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Chitosan: A Bioactive Polysaccharide in Marine-Based Foods

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1. Introduction

Since people are increasingly conscious of the relationship between diet and health, the consumption of marine-based foods has been growing continuously. Consumers identified seafoods as nutritious and complete foods. Hence, they are perceived them as an excellent source of high quality proteins, valuable lipids with high amounts of PUFA. These compounds are well known to contribute to the enhancement of human health by different alternatives such as reducing the risk of cardiovascular disease, coronary disease and hypertension. Additionally, marine-based food products are easily digested and constitute excellent source of essential minerals. Recently, seafoods have been recognized as nutraceuticals or functional foods. Functional foods, first evolved in Japan in 1980, are defined as foods demonstrating beneficial effect on one or more targeted functions on human organism (Ross, 2000). Marine-based functional foods or nutraceuticals, include omega-3 fatty acids, chitin and chitosan, fish protein hydrolysates, algal constituents, carotenoids, antioxidants, fish bone, shark cartilage, taurine and bioactive compounds (Kadam & Prabhasankar, 2010).

Despite the aforementioned desirable properties, seafood products are highly susceptible to quality deterioration, mainly due to the lipid oxidative reactions, particularly involving polyunsaturated fatty acids (PUFAs). These reactions are enhanced (catalyzed) by the presence of high concentrations of heme and non-heme proteins. These proteins are known to contain iron and other metal ions in their structures (Decker & Hultin, 1992). Moreover, marine-based food quality is highly influenced by autolysis, bacterial contamination and loss of protein functionality (Jeon, Kamil & Shahidi, 2002). More recently, pollution of seafood with different hazardous materials such as refinery, industrial wastes and heavy metals has resulted in elevated concern about the consumption of seafood (Kadam & Prabhasankar, 2010). Additionally, aquaculture industry has increasingly attracted much

attention for the intensive farming of fish and shellfish, mainly due to the depleting of wild fish and shellfish stocks worldwide. However, this intensive farming entails several difficulties such as stress, which is the most important factor affecting the immunity system of fish (Ledger, Tucker & Walker, 2002). To address the aforementioned problems, the use of chitosan as protective material appears to be a potential alternative.

In nature, chitosan is found in the cell walls of fungi of the class *Zygomycetes*, in the green algae *Chlorella sp.*, yeast and protozoa as well as insect cuticles and especially in the exoskeleton of crustaceans. Chitosan is a deacetylated derivative of chitin, the second abundant polysaccharide in nature after cellulose. In 1811, the French scientist, Henri Braconnot first discovered chitin in mushroom. In 1820, chitin was isolated from insect cuticles (Bhatnagar & Sillanpa, 2009). In 1859, Rouget reported finding chitosan after boiling chitin in potassium hydroxide (KOH). This treatment rendered the material soluble in organic acids. Hoppe-Seyler named it chitosan in 1894 (Khor, 2001). Chemically, chitosan is a high molecular weight, linear, polycationic heteropolysaccharide consisting of two monosaccharides: N-acetyl-glucosamine and D-glucosamine. They are linked by β -(1 \rightarrow 4) glycosidic bonds. The relative amount of these two monosaccharides in chitosan vary considerably, yielding chitosans of different degrees of deacetylation varying from 75% to 95%, molecular weight in the range of 50-2000 KDa, different viscosities and pKa values (Tharanathan & Kittur, 2003). In addition, chitosan has three functional moieties on its backbone; the amino group on the C2, the primary and secondary hydroxyl groups on the C3 and C6 positions, respectively. These functional groups play important roles in different functionalities of chitosan. The amino group is the most important among the other moieties, especially in acidic conditions, due to the protonation phenomenon, rendering it able to interact with negatively charged molecules (or sites). Additionally, chitosan polymer interacts with the metal cations through the amino groups, hydroxyl ions and coordination bonds.

Commercially, chitosan is produced from chitin by exhaustive alkaline deacetylation, involving boiling chitin in concentrated alkali for several hours. Because this N-deacetylation is almost never complete, chitosan is classified as a partially N-deacetylated derivative of chitin (Kumar, 2000). From a practical point of view, many of commercial interests and applications of chitosan and its derivatives originate from the fact that this polymer combines several features, such as biocompatibility, biodegradability, nontoxicity and bioadhesion, making it as valuable compound for pharmaceutical (Dias, Queiroz, Nascimento & Lima, 2008), cosmetics (Pittermann, Horner & Wachter, 1997), medical (Carlson, Taffs, Davison & Steward, 2008), food (Shahidi, Kamil & Jeon, 1999; No, Meyers, Prinyawiwatukul & Xu, 2007; Kumar, 2000), textile (El Tahlawy, Bendary, El Henhawy & Hudson, 2005), waste water treatment (Che & Cheng, 2006), paper finishing, photographic paper (Kumar, 2000), and agricultural applications (Hirano, 1996).

Although there have been several prior reviews on the use of chitosan in food applications (No et al., 2007; Shahidi et al., 1999), the use of chitosan in seafood applications, especially its novel application in the form of nanocarriers for bioactive compounds for shelf life extension, has not yet been reported. Recently, a study was published on the use of chitosan

nanoparticle for stability enhancement of vitamin C in rainbow trout diet (Alishahi, Mirvaghefi, Rafie-Tehrani, Farahmand, Shojaosadati, Dorkoosh & Elsabee, 2011). Hence, this chapter attempts to survey the applications of chitosan in various fields of marine-based products.

2. Antibacterial activity

The modern era of chitosan research was heralded by publications in the 1990s, that described the antimicrobial potentials of chitosan and its derivatives, exhibiting a wide spectrum of activities against human pathogens and food-borne microorganisms (Chen, Xing & Park & Kong, 2010; No, Park, Lee & Meyers, 2002; Rabea, Badway, Stevens, Smagghe & Steurbaut, 2003; Raafat, Barga, Haas & Sahl, 2008; Raafat & Sahl, 2009). The first study reporting antibacterial properties was reported by Allan & Hardwiger (1979). They reported that chitosan showed a broad range of activities and a high inactivation rate against both Gram-positive and Gram-negative bacteria, (Allan & Hardwiger, 1979). However, although several studies have been published in this area, the exact mechanism of the antimicrobial activity of chitosan remains ambiguous.

Six major mechanisms have been proposed in the literature, as follows (Kong et al., 2010; Raafat & Sahl, 2009; Rafaat et al., 2008): (1) the interaction between the positively charged chitosan amine groups and the negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents; (2) the activation of several defense processes in the host tissue by the chitosan molecule acting as a water-binding agent and inhibiting various enzymes by blocking their active centers; (3) the action of chitosan as a chelating agent, selectively binding metals and then inhibiting the production of toxins and microbial growth; (4) the formation, generally by high molecular weight chitosan, of an impervious polymeric layer on the surface of the cell, thereby altering cell permeability and blocking the entry of nutrients into the cell; (5) the penetration of mainly low-molecular weight chitosan into the cytosol of the microorganism to bind DNA, resulting in interference with the synthesis of mRNA and proteins; and (6) the adsorption and flocculation of electronegative substances in the cell by chitosan, distributing the physiological activities of the microorganisms, causing their death.. However, it is very important to mention that chitosan is soluble only in acidic media and therefore, the effect of pH on microorganisms must be considered together with the effect of chitosan. Thus, the synergetic effect of chitosan/pH together is probably the most evident explanation of the antimicrobial effect of chitosan.

Complicating the issue, a number of studies aimed at determining the antibacterial activities of chitosan on Gram-positive and Gram-negative bacteria have been reported antithetical outcomes, making their interpretation difficult. More recently, Kong et al. (2010) showed that chitosan and its derivatives are more powerful antibacterial agent against Gram-negative bacteria than against Gram-positive microorganism. Conversely, Raafat and Sahl (2009) reported a study in which they demonstrated that Gram-positive bacteria are more

sensitive to the antibacterial effect of chitosan than Gram-negative bacteria. Therefore, the interpretation of the sensitivity of bacteria to chitosan is quite difficult.

To address this problem, Kong et al. (2010) proposed that the variation in the bactericidal efficacy of chitosan arises from different parameters that must be considered when evaluating the antibacterial activity of chitosan. These factors can be categorized into four classes as follows: (1) intrinsic microbial factors, including microbial species and cell age, (2) intrinsic factors of chitosan molecules, namely, the positive charge density, protonation level of the amine group, the chitosan molecular weight and concentration, hydrophilic/hydrophobic characteristics and chelating capacity of the chitosan molecule; (3) its physical state, i.e., either water soluble or solid chitosan; and (4) environmental factors including the ionic strength of the testing medium, pH, temperature and contact time between chitosan and bacterial cells. In addition, it is worth noting that, despite the widely reported antimicrobial properties of chitosan in the literature, the results are mainly based on in vitro experiments. In real-world applications, it is important to consider that most foods are complex matrices composed of different compounds (proteins, carbohydrates, lipids, minerals, vitamins, salts and others) and many of them may interact with chitosan to varying levels, possibly leading to a loss or enhancement of its antibacterial activity (Devlieghere, Vermeulen, & Debevere, 2004).

Taking into account the above insights about the antibacterial characteristics of chitosan, the following applications of chitosan in seafood products were considered. Due to the high perishability characteristics of marine-based products, there has been an increased interest in the application of chitosan to extend the shelf life of the products. In this context, chitosan has increasingly gained attention as an antibacterial additive in seafood from both seafood processors and consumers, largely due to a desire to reduce the use of synthetic chemicals in seafood preservation. Cao, Xue and Liu (2009) reported that chitosan at 5 g/L extended the shelf life of oyster (*Crossostrea gigas*) from 8-9 days to 14-15 days. They explained that *Pseudomonas* and *Shewanella* are the most prolific microorganisms during cold storage of fish and shellfish and these bacteria can easily be reduced or eliminated with the addition of chitosan at this concentration.

Fish balls have a high water activity and are prone to the growth of microorganisms, with a relatively short shelf life of 4-5 days at a storage temperature of approximately 5 °C. Kok and Park (2007) reported that fish ball shelf life has been increased by adding chitosan which maintained both aerobic and yeast counts at < 1 log CFU/g over 21 days of storage. Kok and Park (2007) also reported that physical state of chitosan is an important parameter for its antibacterial activity. In the dissolved state, chitosan showed excellent antibacterial activity and contributed to the extension of the product shelf life. However, in the study reported by Lopez-Caballero et al. (2005a and b), it was demonstrated that the addition of powdered chitosan to fish patties had no effect on bacterial growth. Roller and Corvill (2000) reported that the spoilage flora was inhibited from log 8 CFU/g in the control sample (without chitosan) to log 4 CFU/g over the 4-week study at 5 °C with the use of chitosan combined with acetic acid in shrimp salad. However, it is important to consider the effect of

acetic acid on the ability of chitosan to extend shelf life. Fernandez-Saiz, Soler, Lagaron and Ocio (2010) studied bacterial growth in two conditions. The first one was a fish soup (ANETO[®] brand, packaged in TetraBrik[®] and fabricated by Jamon Aneto, S. L., Barcelona, Spain). The other medium was a model laboratory growth medium, tryptone soy broth. They reported a significant reduction of the growth of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella spp* when the products were stored in the presence of chitosan. In fish soup and under laboratory conditions, the effect of chitosan in the tested medium at 4, 12, and 37 °C depended significantly on the bacteria type, incubation temperature and food matrix (substrate). The antibacterial effectiveness of chitosan was decreased in the fish soup, suggesting that the constituents of the fish soup had high influence on the antimicrobial efficacy of chitosan. The authors reported that chitosan was probably irreversibly bound by microbial cells or negatively charged compound in the soup and therefore rendered it inactive against the remaining unbound microorganisms. In conclusion, the effect of chitosan-to-cell ratio must be considered. Lopez-Caballero et al. (2005a-b) showed that the addition of chitosan to sausage treated at high pressure yielded a 2-log cycle decrease of total bacterial counts of *Pseudomonas* and *Enterobacteria* at 8-11 days storage. Ye, Neetoo and Chen (2008) stated that chitosan has antibacterial activity that is effectively expressed in aqueous system; however, its antibacterial properties against *L. monocytogenes* in cold-smoked salmon were negligible when chitosan was in the form of an insoluble film. The growth of *L. monocytogenes* in salmon samples wrapped in the chitosan-coated film and plain films was similar. The authors demonstrated that it is possible that chitosan is ineffective in films because it is unable to diffuse through a rigid food matrix such as salmon. Regarding microbial counts, Lopez-Caballero et al. (2005b) showed that chitosan in the soluble state had no significant effect in high-pressure treated cod sausages. However, when chitosan was added in a dry form, higher counts of microorganisms were recorded. This is an indication that chitosan in soluble form contributed to maintaining product safety. The microbial counts in their study were for lactic acid bacteria, *Enterobacteria*, *Pseudomonas* and *Staphylococcus*. Duan, Jiang, Cherian and Zhao (2010) reported that the initial total plate count (TPC) of fresh lingcod was 3.67 log CFU/g, which then rapidly increased to 6.16 and 8.36 log CFU/g on day 6 and day 14, respectively. When chitosan coatings were used, the results showed a 0.15-0.64 reduction in TPC. Moreover, the TPCs of chitosan-coated samples stored under vacuum or modified-atmosphere packaging were significantly lower than those of the control sample during the subsequent cold storage. The combination of chitosan coating and vacuum or modified atmosphere packaging (MAP) resulted in 2.22-4.25 reductions in TPC for the first 14 days of cold storage. The TPCs of chitosan-coated and MPA samples were lower than 10⁵ CFU/g even after 21 days of cold storage. This result indicated a significant delay of microbial spoilage. Qi, Zhang and Lan-Lan (2010) reported that because non-fermenting Gram-negative bacteria are dominant in the initial microbial flora of fish and shellfish sourced from cold seawater, controlling the growth of these Gram-negative bacteria may be important for the preservation of oysters. They demonstrated that combined treatment with chitosan and ozonated water had better antibacterial effect than either treatment alone. When only aerobic plate count was measured, the authors showed that the product shelf life with the combination of chitosan with ozonated water was at least

20 days, whereas it was only 8 days for the control sample, 10 days for the ozonated samples, and 14 days for the chitosan-treated samples. Duan, Cherian and Zhao (2009) indicated that fish oil incorporated in chitosan coatings lowered significantly the total and psychrotrophic counts in frozen lingcod fillets over three months cold storage. Ojagh, Rezaei, Razavi and Hosseini (2010) also reported that chitosan coatings enriched with cinnamon oil decreased effectively total viable counts and psychrotrophic bacteria in rainbow trout (*Oncorhynchus mykiss*) during 16-day cold storage.

3. Antioxidant activity

Seafood is considered as excellent sources of functional foods for balanced nutrition favorable for promoting good health. The beneficial health effects of marine foods are ascribed to their lipids, mainly the long-chain omega-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Newton, 2001). However, these valuable compounds in seafood are highly sensitive to oxidative reactions and development of off-flavor even during cold storage (Cadwalladar & Shahidi, 2001). It has been proposed that lipid oxidation in fish and shellfish may be initiated and propagated by a number of mechanisms, namely autooxidation, photosensitized oxidation, lipoxygenase, peroxidase and microsomal enzymes (Hsieh & Kinsella, 1989). Additionally, fish and shellfish muscles contain protein-bound iron compounds, for example, myoglobin, hemoglobin, ferritin, transferrin and haemosiderin, as well as other metals. This is a factor that plays an important role in initiating lipid oxidation in marine-based products (Decker & Hultin, 1992). Castell, Maclean and Moore (1965) showed that the relative pro-oxidant activity of metal ions in fish and shellfish muscles decreased in the order of $\text{Cu}^{+2} > \text{Fe}^{+2} > \text{Cd}^{+2} > \text{Li}^{+2} > \text{Mg}^{+2} > \text{Zn}^{+2} > \text{Ca}^{+2} > \text{Ba}^{+2}$. The iron-bound proteins and other metal ions may be released during the storage period and can thus activate and/or initiate lipid oxidative reactions (Decker & Hultin, 1992).

Along with the growing consumer demand for seafood devoid of synthetic antioxidants, chitosan has been a booming antioxidant agent in fish and shellfish. The antioxidant activity of chitosans of different viscosities (360, 50 and 14 cP) in cooked, comminuted flesh of herring (*Clupea harengus*) was investigated (Kamil, Jeon and Shahidi, 2002). The oxidative stability of fish flesh during cold storage at 4 °C with the addition of chitosan at concentrations of 50, 100 and 200 ppm was compared with that of fish treated with conventional antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (THQ) (all at 200 ppm). Among the three chitosan samples tested, the 14 cP chitosan was the most effective in preventing lipid oxidation. The formation of thiobarbituric acid test reactive substances (TBARS) in herring samples containing 200 ppm of 14 cP chitosan was reduced by 52% as after 8 days of storage compared with that the control sample without chitosan. At a chitosan concentration of 200 ppm, the 14 cP chitosan exerted an antioxidant effect similar to that of commercial antioxidants in reducing TBARS values in comminuted herring flesh. A study by Kamil, Jeon and Shahidi (2002) indicated that the antioxidant capacity of chitosan added to fish

muscle depends on its molecular weight (MW) and concentration in the product. Similarly, Kim and Thomas (2007) observed that the antioxidative effects of chitosan in salmon depended on its molecular weight (tested at MW = 30, 90 and 120 kDa) and concentration (evaluated at 0.2%, 0.5% and 1% w/w). The authors reported that the 30 kDa chitosan showed the highest radical-scavenging activity. The scavenging activities of chitosan were increased by increasing its concentration. However, varying the concentration showed no significant effects when 120 kDa chitosan was used. Ahn and Lee (1992) studied the preservative effect of chitosan film on the quality of highly salted and dried horse mackerel. The product was prepared by soaking the fresh horse mackerel in 15% salt solution for 30 min, coating with or without (control) chitosan, and drying for 3h at 40 °C in a hot air dryer. During cold storage at a temperature of 5 °C for 20 days, the chitosan-coated samples had lower TBARS and peroxide values (PV) than the control samples. Similarly, Lopez-Caballero et al. (2005) also found that coating codfish patties with a chitosan-gelatin blend considerably lowered lipid oxidation. However, being non soluble in powder form at neutral pH, chitosan had no effect on the prevention of lipid oxidation. Shahidi, Kamil and Jeon (2002) reported that chitosans with different molecular weights and viscosities (14, 57 and 360 cP) were effective in controlling the oxidation of lipids in comminuted cod (*Gadus morhua*) following cooking. Both PV and TBARS values were reduced as a result of treatment of the fish prior to cooking with 50, 100 and 200 ppm of 14, 57, and 360 cP chitosans. Inhibition of the oxidation was concentration-dependent and was the highest for the 14 cP chitosan. The authors stated that the antioxidant activity of chitosans of different viscosity in cooked comminuted cod may be attributed to their metal-chelating capacities. Chitosans with different viscosities were found to protect cooked cod from oxidation at various levels. The observed differences were presumably due to differing degrees of deacetylation and molecular weights of the chitosan molecules. In the study reported by Qin (1993), it has been indicated that the ion-chelating ability of chitosan is strongly affected by the degree of deacetylation. The highly acetylated chitosan has very little chelating activity. In addition, high molecular weight chitosan has a compact structure and the effect of intramolecular hydrogen bonding is stronger, which weakens the activities of the hydroxyl and amino groups. As result, the probability of the exposure of these active moieties may be restricted, resulting in less radical scavenging activity. Obviously, low molecular weight chitosan exhibits higher hydroxyl radical scavenging activity, which is partially attributable to its metal chelating ability. The Fe^{+2} chelating ability of chitosan is mainly attributed to the presence of amino groups, which contain free electron pairs that contribute to form chitosan/ Fe^{+2} complex. The Fe^{+2} chelating ability of low molecular weight chitosan is more pronounced than that of high molecular weight chitosan. Consequently, the amino groups in chitosan can react with free radicals to form additional stable macroradicals. Therefore, the active hydroxyl and amino groups in the polymer chains are the origin of the scavenging ability of chitosan (Jeon, Shahidi & Kim, 2000; Feng, Du, Li, Hu & Kennedy, 2008; No et al., 2007). Kamil et al. (2002) explained that in the charged state (protonated amino groups), the cationic groups of chitosans impart intramolecular electric repulsive forces. This

phenomenon may be responsible for lesser chelating ability of high viscosity (high Mw) chitosans. Jeon et al. (2002) demonstrated that the antioxidant activity of chitosan is also effective when it is applied as a protective film. In this kind of application, it retards lipid oxidation by acting as a barrier against oxygen penetration. Sathivel, Liu, Huang and Prinyawiwatukul (2007) showed that the TBARS value of coated pink salmon (*O. gorbuscha*) fillets glazed with chitosan (1.3 mg MDA(malondialdehyde)/kg sample) was significantly lower than that of fillets glazed with lactic acid (3 mg MDA/kg sample) or distilled water (1.8 mg MDA/kg sample). The results indicated that chitosan glazing was more effective at reducing lipid oxidation among the studied alternatives. Sathivel (2005) also reported that the TBARS value of pink salmon fillets coated with 1% and 2% chitosan was significantly lower than in both the control sample and protein-coated product after 3 months of frozen storage. The author stated that a higher the concentration of chitosan, resulted in a lower TBARS value. The latter implies that the antioxidant effect of chitosan in the coating state is highly correlated to the coating material thickness, thereby hindering the entrance of oxygen into pink salmon fillet and initiating the oxidative process. Moreover, the primary amino groups of chitosan would form a stable fluorosphere with volatile aldehydes such as malondialdehyde which are derived from breakdown of fats during lipid oxidation (Weist & Karel, 1992).

Duan et al. (2009; 2010) showed that the combination of chitosan with modified atmosphere packaging enhanced the lipid stability of lingcod (*O. elongates*) within 21 days of cold storage. When applied on the surface of lingcod fillets, chitosan coatings may act as a barrier between the fillet and the surrounding atmosphere. This is mainly due to the good oxygen barrier properties of chitosan films, which slow down the diffusion of oxygen from the surrounding air to the surface of fillet and retard lipid oxidation (Aider, 2010). Additionally, Ojagh et al. (2010) reported that chitosan coatings enriched with cinnamon oil could suitably delay lipid oxidation in the refrigerated rainbow trout during 16 days of storage and markedly reduced the TBARS and PV values as compared with the control product. Mao and Wu (2007) showed that lipid oxidation of kamabako gel from grass carp (*Ctenopharyngodon idellus*) significantly decreased when a 1% chitosan solution was added.

4. Bioactive coatings

The modern marine-related food industries are encountering challenges and require for specific alternatives to surmount them. Among these, issues related to seafood packaging for products with a short shelf life are of pivotal importance. Although the utilization of conventional packaging materials such as plastics and their derivatives are effective for seafood preservation, they create serious and hazardous environmental problems, a situation which presents the seafood industry as a source of pollution and social concerns. This problem requires that all stakeholders in this industry and especially scientists specializing in the food engineering and packaging field to seek alternatives to address this serious problem related to the packaging material. A non-negligible aspect, which is the total cost of the final product, is also related to the packaging materials because it is well

known that the contribution of the packaging to the product total cost is highly significant. So, the search for more economical packaging materials is a very important subject in the seafood industry (Aider, 2010).

Edible bio-based films have been investigated for their abilities to avoid moisture or water absorption by the seafood matrix, oxygen penetration to the food matrix, aroma loss and solute transport out of the product (Dutta, Tripathi, Mehrotra & Dutta, 2009). Based on this consideration, one of the most perspective active bio-film is the one based on chitosan. More recently, two review studies have reported the application of chitosan as bioactive film in the food industry (Aider, 2010; Dutta et al., 2009). Chitosan film, like many other polysaccharide based films, tend to exhibit resistance to fat diffusion and selective permeability to gases. However, they have a serious lack in terms of resistance to water and water vapor transmission. This behavior is mainly due to the strong hydrophilic character of these biopolymers, a property that leads to high interaction with water molecules (Bordenave, Grelier, & Coma, 2007). Owing to this, polymer blending or biocomposites and multilayer systems are potential approaches to prepare chitosan based bioactive coatings or films with desirable characteristics. In this context, Ye et al. (2008) stated that since edible film formed by chitosan is brittle and does not have good mechanical properties, coating chitosan onto a plastic film would overcome this problem. These authors have used chitosan-coated plastic films in which they have incorporated five antibacterial agents, namely nisin, sodium lactate (SL), sodium diacetate (SD), potassium sorbate (PS) and sodium benzoate (SB) as a novel antibacterial edible film against *Listeria monocytogenes* on cold-smoked salmon. This approach solved problems related to food safety since it is well known that *L. monocytogenes* could grow to high levels on cold-smoked salmon, even at normal refrigeration temperature. The risk related to *L. monocytogenes* is particularly high at abusive storage temperatures. Chitosan-coated films containing 4.5 mg/cm² SL, 4.5 mg/cm² SL-0.6mg/cm² PS and 2.3 mg/cm² SL-500 IU/cm² nisin were the most effective treatments against *L. monocytogenes* at ambient temperature. These treatments showed long term antilisterial efficacy during refrigerated storage on vacuum-packed cold-smoked salmon. However, it is important to consider the fact that since antibacterial activity of chitosan may be negligible when it is in the form of insoluble films. Under this state, chitosan is ineffective because it is unable to diffuse through a rigid food matrix such as salmon. Sathivel et al. (2007) showed that skinless pink salmon (*Oncorhynchus gorbuscha*) fillets glazed with chitosan at a solution concentration of 1% (w/w) had significantly ($p < 0.05$) higher yield and thaw yield than the lactic acid-glazed and distilled water-glazed fillets. This behavior was valid although those fillets all had similar moisture content after thawing. In addition, the rheological study showed that chitosan has pseudoplastic and viscoelastic characteristics. The glass transition temperature for the chitosan film was observed at 80.23 °C. The oxygen, carbon dioxide, nitrogen and water vapor permabilities of the chitosan film were 5.34 10⁻² (cm³/m day atm), 0.17 (cm³/m day atm), 0.03 (cm³/m day atm) and 2.92 10⁻¹⁰ (g water m/m² Pas), respectively. The authors demonstrated that despite the good barrier properties of chitosan against oxygen, it maintained low water vapor transmission because of their hydrophilic nature. Likewise, they stated that chitosan film showed shear thinning and

viscoelastic characteristics and temperature dependent viscosity, which allowed uniform glazing on the salmon fillets and prevented rupturing of chitosan glazing during solidification when the glazed fillets were frozen. Therefore, chitosan glazing applied on the surface of the pink salmon fillets might have acted as a barrier between the fillets and the air surrounding, thus slowing down the diffusion of oxygen from the surrounding air into the fillets. Kester and Fennema (1986) reported that chitosan coatings might act as moisture-sacrificing agents of moisture barriers. Thus, moisture loss from the product could be delayed till the moisture contained within the chitosan coating had been evaporated. Sathivel (2005) highlighted that pink salmon fillets coated with chitosan resulted in significantly higher yield, thaw yield, similar drip loss and cook yield, higher moisture content after thawing, less moisture loss than the control samples and somewhat less than protein-coated products. Besides, there were no significant ($p < 0.05$) effects of coating on color parameters (a^* , b^* and L^* values) for cooked fillets after three months frozen storage. Lopez-Caballero et al. (2005) used chitosan as a material to form a chitosan-gelatin coating for cod patties. They showed that the use of chitosan either as a coating or a powdered ingredient did not affect the product lightness at the end of the storage period. However it resulted in an increase of the product yellowness (b -color parameter). The chitosan coating increased the patty elasticity, whereas the addition of powdered chitosan to the patty mixture increased the other rheological parameters such as gumminess, chewiness, cohesiveness and adhesiveness. Moreover, the coating did prevent spoilage of cod patties as reflected by a decrease in total volatile basic nitrogen (TVBN). Conversely, none of these effects on the bacterial spoilage were observed when the chitosan was added to the patty mixture in a powdered form. Ultimately, the authors reported that the coating had good sensory properties, melted away on cooking and hence did not impart any taste to the product. They provided protection by delaying spoilage. Duan et al. (2010) produced chitosan-krill oil coating and used it in modified atmosphere packaging to extend the shelf life of Lingcod fillets. They reported that chitosan-krill oil coating increased total lipid and omega-3 fatty acid contents of the lingcod by about 2-fold. The reduced chemical changes were reflected by the TVBN values and did not change the color of the fresh fillets, did not affect consumer's acceptance of both raw and cooked lingcod fillets. Consumers preferred the overall quality of chitosan-coated, cooked lingcod fillets over the control. The preference was based on the product firm texture and less fishy aroma and flavor. Considering the lower cost of vacuum packaging, it could be applied in combination with chitosan coatings to maintain the omega-3 fatty acid content and extend shelf life of fresh lean fish such as lingcod. Duan et al. (2009) also showed that fish oil incorporated to chitosan coating decreased the drip loss of frozen samples by 14.1-27.6%. This coating also well fortified the omega-3 fatty acids in lean fish. Cao et al. (2009) and Qi et al. (2010) showed that the chitosan coating could surprisingly increase the shelf life of highly perishable pacific oyster (*C. gigas*) during 21 days storage. This affirmation was based on TVBN, pH values and sensory evaluation of pacific oyster. They stated that the discrepancies between their results and others were derived from the differences in chemical composition of fish and shellfish in which oyster contains significant levels of carbohydrate (glycogen) and a lower total quantity of nitrogen. Ojagh et al. (2010) synthesized chitosan coatings enriched with cinnamon oil to extend the shelf life of refrigerated rainbow trout and showed that sensory

characteristics and TVBN of the end product were drastically improved as the coating was employed on rainbow trout fillets within 16 days cold storage. Similarly, Lopez-Caballero et al. (2005) stated that the addition of dry chitosan led to a noticeable increase in elasticity and product yellowness when cod sausages were enriched with chitosan solution. The TVBN remained stable during 25 days storage and the product elasticity was reinforced.

5. Effluent treatment

The use of chitosan as a coagulating agent for removing suspended solids from various processing streams has been widely investigated including cheese whey and dairy wash water, in the processing of poultry and seafood products (Kumar, 2000; Savant, 2001; Savant & Torres, 2000; Savant & Torres, 2003; Shahidi et al., 1999). Chitosan at a concentration of 10 mg/L reduced up to 98% the total suspended solids in shrimp processing wastewater (Bough, 1976). Protein recoveries from surimi wash water (SWW) using 150 mg/L chitosan-alginate complex per liter SWW at mixing ratio of 0.2 resulted in 78-94% adsorption after 24 h (Wibowo, 2003). This result was higher than the one obtained by using 50 mg/L, which yielded 81-90 % protein adsorption in the same treatment time (Savant, 2001). These reported findings suggest that reaction time and chitosan concentration play an important role in reducing total suspended solids and lowering solution turbidity. Moreover, the differences in molecular weights (MW) and degree of deacetylation (DD) between chitosan samples could explain the significant differences in protein recovery capacity. At the lowest concentration (20 mg/L SWW) tested in the study reported by Wibowo (2003), the experimental chitosan gave higher protein recovery than a commercial sample, which required a 5-fold higher concentration for the same effectiveness. This finding has commercial implications as it would reduce processing costs and the chitosan content in the solids recovered by the treatment (Wibowo, Velazquez, Savant & Torres, 2007a). If implemented commercially, the chitosan-alginate complex may be an effective alternative not only for the recovering of soluble proteins that would otherwise be discarded into the environment, but also as an economically viable downstream process over expensive, commercial membrane treatments and their limited use due to fouling (Savant, 2001). Surimi wash water protein (SWWP) was precipitated by using a chitosan-alginate complex. The precipitate had a crude protein content of 73.1 % and a high concentration of essential amino acids (3% histidine, 9.4% lysine, 3.7% methionine, and 5.1% phenylalanine). In a rat-feeding trial, SWWP as a single protein source showed higher modified protein efficiency and net protein rations than the casein control. Blood chemistry analysis did not reveal any deleterious effect from the full protein substitution or the chitosan in SWWP (Wibowo, Savant, Gherian, Velazquez & Torres, 2007b; Wibowo, Velazquez, Savant & Torres, 2005). Moreover, Guerrero, Omil, Mendez & Lema (1998) showed that the utilization of chitosan at a concentration of 10 mg/L and pH 7 in the process of coagulation-flocculation followed by centrifugation in fish-meal factory effluents decreased the total suspended solids up to 85%. The most important mechanisms explaining the chitosan effectiveness in seafood plant effluents treatment was mainly attributed to its positive charge and interaction with negatively charged compounds in the effluents such as protein. Furthermore, the hydroxyl

groups on the chitosan molecule contribute to increase the precipitation of proteins and other suspended solids in the seafood plant effluents (Savant, 2001; Wibowo et al., 2007a,b).

6. Gelling enhancer

Surimi is a refined fish protein product prepared by washing mechanically deboned fish to remove blood, lipids, enzymes and sarcoplasmic protein. The myofibrillar proteins are concentrated in the resulting product and form an elastic gel when solubilized with NaCl and heated (Mao & Wu, 2007). Gel forming properties of myofibrillar proteins are quickly lost by degradation by the action of endogenous proteolytic enzymes if fish is not processed into surimi immediately. The utilization of frozen fish flesh for surimi production is unsuitable due to the rapid loss of protein functionality by freeze denaturation. High quality surimi is produced from fresh, unfrozen fish. Thus, processing at sea has been required in order to obtain high quality surimi. However, the cost of the processing at sea is much higher compared to the land-base processing (Lanier, Manning, Zetterling & Macdonald, 1992). In order to prepare a strong and elastic gel from fish species with low commercial value, low quality surimi is produced onshore with the aid of gel-forming biopolymers such as starch. In this way, chitosan is a good option to be incorporated into the products to improve their techno-functional quality (Kataoka, Ishizaki, & Tanaka, 1998; Mao & Wu, 2007; Li & Xia, 2010). Overall, gel-forming ability of surimi depends on both intrinsic and extrinsic factors, namely fish species, physio-chemical properties of muscle proteins, the presence of endogenous enzymes such as proteinase and transglutaminase, and the conditions used in the product processing (Benjakul, Visessanguan, Phatchrat & Tanaka, 2003). The strength of gels prepared from low quality walleye Pollock (*Theragra chalcogramma*) was almost doubled by the addition of 1.5% chitosan when salted surimi pastes were set below 25 °C. The polymerization of myosin heavy chain accelerated in the presence of 1.5% chitosan (Kataoka et al., 1998). Along with chitosan, endogenous transglutaminase (TGase) played an important role in the formation of gel. The addition of TGase inhibitor to the salted walleye Pollock surimi inhibited the gel enhancement by chitosan. The mechanisms of chitosan effect on enhancing the gel formation is not clear. However, the participation of hydrophobic interactions, hydrogen bondings, and electrostatic interactions during the setting process has been proposed as a possible mechanism by which chitosan can enhance the formation of cross-linked myosin heavy chain components during their polymerization by endogenous enzymes (Benjakul et al., 2003; Kataoka et al., 1998; Li & Xia, 2010; Mao & Wu, 2007). Benjakul et al. (2003) reported that barred garfish (*Hemiramphus far*) surimi gel showed an increase in the breaking force when 1% chitosan was added. However, gel-forming ability of surimi containing chitosan was inhibited in the presence of EDTA due to the chelating of calcium ions that are necessary for TGase activity. Owing to this, the enhancing effect of chitosan was possibly mediated through the action of endogenous TGase during product processing, resulting in the formation of protein-protein and protein-chitosan conjugates. In conjunction with processing and the addition of calcium ions, TGase may play an important role in the cross-linking of protein-protein and protein-chitosan conjugates by means of the amino groups of

chitosan as the acyl acceptor. Conversely, chitosan did not substantially modify the rheological and microstructural properties of horse mackerel gels (*Trachurus spp.*). Also, it had a slight reduction in gel elasticity obtained under high-pressure conditions (Gomez-Guillén, Montero, Sole & Perez-Mateos, 2005). Kok and Park (2007) stated that in the threadfin bream (*Nemipterus spp.*) surimi, the balance of protein-chitosan and protein-protein conjugates determined the surimi gel strength. Similarly, Mao and Wu (2007) showed that in the presence of chitosan in kamaboko gel of grass carp (*Ctenopharyngodon idellus*), protein-chitosan conjugates would be formed between the reactive amino groups of glucosamine and the glutaminy residue of the myofibrillar proteins. The bonds between chitosan and myofibrillar proteins could be associated with the improvement of texture properties in the gels with final structure formed by both covalent and non-covalent interactions. The effect would be also due to some modifications of the endogenous TGase activity. More recently, Lia and Xia (2010) showed that molecular weight and degree of deacetylation (DD) of chitosan have different impacts on gel properties of salt-soluble meat proteins from silver carp. The gel containing chitosan with DD of 77.3% showed the highest penetration force and storage modulus. The penetration forces of gels increased with increasing the amount of molecular weight of chitosan incorporated in the gel. The interaction between chitosan and salt-soluble meat proteins was mainly stabilized by the electrostatic interactions and hydrogen bonds.

7. Encapsulation

Nowadays, the value of functional foods and bioactive compounds are increasing due to the awareness and consciousness of people about it. Despite this fact, many of these compounds are so much sensitive to environmental factors such as oxygen, light, and temperature. In addition, being incorporated into foods and drugs in delivery systems, these bioactive components are hydrolyzed by harsh conditions in the gastrointestinal tracts (Alishahi et al., 2011). Schep, Tucker, Young, Ledger and Butt (1999) stated that many of oral delivery systems of bioactive compounds in aquaculture met the three major barriers through the gastrointestinal tract, involving the enzymatic barriers from the host luminal and membrane bound enzymes, immunological cells present within both the enterocytes and underlying connective tissue and the physical barrier of the epithelial cells. Based on this consideration, the encapsulation of bioactive compounds and functional foods could be a promising way to overcome these problems. Encapsulation is a process in which thin films, generally of polymeric materials, are applied to little solid particles, liquids or gas droplets. This method is used to entrap active components and release them under controlled conditions (Deladino, Anbinder, Navarro & Martino, 2008). Several materials have been encapsulated for the use in the food industry such as vitamins, minerals, antioxidants, colorants, enzymes and sweeteners (Shahidi & Han, 1993). Chitosan can act as an encapsulating agent because of its non-toxicity, biocompatibility, mucus adhesiveness and biodegradability (Alishahi et al., 2011; Kumar, 2000). Recently, Alishahi et al. (2011) showed that chitosan/vitamin C nanoparticle system successfully increased the shelf life and delivery of vitamin C during 20 days storage of rainbow trout. They showed that shelf life of vitamin C significantly ($p <$

0.05) increased in rainbow trout feed till 20 days at ambient temperature, while the control which was feed by vitamin C alone, drastically lost its vitamin C content during few days at ambient temperature. Moreover, the controlled release behavior of vitamin C, in vitro and in vivo, showed that vitamin C was released in the gastrointestinal tract of rainbow trout in the controlled manner (up to 48 h) and chitosan nanoparticles could well maintain vitamin C against harsh conditions, acidic and enzymatic hydrolysis, in the gastrointestinal tract of rainbow trout. Also, Alishahi et al. (2011) showed that the chitosan nanoparticles containing vitamin C could significantly ($p < 0.05$) induce the non-specific immunity system of rainbow trout, as compared with the control. RajeshKumar, VenKatesan, Sarathi, Sarathbabu, Thomas and Anver Basha (2009) demonstrated that chitosan nanoparticles are able to encapsulate DNA and then favorably incorporated into shrimp feed to protect them from white spot syndrome virus. Their results showed that these nanoparticles increased the survival rates of shrimp against white spot syndrome during 30 days post-treatment. Likewise, RajeshKumar, Ishaq Ahmed, Parameswaran, Sudhakaran, Sarath Babu and Sahl Hameed (2008) incorporated chitosan nanoparticles containing DNA vaccine into Asian sea bass (*Lates calcarifer*) feed. Their results indicated that the sea bass orally vaccinated with chitosan-DNA (pVAOMP38) complex showed moderate protection against experimental *Vibrio anguillarum* infection. Similarly, Tian, Yu and Sum (2008) reported that chitosan microspheres loaded with plasmid vaccine was interestingly used to orally immunize Japanese flounder (*Paralichthys olivaceus*). They explained that the release profile of DNA from chitosan microspheres in PBS buffer (pH 7.4) was up to 42 days after intestinal imbibitions. Aydin and Akbuga (1996) showed that salmon calcitonin, available for clinical use, was suitably encapsulated in chitosan beads and the results confirmed that salmon calcitonin-loaded chitosan beads could be prepared by gelling the cationic chitosan with the anionic counterpart providing a controlled release property. Also, shark liver oil could be efficiently encapsulated in calcium alginate beads coated with chitosan in order to mask its unpleasant taste (Peniche, Howland, Carrillo, Zaldivar & Arguelles, 2004). The chitosan coating allowed controlling the permeability of capsules and avoiding leakage. The shark liver oil loaded chitosan/calcium alginate capsules were initially resistant to the acid environment of the stomach. But after 4 h at the intestinal pH (7.4), the capsule wall weakened and thereby was able to be easily deteriorated and disintegrated by the mechanical and peristaltic movements of the gastrointestinal tract. Likewise, Klinkesorn and McClements (2009) stated that the encapsulation of tuna oil droplets with chitosan affected their physical stability and digestibility when they were passed through an in vitro digestion model containing pancreatic lipase. The amount of free fatty acids released from the emulsions decreased as the concentration of chitosan increased. However the release was independent of chitosan Mw. These results showed that chitosan was able to reduce the amount of free fatty acids released from the emulsion, which may be attributed to a number of different physiological mechanisms, including formation of a protective chitosan coating around the lipid droplets, direct interaction of chitosan with lipase, or fatty acid binding by the chitosan. Also, they showed that pancreatic lipase was able to digest chitosan and release glucosamine, having important implications for the utilization of chitosan coatings for the encapsulation, protection and delivery of Omega-3 fatty acids. They suggested that

encapsulation with chitosan could be used to protect emulsified polyunsaturated lipids from oxidation during storage. However, they will release the functional lipids after they are consumed. Industrially, tuna oil encapsulation with chitosan using ultrasonic atomizer was shown to be the promising technique in the near future (Klaypradit & Huang, 2008).

8. Conclusions

Chitosan, a deacetylated derivative of chitin, has attracted a great attention in the seafood industry due to its non-toxicity, biodegradability, biocompatibility and mucus adhesiveness properties. Chitosan has different characteristics such as antibacterial, antioxidant, film-forming ability, gel enhancer, encapsulating capacity, tissue engineering scaffold, wound dressing, and coagulating agent. Upon knowing these, chitosan could successfully be incorporated into seafood products for both seafood quality and human health enhancement. Regarding its outstanding characteristics, chitosan would be used as functional ingredients in marine-based products and it merits further researches in the future.

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