetic nanoparticles, and at the same time promotes the immobilization of the enzyme by covalent bonding with the help of the reactive amino and hydroxyl groups on the chitosan molecule, significantly improved the activity and stability of the enzyme. The turbidity was significantly reduced in the clarification of food and beverage, and the immobilized enzyme still showed excellent reusability after repeated use [96–99].

Chitosan is one of the popular candidates for natural food preservatives in the food processing industry due to its excellent antimicrobial and antioxidant properties [100]. However, food matrices may limit the antimicrobial capacity of chitosan. Fernandes et al. [101] point out that the antimicrobial property of chitosan observed in apple juice are higher than that in milk, while adding chitosan to apple juice produces an odor, such as astringency and aftertaste-the amplitude of this flavor increases as the molecular weight of chitosan increases. This is because chitosan can only be dissolved in acidic solvents, and the use of acid chitosan in food may affect odor, taste and acid degradation. Chantarasataporn et al. [102] proposed water-based chitosan in the form of oligochitosan and nanowhisker chitosan as a novel food

Table 1
Application of functionalized chitosan to clarify food and beverage.

Polysaccharides	Additives	Beverage	Research findings	References
CS 0.03 %–0.1 %(*w*/*v*)	Pectinex 3× L	Passion fruit Juice	•Juice turbidity reduced by nearly 100 %.	[15]
CS 0.5 % (*w*/*v*)	Alginate Lignin	Apple juice; Grape juice; Orange juice; Pomegranate juice	•Juice turbidity decreased by 84.02 %, 57.84 %, 86.14 % and 82.13 % respectively.	[16]
CS-Magnetic Nanoparticles 0.25 % (*w*/*v*)	Rohapect 10 L	Grape juice; Apple juice; Orange juice	•100 % reduction in juice turbidity. •After 25 cycles of repeated use, the final residual activity of the enzyme was 85 %.	[96]
CS-Magnetic Nanoparticles 0.5 % (*w*/*v*)	Naringinase Pectinase	Grapefruit juice	•The juice turbidity was reduced by about 52 %, and the pomelo peel content was reduced by about 85 %. •The immobilized enzyme showed excellent reusability over 7 cycles. •The two enzymes retained 64 % and 85 % of their residual activity, respectively, after 30 days of storage.	[97]
CS-Magnetic Nanoparticles 3 % (*w*/*v*)	Tannase	Black tea; Green tea	•Turbidity decreased by 72.17 % and 47.2 %, respectively. •55.0 % enzymatic activity remaining after 8 cycles.	[98]
CS 1.5 % (*w*/*v*)	Calcium alginate Polydopamine	Apple juice	•The clarification ability of chitosan was retained. •The removal efficiencies of difenoconazole and empyrean were 66.5 % and 51.3 %, respectively.	[99]
CS 0.5 (*w*/*v*)	Moringa seeds	Green tea Extract	•The turbidity and solid content of polyphenols were reduced by 95 %, 16 %, and 18 %, respectively.	[95]

preservative, which does not require chemical modification or the use of organic solvents and exhibits good stability in the water as a colloidal solution. In the minced pork model, water-based chitosan effectively delayed the oxidation of biogenic amines, lipids and proteins in meat products, and extended their shelf life. This opened up new research ideas for chitosan as a suitable food safety preservative for processed meats such as sausages and minced pork.

Furthermore, some studies have demonstrated that functionalized chitosan can also be used as an encapsulant for flavor encapsulation, which can effectively maintain the flavor during food storage and processing, and meanwhile, maintain the desired structure and texture better [17,103]. Gong et al. [104] constructed emulsified microcapsules by using ultrasound-assisted cross-linking polymerization of chitosan and pectin. The aqueous solution of chitosan and pectin forms amide bonds to encapsulate the hydrophobic cinnamaldehyde, which endows the cinnamon flavor with thermal stability, making it more suitable for bakery products. Zhu et al. [105] microencapsulated vanilla essential oil with jackfruit seed starch, chitosan and β-cyclodextrin, and their experiments proved that chitosan has storage stability and sustained release potential as an encapsulating agent. Therefore, the use of chitosan as the shell material for flavor encapsulation such as microcapsules also has good application potential in food industry processing.

5.2. Food packaging

As mentioned in the previous sections, there are a large number of amine groups in the chitosan chain formed by the deacetylation of the chitin structure, and the properties of chitosan such as antibacterial, antioxidant and solubility are related to its deacetylation degree. The higher the DD, the stronger the electrostatic repulsion between chitosan chains, which inhibits flocculation, and the higher the viscosity of high DD chitosan, which would also help prevent particles from coalescence by slowing down film drainage [106]. In addition, the antibacterial activity of chitosan films is also related to DD, and chitosan with higher DD is generally considered to have higher antibacterial activity [31]. On the other hand, the unique ion-binding properties of chitosan make it possible to combine with polymers, metal ions or nanoparticles to form complexes as active ingredients, which can be used in active and antibacterial food packaging to preserve food products effectively [107]. The gelling or crosslinking properties are also one of important reasons for the applications of chitosan in food packaging. Once the gelled chitosan is formed into a film, it exhibits good water and moisture resistance, and the film becomes a material suitable for food packaging [108].

Chitosan is a unique natural biopolymer, its biodegradability and edibility make it a hotspot in food packaging applications, usually in the form of packaging films and food coatings [109]. This section discusses some of the research works on chitosan films and coatings in food packaging applications. Fig. 6 summarizes some representative chitosan films or coatings based on different formulations and functions.

5.2.1. Application of flexible chitosan packaging films

The methods for preparing flexible chitosan films include: melt extrusion [110], layer-by-layer assembly [111], coating [112] and direct casting [113] as shown in Fig. 7. Among them, the solution casting method is one of the most widely used methods for preparing chitosan-based films. Briefly, this method is mainly to dissolve, mix chitosan and other components, and pour the prepared solution into plates, and then dry the composite under certain temperature, humidity to evaporate the solvent and finally pick the films out of plates [114]. However, the application of pure chitosan in food packaging is limited due to its high sensitivity to humidity and moisture. Furthermore, chitosan cannot be directly processed by industrial methods such as extrusion and molding, because it is not thermoplastic and will degrade before the melting temperature. To overcome these limitations, scientists have made countless attempts, the main research directions are as follows: mixing chitosan with other natural or synthetic polymers, or adding active and functional substances, such as metal ions, fillers, plasticizers, cross-linking agents, natural essential oils, which can effectively improve the application value of chitosan in food packaging. [10,115–118].

The mechanical properties of chitosan composite films were enhanced by adding various plasticizers and metal nanoparticles, such as copper oxide (CuO) and zinc oxide (ZnO). In a recent study, the function of chitosan films was enhanced by adding ZnO nanoparticles

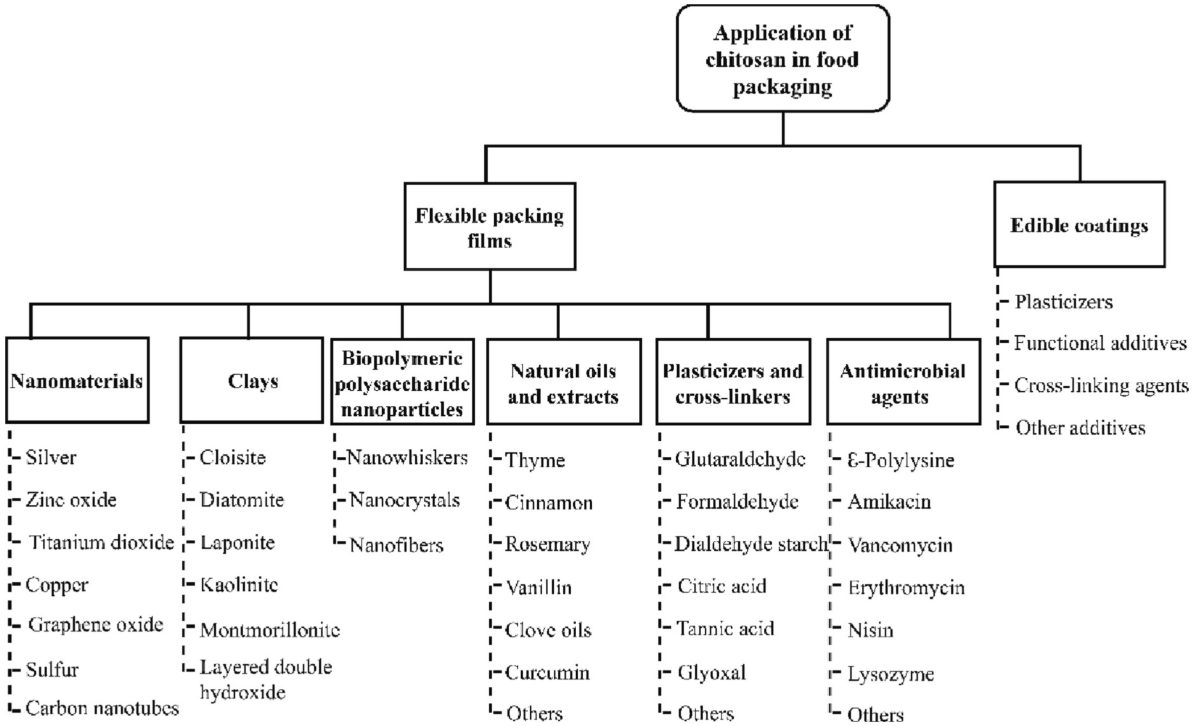

Fig. 6. Chitosan films or coatings based on different formulations and functions.

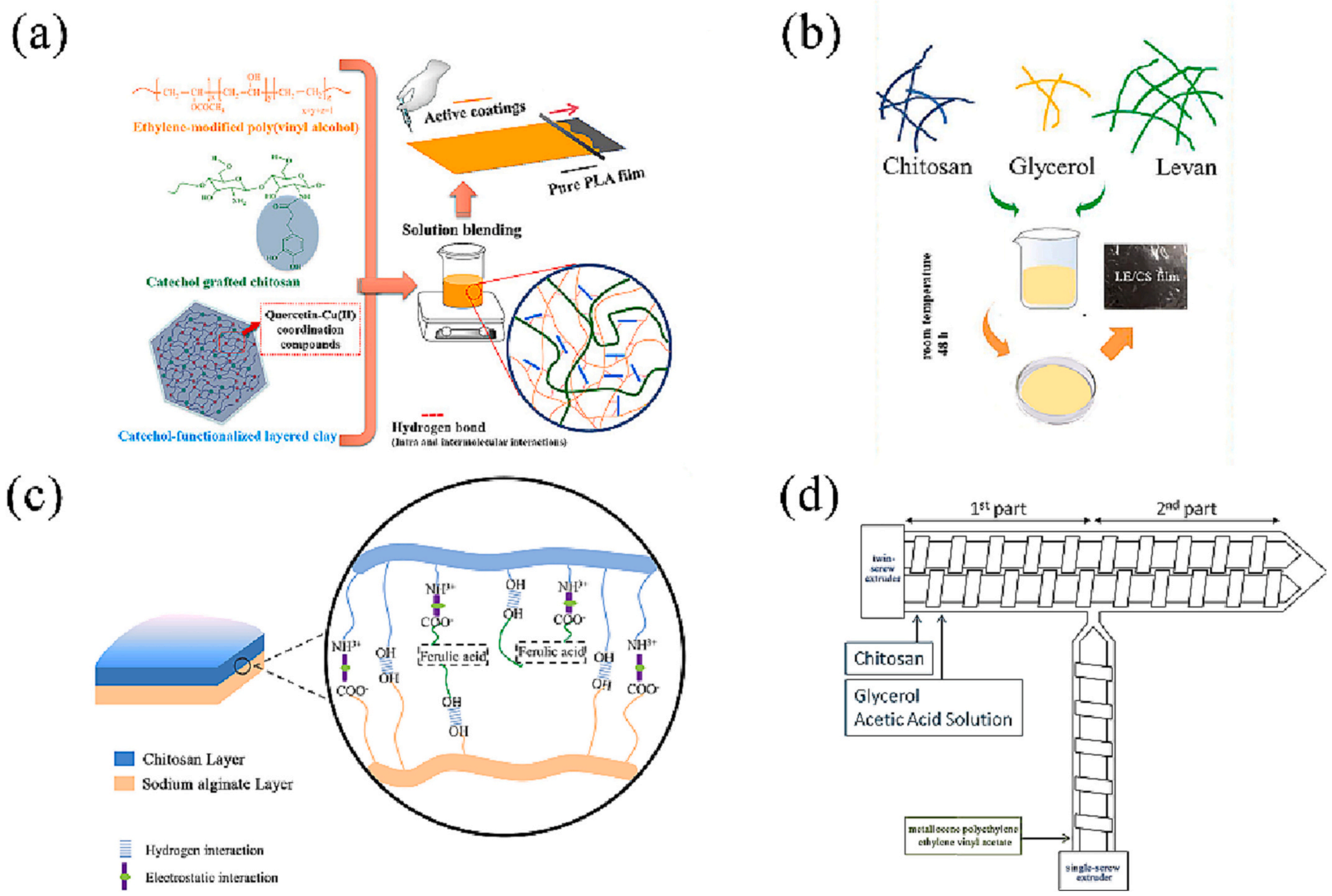

Fig. 7. Schematic diagrams of chitosan composite films prepared by different methods: (a) coating [112], (b) Direct casting [113], (c) Layer-by-layer assembly [111], (d) one-step extrusion [110].

and antioxidants from bamboo leaves (AOB), the addition of the former enhanced its antibacterial activity and the latter remarkably improved its antioxidant activity. Structural characterization results showed that chitosan, ZnO nanoparticles and AOB had good compatibility with each other, thus improving the mechanical strength and light transmittance of chitosan films. Meanwhile, the agar diffusion method was used to confirm that the synergistic effect of AOB and ZnO nanoparticles enhanced the antibacterial activity of chitosan films against *Escherichia coli* and *Staphylococcus aureus* [119]. Benucci et al. [120] proposed an ordered intercalation structure with strong interfacial adhesion and ionic bonds in chitosan-clay dispersions, which improved the mechanical properties of chitosan films. This was also confirmed by Rodrigues' research by adding ZnO and montmorillonite to increase the tensile strength and elongation at break of the chitosan films [121]. The addition of montmorillonite promoted the dispersion of ZnO in the chitosan matrix, and ZnO could reduce the interfacial defects and promote the coupling between chitosan molecules, thereby improving the tensile strength and elongation at break of the film. Unlike clay, some natural reinforcing materials (such as cellulose, lignin) can not only improve the mechanical performance of chitosan films, but also have potential biological activity. Vijayakumar et al. [122] prepared chitosan-based lignin nanoparticle nanocomposite films, and pointed out that when the concentration of lignin nanoparticles was 15 %, the water vapor transmission rate of the composite films decreased, the tensile strength increased, and the UV-blocking properties increased with increasing lignin nanoparticle concentration. In addition, the antioxidant and antibacterial activity of the composite film were also significantly enhanced, making it a potential active food packaging material (Fig. 8 (B—C)) [11]. Some studies have tried adding essential oils into chitosan films, which also imparted biological activity to the films to improve the shelf life of food, and the expected results have been achieved so far [10–13]. Gasti et al. [14] developed chitosan-ZnO hybrid nanoparticles loaded with clove essential oil and incorporated them into a chitosan-pullulan composite matrix to prepare high-sensitivity antibacterial and antioxidant film. The composite membrane has improved barrier properties, enhanced mechanical properties, and antioxidant activity, as well as strong antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The experimental results showed that the shelf life of chicken can be extended by up to 5 days when stored at 8 ± 2 °C (Fig. 8).

Food safety has always been a concern of consumers, and these developed chitosan films not only have the advantages of small size, lightweight, high safety, and low cost, but also can detect changes in food during storage (such as pH, microbial enzymes and microbial metabolism), effectively extending the shelf life of food, and showing good potential in smart food packaging.

5.2.2. *Edible coatings*

The coating is a method of preserving and storing food by spreading, spraying and impregnating chitosan solution. The coating is an effective way to limit microbial growth and extend the shelf life of food products while maintaining their safety and quality. Currently, many works have introduced chitosan-based edible coatings on unprocessed or lightly processed fruit, vegetable, fish and meat products, including the addition of fillers, antioxidants, antimicrobials, essential oils [123–137]. Studies have confirmed that the chitosan-based composite coating can inhibit the respiration rate, rot index and weight loss of fruits and vegetables during storage, and enhance the antioxidant and antibacterial

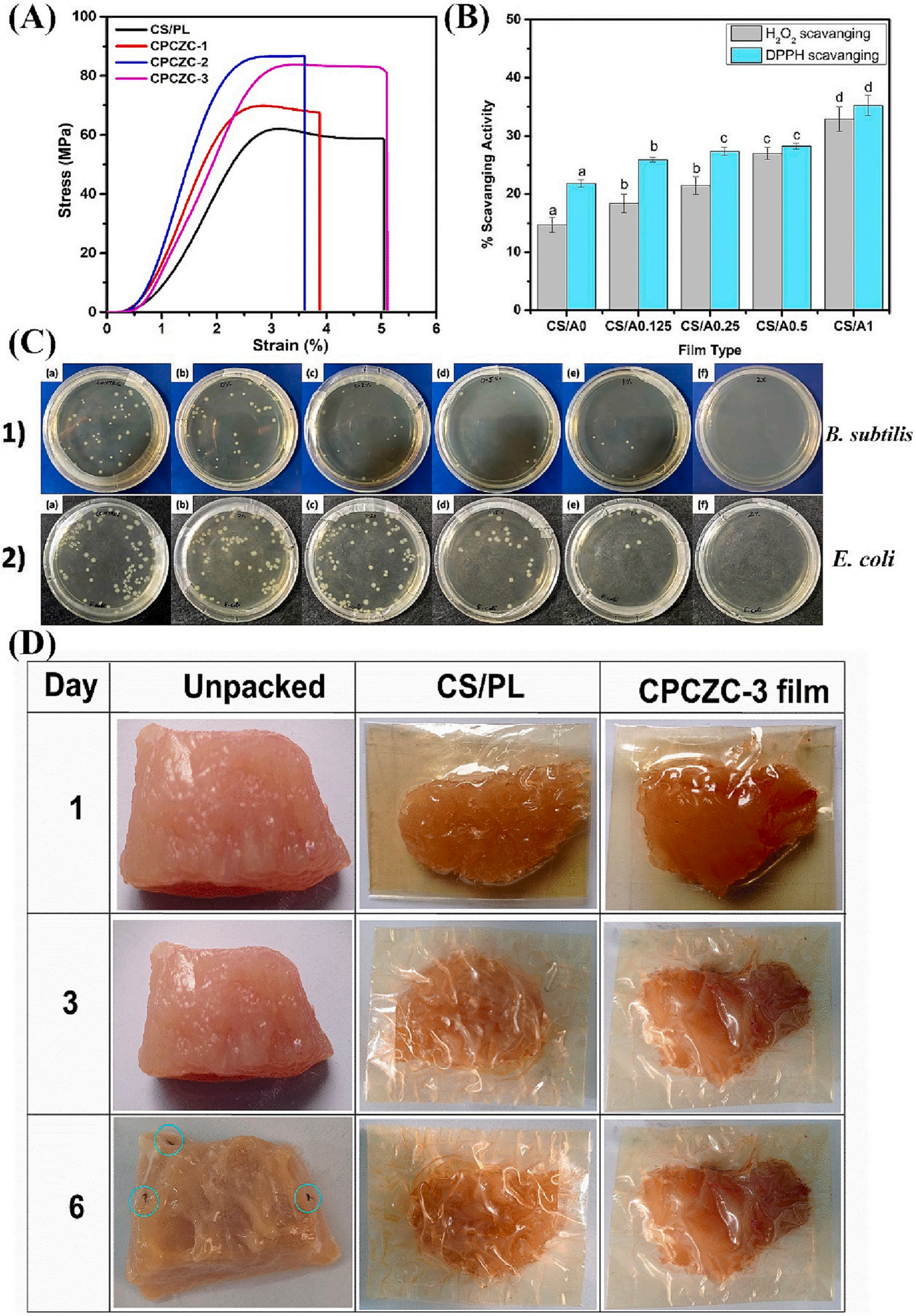

Fig. 8. (A) Stress-strain curve of prepared CS/PL and CPCZC films. (B) Antioxidant activity of the neat and modified chitosan films. (C) Total bacterial colony counts of *B. subtilis* and *E. coli*, for control (a), CS/A0 (b), CS/A0.125 (c), CS/A0.25 (d), CS/A0.5 (e), and CS/A1 (f). (A0, A0.125, A0.25, A0.5, A1 indicate that the ratio of CS to Apricot kernel essential oil was 1:0, 1:0.125, 1:0.25, 1:0.5, 1:1 (*w*/*v*)) [11]. (D) Chicken packaging test at 8 ± 2 °C [14].

activity. The research of Luksiene et al. [8] confirmed that the chlorophyll chitosan coating can effectively reduce the loss of water, color, titratable acidity and ascorbic acid content of strawberries and presented high antibacterial ability, preservation test showed that the shelf life of strawberry was extended by 3 days. The study of Xing et al. [7] mixed chitosan with nano-TiO_2 to prolong the shelf life of the whole mango. In addition, the chitosan composite coating has a strong inhibitory effect on the lipid oxidation process during the storage of meat products. Shen et al. [9] used chitosan as the carrier and trilogy can phosphate (TPP) as the crosslinking agent to prepare chitosan nanoparticles by ion crosslinking method, and then load curcumin (Cur) to get curcumin nanoparticles (CMC-CNPs). The experiments showed that the CMC-CNPs edible coating had excellent antioxidant capacity, and the application on fresh pork showed that the coating had a significant inhibitory effect on lipid oxidation. It can be effectively used in the packaging of fat-rich foods. Table 2 briefly summarizes the application of chitosan composite coatings in protecting food quality and shelf life.

These studies support the potential role of chitosan coatings in extending the shelf life of foods and delaying food spoilage. However, in the modification of its structure or function, care should be taken not to affect its edibility, appearance acceptability and environmental friendliness, so that it has better application value in food preservation.

5.3. *Food ingredients*

As a food-grade hydrophilic biopolymer, CS has been widely used as an antimicrobial agent in the food industry [138,139]. Khan et al. [140] evaluated nisin-loaded chitosan-monomethyl fumaric acid (CM-N) nanoparticles as a novel direct food additive. The results confirmed that CS-N had good antibacterial properties, and the bacterial count in orange juice was significantly reduced. In addition, the excellent emulsifying properties of chitosan and mucosal adhesion enhance the bioavailability of encapsulated compounds. Chitosan-based encapsulation systems protect labile compounds such as terpenes, carotenoids, and anthocyanins and probiotics from environmental conditions and have potential applications in functional beverages [141,142]. Huang et al. [143] developed flaxseed oil and quercetin co-loaded liposome-chitosan hydrogel, which effectively enhanced photostability and improved the solubility of lipophilic nutrients. In vitro simulated digestion has shown that hydrogel beads can improve the stability of gastrointestinal liposomes, inhibit the rapid release of fatty acids. This work provided a research basis for the development of functional foods with nutritional bioactive substances.

Many diet foods based on chitosan are continuously reported [144,145]. For example, chitosan can react with acidic polysaccharides (such as carboxymethyl cellulose, pectin and carrageenan) to form polysaccharide complexes with fleshy tissue structures, which can be processed into artificial meat. This kind of food provides health functions to some extent. They can improve human immunity, discharge excess fat, and prevent obesity [145].

Table 2
Chitosan-based composite coatings for extending the shelf life of foods.

Biopolymer	Additives	Application in Food	Main effects of the coating	References
CS 1 % (w/v)	Nano TiO$_2$	Mango	• The fruit rot index of the composite coating decreases, delayed the peak respiration and enhances the hardness of the fruit. The total soluble solids content was reduced, peroxidase and polyphenol oxidase activity, and total phenol and flavonoid content were higher than the control group.	[7]
CS 1 % (*w/v*)	Silver nanoparticles	Melon	• The respiration rate of the fruit treated with the composite coating was reduced, the total vitamin C content was the highest, and the shelf life of the fruit could be extended by more than 13 days at 5 °C.	[124]
CS 1 % (w/v)	Montmorillonite	Tangerine fruits	• The composite coatings showed more efficient and prolonged effects to maintain the nutrient content, and to reduce the water loss, respiration rate as well as accumulated decay rate of fruits	[123]
CS 1 % (*w/v*)	Chlorogenic Acid	Peach	• The firmness, soluble solids, titratable acidity and L-ascorbic acid content of peaches remained well, and inhibited the increase of weight loss, decay index, and respiration rate of peaches stored at 20 °C for 8 days.	[126]
CS 1 % (*w/v*)	Ascorbic acid	Plums	• The composite coating significantly reduced weight loss, maintains fruit firmness, membrane integrity, inhibits increased respiration rate, reduces color characteristics, and reduced pectin methylesterase and polygalacturonase acid activity. • Coating significantly increased superoxide dismutase, peroxidase, and catalase activities and decreased polyphenol oxidase activities and superoxide free radicals and malondialdehyde content, which reduced oxidative stress.	[127]
CS 1.5 %(*w/v*)	Citric acid Licorice extract	Japanese sea bass	• The composite coating could preserve the quality of fish fillets by inhibiting lipid oxidation and microbial growth and thus extend the shelf life.	[128]
CS 2 % (*w/v*)	Gallic acid	Fresh pork	• The composite coated samples had low levels of lipid oxidation and myoglobin oxidation and exhibited proprotein oxidation effects.	[135]
CS 0.4 %(w/v)	ε-polylysine Ascorbic acid	Pork	•Coating treated pork inhibited bacterial growth, increased pH and total volatile alkaline nitrogen (TVB-N) and thiobarbituric acid reactive species, decreased red index of pork nuggets, and prolonged shelf life.	[136]
CS 2 % (*w/v*)	ε-polylysine Sodium alginate	Farmed pufferfish	•Coating protected the myofibrils of farmed puffer fish and inhibited the growth of microorganisms.	[129]
CS 0.5 %(*w/v*)	Alginate	Japanese pear	•Effectively reduced fruit respiration and ethylene productivity, inhibited the loss of pulp firmness, and prevented peel color change for 21 days at 20 °C.	[130]
CS 1 % (*w/v*)	Alginate Resveratrol powder	Sea bass fillets	•Coating inhibited microbiological growth and retarded oxidation of fillets.	[131]
CS 1 % (*w/v*)	Chlorophyllin	Strawberries	• The composite coating in the presence of light prolonged the shelf-life of strawberries by 3 days without any negative impact on the visual quality and color, saving weight losses, water content and antioxidant activity.	[8]
CS 1 % (w/v)	Glucose	Grapes	• The composite coating reduced the rot, weight loss and respiration rate of grapes, effectively maintained the fruit texture such as berry hardness, elasticity, cohesion and chewiness, improved the postharvest quality of table grapes, and extended the shelf life of grapes.	[132]
CS 0.05 % (*w/v*)	Byrsonima crassifolia extract	Bell pepper	• The composite coated samples showed a 30 % the weight loss and 15 % the change of color after 21 days of storage, the phenolic content, carotenoids and reducing capacity were increased by 18 % and the microbiological activity was reduced by 85 %.	[133]
CS 2 % (w/v)	Monolaurate	Grass carp fillets	•Coating inhibited microbial growth, nucleotide breakdown, formation of alkaline components and textural deterioration, and were effective in maintaining sensory acceptability across groups.	[137]
CS 1 % (*v/v*)	Curcumin Tripolyphosphate	Pork	• The composite coating could significantly inhibit the lipid oxidation of fresh pork, and improved the quality and shelf life of fresh pork stored at 4 ± 1 °C for 15 days.	[9]
CS 2 % (w/v)	*Thymus capitatus* essential oil	Strawberry	• The composite coating delayed the loss of physicochemical and antioxidant properties, due to protection against the microbial development of aerobic mesophylls, molds, and yeasts.	[125]
CS 1.5 % (*w/w*)	Rosemary Oregano essential oils	Goat‘s milk cheese	• The composite coating improved the microbial safety of the cheese, reduced the lipolytic and proteolytic activities, and the cheese had a better sensory evaluation in terms of aroma and flavor.	[134]

5.4. Safety concerns of functionalized chitosan

Although chitosan has been classified as "generally recognized as safe" by the U.S. Food and Drug Administration [146]. After functionalizing and modifying chitosan, food contact materials with excellent antibacterial, mechanical, barrier and other functional properties can be prepared to extend the shelf life of food and maintain the high quality of food. Migration in food materials is seen as a mass transfer activity through which low molecular weight integrators of contact materials are released into the food [147]. Therefore, the development of new food packaging materials requires investigating migration events to ensure food safety and integrity. At present, many studies have discussed migration studies of functionalized chitosan complex materials, and the results show that the migration amount is relatively low compared to other migration rates and is below the acceptable limit [147–149]. However, there have been few studies on the in vivo toxicity of chitosan complex materials to humans and animals after application in food. Therefore, more research is needed to comprehensively elucidate the migration of chitosan complex materials to food, its mechanisms, diffusion processes, toxicological and morphological characteristics to determine risk assessment and food safety for food.

However, studies have shown that when animals and humans ingest chitosan, the number of intestinal flora in the digestive system changes. Egan et al. [150] found a decrease in the number of *Firmicutes* and *Lactobacillus spp* in pigs fed chitosan. In humans, two weeks of chitosan intake in the gut significantly increases the number of *Bacteroides*, which is associated with various soft tissues and other infections, so an increase in the number of *Bacteroides* may be harmful to health [151]. In summary, although functionalized modified chitosan has the potential as a high-quality food ingredient, its potential harm to animals and humans also needs to be further confirmed by researchers.

6. Conclusion and prospects

This review provides the application of functionalized chitosan in food. The excellent biocompatibility, biodegradability and modifiability of functionalized chitosan make its structure and application more diversified. However, there are still many problems and challenges to be solved in the transition from the laboratory stage to industrial production. (1) In the preparation of chitosan-based composites, the use of chemical reaction reagents should be avoided to reduce human poisoning caused by food contamination. (2) At present, most functionalized chitosan derivatives face high production cost and lack of versatility. Therefore, researchers still need to explore and develop economical and functional chitosan for food-related applications. (3) Although a large number of functionalized chitosan derivatives have significant chemical, biological and functional advantages over unmodified chitosan, the exact mechanism of their Significant and the assessment of safety risks remain mysterious.

However, research on functionalized chitosan offers valuable options for food processing, food packaging and food ingredients. With the requirements of environmental protection and sustainable development, it will provide broad prospects and hope for the application of functionalized chitosan in food. We can boldly speculate that functionalized chitosan is a promising candidate for a future alternative to non-degradable plastics.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work was supported by the Plan of Action for Scientific and Technological Innovation of Science and Technology Commission of Shanghai Municipality (23N31900200).

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