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Review Article

A Review on Chitosan: Ecofriendly Multiple Potential Applications in the Food Industry

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Abstract

Recently, increasing attention has been paid to development of new functional materials for the environment and health, due to the huge waste produced annually by the shellfish processing industry and the absence of waste management which lead to environmental and human health negative effects. The extraction of chitin from crustaceans' shells may be a solution to minimize the waste and to produce chitosan, a valuable compound which possess biological properties (e.g., anti-cancer, antioxidant, and immune-enhancing) with application in several fields (e.g., food, medical, pharmaceutical, cosmetic, chemicals, agricultural crops, and textile). On the basis of the results of different studies, chitosan, a natural bio-degradable and non- toxic bio-polysaccharide derived from chitin, has potential to be used as natural antimicrobial. Chitosan has exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms, including fungi, Gram-positive and Gram negative bacteria. The aim of the present review was to summarize the most important information on chitin and chitosan as ecofriendly materials from its bioactivity point of view and to highlight various aspects such as characteristics with their potential applications especially focusing on the food industry field.

Keywords: Shellfish, chitin, chitosan, antimicrobial, antioxidant

Introduction

Disposal of waste produced annually by the shellfish processing industries exemplify a practical challenge. Whereas, by-products represents with approximately 75% of the total weight of crustaceans (shrimp, crabs, prawns, lobster, cuttlefish and krill) (Kuddus & Ahmad, 2013) and often, seafood wastes are thrown away at sea, burned, or left out to spoil (Xu *et al.,* 2013). So, the extraction of chitin from crustaceans' shells and its use as is or after further processing may be a way to minimize the waste and to produce valuable compounds with remarkable biological properties and application in different fields (Hamed *et al.,* 2016).

Chitosan; a linear polysaccharide consisting of (1, 4)-linked 2- amino-deoxy-β-D-glucan, is a deacetylated derivative of chitin and is considered a bio based environmental friendly material, which is the second most abundant polysaccharide found in nature after cellulose and estimated to be produced annually almost as much as 10 billion tons (Peter, 1995; Caner & Cansiz, 2007; Fernandez-Saiz *et al.,* 2009; Revathi *et al.,* 2012; Du *et al.,* 2014). Chitosan is a highly insoluble material like cellulose in its solubility and low chemical reactivity. It is a white, hard, inelastic, nitrogenous polysaccharide. Chitosan is prepared commercially by alkaline deacetylation of chitin obtained from the exoskeletons of marine crustaceans, insects, mushroom, fungi and algae cell walls. Chitosan and its derivatives are renewable, biocompatible, biodegradable, and non-toxic compounds that have many biological activities such as: anti-cancer (Salah *et al.,* 2013), antioxidant (Yen *et al.,* 2008), anti-microbial (Goy *et al.,* 2009), anticoagulant (Vongchan *et al.,* 2003), antihypertensive, antidiabetic, antiobesity, antiallergic, antiinflammatory, neuroprotective and matrix metalloproteinases inhibitory effects (Ngo *et al.,* 2015).

Chitosan has a lot of applications in agriculture, coating of seeds for improved yield and protection from fungal diseases (Hirano, 1997; Knorr, 1984; Shahidi *et al.,* 1999), biotechnology processes, in production of cell and enzyme immobilization matrices, healing-accelerating sutures and coverings, contact lenses, and artificial skin (Begin & Calsteren, 1999), waste water purification (Knorr, 1991), and chelation of metals (Shahidi *et al.,* 1999). Chitosan is used to form gels, films, beads, and fibers (Guibal, 2004; Sankararamakrishnan *et al.,* 2006; Zivanovic *et al.,* 2007), and in medicine as wound dressings (Koide, 1998). Chitosan has been researched for use in the food industry in various fields, including as an antimicrobial, antioxidant, a thickening agent in beverages, a clarifying agent in juices, and as a packaging material (Devlieghere *et al.,* 2004; Shahidi *et al.,* 1999; Xie *et al.,* 2001). The health care industry uses about 65% of total chitosan, agriculture 12%, waste and water treatments 7%, the food and beverage industry 6%, and immobilization and biotechnology 5% (Knorr, 1991; Li *et al.,* 1997).

Chitosan is being extensively used in the pharmaceutical industry, paper making, photography, solid state batteries, chromatography, dietary supplements and animal feed (Skjak-Braek *et al.,* 1989; Gupta & Ravi Kumar, 2000; Uhrich, *et al.,* 1999). Some of research has been conducted to use chitosan as a drug delivery vehicle, especially for the treatment of colon diseases, such as ulcerative colitis and Crohn's disease and as a dietary supplement for lowering cholesterol and controlling overweight (Kumar *et al.,* 2004). Chitosan, as a potential food preservative of natural origin, has been approved by the United States Food and Drug Administration (USFDA) as a Generally Recognized as Safe (GARS) food additive (USFDA, 2013). Therefore, in this article, summarizes the recent studies for the different potential applications of chitosan for improving quality and shelf life of foods.

Chitosan structure and physicochemical characterization

Chitosan has three types of reactive functional groups; an amino group as well as both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Youssef *et al.,* 2015). It is structurally similar to cellulose, but it is an aminopolymer and has acetamide groups at the C-2 positions in place of the hydroxyl groups. The structures of cellulose, chitin and chitosan are shown in Figure1. Cellulose is a homopolymer, while chitin and chitosan are heteropolymers. Chitin and chitosan are of commercial interest due to their high percentage of nitrogen (6.89%) compared to synthetically substituted cellulose (1.25%) (Kumar, 2000).

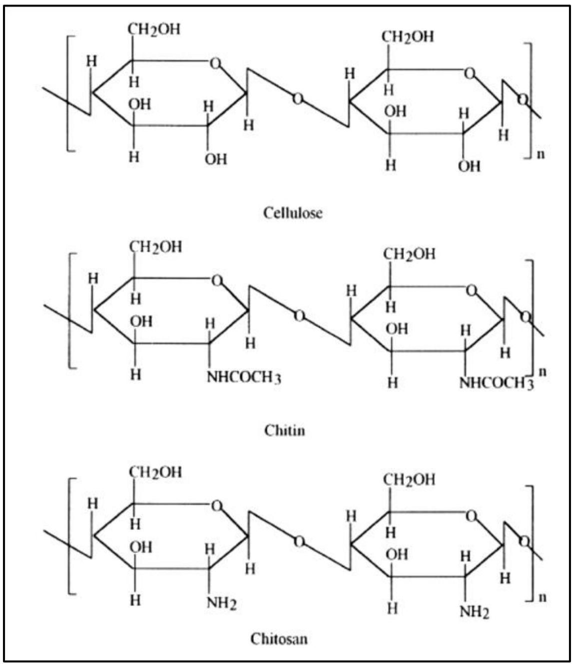

Figure 1: Chemical structure of cellulose, chitin and chitosan

Chitosan is a high molecular weight cationic linear polysaccharide that contains copolymers of D-glucosamine (deacetylated units) and N-acetyl-D-glucosamine (acetylated units) linked by β(1, 4) glycosidic bonds. This biopolymer is obtained by the partial deacetylation of chitin. Chitosan is considered to be polycationic, nontoxic, biocompatible, and biodegradable (Zhang *et al.,* 2010; Teng, 2011; Yuan *et al.,* 2011; Anaya *et al.,* 2013;Younes *et al.,* 2014; Reesha *et al.,* 2015; Yuan *et al.,* 2016). The degree of deacetylation is generally defined as the glucosamine/N-acetyl glucosamine ratio, which goes up as chitin is converted to chitosan. Therefore, when the percentage of N-acetyl glucosamine is higher than glucosamine, the biopolymer is called chitin and when the percentage of glucosamine exceeds N-acetyl glucosamine the compound is called chitosan (Khor & Lim, 2003; Ramírez *et al.,* 2010; Viarsagh *et al.,* 2010). The most important physicochemical characteristics of chitosan are its degree of deacetylation (DDA) and the molecular weight. The DDA has influence on all the physicochemical properties such as molecular weight, viscosity, solubility, etc. This parameter can also influence the solubility of the polymer in organic or aqueous solvents, where by increasing the DDA, the solubility increases. While molecular weight affects antibacterial properties (No *et al.,* 2002; Liu *et al.,* 2006).

Chitin is insoluble in most organic solvents, while chitosan is insoluble at neutral and alkaline pH in aqueous solution but it is readily soluble in dilute acids (pH<6-6.5) such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid along with dilute HCl. This is because chitosan can be considered a strong base as it possesses primary amino groups (No *et al.,* 2007; Sogias *et al.,* 2010; Tungtong *et al.,* 2012). The presence the amino groups are highly advantageous, providing distinctive functions and conducting modification reactions. So, chemical modifications of these groups have provided numerous useful materials in different fields of application (Crini & Badot, 2008; Kablan *et al.,* 2008). At low pH, these amines get protonated and become positively charged and that makes chitosan a water-soluble cationic polyelectrolyte. On the other hand, as the pH increases above 6, chitosan's amines become deprotonated and the polymer loses its charge and becomes insoluble. This unique property makes it appropriate for a wide range of applications in foods, cosmetics, and pharmaceuticals (Ferraro *et al.,* 2010).

Extraction of Chitin

There are two types of methods are used to obtain chitin: chemical and biological (microbial) methods (Figure 2). The chemical methods are the most commonly used treatment commercially, although it has several defects such as: uneconomical, eco-unfriendly, and negatively, those affect the physico-chemical properties of chitin. Another drawback of chemical chitin purification is that the removed proteins and minerals, although potentially valuable supplements for human foods and animal feeds, are sufficiently damaged that they are no longer appropriate for these applications (Arbia *et al.,* 2013; Hamed *et al.,* 2016). Therefore, the interest in biological extraction is increasing since it is a safer and cheaper treatment for chitin recovery. But till now, it has been limited to laboratory scale studies (Kaur & Dhillon, 2013).

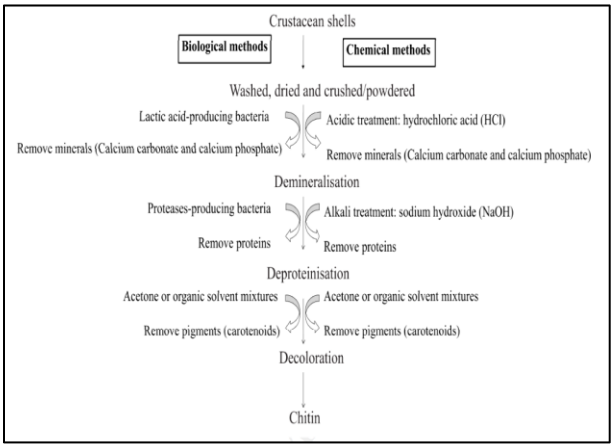

Figure 2: Chitin extraction by chemical and biological methods

Chemical methods

After obtaining the shells from different sources, they are washed, dried, and grounded into a powder (Abdou *et al.,* 2008). The traditional chemical methods involve three steps: demineralisation, deproteinisation, and decoloration. The first step consists of dissolving the powdered raw material with an acidic treatment with hydrochloric acid, the preferred reagent. The purpose is to remove mineral constituents (calcium carbonate and calcium phosphate). Proteins are then extracted from the decalcified shells by treating them with dilute aqueous sodium hydroxide (deproteination); crude chitin is then obtained. A decoloration step is added if a colorless product is wanted. Acetone or an organic solvent mixture is used to remove the pigments such as carotenoids. This yields a partially deacetylated chitin, which may then be further deacetylated to chitosan. This chitin is termed α-chitin because of its crystal structure. Treatment of this chitin with 50% NaOH for 1–3 h at 120°C gives a 70% deacetylated chitin (chitosan), which is soluble in many dilute acids. Repeating this step can give deacetylation values up to 98% (Abdulkarim *et al.,* 2013; Benhabiles *et al.,* 2012; Mohammed *et al.,* 2013). Chitosan, obtained from the alkaline deacetylation of chitin, is a functional polysaccharide with great potential in food applications and packaging requirements (Fernández-Pan *et al.,* 2015).

Biological methods

To avoid acidic and alkali treatments that could be a source of environmental problems, biological treatments offer an alternative way to extract chitin from crustacean shells. Lactic acid-producing bacteria have been used for demineralization of crustacean shells. In fact, the lactic acid produced by bacteria reacts with the calcium carbonate component in the biomass waste resulting in the formation of calcium lactate, which can be precipitated and removed by washing (Arbia *et al.,* 2013).

For the deproteinisation, proteases from bacteria will eliminate proteins. The biological treatment consists in a fermentation of the crustacean bio waste by different species of lactic or non-lactic bacteria such as *Lactobacillus plantarum*, *Pseudomonas aeruginosa* K-187, *Serratia marcescens* FS-3, or *Bacillus subtilis* (Jo *et al.,* 2008). Two other bacteria *Bacillus cereus* and *Exiguobacterium acetylicum* have shown high activity with both the deproteinisation and demineralization steps. The fermentation of shrimp shell wastes resulted in 97.1 and 92.8% deproteinisation, and 95 and 92% demineralisation, respectively (Sorokulova *et al.,* 2009).

Food applications of chitosan

Chitin and its derivatives are known to have wide range of biological activities including antimicrobial effects, antioxidant effects, clarification and de-acidification of fruit juices and antimicrobial edible packaging that could be used in the food industry to improve food safety, quality, and shelf-life.

Antimicrobial activity of chitosan

Chitosan and its derivatives have attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of foodborne filamentous fungi, yeast, and bacteria (Sethulekshmi, 2014; Barikani *et al.,* 2014; Severino *et al.,* 2015; Ali *et al.,* 2015; Yang *et al.,* 2015; Lekjing, 2016). The mechanism of the antimicrobial activity of chitosan and their derivatives has not yet been fully elucidated, but several hypotheses have been proposed. The most feasible hypotheses are a change in cell permeability barrier due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes (**Figure 3**). This interaction leads to the leakage of proteinaceous and other intracellular constituents (Fang *et al.,* 1994; Chen *et al.,* 1998; No *et al.,* 2007; Xing *et al.,* 2009; Li *et al.,* 2015; Chien *et al.,* 2016).

Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth (Cuero *et al.,* 1991; Li *et al.,* 2010; Chien *et al.,* 2016; Yuan *et al.,* 2016). It also activates several defense processes in the host tissue (El Ghaouth *et al.,* 1992), acts as a water binding agent and inhibits various enzymes (Young *et al.,* 1982). Binding of chitosan with DNA and inhibition of mRNA synthesis occurs via chitosan penetrating the nuclei of the micro-organisms and interfering with the synthesis of mRNA and proteins (Sudharshan *et al.,* 1992; Devlieghere *et al.,* 2004; Chen *et al.,* 2005; El-tahlawy *et al.,* 2005).

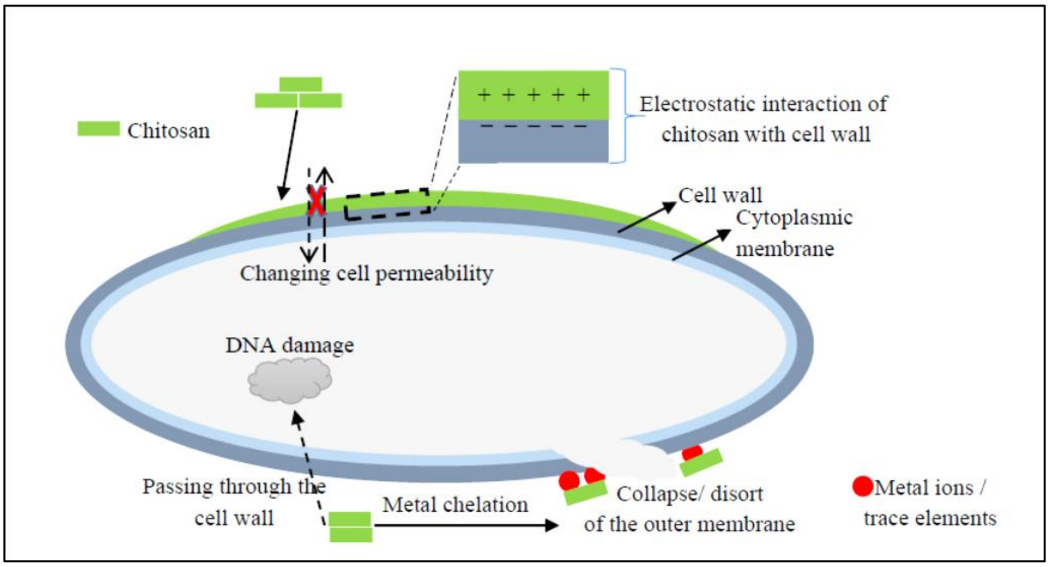

Figure3: Schematic representation of antimicrobial mechanisms of chitosan and its derivatives

Chitosan on the surface of the cell can form a polymer membrane which prevents nutrients from entering the cell (Zheng *et al.,* 2000; Helander *et al.,* 2001; Liu *et al.,* 2004) or acts as an oxygen barrier which can inhibit the growth of aerobic bacteria (Yuan *et al.,* 2016). Chitosan generally has antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi, while it has stronger bactericidal effects against bacteria rather than against fungi (Ziani *et al.,* 2009; Kong *et al.,* 2010).

The antibacterial effects of chitosan and chitosan oligomers are dependent on several factors such as its molecular weight, degree of deacetylation (DD), source, derivatives, pH, concentration, type of microorganism, etc., which should be considered before being applied (Chien *et al.,* 2016; Hosseinnejad & Jafari 2016). Chitosan has been successfully used to prolong shelf-life of longan fruit, fresh cut broccoli, and raspberry and many other fruits and vegetables (Jiang & Li, 2001; Moreira*et al.,* 2011; Tezotto-Uliana *et al.,* 2014). The addition of chitosan to food inhibits microorganisms' growth and avoids poor appearance, off flavors, and economic losses. El-Diasty *et al.*, (2012) noticed that by adding chitosan to cheese it improved its mycological quality. Mould and yeast growth were inhibited and shelf-life was extended. Devlieghere *et al.*, (2004) showed that native chitosan was significantly more active against *Candida lambica* at pH 4.0 than at pH 6.0. Also, it was demonstrated by Roller and Covill (1999) that inhibitory properties of chitosan against *Mucorracemosus* were greater at lower pH.

No *et al.,* (2002) revealed that 0.1% chitosan (MW = 1671, 1106, 746, 470, 224 and 28 kDa) showed stronger bactericidal effects against grampositive bacteria than gram-negative bacteria. For gram-negative bacteria, chitosan of 746 kDa appeared most effective against *E. coli* and *Pseudomonas fluorescens*, compared with chitosan 470 kDa against *Salmonella Typhimurium* and *Vibrio parahaemolyticus.* Recently, Younes *et al.*, (2014) demonstrated that antibacterial activity was further enhanced for gram-negative bacteria with decreasing MW, whereas, opposite effect was observed with the gram positive bacteria. Concerning the antifungal activity, the influence of chitosan characteristics was dependent on the particular type of fungus.

Antioxidative properties of chitosan

Reactive oxygen species (ROS) such as H_2O_2, hydroxyl radicals, and superoxide lead to oxidative stress which is correlated with various pathologies: cancer (Manda *et al.,* 2009), cardiovascular disease (Zhang *et al.,* 2010), premature aging (Cui *et al.,* 2012), rheumatoid arthritis, and inflammation (Filippin *et al.,* 2008). Chitosan and several of its derivatives, which being safe and non-toxic have shown antioxidant effects (Park & Kim, 2010). Thus it could be added as an ingredient for the production of functional food which could prevent age-related and diet-related diseases retarding the progress of numerous chronic diseases (Kerch, 2015).

The oxidation of highly unsaturated food lipids causes off-favors and rancidity. Usually, synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) are used. However, because of potential health hazards associated with such compounds (Younes & Rinaudo, 2015), more safe and natural antioxidants have been preferred; especially if a lack of toxicity can be demonstrated (Harish Prashanth & Tharanathan, 2007).

The addition of 1% chitosan resulted in a decrease of 70% in 2-thiobarbituric acid reactive substances (TBARS) values of meat after 3 days of storage at 4°C.Chitosan's inhibition seems to be related to its chelation of the free iron that is released from the heme proteins of meat during heat processing (Tharanathan & Kittur, 2003).

The highly unsaturated fatty acids commonly found in seafood are particularly sensitive to oxidative change during storage. This oxidation is catalyzed by the high concentrations of pro-oxidants such as hemoglobin and metal ions in the fish muscle (No *et al.,* 2007). Treatment of herring fish samples with chitosan, however, showed lower peroxide values and total volatile aldehydes than the untreated samples. The antioxidant effect of chitosan depends on its molecular weight, concentration, and viscosity (Kamil*et al.,* 2002). Chitosan with different viscosity were used (360, 57, and 14 cP) to treat the fish samples. The corresponding viscosity average molecular weights were 1.8 x 10^6, 9.6 x10^5, and 6.6 x 10^5 Da. All three biopolymers showed antioxidant effects, i.e., they lowered peroxide values, TBARS, and total volatile aldehydes. However, the low viscosity chitosan (14 cP) showed the strongest antioxidative effect (Lin & Chou, 2004). Chitosans may retard lipid oxidation by chelating ferrous ions present in the fish model system, thus eliminating prooxidant activity of ferrous ions or preventing their conversion to ferric ion (No *et al.,* 2007).

Kim & Thomas (2007) also observed similar results with chitosan with different molecular weights (30, 90, and 120 kDa) in Atlantic salmon (*Salmosalar*). The antioxidant activity was measured as decreased TBARS and increased 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity. The scavenging effect of chitosan was compared to that of BHT and an equivalent efficiency of 85% was obtained. Moreover, the lowest molecular weight chitosan (30 kDa) showed the highest activity (Hamed *et al.,* 2016).

Clarification and deacidification of fruit juices

Processing of clarified fruit juices commonly involves the use of clarifying agents, including gelatin, bentonite, silica sol, tannins (Simpson et al., 1994), potassium caseinate and poly-vinyl pyrrolidone (Spagna *et al.,* 1996). Chitosan can be used as a clarifying agent due to its polycationic behavior inacidic media since protonated chitosan interacts with negatively compounds through electrostatic interactions. Chitosan has been used as clarifying agent of fruit juices (Chatterjee *et al.,* 2004; Ghorbel-Bellaaj *et al.,* 2012;Cesar *et al.,* 2014), wines (Chagas *et al.,* 2012),beer (Gassara *et al.,* 2015), and tea (Rao *et al.,* 2011). Chitosan salts, which carry astrong positive charge, have been shown to be effective as dehazing agents; they may also be used to control acidity in fruit juices (Imeri and Knorr, 1988). Chitosan is a good clarifying agent for grapefruit juice either with or without pectinase treatment (Spagna *et al.,* 1996) and highly effective fining agent for apple juice, which can afford zero turbidity products with 0.8 kg/m^3 of chitosan (Soto-Perlata *et al.,* 1989). In a similar study, Spagna *et al.,* (1996) observed that chitosan has a good affinity for polyphenolic compounds such as catechins, proanthocyanidins, cinnamic acid and their derivatives that can change the initial straw-yellow colour of white wines into deep golden-yellow colour due to their oxidative products. By adding chitosan to grapefruit juice at a concentration of 0.015 g/mL, total acid content was reduced by about 52.6% due to decreasing the amount of citric acid, tartaric acid, malic acid, oxalic acid and ascorbic acid, by 56.6, 41.2, 38.8, 36.8 and 6.5%, respectively (Rwan and Wu 1996). Martín-Diana et al., (2009) incorporated chitosan in un-pasteurized orange juice and evaluated of quality and nutritional markers. Their results recommend the use of chitosan at concentrations up to 1g L^{-1} to extend quality and preserve ascorbic acid and carotenoids during storage time of fresh orange juice, thus avoiding the use of standard thermal treatments which produces a negative impact on the nutritional value.

Antimicrobial edible packaging

Considering the health tendency of the modern food technology, the use of bio-based active films as packaging materials is very important. The antibacterial properties of chitosan have also been used as active edible packaging (Hamed *et al.,* 2016). Biofilms have been formed from chitosan that allow long term storage of food products. Antimicrobial coating of vegetables, fruits, grains, and fish retard microbial invasion as chitosan acts as a protective barrier to enhance the sensory and nutritional quality of the food (Sinha *et al.,* 2014; Aranaz *et al.,* 2009). There are many mechanisms involved in extending shelf life of food by coating films. These include controlled moisture transfer between food and surrounding environment, controlled release of chemical agents like antimicrobial substances, antioxidants, reduction of oxygen partial pressure in the package that results in a decreased rate of metabolism, controlled rate of respiration, high impermeability to certain substances like fats and oils, temperature control, structural reinforcement of food and coat flavor compounds and leavening agents in the form of microcapsules (Shahidi *et al.,* 1999). Besides being a protective barrier, edible biopolymer films can be used as carriers of bioactive compounds to enhancing food quality. Polymeric bioactive films could be combined with different antimicrobial agents such as organic acids, bacteriocins (nisin and lacticin), plant extracts (thymol, p-cymene, and cinnamaldehyde), proteins (e.g., conalbumin), antibiotics, fungicides, and chelating agents (EDTA) to reduce food spoilage by pathogenic microorganisms and enhance shelf-life (Dutta *et al.,* 2012). Chitosan-based edible films are biodegradable and can be consumed along with the product in the package. Moreover, they form transparent films with good mechanical properties that constitute a shell protecting the appearance and the quality of the food inside (Bourbon *et al.,* 2011; Park *et al.,* 2013). A numbers of investigations have shown that pure chitosan coating exhibited antibacterial activity in shrimp during iced or refrigerated storage, refrigerated cod and herring, refrigerated silver carp fillets, refrigerated chicken breast fillets and meat fillets, skinless frankfurters and cooked pork sausages in refrigerated storage (Jeon *et al.,* 2002; Khanjari *et al.,* 2013; Latou *et al.,* 2014; Ramezani *et al.,* 2015; Upadhyay *et al.,* 2015; Wang *et al.,* 2015; Lekjing, 2016). Özdemir & Gökmen (2017) found that by using coating mixture of 1% chitosan and %1 ascorbic acid to pomegranate arils extends its shelf-life by improving microbiological safety, preserving nutritional and sensory quality. Antimicrobial films have been prepared by including various organic acids and essential oils in a chitosan matrix, and the ability of these bio-based films to inhibit the growth of indigenous (lactic acid bacteria and *Enterobacteriaceae*) or inoculated bacteria (*Lactobacillus sakei* and *Serratia liquefaciens*) onto the surfaces of vacuum-packed cured meat products have been investigated (Aider, 2010).

Conclusion

Chitosan and its derivatives are valuable compounds that meet the needs of consumers for natural products that impact positively on health. Therefore, they can be applied in food industrial applications as natural preservatives or for active packaging due to their antimicrobial and antioxidant properties, allowing extending the shelf-life while decreasing food waste entailing environmental and economic benefits. These positively charged polysaccharides are also useful for many food applications such as beverages clarification and encapsulation of bioactive compounds. Above all, chitosan and its derivatives are eco-friendly. The shellfish processing industries are a large source of by-products, predominantly chitosan. Despite their potential value, crustacean shell wastes are still underutilized and their use in all of the above food applications needs to be further researched. At the same time, the various industries need to be encouraged to incorporate many of these applications commercially due to, their technology readiness level for the majority of the applications are still underutilized.

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