CHAPTER 7

Chitosan as Potential Marine Nutraceutical

Jae-Young Je[*,1] and **Se-Kwon Kim**[†]

Contents

Abstract

Chitosan, the most abundant marine mucopolysaccharide, is derived from chitin by alkaline deacetylation, and possesses versatile biological properties such as biocompatibility, biodegradability, and a non-toxic nature. Due to these characteristics, considerable attention has been given to its industrial applications in the food, pharmaceutical, agricultural, and environmental industries. Currently, chitosan can be considered as a potential marine nutraceutical because its remarkable biological activities have been investigated and reported, in order to exploit its nutraceutical

* School of Food Technology and Nutrition, Chonnam National University, Yeosu, Republic of Korea
† Department of Chemistry and Marine Bioprocess Research Center, Pukyong National University, Busan, Republic of Korea
1 Corresponding author: Jae-Young Je, *E-mail address:* jjy1915@jnu.ac.kr

Advances in Food and Nutrition Research, Volume 65
ISSN 1043-4526, DOI: 10.1016/B978-0-12-416003-3.00007-X

properties. This chapter, therefore, reviews the biological activities of chitosan including antioxidant, hypocholesterolemic, antimicrobial, and anti-inflammatory activities, as well as its mode of action in each activity.

I. INTRODUCTION

Chitin, a naturally occurring biopolymer, consists of 2-acetamido-2-deoxy-(1-4)-β-D-glucopyranose residues (*N*-acetyl-D-glucosamine units) and is a well-known mucopolysaccharide and distributed in the shell of crustaceans, in the cuticle of insects, and in the cell wall of some fungi and microorganisms. Chitin has a strong intra- and intermolecular hydrogen bonding network, which provides the water-insoluble property of chitin. Chitin production is estimated as approximately 1×10^9 metric tons annually, and it is the second most abundant natural biopolymer. A great attention has been paid to its biomedical applications such as a drug carrier, an antitumor agent, and a wound-healing agent because of its useful biological properties including nontoxicity, biocompatibility, biodegradability, hemostatic activity, and wound healing (Muzzarelli, 1994).

Chitosan is also a natural cationic polymer derived by deacetylation of chitin under alkaline conditions. Chitosan is a linear polysaccharide consisting of 2-amino-2-deoxy-(1-4)-β-D-glucopyranose residues (D-glucosamine units). Due to the deacetylation of chitin, chitosan can be dissolved in water by making salt with various acids on the amino group of D-glucosamine units. Further, chitosan can be dissolved in water alone as it has a partially acetylated degree of about 50% D-glucosamine units (Aiba, 1989).

Major applications of chitosan were previously focused on sludge dewatering, food processing, and metal ion chelation until the mid-1980s. Further, it has received considerable attention for its commercial applications in agriculture, chemistry, biomedical, food, cosmetic, and biotechnology industries (Alasalvar *et al.*, 2002; Knorr, 1984; Kurita, 2006). In recent years, however, its numerous biological activities were documented such as antioxidant (Park *et al.*, 2004a), antibacterial (Allan and Hadwiger, 1979; Hirano and Nagao, 1989; Jeon and Kim, 2000; Jeon *et al.*, 2001), hypocholesterolemic (Sugano *et al.*, 1992), and immune-stimulating (Jeon and Kim, 2001), and due to these biological properties, chitosan has received much attention for development as a potential marine nutraceutical. This chapter, therefore, deals with producing chitosan from chitin, and will overview the biological properties of chitosan and its derivatives as potential marine nutraceuticals.

II. PREPARATION OF CHITOSAN

Due to strong hydrogen bonds, chitin is insoluble in water as well as in most solvents, making it difficult to apply in industry. Chitin can be converted into chitosan by enzymatic means or alkali deacetylation, this being the most utilized method. Chitosan is soluble in some organic acids such as lactic acid and acetic acid, as well as in inorganic acids. In addition, chitosan salts can be water-soluble depending on factors such as pH and degree of acetylation (Illum, 1998).

A. Chitosan from crustacean chitin

Several sources have been used for the production of chitosan, and the most exploited sources of chitin are the processing waste of shellfish and marine crustaceans, especially crab, lobster, shrimp, krill, oysters, and squid (Rasmussen and Morrissey, 2007). Generally, marine crustacean shells contain around 15–40% chitin (dry weight) with proteins and calcium carbonate (Kurita, 2006). To isolate chitin from natural resources, other components such as cellulose, mannan, polygalactosamine, glucan as well as proteins, pigments, and minerals (especially calcium carbonate) must be removed during extraction procedures. Generally, decalcification of samples is conducted by using dilute hydrochloric acid followed by deproteination in dilute aqueous sodium hydroxide solution. Then the decalcified and deproteinated product undergoes decolorization using 0.5% $KMnO_4$ and oxalic acid. Finally, chitosan can be obtained from chitin followed by deacetylation of the chitin using a hot concentrated sodium hydroxide solution (Fig. 7.1).

B. Chitosan from other sources

Shell wastes from shrimp, crab, and lobster processing industries are the traditional source of chitin. However, commercial production of chitosan by deacetylation of crustacean chitin with a strong alkali appears to have limited potential for industrial acceptance because of seasonal and limited supply, difficulties in processing, particularly with the large amount of waste of concentrated alkaline solution causing environmental pollution, and inconsistent physico-chemical properties (Chatterjee *et al.*, 2005).

Because the biological properties of chitosan are dependent on its physico-chemical properties such as molecular weight (MW) and degree of deacetylation (DD), uniform physico-chemical properties are a prerequisite to specific industrial applications. As an alternative method, fermentation technology for chitosan preparation from fungal cell walls has received much attention as an eco-friendly pathway. Rane and

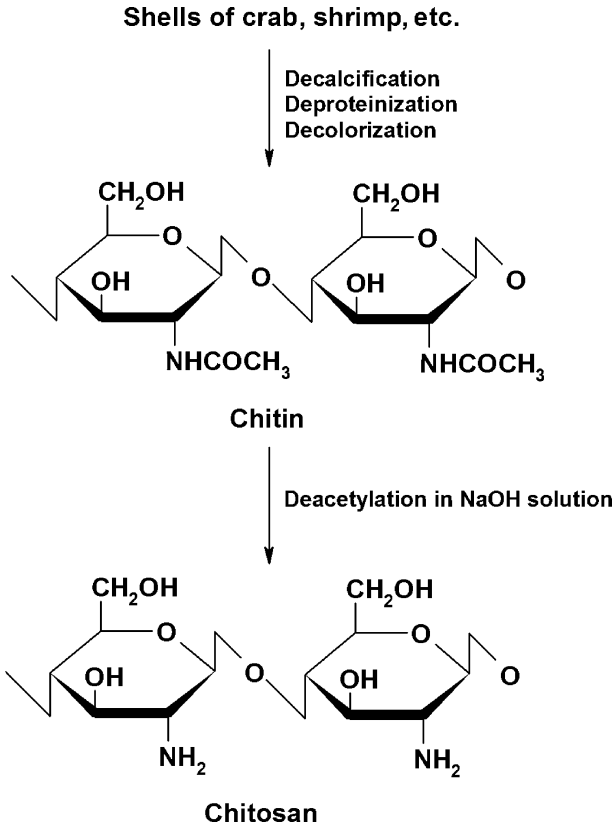

FIGURE 7.1 Preparation of chitosan from chitin.

Hoover (1993) described the production of chitosan from mycelia of *Absidia coerulea*, *Mucor rouxii*, *Gongronella butieri*, *Phycomyces blakesieeanus*, and *Absidia blakesleeana*. *A. coerulea* ATCC 14076 and NRRL 1315 were the best producers of chitosan among the strains examined. Crestini *et al.* (1996) also examined the production and isolation of chitosan through submerged and solid fermentation of *Lentinus edodes*. Chatterjee *et al.* (2005) determined the optimum conditions for the production of chitosan from *M. rouxii*, and investigated the MW, protein content, and DD of the isolated chitosan. Suntornsuk *et al.* (2002) utilized food processing by-products for the growth of fungal strains including *Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Zygosaccharomyces rouxii* TISTR5058, and *Candida albicans* TISTR5239, and their chitosan production yields were in the range of 0.4–4.3 g/kg of soybean residue and 0.5–1.6 g/kg of mungbean residue. These fungal approaches have benefits of easy handling, harvesting, and control in producing high quality

chitosan, as well as different types of chitosans produced by diverse strains with individual environmental and nutritional conditions (Tajdini *et al*., 2010; Wang *et al*., 2008).

III. HEALTH BENEFITS OF CHITOSAN AND ITS DERIVATIVES

Because it has a variety of biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and immune-stimulating effects, chitosan offers a number of uses in food, cosmetic, biomedical, and pharmaceutical industries. In this section, the nutraceutical properties of chitosan and its derivatives are discussed.

A. Antioxidant activity

Reactive oxygen species (ROS) are produced by abnormal metabolic processes as well as sunlight, ultraviolet light, and chemical reactions, and can change the structure of DNA, membrane lipids, and protein, which may result in diseases such as cancer, aging, inflammation, and atherosclerosis (Moskovitz *et al*., 2002). Generally, ROS are effectively eliminated by antioxidant enzymes and compounds in our body. However, over production of ROS can cause considerable damage to biomacromolecules by ROS-mediated oxidative stress. The term antioxidant is defined as any substance, that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Park *et al*., 2001). Therefore, supplementation of antioxidants, which can overcome oxidation-mediated problems, may prevent the body from a set of diseases by directly quenched ROS. There has been a growing interest to develop natural antioxidant compounds from many sources to overcome the ROS-mediated deleterious effects in biological systems. Numerous natural antioxidants such as tocopherols, ascorbic acid, polyphenols, and peptides are marketed around the world.

A number of studies have examined the antioxidant activities of chitosan from various sources. Park *et al*. (2004a) prepared three kinds of partially deacetylated hetero-chitosans such as 90% deacetylated, 75% deacetylated, and 50% deacetylated chitosan from crab chitin, and their antioxidant properties were measured using electron spin resonance spectrometry. Park and coworkers found that their antioxidant activities were dependent on the DD, and the 90% deacetylated chitosan showed the highest free radical scavenging activities. Yen *et al*. (2008) also found that a sample with more amino groups at the C-2 position showed the highest antioxidant activity. Tomida *et al*. (2009) examined the protective effects of seven different MW chitosans on plasma protein from oxidation by peroxyl radicals. In the ability to protect plasma protein from

oxidation, it was found that the low MW chitosan was more effective in preventing the formation of carbonyl groups in plasma protein exposed to peroxyl radicals.

In vivo studies were also conducted by several researchers. Anraku *et al*. (2009) examined the antioxidant effects of water-soluble chitosan in normal subjects by measuring the reduction of indices of oxidative stress. Treatment with chitosan for 4weeks produced a significant decrease in levels of plasma glucose and the atherogenic index, and led to an increase in high-density lipoprotein cholesterol (HDL-C). Chitosan treatment also lowered the ratio of oxidized to reduced albumin and increased total plasma antioxidant activity. Further, Anraku *et al*. (2011) proved the antioxidant effects of high MW chitosan in normal volunteers, and the obtained results were consistent with previous results observed by Anraku *et al*. (2009).

B. Hypocholesterolemic effects

Obesity and high cholesterol levels are major factors influencing human health and can lead to increased risk of cardiovascular disease, type II diabetes, and some cancers. The hypocholesterolemic action of chitosan can be explained by decreased cholesterol absorption through entrapment of bile acids and interference with bile acid absorption. It is well-known that bile acids are produced from cholesterol, which is a major route of cholesterol metabolism, and about half of the synthesized cholesterol in our body is used for bile acid synthesis. About 90% of excreted bile acids are reabsorbed and recycled in enterohepatic circulation that moves bile acids to the liver and the gallbladder. Due to the negatively charged form of bile acids, chitosan can form complexes with bile acids due to its positively charged form, thereby bile acids are subsequently removed from reabsorption and recycling systems, and fewer bile acids are returned to the liver. This increases the synthesis of bile acids in the liver from the oxidation of cholesterol, thereby decreasing the total blood cholesterol level (Lee *et al*., 1999).

A number of studies have revealed that chitosan can reduce plasma and liver triacylglycerol (TG) and total cholesterol levels *in vitro* and *in vivo*. Recently, the effects of the DD and the viscosity-average molecular weight (Mv) of chitosan samples on their fat-binding and cholesterol- and bile acid-binding capacities *in vitro* were evaluated (Liu *et al*., 2008a,b; Zhou *et al*., 2006). The fat-binding capacity of chitosan increased with increasing DD and Mv values, whereas cholesterol-binding ability was not affected by the values of DD and Mv. In addition, high Mv chitosan samples showed the greatest bile acid-binding ability but were not affected by the DD value.

In vivo studies also showed that chitosan has hypocholesterolemic and hypolipidemic activities (Nagyvary *et al*., 1979; Sugano *et al*., 1978, 1980).

It was found that chitosan with a relatively low MW (5–120 kDa) and 79% DD had the greatest cholesterol-lowering effects, and short oligomers seemed to be ineffective (Sugano *et al.*, 1988; Trautwein *et al.*, 1997; Ylitalo *et al.*, 2002). Rats fed diets with high DD chitosan exhibited significantly lowered plasma triglyceride, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) levels as well as elevated high-density lipoprotein cholesterol (HDL-C) levels. Moreover, food-efficiency ratio decreased with increasing DD (Li *et al.*, 2007; Liu *et al.*, 2008b). In addition, liver hepatic and lipoprotein lipase activities were reduced by chitosan, which indicated that it could regulate lipase activity; chitosan did not only prevent hyperlipidemia induced by long-term administration of a high-fat diet but also reduced serum lipid levels and liver-fat accumulation in hyperlipidemic rats. Xu *et al.* (2007) also investigated the effects of chitosan on lipid metabolism in hyperlipidemic rats. Chitosan lowered total cholesterol levels, LDL-C in plasma, and total cholesterol and total triglycerides in the liver, and increased fecal bile acid excretion, but did not change levels of triglyceride and HDL-C in plasma. It was also found that hepatic LDL receptor mRNA levels were increased by chitosan.

Bokura and Kobayashi (2003) suggested that in the digestive system, chitosan acts by forming a gel in the intestinal tract, which entraps cholesterol and lipids, and ingested chitosan develops an HCl layer in the stomach. Chitosan particles form agglomerates with cholesterol and fatty acids in the duodenum, which resulted in inhibiting cholesterol adsorption from the gastrointestinal tract. In a randomized, double-blind, and placebo-controlled clinical study, oral administration of chitosan reduced serum total cholesterol, especially in elderly women, while chitosan exhibited mild effects of reducing total and LDL cholesterol after 8 weeks (Bokura and Kobayashi, 2003).

Several researchers suggested a possible mechanism with regard to the hypocholesterolemic activity of chitosan as follows. Tai *et al.* (2000) explained that the possible mechanism of the hypocholesterolemic activity of chitosan is similar to dietary fiber. Chitosan has high positive charge densities at low pH (6.5); therefore, chitosan is positively charged in gastric acid, which allows it to make complexes between positively charged chitosan and negatively charged cholesterol, fatty acids, and bile acids. The complexes then move into the intestines under an alkaline condition and change into an insoluble gel form. When the complexes change into the gel form, they cannot be hydrolyzed by pancreatic lipase and are excreted in the feces. A number of studies had also reported that chitosan increased fecal excretion of cholesterol and bile acids in animals and humans (Imonek and Bartonova, 2005; Lengsfeld *et al.*, 1999; Yao and Chiang, 2006). Due to the electrostatic interaction between chitosan and negatively charged cholesterol, fatty acids, and bile acids, higher DD chitosan shows the highest hypocholesterolemic activity but viscosity is

not the major factor influencing the hypocholesterolemic effects of chitosan in the upper gastrointestinal tract.

C. Antimicrobial activity

Many studies have been conducted on the antimicrobial activity of chitosan since Allan and Hadwiger (1979) first reported that chitosan and its derivatives had broad-spectrum antimicrobial effects. The group of researches proved that chitosans are capable of inhibiting the growth of some microorganisms including bacteria, yeasts, and fungi.

Jeon *et al*. (2001) prepared 89% deacetylated chitosan, and the MIC values of chitosan against Gram-negative and Gram-positive bacteria were less than 0.06%. No *et al*. (2002) also examined the antibacterial activities of six chitosans and six chitosan oligomers with different MWs against four Gram-negative (*Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, and *Vibrio parahaemolyticus*) and seven Gram-positive bacteria (*Listeria monocytogenes*, *Bacillus megaterium*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus bulgaricus*). The MICs of the chitosans ranged from 0.05% to more than 0.1% depending on the bacterial species and the MW of each chitosan, and the activity was higher than that of chitosan oligomers. Seo *et al*. (1992) tested the effects of chitosan on the growth of 11 different bacteria and found that the MIC values of the chitosan ranged from 10 to 1000 ppm. Among the organisms tested, the growth of *E. coli*, *P. fluorescens*, *B. cereus*, and *S. aureus* was inhibited by chitosan concentrations of 20, 500, 500, and 1000 ppm (i.e., 0.002, 0.05, 0.05, and 0.1%, respectively), respectively. Uchida *et al*. (1989) previously reported that the MIC values of chitosan for *E. coli* and *S. aureus* were 0.025% and 0.5%, respectively. According to the literature, the antimicrobial activity of chitosan is influenced by a number of factors such as the type of chitosan, molecular mass, and some of its other physico-chemical properties (Shibasaki *et al*., 1994; Uchida *et al*., 1989). In recent years, the antimicrobial activity of chitosan against methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) was investigated by several researchers. Lee *et al*. (2009) prepared β-chitosan (685 kDa, 20 cps) from arrow squid pens and evaluated it against MRSA and VRSA, and found a MIC value of 0.625 mg/mL against MRSA and VRSA, respectively. Park *et al*. (2009) also prepared chitosan (P6-MMC) with an average MW of 110 kDa from high MW chitosan by enzymatic hydrolysis, and evaluated its antimicrobial activity against MRSA. It was observed that P6-MMC exhibited superior anti-MRSA activity in a dose-dependent manner compared to native chitosan.

So far, a tremendous amount of literature supports the essential importance of the polycationic structure of chitosan in antimicrobial

activity. A higher positive charge leads to electrostatic interaction between chitosan and negatively charged cell walls. A high DD shows a higher positive charge in chitosan. Park *et al*. (2004b) proved that high DD chitosan revealed a higher antimicrobial activity than low DD chitosan. Takahashi *et al*. (2008) also provided evidence that a higher DD with more positive charge was especially successful in inhibiting the growth of *S. aureus*, suggesting the antibacterial activity of chitosan toward *S. aureus* was enhanced with increasing DD. These results suggest that a free amino group plays a pivotal role in the antimicrobial activity. However, numerous studies on the bactericidal activity of chitosan have generated ambiguous results concerning the correlation between bactericidal activity and chitosan MW. Several reports revealed that high MW chitosan showed higher antimicrobial activity than low MW chitosan because high MW chitosan has more positive charge. On the other hand, low MW chitosan showed higher inhibitory activities against *Fusarium oxysporum*, *Phomopsis fukushi*, and *Alternaria alternata* than high MW chitosan (Hirano and Nagao, 1989). Jeon and Kim (2000) also showed that low MW chitosan with MW of 5000–10,000 Da had stronger antimicrobial activity than high MW chitosan. Chitosan is known to have chelating ability for various trace metal ions including Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Mg^{2+}, and Cu^{2+} in acid conditions. It is well known that the cell walls of microorganisms, especially the outer membrane of Gram-negative bacteria, are stabilized by interactions between metal ions and cell wall molecules such as lipopolysaccharides. Therefore, the chelating of chitosan toward trace metal ions disrupts the integrity of the outer membrane, resulting in loss of the barrier function or blocking of the nutrient flow with concomitant bacterial death due to depletion of nutrients.

Currently, chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively (KFDA, 1995; Weiner, 1992), and is mostly applied as a food additive or preservative and as a component of packaging material. In addition, the antimicrobial characteristics of chitosan present a profitable potential for developing functional food ingredients; however, they may prove to be useful in nutraceuticals for the ability to stimulate host defenses against a variety of bacteria, fungi, and yeast (Lim and Hudson, 2003).

D. Anti-inflammatory activity

Inflammation involving a wide variety of physiological and pathological processes is a kind of defense mechanism in an organism and is initiated by the invasion of pathogens or by tissue injury caused by biological, chemical, or physical damage. Inflammation is largely divided into acute and chronic inflammation. Acute inflammation is a short-term normal response, whereas chronic inflammation may progress from acute

inflammation if the stimuli persist, causing individual tissue damage (Drayton *et al*., 2006). Usually, leukocytes including macrophages, neutrophils, and eosinophils lead to tissue repair by the production of a variety of inflammatory mediators in order to remove the cause of inflammation. Among the cellular responses, the activation of macrophages is essential to the initiation and continuation of defensive reactions. Once they are stimulated by stimuli, macrophages released proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6, as well as inflammatory mediators including nitric oxide and prostaglandin E_2 (PGE_2), which are synthesized by inducible nitric oxide and cyclooxygenase-2 (COX-2), in order to enhance the defense capacity. Secretion of the inflammatory mediators against invading pathogens is essential for innate immunity and promotes the actions of the specific immune system; however, overproduction of macrophage-derived mediators are involved in the causation of many human diseases including rheumatoid arthritis, asthma, atherosclerosis, and endotoxin-induced multiple organ injury (Guslandi, 1998; Ritchlin *et al*., 2003; Simons *et al*., 1996). Therefore, anti-inflammatory agents reduce the magnitude of the released mediators by inflammatory response, and several nutraceuticals such as ω-3 PUFA, soy isoflavones, plants, and spices are being discovered.

Although a number of studies regarding the biological properties of chitosan have been performed, a few on its anti-inflammatory activities have recently been published. Ueno *et al*. (2001) described that chitosan promotes phagocytosis and the production of osteopontin and leukotriene B by polymorphonuclear leukocytes; activities such as phagocytosis; the production of IL-1, transforming growth factor b1, and platelet-derived growth factor by macrophages; and the production of IL-8 by fibroblasts, enhancing immune responses. Chitosan was also able to prevent pulmonary inflammation by inhibiting type 2 helper T cells and reducing levels of IL-4 and IL-5, and water-soluble chitosan inhibited the secretion of both IL-8 and TNF-α from mast cells (Hall *et al*., 2002; Kim *et al*., 2004). Since mast cells are necessary for allergic reactions and have been implicated in a number of neuroinflammatory diseases, these results suggest that chitosan has the potential to reduce the allergic inflammatory response. Chou *et al*. (2003) also found that chitosan inhibited PGE_2 formation and COX-2 induction in LPS-stimulated RAW264.7 macrophages. A high amount of PGE_2 derived from COX-2 induced by many proinflammatory mediators including TNF-α, IL-1β, and LPS has been implicated in the pathogenesis of sepsis and inflammation (Futaki *et al*., 1997; Hammond *et al*., 1999). In addition, treatment of a selective COX-2 inhibitor suppressed PGE_2 as well as normalized proinflammatory cytokine formation and subsequently improved survival after trauma, suggesting that inhibition of PGE_2 overproduction by suppressing COX-2 expression may be a potential pharmacologic target to treat

inflammatory diseases (Mark Strong *et al*., 2001). In another study, Kim *et al*. (2002) investigated the anti-inflammatory effect of water-soluble chitosan in human astrocytoma cells activated by amyloid β peptide and IL-1β, because a chronic inflammatory response associated with β-amyloid and IL-1β is responsible for the pathology of Alzheimer's disease. After stimulation by β-amyloid and IL-1β, the production of proinflammatory cytokines was amplified; however, the production of TNF-α and IL-6 was significantly inhibited by pretreatment of water-soluble chitosan in the human astrocytoma cells. As described above, chitosan may be able to modulate some inflammatory cytokines as well as inflammatory gene expression; therefore, chitosan could be used as an ingredient in functional foods for the prevention or alleviation of inflammatory diseases.

E. Other biological properties

Chitosan has also been reported to prevent increases in blood pressure. A high level of sodium chloride in the diet has been shown to increase blood pressure in human and animal models of hypertension (Boon and Aronson, 1985). In addition, the ingestion of nonchloride sodium salt did not increase blood pressure, but an elevation of blood pressure was observed with the ingestion of chloride salts. These results suggest that chloride ions play an important role in the elevation of blood pressure in human and animal models of hypertension. Okuda *et al*. (1997) proved that chitosan could reduce blood pressure with the administration of a high-salt diet containing chitosan, and serum angiotensin-converting enzyme was also significantly reduced by chitosan in stroke-prone spontaneously hypertensive rats.

The immunostimulating activity of chitosan has also been reported. A 70% DD chitosan showed immunostimulating effects by activating macrophages and natural killer cells in rats when infected with *E. coli* and *Sendai virus* (Nishimura *et al*., 1984). Chang *et al*. (2004) also reported the immune-enhancing effects of chitosan as a novel adjuvant to an inactivated influenza vaccine, and the antibody content in serum remarkably increased the antiviral defenses of mice.

Further, the antiulcer effect of chitosan was investigated. Anandan *et al*. (2004) induced ulcers in rats by HCl–ethanol, and investigated the effects of ulcers in the presence of chitosan. Significant increases were observed in the volume and acidity of gastric fluid and in the level of lipid peroxidation, along with significant decreases in glutathione levels, activity of antioxidant enzymes, and peptic activity in the gastric mucosa. However, the ulcerogenic effects of HCl–ethanol were decreased in the presence of chitosan to normal levels of gastric activity and peptic activity.

IV. FUTURE PROSPECTS

Chitosan is a deacetylated form of chitin from the exoskeletons of marine crustaceans, shellfish, and some fungi. Due to its biocompatible, biodegradable, and nontoxic nature, considerable interest and attention have been focused on chitosan to develop nutraceuticals. According to past research, chitosans exhibit a variety of biological activities including antioxidant, hypocholesterolemic, antimicrobial, immune-stimulating, and anti-inflammatory activities that give them the potential to be highly effective nutraceuticals. However, it should be noted that the described biological activities of chitosan were dependent on its MW and DD, and not all of these biological activities are displayed in the same chitosan. Prior to potential application of chitosan as a nutraceutical, special types of chitosan displaying special bioactivities should be developed. Moreover, high MW chitosans are water-insoluble, and are difficult to absorb in the intestine, which is a major limitation for applications. In addition, the exact mechanism of each biological activity of chitosan should be understood in molecular-level detail. Although a tremendous amount of research on chitosan with regard to exploiting it as a nutraceutical has been conducted, some problems still remain unsolved; therefore, solutions to these problems may help accelerate its future applications.

REFERENCES

Aiba, S. (1989). Studies on chitosan: 2. Solution stability and reactivity of partially N-acetylated chitosan derivatives in aqueous media. *Int. J. Biol. Macromol.* **11**, 249–252.

Alasalvar, C., Shahidi, F., and Quantick, P. (2002). Food and health applications of marine nutraceuticals: A review. *In* ''Seafoods: Quality, Technology, and Nutraceutical Applications'', (C. Alasalvar and T. Taylor, Eds), pp. 175–204. Springer-Verlag, Berlin.

Allan, G. R. and Hadwiger, L. A. (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. *Exp. Mycol.* **3**, 285–287.

Anandan, R., Mathew, S., and Viswanathan, N. P. G. (2004). Antiulcerogenic effects of chitin and chitosan on mucosal antioxidant defense system in HCl-ethanol induced ulcer in rats. *J. Pharm. Pharmacol.* **56**, 265–269.

Anraku, M., Fujii, T., Furutani, N., Kadowaki, D., Maruyama, T., Otagiri, M., Gebiciki, J. M., and Tomida, H. (2009). Antioxidant effects of a dietary supplement: Reduction of indices of oxidative stress in normal subjects by water-soluble chitosan. *Food Chem. Toxicol.* **47**, 104–109.

Anraku, M., Fujii, T., Kono, T., Kojima, E., Hata, T., Tabuchi, N., Tsuchiya, D., Goromaru, T., Tsutsumi, H., Kadowaki, D., Maruyama, T., Otagiri, M., *et al*. (2011). Antioxidant properties of high molecular weight dietary chitosan in vitro and in vivo. *Carbohydr. Polym.* **83**, 501–505.

Bokura, H. and Kobayashi, S. (2003). Chitosan decreases total cholesterol in women. A randomized, double-blind, placebo-controlled trial. *Eur. J. Clin. Nutr.* **57**, 721–725.

Boon, N. A. and Aronson, J. K. (1985). Dietary salt and hypertension: Treatment and prevention. *Br. Med. J.* **290**, 949–950.

Chang, H. Y., Chen, J. J., Fang, F., and Chen, Z. (2004). Enhancement of antibody response by chitosan, a novel adjuvant of inactivated influenza vaccine. *Chin. J. Biol.* **17**, 21–24.

Chatterjee, S., Adhya, M., Guha, A. K., and Chatterjee, B. P. (2005). Chitosan from *Mucor rouxii*: Production and physico-chemical characterization. *Process Biochem.* **40**, 395–400.

Chou, T. C., Fu, E., and Shen, E. C. (2003). Chitosan inhibits prostaglandin E2 formation and cyclooxygenase-2 induction in lipopolysaccharide-treated RAW 264.7 macrophages. *Biochem. Biophys. Res. Commun.* **308**, 403–407.

Crestini, C., Kovac, B., and Sermanni, G. G. (1996). Production and isolation of chitosan from submerged and solid state fermentation from *Lentinus edodes*. *Biotechnol. Bioeng.* **50**, 207–210.

Drayton, D. L., Liao, S., Mounzer, R. H., and Ruddle, N. H. (2006). Lymphoid organ development: From ontogeny to neogenesis. *Nat. Immunol.* **7**, 344–353.

Futaki, N., Tajahashi, S., Kitagawa, T., Yamakawa, Y., Tanka, M., and Higuchi, S. (1997). Selective inhibition of cyclooxygenase-2 by NS-398 in endotoxin shock rats in vivo. *Inflamm. Res.* **46**, 496–502.

Guslandi, M. (1998). Nitric oxide and inflammatory bowel diseases. *Eur. J. Clin. Invest.* **28**, 904–907.

Hall, G., Lund, L., Lamb, J. R., and Jarman, E. R. (2002). Kinetics and mode of peptide delivery via the respiratory mucosa determine the outcome of activation versus TH2 immunity in allergic inflammation of the airways. *J. Allergy Clin. Immunol.* **110**, 883–890.

Hammond, R. A., Hannon, R., Frean, S. P., Armstrong, S. J., Flower, R. J., and Bryant, C. E. (1999). Endotoxin induction of nitric oxide synthase and cyclooxygenase-2 in equine alveolar macrophages. *Am. J. Vet. Res.* **60**, 426–431.

Hirano, S. and Nagao, N. (1989). Effects of chitosan, pectic acid, lysozyme and chitinase on the growth of several phytopathogens. *Agric. Biol. Chem.* **53**, 3065–3066.

Illum, L. (1998). Chitosan and its use as a pharmaceutical excipient. *Pharm. Res.* **15**, 1326–1331.

Imonek, J. and Bartonova, H. (2005). Effect of dietary chitin and chitosan on cholesterolemia of rats. *Acta Vet. Brno* **74**, 491–499.

Jeon, Y. J. and Kim, S. K. (2000). Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. *Carbohydr. Polym.* **41**, 133–141.

Jeon, Y. J. and Kim, S. K. (2001). Potential immuno-stimulating effect of antitumoral fraction of chitosan oligosaccharides. *J. Chitin Chitosan* **6**, 163–167.

Jeon, Y. J., Park, P. J., and Kim, S. K. (2001). Antimicrobial effect of chitooligosaccharides produced by bioreactor. *Carbohydr. Polym.* **44**, 71–76.

KFDA (1995). Food Additives Code. Korea Food and Drug Administration, Seoul p. 449.

Kim, M. S., Sung, M. J., Seo, S. B., Yoo, S. J., Lim, W. K., and Kim, H. M. (2002). Water-soluble chitosan inhibits the production of pro-inflammatory cytokine in human astrocytoma cells activated by amyloid β peptide and interleukin-1β. *Neurosci. Lett.* **321**, 105–109.

Kim, M. S., You, H. J., You, M. K., Kim, N. S., Shim, B. S., and Kim, H. M. (2004). Inhibitory effect of water-soluble chitosan on TNF-α and IL-8 secretion from HMC-1 cells. *Immunopharmacol. Immunotoxicol.* **26**, 401–409.

Knorr, D. (1984). Use of chitinous polymers in food-a challenge for food research and development. *Food Technol.* **38**, 85–97.

Kurita, K. (2006). Chitin and chitosan: Functional biopolymers from marine crustaceans. *Mar. Biotechnol.* **8**, 203–226.

Lee, J. K., Kim, S. U., and Kim, J. H. (1999). Modification of chitosan to improve its hypocholesterolemic capacity. *Biosci. Biotechnol. Biochem.* **63**(5), 833–839.

Lee, S. J., Kim, E. K., Moon, S. H., Jeon, B. T., Park, P. J., and Song, M. D. (2009). Antimicrobial activity of β-chitosan against VRSA and MRSA. *J. Chitin Chitosan* **14**, 130–133.

Lengsfeld, H., Fleury, A., Nolte, M., Piquerez, J. C., Hadvary, P., and Beglinger, C. (1999). Effect of orlistat and chitosan on faecal fat excretion in young healthy volunteers. *Obes. Res.* **7**(Suppl. 1), 50S.

Li, L., Zhang, J. L., Liu, J. N., and Xia, W. S. (2007). Effects of chitosan on serum lipid and fat liver. *Chin. J. Mar. Drugs* **26**, 7–9.

Lim, S. H. and Hudson, S. M. (2003). Review of chitosan and its derivatives as antimicrobial agents and their uses as textile chemicals. *J. Macromol. Sci.* **43**, 223–269.

Liu, J. N., Xia, W. S., and Zhang, J. L. (2008a). Effects of chitosans physico-chemical properties on binding capacities of lipid and bile salts in vitro. *Chin. Food Sci.* **29**, 45–49.

Liu, J. N., Zhang, J. L., and Xia, W. S. (2008b). Hypocholesterolemic effects of different chitosan samples in vitro and in vivo. *Food Chem.* **107**, 419–425.

Mark Strong, V. E., Mackrell, P. J., Concannon, E. M., Mestre, J. R., Smyth, G. P., Schaefr, P. A., Stapleton, P. P., and Daly, J. M. (2001). NS-398 treatment after trauma modifies NF-kappa B activation and improves survival. *J. Surg. Res.* **98**, 40–46.

Moskovitz, L., Yim, M. B., and Chock, P. B. (2002). Free radicals and disease. *Arch. Biochem. Biophys.* **397**, 354–359.

Muzzarelli, R. (1994). *In vivo* biochemical significance of chitin-based medical items. *In* ''Polymeric Biomaterials'', (S. Dumitriu, Ed.), pp. 179–197. Marcel Dekker, New York.

Nagyvary, J. J., Falk, J. D., Hill, M. L., Schmidt, M. L., Wilkins, A. K., and Bradbury, E. L. (1979). The hypolipidemic activity of chitosan and other polysaccharides in rats. *Nutr. Rep. Int.* **20**, 677–684.

Nishimura, K., Nishimura, S., Nishi, N., Saiki, I., Tokura, S., and Azuma, I. (1984). Immunological activity of chitin and its derivatives. *Vaccine* **2**, 93–99.

No, H. K., Park, N. Y., Lee, S. H., and Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Food Microbiol.* **74**, 65–72.

Okuda, H., Kato, H., and Tsujita, T. (1997). Antihypertensive and antihyperlipidemic actions of chitosan. *J. Chitin Chitosan* **2**, 49–59.

Park, P. J., Jung, W. K., Nam, K. S., Shahidi, F., and Kim, S. K. (2001). Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. *J. Am. Oil Chem. Soc.* **78**, 651–656.

Park, P. J., Je, J. Y., and Kim, S. K. (2004a). Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. *Carbohydr. Polym.* **55**, 17–22.

Park, P. J., Je, J. Y., Byun, H. G., Moon, S. H., and Kim, S. K. (2004b). Antimicrobial activity of hetero-chitosans and their oligosaccharides with different molecular weights. *J. Microbiol. Biotechnol.* **14**, 317–323.

Park, J. K., Moon, H. R., Kim, W. J., Kim, S. M., and Park, Y. I. (2009). Preparation and antimicrobial activity of medium-molecular weight chitosan. *J. Chitin Chitosan* **14**, 138–142.

Rane, K. D. and Hoover, D. G. (1993). Production of chitosan by fungi. *Food Biotechnol.* **7**, 11–33.

Rasmussen, R. S. and Morrissey, M. T. (2007). Chitin and chitosan. *In* ''Marine Nutraceuticals and Functional Foods'', (F. Shahidi and C. Barrow, Eds), pp. 155–182. CRC Press, New York.

Ritchlin, C. T., Haas-Smith, S. A., Li, P., Hicks, D. G., and Schwarz, E. M. (2003). Mechanisms of TNF-α and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. *J. Clin. Invest.* **111**, 821–831.

Seo, H., Mitsuhashi, K., and Tanibe, H. (1992). Antibacterial and antifungal fiber blended by chitosan. *In* ''Advances in Chitin and Chitosan'', (C. J. Brine, P. A. Sanford, and J. P. Zikakis, Eds), pp. 34–40. Elsevier Applied Science, London and New York.

Shibasaki, K., Sano, H., Matsukubo, T., and Takaesu, Y. (1994). Effects of low molecular chitosan and pH changes in human dental plague. *Bull. Tokyo Dent. Coll.* **35**, 33–39.

Simons, R. K., Junger, W. G., Loomis, W. H., and Hoyt, D. B. (1996). Acute lung injury in endotoxemic rats is associated with sustained circulating IL-6 levels and intrapulmonary CINC activity and neutrophil recruitment-role of circulating TNF-α and IL-β? *Shock* **6**, 39–45.

Sugano, M., Fujikawa, T., Hiratsuji, Y., and Hasegawa, Y. (1978). Hypocholesterolemic effects of chitosan in cholesterol-fed rats. *Nutr. Rep. Int.* **18**, 531–537.

Sugano, M., Fujikawa, T., Hiratsuji, Y., Nakashima, K., Fukuda, N., and Hasegawa, Y. (1980). A novel use of chitosan as a hypocholesterolemic agent in rat. *Am. J. Clin. Nutr.* **33**, 787–793.

Sugano, M., Watanabe, S., Kishi, A., Izumi, M., and Ohtakara, A. (1988). Hypocholesterolemic action of chitosans with different viscosity in rats. *Lipids* **23**, 187–191.

Sugano, M., Yoshida, K., Hashimoto, H., Enomoto, K., and Hirano, S. (1992). Hypocholesterolemic activity of partially hydrolyzed chitosan in rats. *In* ''Advances in Chitin and Chitosan'', (C. J. Brine, P. A. Sanford, and J. P. Zikakis, Eds), pp. 472–478. Elsevier Applied Science, London and New York.

Suntornsuk, W., Pochanavanich, P., and Suntornsuk, L. (2002). Fungal chitosan production on food processing by-products. *Proc. Biochem.* **37**, 727–729.

Tai, T. S., Sheu, W., Lee, W. J., Yao, H. T., and Chiang, M. T. (2000). Effect of chitosan on plasma lipoprotein concentrations in type 2 diabetic subjects with hypercholesterolemia. *Diabetes Care* **23**, 1703–1704.

Tajdini, F., Ali Amini, M., Nafissi-Varcheh, N., and Ali Faramarzi, M. (2010). Production, physiochemical and antimicrobial properties of fungal chitosan from *Rhizomucor miehei* and *Mucor racemosus*. *Int. J. Biol. Macromol.* **47**, 180–183.

Takahashi, T., Imaia, M., Suzukia, I., and Sawai, J. (2008). Growth inhibitory effect on bacteria of chitosan membranes regulated by the deacetylation degree. *Biochem. Eng. J.* **40**, 485–491.

Tomida, H., Fujii, T., Furutani, N., Michihara, A., Yasufuku, T., Akasaki, K., Maruyama, T., Otagiri, M., Gebicki, J. M., and Anraku, M. (2009). Antioxidant properties of some different molecular weight chitosans. *Carbohydr. Polym.* **344**, 1690–1696.

Trautwein, E. A., Jurgensen, U., and Erbersdobler, H. F. (1997). Cholesterol-lowering and gallstone-preventing action of chitosans with different degrees of deacetylation in hamsters fed cholesterol-rich diets. *Nutr. Res.* **17**, 1053–1065.

Uchida, Y., Izume, M., and Ohtakara, A. (1989). Preparation of chitosan oligomers with purified chitosanase and its application. *In* ''Chitin and Chitosan'', (G. Skjak-Braek, T. Anthonsen, and P. Standford, Eds), pp. 373–382. Elsevier Applied Science, United Kingdom.

Ueno, H., Mori, T., and Fujinaga, T. (2001). Topical formulations and wound healing applications of chitosan. *Adv. Drug Deliv. Rev.* **52**, 105–115.

Wang, W., Du, Y., Qiu, Y., Wang, X., Hu, Y., Yang, J., Cai, J., and Kennedy, J. F. (2008). A new green technology for direct production of low molecular weight chitosan. *Carbohydr. Polym.* **74**, 127–132.

Weiner, M. L. (1992). An overview of the regulatory status and of the safety of chitin and chitosan as food and pharmaceutical ingredients. *In* ''Advances in Chitin and Chitosan'', (C. J. Brine, P. A. Sanford, and J. P. Zikakis, Eds), pp. 663–670. Elsevier Applied Science, London and New York.

Xu, G., Huang, X., Qin, L., Wu, J., and Hu, Y. (2007). Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. *Asia Pac. J. Clin. Nutr.* **16**, 313–317.

Yao, H. T. and Chiang, M. T. (2006). Effect of chitosan on plasma lipids, hepatic lipid and fecal bile acid in Hamsters. *J. Food Drug Anal.* **14**, 183–189.

Yen, M. T., Yang, J. H., and Mau, J. L. (2008). Antioxidant properties of chitosan from crab shells. *Carbohydr. Polym.* **74**, 840–844.

Ylitalo, R., Lehtinen, S., Wuolijoki, E., Ylitalo, P., and Lehtimaki, T. (2002). Cholesterol-lowering properties and safety of chitosan. *Arzneimittelforschung* **52**, 1–7.

Zhou, K., Xia, W., Zhang, C., and Yu, L. (2006). In vitro binding of bile acids and triglycerides by selected chitosan preparations and their physicochemical properties. *LWT Food Sci. Technol.* **39**, 1087–1092.