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Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives

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ABSTRACT

Background: Demand for healthy and safe food with minimal use of synthetic inputs (including synthetic preservatives) is increasing rapidly. Plastic polymers being hazardous to the environment, significant efforts have been devoted to evaluate various bio-based polymers as alternatives to synthetic plastic packaging. Chitin and its deacetylated derivative, chitosan, is primarily a by-product of crustacean, fish and seafood processing and handling. Chitosan possesses antimicrobial activities and film forming property, making them attractive biopolymers for food packaging and food preservation applications applied through spraying, dipping, coating, or wrapping by films.

Scope and approach: This comprehensive review of contemporary research focuses on applications of chitosan and chitosan based nanocomposites in the area of food packaging and preservation. It includes different properties and functionalities of chitosan, various blends and nanocomposites of chitosan, their fabrication techniques, and applications in shelf life extension of fruits, vegetables, meat and fish products.

Key findings and conclusions: Chitosan is an attractive alternative to synthetic plastics polymers due to its biodegradability, antimicrobial activity, and film forming properties. Incorporation of nanomaterials into chitosan based food-packaging systems can prevent the growth of spoilage and pathogenic microorganisms, improve food quality and safety, and extend shelf-life of food. It has been reported that applications of chitosan-based films or coatings or treatments have resulted in shelf life extension of fresh produce, meat products, bread, and dairy products such as cheese which has been highlighted.

1. Introduction

Consumer demands for varieties of food throughout the year, and preference for convenience have encouraged unprecedented growth of new developments in food packaging to ensure availability of safe and healthy food. The primary function of food packaging is to separate food item(s) from the surrounding environment minimizing or preventing exposure to spoilage factors including the effects of microorganisms, oxygen, temperature and humidity to avoid or delay loss of quality and nutrition, thus extending the shelf-life. However, food packaging systems perform several other important functions such as providing convenience, communication with consumers, and marketing of the packaged product. Petroleum-based synthetic plastic(s) have been dominating the food packaging industries consisting 37% of the total market for food packaging materials due to their comparatively low cost, low transportation, lighter weight, high mechanical strength and rigidity, good barrier properties, ability for heat sealing, and shape versatility (Muller, González-Martínez, & Chiralt, 2017). However, these synthetic plastics have harmful effects on the environment (Cox et al., 2019). Healthy and safe food free from synthetic chemicals (preservatives) has become one of the key challenges for food manufacturers and food businesses. Over the last decade, consumers' increasing concerns for health and environment have intensified the focus of researchers on the use of bio-based packaging materials as an alternative to synthetic plastic polymer(s) in food packaging (Rodríguez-Rojas, Arango Ospina, Rodríguez-Vélez, & Arana-Florez, 2019). Such biodegradable alternatives include polysaccharides such as cellulose, starch, chitosan, agar, and proteins such as casein as potential replacements to plastic polymers (Fabra, López-Rubio, & Lagaron, 2014). Bioplastics degrade in the presence of appropriate moisture and temperature conditions and availability of oxygen without producing any toxic residues. Some biopolymers degrade in just a few weeks, whereas

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degradation of synthetic polymers takes several years, depending on the type and origin of the polymer (Barboza, Dick Vethaak, Lavorante, Lundebye, & Guilhermino, 2018; Hasırcı, Lewandrowski, Gresser, Wise, & Trantolo, 2001).

Antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed, safe food that are free from synthetic preservative(s). Antimicrobial packaging comprises, packaging technique(s) such as modified atmosphere packaging or packaging materials containing antimicrobial agents such as natural antimicrobials, nanomaterials, etc., used to control growth and to reduce activities of microbes. The primary goals of an antimicrobial packaging system are; food safety assurance, food quality maintenance and shelf-life enhancement. In recent year, various efforts have been made to develop biopolymer based antimicrobial packaging materials to improve food shelf life and quality. In biopolymers, chitosan (CS) has been emerging as a promising material for antimicrobial applications in food industry as it is biodegradable, nontoxic and biocompatible, as well as lower cost and abundant availability in nature (Perinelli, Fagioli, Campana, Lam, Baffone, Palmieri, et al., 2018; Silberbauer & Schmid, 2017). Blended films of chitosan with other biopolymers such as polysaccharides or proteins have been fabricated using solution-casting, layer-by-layer, extrusion and other techniques, and studied for their physicochemical, functional and antimicrobial properties for applicability as food packaging materials. Chitosan composites with numerous natural antioxidants, antimicrobial components, and nanomaterials have also attracted significant research focus in recent years. The main themes of this review include the recent advancement in chitosan and chitosan based nanocomposite, their fabrication and applications in food packaging and preservation for shelf life extension of fruits, vegetables, meat and fish products (Fig. 1).

2. Chitosan and its properties

2.1. Source and structure

Chitin is the second-most abundant polysaccharides on Earth after cellulose and consists of three kinds of reactive functional groups, an amino group at C-2, and both the primary and secondary hydroxyl groups at the, C-3, and C-6 positions, respectively. Chitin is mainly available in marine invertebrates and insects as a major constituent of the exoskeleton, and in certain fungi as a component of the cell walls. Marine invertebrates such as crabs, shrimps, lobsters and oysters are consumed every year as sources of protein rich sea food. However, the external shells and other non-edible parts of these crustaceans constitute about half of the body mass, and are usually discarded as waste (Hamed, Özogul, & Regenstein, 2016). These discarded wastes are prominent source of chitin.

Fig. 1. Various food packaging applications of chitosan.

Chitosan (CS) is a polysaccharide of N-acetyl D-glucosamine and D-glucosamine units and it is mainly obtained by the partial deacetylation of chitin (Fig. 2) (Kumar, Ye, Dobretsov, & Dutta, 2019a; Shahidi, Arachchi, & Jeon, 1999). Chitosan is commercially obtained through partial deacetylation of chitin, leading to the formation of N-acetyl-glucosamine and D-glucosamine copolymer. Chitosan is a soluble form of chitin, and has been used in various industrial applications including uses in food preservation and packaging (Kausar, 2017; Kumar, Ye, Dobretsov, & Dutta, 2019b; Mihai & Popa, 2015).

2.2. Degree of deacetylation (DDA) and solubility

The degree of deacetylation (removal of an acetyl group) is one of the significant chemical characteristics of chitosan. During deacetylation, the acetyl (-C_2H_3O) group is replaced by amino (-NH_2) group from the polymer chain resulting in the formation of N-acetyl-glucosamine and D-glucosamine copolymer. Copolymers with more than 50% D-glucosamine units are typically termed as chitosan. The degree of deacetylation (DD) of chitosan is defined as the ratio of D-glucosamine units to the sum of D-glucosamine and N-acetyl D-glucosamine units present in the chain. Thus, 70% DD of chitosan would mean that the chitosan contains 70% of D-glucosamine units and 30% of N-acetyl-glucosamine units in the polymer chain. The solubility of chitosan mainly depends on the pH of the solution and the DD of chitosan. The pKa value of amino group in chitosan is about 6.3, thus chitosan is soluble in slightly acidic solutions (Szymańska & Winnicka, 2015). Amino (-NH_2) groups of chitosan is protonated upon dissolution at pH 6 or below, and it forms cationic amine groups (-NH_3^+), increasing intermolecular electric repulsion and resulting in a polycationic soluble polymer.

2.3. Antimicrobial activity

Chitosan is a natural biopolymer that has good antimicrobial activity against various microorganisms such as Gram-positive and Gram-negative bacteria, filamentous fungi and yeast (Hosseinnejad & Jafari, 2016; Y.; Pan, Huang, Shi, Zhan, Fan, Pan, et al., 2015; Raafat & Sahl, 2009). Many researchers have demonstrated the antimicrobial properties of chitosan, but the actual mechanisms behind it is not yet clear (Raafat & Sahl, 2009). Several mechanisms responsible for antimicrobial activities has proposed but the most acceptable ones are – (i) interaction of negatively charged microbial cell membranes with positively charged amine groups in chitosan altering the membrane barrier properties, that lead to leakage of intracellular contents, and ultimately cell death (Hosseinnejad & Jafari, 2016). (ii) The second proposed mechanism is based on chelating properties of chitosan. Chitosan selectively binds with metals, inhibiting various metabolic enzymes of microbial cells by blocking their active centres, and reducing microbial growth. (iii) Molecular weight of chitosan can also influence antimicrobial action (C. Qin et al., 2006; Zheng & Zhu, 2003). High-molecular-weight chitosan can form an impervious polymeric layer on the surface of the microbial cell changing the cell permeability and ultimately blocking the entry of nutrients into the cell, whereas, low-molecular-weight chitosan could penetrate into the cytosol, and bind with DNA of the cells, resulting in interference with the synthesis of mRNA and proteins leading to cell death (Hosseinnejad & Jafari, 2016).

In acidic conditions (pH $>$ 6.3), polycationic nature of chitosan arises from the positively charged amino groups of the chitosan polymer which then interact with negatively charged components on

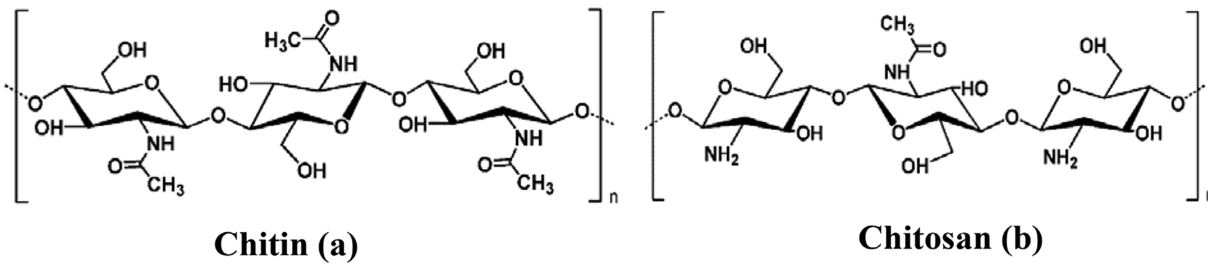

Fig. 2. Chemical structure of (a) chitin and (b) chitosan.

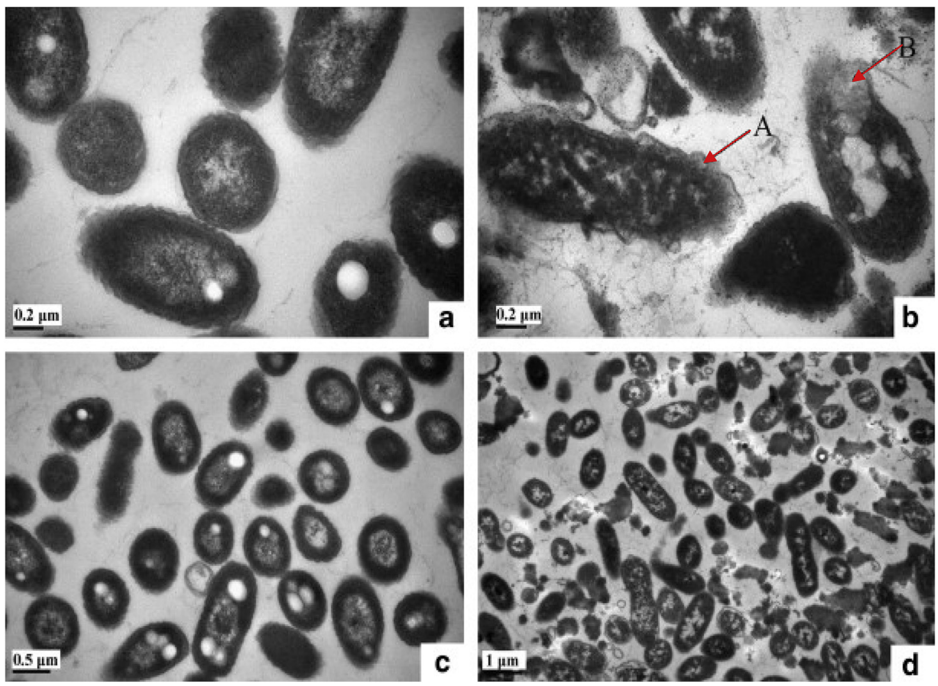

Fig. 3. TEM images of *Burkholderia seminalis* treated with (a, c) buffer (control) and (b, d) 2.0 mg/mL chitosan; (A: additional layer; B: membrane damage); Reprinted with permission (Lou et al., 2011).

microbial cell membranes that lead to cell death, which is the most accepted mechanism (X.-f. Li et al., 2010). This hypothesis was further supported by Lou et al., 2011, investigating the antibacterial activity of chitosan against apricot fruit rot pathogen *Burkholderia seminalis* (Fig. 3). They suggested that due to electrostatic interaction, the positively charged chitosan adhered to the negatively charged microbial cell membrane (carbonyl and phosphoryl groups of the phospholipid components) resulting in leakage of bacterial cell membranes, and rupture of bacterial cell (Lou, Zhu, Muhammad, Li, Xie, Wang, et al., 2011). The antimicrobial activity of chitosan is mainly influenced by molecular weight (MW) and degree of deacetylation (DD) of chitosan among several other physico-chemical properties (Kumar et al., 2019b; Verlee, Mincke, & Stevens, 2017).

2.4. Antioxidant activity

Food produces are frequently exposed to different ways of oxidative deterioration. Antioxidant activities of chitin, chitosan, and their derivatives are well-documented (Xie, Xu, & Liu, 2001), wherein free-radical scavenging ability and chelation of metal ions give these biopolymers their antioxidant activities (Anraku, Gebicki, Iohara, Tomida, Uekama, Maruyama, et al., 2018; Yen, Yang, & Mau, 2008). Many researchers have reported significant improvements in antioxidant activities, including free-radical scavenging and metal ion chelation with increased degree of deacetylation of chitin (Sabaghi, Maghsoudlou, & Habibi, 2015). In view of increased consumer concerns regarding synthetic antioxidant(s) as food additives, chitosan itself has found application as alternative antioxidant(s) in food formulations (Ngo & Kim, 2014; Sabaghi et al., 2015).

The antioxidant values of chitosan can be further improved by adding natural antioxidants derived from plant polyphenols (Genskowsky et al., 2015; Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, & Fernández-López, 2013; Siripatrawan, 2016). The polyphenolic compounds in green tea (*Camellia sinensis*) e.g. catechins, theaflavins, and the arubigenes, have good antioxidant activities. Siripatrawan and Harte (2010), reported that chitosan films (2% v/v of chitosan with 95% degree of deacetylation in 1% acetic acid) incorporated with green tea extract showed improved mechanical properties (e.g. tensile strength, elongation at break) of the composite film (Siripatrawan & Harte, 2010). Relatively poor moisture barrier properties of chitosan film was also reported to improve upon the incorporation of plant polyphenols like tea extract, as these antioxidant compounds interact and establish hydrogen and covalent bonds with reactive groups of chitosan (L. Wang, Dong, Men, Tong, & Zhou, 2013). Several other publications documented effectiveness of antioxidant activity of chitosan films or coatings incorporated with many other plant-based flavonoids e.g. procyanidins from grape seed extract (Mannozzi et al., 2018), pomegranate peel extract (Alsaggaf, Moussa, & Tayel, 2017), apple peel extract (Riaz, Lei, Akhtar, Wan, Chen, Jabbar, et al., 2018), and thyme extracts (Talón et al., 2017). Essential oil (EO) components are aromatic, bioactive, antioxidant terpenoids derived from plant-based sources which has been extensively studied as natural food preservative(s), but with limited application, as their addition may compromise desirable flavor of the food item (Siripatrawan, 2016). However, thymol from thyme, carvacrol from thyme and oregano, cinnamaldehyde from cinnamon, and eugenol from clove are common EO-derived components that are evaluated by many researchers as natural food preservative(s) (Yuan, Chen, & Li, 2016). Numerous publications have reported improved antioxidant activities of chitosan films or coatings as a result of incorporation of terpenoids in EO components that exert free-radical scavenging, transition-metal-chelating, singlet-oxygen-quenching activities, and/or act as hydrogen donors (Valizadeh, Naseri, Babaei, Hosseini, & Imani, 2019; T.; Xu et al., 2019).

3. Chitosan and its blend with other biopolymers

Several biopolymers have been studied for the development of biodegradable, edible, and antimicrobial films for their food packaging applications (Elsabee & Abdou, 2013). Such biopolymers include starch, cellulose, their derivatives, gums, proteins (animal or plant-based) and lipids (Muxika, Etxabide, Uranga, Guerrero, & de la Caba, 2017). In order to apply chitosan in the form of films and coatings, and in order to improve its functionality as a food packaging material (Zhu, Wu, & Sun, 2019), it has to be blended with some other biopolymers such as polysaccharides, proteins, and lipids. In general, polysaccharide blends have several advantages compared to blends with protein(s) and/or lipid(s), as the films are of low material cost, the resources for the films are abundant, they are relatively stable, and have better heat sealability and water solubility (K. Li, Zhu, Guan, & Wu, 2019).

3.1. Chitosan blends with other carbohydrates

Chitosan is blended with different polysaccharides such as alginates, starch, carrageenan, pectin, and even bacterial cellulose. Both chitosan and alginate are polysaccharides of aquatic origin, compatible to each other, and the blend(s) between the two are more suitable for food packaging application(s) than blends of chitosan with protein or lipid polymer(s) (K. Li, Zhu, et al., 2019). Alginates are polyanionic, linear, binary copolymers of β-(1–4) linked D-mannuronic acid and α-(1–4)-linked L-guluronic acid (Dumont, Villet, Guirand, Montembault, Delair, Lack, et al., 2018). Alginates gel via ionotropic gelation at pH values below the pK_a of alginate (acid gelation). Considering the electrostatic association of chitosan and alginates, several researchers exploited the polyelectrolyte complexation occurring when both polyelectrolytes are charged. However, for large-scale manufacturing and applications, blending is the most effective and efficient way of film formation. Dumont et al. (2018) used wet-spinning process to coat alginate fibers with chitosan, followed by subsequent characterization of the blended film, including its antimicrobial properties. Blends of chitosan and alginate possess biodegradability, biocompatibility, low toxicity and low immunogenicity. Such blended film possesses good barrier properties against lipids and gases, while it is relatively inferior barrier against water vapor (Elsabee & Abdou, 2013).

Starch from different sources such as rice, corn, and cassava are most well-studied in blends with chitosan (Bourtoom & Chinnan, 2008). It has been found that starch with higher amylose content blends well due to stable hydrogen bond formation with chitosan (Y. X. Xu, Kim, Hanna, & Nag, 2005). Both the studies used starch gelatinization followed by solution casting in chitosan solution to prepare the blended film. 40% sorbitol or 25%(w/w) glycerine, respectively, were added to the blended polymer as a plasticizer to improve mechanical properties of the blended films. Beside blends, chitosan-coated starch film was also prepared and surface characteristics such as hydrophobicity, mechanical properties such as tensile strength, thermal stability, functionalities such as water vapor transmission rate (WVTR), and other barrier properties were studied (Elsabee & Abdou, 2013). In general, increasing chitosan content in the blend improved tensile strength, while increased starch content led to increase in elongation at break of the blended film.

Carrageenan is anionic linear polysaccharides extracted from red seaweed. This polysaccharide has already been applied as coating(s) on fresh fruits and vegetables to minimise oxidative deterioration and moisture losses (Lin & Zhao, 2007). Like alginates, carrageenan also interact with chitosan to form a polyelectrolyte complex (Spagnuolo, Dalgleish, Goff, & Morris, 2005). Majority of research on this blend of chitosan has been focused on encapsulation applications in food packaging and pharmaceuticals. A few studies on films developed by κ-carrageenan-chitosan complex reported poor thermal stability and inferior moisture barrier properties of the blended films (Shahbazi, Rajabzadeh, Ettelaie, & Rafe, 2016). Perhaps, this has been the basis of lack of published literature on application of such blended films for food packaging. However, κ-carrageenan-chitosan blending in an organic acid solvent system (e.g. in acetic, lactic, citric, and ascorbic acids) has been reported to improve tensile strength and barrier properties of the blended film due to polyelectrolyte behaviours of κ-carrageenan and chitosan with opposite ionic charges (El-Hefian, Nasef, & Yahaya, 2010; Park, Lee, Jung, & Park, 2001).

Both chitosan and pectin have been explored at length for their film forming and subsequent applications of such films. However, blending of the two natural polymers has been studied as a mechanism to address the challenges of poor barrier and mechanical properties of the individual biopolymer films (Younis & Zhao, 2019). It has been reported that although chitosan-pectin blended film has better appearance (transparency) and mechanical properties, the blending does not influence the barrier properties. Beside these traditional polysaccharides, chitosan-blended films of with some of the 3rd generation carbohydrate polymers, including poly lactic acid and microbial cellulose, are being studied for their potential application(s) in food packaging (Andrade-Del Olmo, Pérez-Álvarez, Hernáez, Ruiz-Rubio, & Vilas-Vilela, 2019; Panariello, Coltelli, Buchignani, & Lazzeri, 2019; Ye, Wang, Lan, Qin, & Liu, 2018). Olmo et al. (2019) used layer-by-layer method to fabricate multilayer, blended film by depositing chitosan and β-cyclodextrin incorporated with carvacrol on PLA blended with ZnO nano particles. Ye et al. (2018) blended tea polyphenol and chitosan with PLA film prepared by stretching method. The former reported increased antimicrobial effect, while the latter reported enhanced heat-sealing of the blended film.

3.2. Chitosan blends with proteins

Chitosan-based blended films with protein biopolymers such as collagen, gelatin and caseinate are quite well-studied. Gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility, and their blends i.e. both chitosan-gelatin blends and chitosan-gelatin bi-layer film, have been studied (M. Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011). Both types of blending result in films showing improved barrier properties against water vapor permeability, and lead to significant improvement in elongation at break. For blending with chitosan, gelatin from fish or seafood or bovine origin has been most commonly used (Elsabee & Abdou, 2013). Solution casting of aqueous solutions of chitosan and gelatin (at 60 °C and pH 4) is a simple technique for preparation of the blended film.

Collagen has unique ability to form insoluble fibers with high tensile strength and stability making its application(s) appropriate in cosmetic, pharmaceutical, tissue engineering and biomedical industries (Bhuimbar, Bhagwat, & Dandge, 2019). Traditionally, collagen is a meat processing by-product, but has been derived from fish processing wastes in recent years (Silva et al., 2014). Like chitosan-gelatin blend, chitosan-collagen blended film can be prepared by solution casting method using 1:1 mixture of the two biopolymers. The blending occur at molecular level, as hydrogen-bond network alters collagen structure to completely mix with chitosan (Sionkowska et al., 2006). However, the blended film show reduced mechanical properties compared to collagen films, and hence have limited food packaging applications.

Chitosan being a positively charged polysaccharide, forms complex with carboxylic groups of casein through electrostatic interaction (Celli, Ravanfar, Kaliappan, Kapoor, & Abbaspourrad, 2018). Simple solution-casting method is useful for preparation of chitosan-sodium caseinate blended films, while addition of glycerol as plasticizer in the blend improves mechanical properties such as tensile and impact strength of the films significantly (Mariana Pereda, Aranguren, & Marcovich, 2007). Chitosan-casein blended films are often used as edible food packaging material (Elsabee & Abdou, 2013). Beside excellent thermoformability and film-forming properties, this blend also possesses improved barrier against moisture (H. Wang, Qian, & Ding, 2018).

4. Polymeric antimicrobial composites of chitosan and its blend

Chitosan itself has good antimicrobial activities against several types of microorganisms (Verlee et al., 2017). However, especially for food preservation and packaging applications, further improvement in antimicrobial properties of chitosan is desirable. In order to enhance its antimicrobial properties, natural antimicrobials and antimicrobial nanostructures (metal and metal oxide nanoparticles) have been used (Matharu, Ciric, & Edirisinghe, 2018).

4.1. Natural antimicrobials

Natural antimicrobials are mainly obtained from animal, plant, and microbial sources (T. Huang, Qian, Wei, & Zhou, 2019). Natural antimicrobials are biocompatible and biodegradable in nature, and have low toxicity, and their antimicrobial activity are comparable to the synthetic counterpart, exhibiting antimicrobial activity against broad range of microorganisms including spoilage microorganisms. Natural antimicrobials are used in food for three main reasons: to control natural spoilage processes (i.e., food preservation), to maintain/improve nutritive value (i.e., food quality) and to minimise/stop the growth of microorganisms (i.e., food safety) (Davidson, Cekmer, Monu, & Techathuvanan, 2015; Fortunati, Mazzaglia, & Balestra, 2019). The most commonly used commercially available natural antimicrobials include plant-derived essential oils, antimicrobials derived from microbiological sources (e.g., bacteriocin) and derived from animals (e.g., lysozyme from egg cell) (T. Huang et al., 2019). In fact, natural antimicrobials can help to maintain appearance, taste, quality, and enhance shelf life by preserving nutrition profile of food.

Plants extract such as essential oil have excellent antioxidant activities, along with the antimicrobial properties, used in the fabrication of antimicrobial composites for food packaging and preservation application (Pisoschi et al., 2018). Essential oil mainly consists of low-molecular weight aliphatic and aromatic secondary metabolite in which terpenes (i.e., p-cymene, limonene, or pinene), terpenoids (i.e., thymol, carvacrol, or menthol), and phenylpropenes (i.e., eugenol, cinnamaldehyde, or vallinin) are major compounds. It has been reported that combining chitosan (CS) with essential oils (EOs) from clove, oregano, cinnamon, or eucalyptus enhanced antimicrobial activity in food packaging applications (Yuan et al., 2016). Such antimicrobial activity resulted in the development of chitosan-based active packaging concepts that reduced, inhibited, or delayed growth of microorganisms on food surface or in food, increasing its postharvest/post-manufacturing shelf life (Ponce, Roura, & Moreira, 2016). In Table 1, we have summarized the published reports of preservation of many food items such as fresh fruits (Kumar, Boro, Ray, Mukherjee, & Dutta, 2019), mushroom (Jiang, Feng, & Li, 2012), sliced bread (Passarinho et al., 2014), and muscle food products such as pork (Lu, Shao, Cao, Ou, & Pan, 2016). Franssen & Krochta (2003) also reported that combining polypeptides such as lysozyme, peroxidase, lactoferrin, and nisin with edible coating/film/spray/dip of chitosan may further extend shelf life of food products and/or enhance their safety.

4.2. Metal and metal oxide nanostructures based antimicrobials

Metal nanoparticles, such as silver (Ag) and copper (Cu) nanoparticles are well established broad spectrum antimicrobial agent due to its high efficiency against Gram-negative and Gram-positive bacteria, fungi, protozoa, and certain viruses (Hoseinnejad, Jafari, & Katouzian, 2018; Kumar, Bhattacharya, Singh, Halder, & Mitra, 2017; Kumar, Singh, Halder, & Mitra, 2016; Kumari, Brahma, Rajak, Singh, & Kumar, 2016). Antimicrobial nanomaterials can electrostatically bind with microbial cell walls to change the membrane potential leading to membrane damage and consequently to impaired respiration, imbalance of transport, interrupted energy transduction and cell lysis that can eventually lead to cell death (Pelgrift & Friedman, 2013). Another mechanism for nanostructured antimicrobial is based on the production of reactive oxygen species (ROS) by nanomaterials. Dependent on the physico-chemical properties of nanomaterials as well as the types of bacteria, some nanostructure can stimulate inflammation that could chemically/catalytically convert less toxic oxidants, such as superoxide and hydrogen peroxide, into more reactive free radicals, such as hydroxyl radicals. In addition, upon interactions with light as one of the environmental factors, a variety of semiconductor nanomaterials can generate ROS through the reaction between excited electron-hole and water molecules, resulting in the toxicity to microorganisms. ROS can cause severe oxidative stress, leading to DNA/RNA damage, lipid peroxidation, protein alteration, enzyme inhibition that lead to microbial cell death (X. Pan et al., 2010; Sathe, Richter, Myint, Dobretsov, & Dutta, 2016). Other effects of antimicrobial nanomaterials involve direct inhibition of essential microbial enzymes, production of nitrogen reactive species (NRS) and induction of programmed cell death (Dizaj, Lotfipour, Barzegar-Jalali, Zarrintan, & Adibkia, 2014).

Particle size, shape, surface charges, and surface groups of nanostructures mainly influence the ROS generation and antimicrobial activity. Smaller sized nanostructures can easily penetrate cell membranes and other biological barriers. The amount of uptake decreases with the increase in particle size (Dizaj et al., 2014; Sathe et al., 2017). Furthermore, release of metal ions from nanomaterials, such as Ag^+, Cu^+ and Zn^{2+} ions, can react with molecular oxygen generating superoxide radicals and other ROS, which then exert antimicrobial effects (Joe et al., 2018).

In the bottom-up approach of silver nanoparticles (AgNPs) synthesis, chemical and biological methods including green synthesis have been mostly applied for food applications (Roy, Gaur, Jain, Bhattacharya, & Rani, 2013; Swargiary, Mitra, Halder, & Kumar, 2019). AgNPs can also be synthesized in-situ in chitosan matrix. Chitosan has good affinity toward Ag^+ ions due to existence of amine and hydroxyl groups, and are capable to reduce Ag^+ ions into AgNPs under alkaline conditions (Boufi, Vilar, Ferraria, & Botelho do Rego, 2013; Lu, Lu, Zou, Liu, Rong, Li, et al., 2017). Metal oxide nanoparticles are attractive for a large variety of industrial applications. Metal oxide nanomaterials are commonly used as nano-filler into biopolymer matrix, basically to improve the antimicrobial activities, mechanical and barrier properties of the films (Dizaj et al., 2014; Galstyan, Bhandari, Sberveglieri, Sberveglieri, & Comini, 2018). Metal oxide nanoparticles such as zinc oxide (ZnO), titanium dioxide (TiO_2), copper oxide (CuO), have been reported to have good antibacterial activity, owing to the generation of reactive oxygen species (ROS) (André, Zamperini, Mima, Longo, Albuquerque, Sambrano, et al., 2015; Dizaj et al., 2014; Joe et al., 2018; Sathe et al., 2017). ZnO and TiO_2 are photocatalytic materials, and have been extensively used as antibiofouling agents (Al-Naamani, Dobretsov, Dutta, & Burgess, 2017; Kumar et al., 2019b). They promote peroxidation of the phospholipids present in microbial cell membranes leading to loss of membrane integrity. A summarized list of metal and metal oxides nanoparticles that have been commonly used in antimicrobial nanocomposites for food packaging applications is given in Table 2.

Table 1
Natural antimicrobial compounds applied in chitosan-based composite for antimicrobial food packaging.

Natural antimicrobial	Concentration applied	Tested microorganisms	Applied on food item(s)	References
Agro-industrial residue (AIR) extracts	AIR - 0.84% and 1.90% (v/v) and CS - 2% (w/v)	Mesophilic aerobic counts and Psychrotrophic aerobic counts	Chicken product	Serrano-León, Bergamaschi, Yoshida, Saldaña, Selani, Rios-Mera et al. (2018)
Apple peel polyphenols (APP)	APP - 0.25, 0.50, 0.75 and 1.0% (w/w) CS - 2% (w/v)	*Bacillus cereus, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus*	Food packaging	Riaz et al. (2018)
Apricot Kernel essential oil (AKEO)	CS:AKEO; 1 : 0–1 (w/v) CS- 2% (w/v)	*Bacillus subtilis, E. coli,* and fungi	Sliced bread	Priyadarshi et al. (2018)
Bamboo vinegar (BV)	BV - 2% (v/v) CS - 2% (w/v)	Lactic acid bacteria, Enterobacteriaceae spp, and *Pseudomonas* spp.	Ready to cook pork chops	(H. Zhang, He, Kang, & Li, 2018)
Cinnamon essential oil (CEO)	CS: CEO weight ratio of 1:0.8	Yeasts and molds, Enterobacteriaceae spp, *S. aureus,* Lactic acid bacteria (LAB)	Beef patties	Ghaderi-Ghahfarokhi, Barzegar, Sahari, Ahmadi Gavlighi, and Gardini (2017)
Cinnamon essential oil (CEO)	CEO - 10% (w/w) CS - 1% (w/v) GL - 1% (w/v)	*E. coli* and *S. aureus*	Food packaging	Guo, Chen, Yang, Wang, Ni, Chen, et al. (2019)
Custard apple leaves (CAL)	CAL - 2/10 (w/v) CS- 2.5%(w/v)	*Colletotrichum gloeosporioides*	Papaya fruit	Bautista-Baños, Hernández-López, Bosquez-Molina, and Wilson (2003)
Lemon essential oil (LEO)	LEO-3%(w/w) CS - 1.0% (w/v)	Fungus, *Botrytis cinerea*	Strawberry fruits	Perdones, Sánchez-González, Chiralt, and Vargas (2012)
Lysozyme (L)	L- NA CS- 2.75% (w/v)	Mesophilic, Psychrotrophic, LAB, yeasts and molds	Halloumi cheese	Mehyar, Al Nabulsi, Saleh, Olaimat, and Holley (2018)
Melissa officinalis essential oil (MOEO)	MOEO - 0.25 and 0.5% (w/v) CS -1.5% (w/v)	*E. coli*	Antimicrobial film	Sani, Pirsa, and Tağı (2019)
Nisin (N)	0.2% (w/w) nisin in 2% (w/v) chitosan solution	Determination of total viable count (TVC)	Pork loin	Cao, Warner, and Fang (2019)
Nisin (N)	N - 0.2% (w/v) CS - 1.5% (w/v)	*Lactobacillus* spp.	Mutton meat	He, Zou, Yang, Xia, Zhou, Zhu, et al. (2016)
Nisin (N)	N - 500 and 1000 ppm CS - 1.4% (w/v)	*Listeria monocytogenes*	Cheese	Divsalar et al. (2018)
Oregano essential oil (OEO)	OEO - 1.0% (v/v) Carboxymethyl chitosan - 1.0% (w/v)	*L. monocytogenes*	Raw chicken meat fillets	Khanjari, Karabagias, and Kontominas (2013)
Oregano essential oil (OEO)	OEO - 5, 10, or 15% on polypropylene sachets	Yeast and molds	Sliced bread	Passarinho et al. (2014)
Plum peel extract (PPE)	PPE - 25% (w/w) CS - 2% (w/v)	*E. coli, S. aureus, Salmonella, and L. monocytogenes*	Multifunctional food packaging	(XinZhang, Liu, Yong, Qin, Liu, & Liu, 2019)
Spirulina Extract (SE)	SE - 0, 2.5, 5, 10, 15 and 20% (w/v) CS - 2% (w/v)	*E. coli, S. aureus, P. aeruginosa, L. monocytogenes, S. typhimurium, B. subtilis* and *B. cereus*	Food packaging	Balti et al. (2017)
Thyme oil (TO)	TO - 1.5% (w/v) CS - 2% (w/v)	Yeasts and molds, *Pseudomonas* spp.	Shiitake mushroom	Jiang, Feng, and Zheng (2011)
Thyme essential oil (TEO)	TEO - 1% (v/v) CS - 2% (w/v)	NA	Rainbow trout fish	Chamanara, Shabanpour, Gorgin, and Khomeiri (2012)

4.3. Fabrication techniques for nanocomposite films/coatings

4.3.1. Solution casting

The solution casting method is one of the most widely used methods for preparing chitosan-based films and coatings because of its convenience. Solution blending is a process of dissolving chitosan and its copolymer in a solution, followed by evaporation (El-Hefian et al., 2010). It is a relatively economical and simple method, during which intermolecular electrostatic and hydrogen bond formation results in the polymer structure (Muxika et al., 2017). However, such intermolecular entanglement also makes the film brittle. Several plasticizers such as polyols like glycerol, sorbitol, and polyethylene glycol, sugars (e.g. glucose and sucrose), and lipids are added to improve mechanical properties of the films (Becerra, Sudre, Royaud, Montserret, Verrier, Rochas, et al., 2016; Rodríguez-Núñez, Madera-Santana, Sánchez-Machado, López-Cervantes, & Soto Valdez, 2013). Although, solution casting is a simple and relatively low-cost method, scaling-up of the film-forming process by this technique may pose challenge(s), and needs further development.

The fabrication process includes several steps: (1) dissolving chitosan in acid solution with planned pH (less than 6.0), (2) blending, mixing, or cross-linking with other biopolymer and functional materials/fillers (such as antimicrobials, antioxidants, plasticizes, etc.) at different volume or mass ratio, (3) stirring for obtaining homogeneous viscous solution, (4) solution filtration, sonication, or centrifugation for removing any remaining insoluble particles and air bubbles, (5) casting or pouring onto level surface (e.g., flat polystyrene tray, glass plates), (6) drying under set temperature, relative humidity, and time, (7) peeling off. Solution casting method is mainly used at laboratory scale to prepare films, and further research is needed in order to analyze the feasibility of its in commercial scale (Basumatary et al., 2018; Naskar et al., 2018).

4.3.2. Layer-by-layer assembly (LBL)

The LBL assembly is a versatile technique that has been extensively explored in nanocomposite films fabrication for efficiently controlling the material properties and functionality, because it can combine functional characteristics of different polymers. It is a method used for fabrication of multi-component films that does not need any sophisticated instrument. In LBL assembly, the surface modification mainly

Table 2
Commonly used metal and metal oxides nanostructures in chitosan based nanocomposites for antimicrobial food packaging.

Nanostructures	Concentration	Target MO	Findings	References
AgNPs	1%, w/w	*Staphylococcus aureus, Escherichia coli, Bacillus cereus, Ps. Aeruginosa* and *Candida albicans*	Interaction of Ag^{+} with negatively charged macromolecule leading to distortion of cell wall, protein denaturation and ultimately to cell death	Salari, Sowti Khiabani, Rezaei Mokarram, Ghanbarzadeh, and Samadi Kafil (2018)
AgNPs (laponite immobilized)	5% (w/w)	*E. coli, S. aureus, Aspergillus niger,* and *Penicillium citrinum*	Reported to show good antimicrobial activity	(Z. Wu, Huang, Li, Xiao, & Wang, 2018)
AgNPs	In-situ AgNPs synthesized	*E. coli, Salmonella typhimurium Aspergillus niger,* and *Penicillium citrinum*	Surface charge on chitosan influences adsorption of AgNPs and thus antimicrobial activities	Hajji et al. (2017)
ZnO NPs	In-situ ZnO NPs synthesized	*E. coli,* and *S. aureus*	Zn^{2+} ions and release of reactive oxygen species (ROS) damage bacterial cell wall	Rahman, Mujeeb, and Muraleedharan (2017)
ZnO NPs	2% (w/w)	*E. coli,* and *C. albicans*	ZnO nanoparticles improved the antimicrobial properties of chitosan	(Y. Wang, Zhang, Zhang, & Li, 2012)
ZnO NPs	0.2–1.0% (w/v)	*S. aureus*	The quality of pork meat during cold storage was reported to improve	Suo et al. (2017)
TiO_2 NPs	10% (w/w)	*E. coli, S. aureus, C. albicans*, and *Aspergillus niger*	Showed higher antimicrobial activity against gram negative than gram positive bacteria, possibly due the thinner cell wall in gram negative bacteria	(Xiaodong Zhang, Xiao, Wang, Zhao, Su, & Tan, 2017)
TiO_2 NPs	25% (w/w)	*E. coli, S. aureus, Salmonella, and L. monocytogenes*	Nanocomposite showed good antioxidant, ethylene scavenging and antimicrobial properties	(Xin Zhang, Liu, Yong, Qin, Liu, & Liu, 2019)
Cu NPs	0.08, 0.10 and 0.12% (w/w)	*Alternaria solani,* and *Fusarium oxysporum*	Nanocomposites found effective to decrease Early blight and Fusarium wilt	Saharan, Sharma, Yadav, Choudhary, Sharma, Pal, et al. (2015)
CuO NPs	1% (v/v)	*E. coli, Pseudomonas aeruginosa,* and *Listeria monocytogenes*	Antimicrobial properties against both Gram-positive and Gram-negative bacteria reported	Almasi, Jafarzadeh, and Mehryar (2018)
CuO NPs (MMT-CuO) nanocomposite	1, 3 and 5% (w/w)	*E.coli, P. aeruginosa, S. aureus, and B.cereus*	Composites showing strong antibacterial activity against food-borne microorganisms reported	Nouri, Yaraki, Ghorbanpour, Agarwal, and Gupta (2018)

depends on the deposition and mutual attraction of alternating polyelectrolytes with opposite net charges onto solid support (Khomutov, 2004; Tu et al., 2019; D.; Zhang, Jiang, Sun, & Zhou, 2017). The polyelectrolytes can be biopolymers such as proteins, polysaccharides, synthetic polymers, etc., that hold net charge. Deposition can be achieved either by submersion of the substrate into alternate polyelectrolyte solutions or by spraying of solutions onto the substrate. Both methods have potential for scalability as high throughput coating technologies. The electrical charge could be changed by pH, which significantly affects the amount of deposition of the polymers, as more biopolymer molecules are required to neutralize the previous layer. LBL deposition can be used to prepare active packaging films and coatings by the incorporation of active agents either between layers or within the structure of an individual polyelectrolyte (Guzmán, Mateos-Maroto, Ruano, Ortega, & Rubio, 2017; Kerch, 2015; Rouster et al., 2019).

4.3.3. Extrusion

Extrusion methods are mainly used for the production of conventional commercial plastic packaging films. Extrusion is often preferred over solution casting methods due to faster processing time and lower energy consumption (Estevez-Areco, Guz, Famá, Candal, & Goyanes, 2019; Hong & Rhim, 2012; Wang, Zhang, et al., 2018). There are various steps involved in extrusion process; preparation of formulations using different composition of raw materials and their mixing, blending the mixture in a extruder in order to pelletize all film-forming ingredients, cutting extrudates into pellets through the pelletizer, drying pellets in a hot-air oven, followed by extruding the pellets into sheets through the second extruder, and finally blowing the mixed resins into a film by a blown film extruder. Extrusion process often provides the films with acceptable mechanical properties and good thermal stability. Although extrusion is a promising approach for the films fabrication, there are a limited number of studies related to the use of this technology on chitosan film (Matet, Heuzey, Ajji, & Sarazin, 2015).

4.3.4. Coatings/spraying

Coating is often applied on the surface of fresh foods such as fruits and vegetables, fish, meat, etc. for enhancing their shelf-life (Vargas, Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2012). Coatings can be performed by spreading, spraying, dipping, or immersing of food materials into chitosan based composite solutions. Spread-coating is performed with the help of some tools, like brush or spatula. The coating is an effective way to restrict the growth of microorganisms that leads to shelf life extension, and thus help maintain the quality of food (Dong, Cheng, Tan, Zheng, & Jiang, 2004; Pushkala, Raghuram, & Srividya, 2013; Yu, Jiang, Xu, & Xia, 2017). The coating process involve several steps; formulation of raw materials by using appropriate proportion of chitosan and fillers such as antioxidants, antimicrobials, strengthening agents, etc.; coating sample preparation with treatment such as mixing, heating, irradiating, and steam flash pasteurizing; disinfection of food samples using solution of sodium hypochlorite; spreading chitosan-based composite solutions onto food to form uniform coating using sterile spreader; drying in certain conditions, such as laminar-flow under ventilation; packaging and storing in ambient or other appropriate conditions, such as vacuum, refrigerated, controlled humidity, and/or other temperature condition(s) (Pushkala et al., 2013). The coating in contact with food surface can influence permeability of gas and the antimicrobial component(s) can gradually migrate from the films onto the food surface, thus offering concentrated protection from external deteriorating factors (Kashiri et al., 2017).

Dipping and/or spraying is a method used for formation of coating/edible coating, in an active packaging system. Dipping techniques involve submerging the food in an already-prepared, acidic chitosan film-forming solution, which may be incorporated with natural preservative(s) and plasticizer(s) to improve effectiveness of the applied coating/film (Alsaggaf et al., 2017). The coating is normally done by immersing or dipping the food in the developed composite solutions for a few minutes to tens of minutes, followed by the drainage of excess solution and subsequent drying of the samples by using forced-air dryers, temperature and humidity controlled chambers or food dehydrators or simply by drying the food using air fans. Various dipping procedures have been used to coat food items with one or several layer(s), but in some reports, double-dipping were recommended over three or more dipping. The dipping or immersing method is simple and convenient, as it does not require sophisticated equipments, and also offers high efficacy of food preservation. Spray-coating is involves similar steps like spread coating except spraying is achieved using compressed air-assisted sprayer (Meng, Qin, & Tian, 2010). Spraying chitosan-based film-forming solution involves application of the treatment solution on food surface using aerosol sprayer (Leceta, Molinaro, Guerrero, Kerry, & de la Caba, 2015; Meng et al., 2010; Wang, Sha, et al., 2018). Although dipping and spraying techniques are simple, relatively inexpensive, and widely applicable in many food processing lines, such treatments may compromise sensory attributes of the treated foods (Muxika et al., 2017). Thus, judicious use of this technique to apply chitosan-based biopolymer coating/film on food is highly recommended.

5. Food packaging applications of films and coatings

5.1. Shelf-life extension of fruits and vegetables

Agricultural produce is perishable in nature and in-particular most of the horticultural commodities have a very short postharvest life. Fruits and vegetables exhibit continuous respiration and transpiration even after harvesting, resulting in short shelf life and loss of quality. The ripening of fruits are considered very complex process, and characterized by intense changes in physiological and biochemical parameters such as, chlorophyll degradation, enzymatic cell wall degradation, changes in sugar content, respiratory activity, ethylene production, and in the levels of aromatic compounds (Lee & Hwang, 2017; Perotti, Moreno, & Podestá, 2014). In addition to that, reactive oxygen species (ROS) are produced during electron transport in the mitochondria that can directly damage cells and reduce the quality of fruits and vegetables, mainly under stress or during fruit ripening when it is released in excess (Ali et al., 2019; Perotti et al., 2014). Therefore, various researchers have searched for natural non-toxic substances that can slow down the process of maturation or reduce the decay of fruits and vegetables after harvest (Table 3). One such study reported by Martínez et al., 2018 on application of edible chitosan based coating containing *Thymus capitatus* essential oil that extended shelf life of strawberry fruit for up to 15 days in cold storage (K. Martínez, Ortiz, Albis, Gilma Gutiérrez Castañeda, Valencia, & Grande Tovar, 2018).

Wrapping/coating of biopolymer-based edible film is an inexpensive, simple, and effective method for avoiding moisture loss thus reducing deterioration and respiration rate. In particular, chitosan is gaining importance especially in food packaging application for fabrication of films and coating due to their inherent antimicrobial activity, film forming ability, and biodegradability (Harish Prashanth & Tharanathan, 2007; Kumar et al., 2019b). Nowadays, chitosan are potentially used in many industrial applications, like food, agriculture, cosmetics, textiles, biomedical, environmental protection, etc. (Hamed et al., 2016; Kumar et al., 2019b). Chitosan based composite incorporated with nanomaterials and/or natural antimicrobials have been found to be very useful in improving shelf life, and maintaining quality of postharvest produce (Xing, Li, Wang, Li, Xu, Guo, et al., 2019). The incorporation of nano-fillers into the chitosan matrix is an effective approach to overcome the drawbacks of poor mechanical and barrier properties, thermal stability, and low transparency, associated with the biopolymer. The addition of nano-fillers (having dimension within 100 nm) to natural biopolymers result in improvement of physicochemical and biological properties. It has been observed that chitosan coating reduces weight losses, firmness and increases antioxidant activity as well as soluble solid contents in fresh fruits and vegetables

Table 3
Chitosan and chitosan based antimicrobial films/coatings for shelf life extension of fruits and vegetables.

Chitosan/chitosan composite film	Concentration of Chitosan and Nano-filler	Fruits/Vegetables	Beneficial effects	References
CS coatings	CS – 0.5% (w/v)	Papaya fruits	Chitosan solutions extended shelf-life of papaya fruits by about 4–7 days, during storage at room temperature	Dotto, Vieira, and Pinto (2015)
CS coatings	CS - 1 to 3%(w/v)	Guava fruits	Chitosan effectively prolonged the quality attributes in guava fruits after harvesting due to increased antioxidant processes, delaying the ripening during room temperature storage	Batista Silva, Cosme Silva, Santana, Salvador, Medeiros, Belghith, et al. (2018)
CS/montmorillonite (MMT)	CS – 1.0% (w/v) MMT - 0.5, 1 and 2% (w/w)	Tangerine fruits	Coating improved shelf-life of tangerine fruits	(D. A. N. Xu, Qin, Ren, & Yu, 2018)
CS-chlorophyllin (Chl) coating	CS – 1.0% (w/v) Chl - 0.05% (w/w)	Strawberries	Coating increased the postharvest-life of strawberries by 3-days	Luksiene and Buchovec (2019)
CS/Gelatin/AgNPs films	CS – 1.8% (w/v) AgNPs - 0.1% (w/w)	Red grapes	Composite film extended shelf-life of red grapes by 14- days	Kumar, Shukla, Baul, Mitra, and Halder (2018)
CS/ascorbic acid (AsA) coatings	CS - 1.0% (w/v) AsA – 40.0 mM	Plums	Coating improved shelf-life and maintained quality of plums during storage	(K. Liu, Yuan, Chen, Li, & Liu, 2014)
CS/Poly-vinyl-pyrrolidine (CS/PVP)/SA	CS – 1.0% (w/v) PVP – 1.0% (w/v) SA - 2 and 4 mM	Guava fruits	Composite treatment improved antioxidant activities and delayed fruit browning	Lo'ay and Taher (2018)
CS/TiO_2 films	CS - 2.5% (w/v) TiO_2 - 10% (w/w)	Red grapes	Red grapes upon wrapping with chitosan film lasted for 15-days, whereas when nanocomposite films were used, mildew occurred on fruit surface after 22 days	(Xiaodong Zhang, Xiao, Wang, Zhao, Su, & Tan, 2017)
CS/TiO_2 films	CS - 2.0% (w/v) TiO_2 -1.0% (w/w)	Tomato fruit	Film delayed the ripening process and changes in quality parameters of tomatoes	Kaewklin, Siripatrawan, Suwanagul, and Lee (2018)
CS/nano ZnO coatings on polyethylene films	CS - 2.0% (w/v)	Okra or Ladyfinger	Chitosan composite coating increased shelf-life of Okra from 5-days to 12 days under room temperature storage	Al-Naamani, Dutta, and Dobretsov (2018)
CS/Bacterial cellulose nanocrystals (BCNC)/ AgNPs films	CS - 1.0% (w/v) AgNPs –1.0% (w/w)	–	Addition of BCNC and/or silver nanoparticles leading to improved physical, mechanical properties and antimicrobial properties of chitosan film	Salari et al. (2018)
CS/Polyurethane (PU)/nano ZnO films	CS/PU ratio - 0.25: 0.75 ZnONPs – 1.0, 3.0 and 5.0% (w/w)	Carrot pieces	Carrot pieces wrapped in composite films showed extended shelf-life up to 9-days	(K, M.P, & G.R, 2019)
CS/Sulfur nanoparticles (SNP) films	CS – 2.67% (w/v) SNP- 2.0% (w/w)	–	Composite films can potentially be used as antimicrobial food packaging material	Shankar, Pangeni, Park, and Rhim (2018)
CS/Pullulan	CS- 0.5% (w/v) Pullulan- 0.5% (w/v)	Papaya	CS/pullulan coatings efficiently improve shelf life and maintained the postharvest quality of fresh papaya	(L. Zhang, Huang, & Zhao, 2019)
CS/Heat treatment	CS- 1.0% (w/v)	Plum Fruits	Chitosan coatings helped in maintaining phytochemical profiles of plum fruits during storage	Chang, Lu, Li, Lin, Qiu, Peng, et al. (2019)

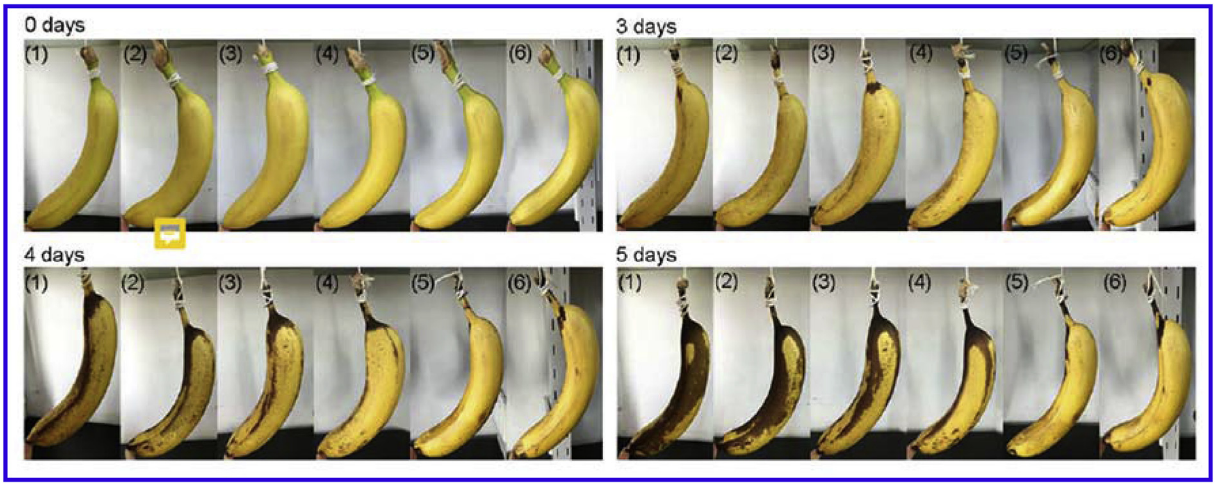

Fig. 4. Appearance of whole banana during storage; (1) uncoated bananas, (2) bananas coated with CMC100, (3) quaternized CS40/CMC60, (4) quaternized CS70/CMC30, (5) quaternized CS90/CMC10 and (6) quaternized CS100 films; Reprinted with permission (Hu et al., 2016).

(Adiletta, Zampella, Coletta, & Petriccione, 2019). Petriccione et al., 2015, reported that 1% and 2% chitosan-coating on strawberry significantly reduced water loss, color loss, losses in titratable acidity and ascorbic acid content, changes in the phenolic, anthocyanin and flavonoid contents (Petriccione et al., 2015). Chitosan coating enhanced the activity of some antioxidant enzymes, preventing flesh browning and reducing membrane damage. In a similar study, quaternized chitosan films blended with carboxymethyl cellulose (CMC) were used to prolong shelf life of whole banana (Hu, Wang, & Wang, 2016). The results showed films containing high % of quaternized chitosan delayed the decay of the fruits (Fig. 4).

Table 3 highlights the research performed by different groups and the results they reported using chitosan-based treatments, alone or in combination with other polymer, for postharvest quality maintenance, reduction of decay, and extension of shelf life of various fruits and vegetables.

5.2. Shelf-life extension of fish and meat products

Foodborne outbreaks are rarely reported and also it is difficult to accurately assess the exact impact of foodborne diseases and outbreak (s) on the consumers. In the United States, meat or muscle food are responsible for quite a significant proportion of foodborne outbreaks (about 500–1500 outbreaks) that have been reported every year between 1998 and 2015 (Clink, 2017). In India, during the last 30 years (1980–2009), only 37 foodborne outbreaks were reported, a vast majority (24 out of 37) of which was due to bacterial pathogen(s) (Rao Vemula, Naveen Kumar, & Polasa, 2012). *Salmonella* spp. has been the major etiological agent, followed by *Vibrio* spp., and *Staphylococcus aureus* in these 24 outbreaks. Meats and fishes are highly perishable foods and are mechanically comminuted, which may incorporate surface contaminants into the inner layers of muscle foods (L. Huang, 2010). Once embedded in the inner layers, foodborne pathogens are relatively more difficult to be inactivated or destroyed by cooking (Laine et al., 2005). Microbial spoilage and oxidation of food are two major problems affecting food quality and shelf-life. The incorporation of antimicrobials and/or anti-oxidants into packaging materials has become popular alternative for addition in synthetic food additives. Due to the potential health risks associated with long-term consumption of some of the synthetic preservative compounds, extensive research has been conducted to use some natural active compounds or some Generally Recognized as Safe (GRAS) nanomaterials as alternatives to synthetic antioxidants/antimicrobials.

There are several reports on the fabrication of chitosan based films and their application to fish and meat products such as pork, chicken, red meat etc. (Y.-Y. Qin, Yang, Lu, Wang, Yang, Yang, et al., 2013; Serrano-León et al., 2018). Siripatrawan & Noipha, 2012, have developed a chitosan based film incorporating green tea extract as an active package for shelf life extension of pork sausages (Siripatrawan & Noipha, 2012). Results obtained during the study showed that pork sausages samples wrapped with films containing green tea extract has lower changes in color, texture, TBA value, sensory characteristics, and microbial growth than those wrapped with chitosan-film and control film, respectively. Finally, they suggested that incorporation of green tea extract into chitosan film could enhance the antioxidant and antimicrobial properties of the film and thus it helps in maintaining the quality and prolongation of shelf-life of the pork sausages. Recently Liu et al., 2019, prepared a chitosan based film incorporated with acetic acid and tested its efficacy in shelf life extension of meat (F. Liu et al., 2019). The chitosan films effectively inhibited the microbial growth in chilled meat during storage period and they found that chitosan films could maintain the quality and freshness of chilled meat for longer times than those of the LDPE films. The results indicated that the microbial growth inhibition of chitosan films on meat was controlled mainly by antibacterial nature of the films. In 2019, Li et al., reported application of chitosan coating containing ε-polylysine and rosmarinic acid on half-smooth tongue sole (*Cynoglossus semilaevis* Günther) fillets maintained their quality during refrigerated storage (N. Li, Liu, Shen, Mei, & Xie, 2019). A brief summary on the chitosan composite films and their applications in quality and shelf-life improvements of fish and meat products are given in Table 4.

6. Conclusion and perspectives

Numerous biopolymers have been studied recently for their suitability in food packaging applications. Chitosan is one of the well-studied biopolymer that has been reported to improve shelf-life of fresh produce, fish and meat products. Incorporation of nanomaterials and phytochemicals into chitosan improve antimicrobial, mechanical and barrier properties of the fabricated films and coatings that lead to improvement in food quality and shelf-life extension. Nanoparticles of silver, zinc oxides and titanium dioxide have been extensively used as the key candidates for the fabrication of chitosan nanocomposite. Other biopolymers such as gelatin, alginate, carrageenan, etc. are used as blends of chitosan for fabrication of composite films and coatings. Chitosan matrices serve as carriers of active compounds such as nanomaterials, essential oil, fruit extracts, and other phytochemicals. The use of chitosan as a biopolymer for food packaging applications prolong shelf-life of various foods and other agricultural products, and simultaneously reduce use of synthetic plastics and additives promoting

Table 4
Chitosan/chitosan based composite films for shelf life extension of fish and meat products.

Chitosan (CS) based films/coatings	% Constituent composition	Meat/Fish and their products	Effects of films	References
CS	CS - 3% (w/v)	Hake (*Merluccius merluccius*) fillets and Sole (*Solea solea*)	Enhanced the quality of fish during refrigerated storage	Fernández-Saiz, Sánchez, Soler, Lagaron, and Ocio (2013)
CS film incorporating green tea extract (GTE)	CS - 2% (w/v) GTE - 20% (w/w)	Pork sausages	Composite film maintained the sausage quality and prolonged shelf-life	Siripatrawan and Noipha (2012)
CS –nanocellulose (NC) films	CS - 1% (w/v) NC - 0.18% (w/w)	Ground meat	Nanocomposite films decreased lactic acid bacterial count in ground meat during storage up to 6-days	Dehnad, Mirzaei, Emam-Djomeh, Jafari, and Dadashi (2014)
CS film with grape seed extract (GSE) and carvacrol microcapsules (CM)	CS – 2.0% (w/v)	Salmon (*Salmo salar*)	Improved shelf-life of refrigerated salmon by 4–7 days	Alves et al. (2018)
CS film incorporated with tea polyphenol (TP)	CS – 2% (w/v) TP - 30% (w/w)	Pork meat patties	Composite film improved microbial shelf-life by 6-days	(Y.-Y. Qin et al., 2013)
CS coating incorporated with citric acid (CA) or licorice extract (LE)	CS – 1.5% (w/v) CA – 0.5% (w/v) or LE - 1.0% (w/v)	Japanese sea bass (*Lateolabrax japonicas*) fillets	Coating retarded lipid oxidation and reduced microbial growth	Qiu, Chen, Liu, and Yang (2014)
CS film incorporated with thinned young apple polyphenols (YAP)	CS – 2% (w/v) YAP-1.0% (w/w)	Grass carp fillets	Composite film extended shelf-life of fish fillets in cold storage conditions	Sun et al. (2018)
CS films incorporating peanut skin (PS) and pink pepper residue (PPR) extract	CS – 2% (w/v) PS - 0.84% and PPR-1.90% (v/v)	Chicken meat	Films maintained chicken quality by controlling lipid oxidation and decreasing microbial count	Serrano-León et al. (2018)
CS –gelatin (GL) film incorporated with grape seed extract (GSE) and *Ziziphora clinopodioides* essential oil (ZEO)	CS/GL- 60:40 GSE - 1 or 2% (w/w) ZEO - 1 or 2% (w/w)	Minced trout fillet	Chitosan composite film enhanced shelf-life of fish fillet upto 11-days during refrigerated storage (4 ± 1 °C)	Kakaei and Shahbazi (2016)
CS and alginate Edible coating with addition of resveratrol powder	CS - 1% (w/v) Alginate - 1.5% (w/v)	Sea bass (*Dicentrarchus labrax*) fillets	Coatings inhibited microbiological growth and retarded oxidation of fillets	(O. Martínez, Salmerón, Epelde, Vicente, & de Vega, 2018)
CS –gelatin (GL) films incorporated with oregano essential oil (OEO)	CS – 1% (w/v) GL – 3% (w/v) OEO- 1 to 4% (w/w)	Grass carp muscle	Film extended shelf-life of fish tissue by 12-days	(J. Wu, Ge, Liu, Wang, Chen, Wang, et al., 2014)
Chitosan based film containing kombucha tea extract (KTE)	KTE - 1 to 3% (w/w)	Minced beef meat	Maintained the quality of beef by retarding microbial growth and extending the shelf-life by 3 days	Ashrafi, Jokar, and Mohammadi Nafchi (2018)
Chitosan-nano ZnO antimicrobial flexible pouches	Chitosan – 2% (w/v) Zinc acetate dihydrate – 1 to 2 g	Raw meat	Prepared pouches showed complete microbial inhibition in raw meat upto 6-days of storage at 4 °C	Rahman et al. (2017)

healthy food and healthy environment. Future research may be devoted on large scale manufacturing and commercialization of chitosan based food packaging systems in order to reduce the burden of synthetic plastics on our society and environments.

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