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Food Research International 39 (2006) 383–393
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

Review

Bioactive compounds from marine processing byproducts – A review

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Received 23 May 2005; accepted 14 October 2005

Abstract

Generation of immense quantity of underutilized marine processing byproducts has long been recognized as wastes and greater efforts were given to use these materials in various applications. With a great number of researches on these byproducts, some biologically active compounds were identified and applicated to the useful compounds for human utilization. Potential applications of proteins, lipids, chitin and minerals in marine bioprocessing leftovers as bioactive materials have increased the value of processing byproducts in recent years. In this review, we have focused on the utilization of marine processing byproducts to screen bioactive compounds and their potential applications.

Keywords: Marine fish processing byproducts; Bioactivity; Bioactive compounds; Nutraceuticals

Contents

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doi:10.1016/j.foodres.2005.10.010

1. Introduction

Marine capture fisheries contribute over 50% of total world fish production and more than 70% of this production has been utilized for processing (FAOSTAT, 2001). As a result, every year a considerable amount of total catch is discarded as processing leftovers and that includes trimmings, fins, frames, heads, skin and viscera. In addition to fish processing, a large quantity of processing byproducts are accumulated as shells of crustaceans and shellfish from marine bioprocessing plants. Recent estimates revealed that current discards from the world's fisheries exceed 20 million tons equivalent to 25% of the total production of marine capture fisheries (FAOSTAT, 2001). Therefore, there is a great potential in marine bioprocess industry to convert and utilize more of these byproducts as valuable products. The term byproducts is however not clearly defined to distinguish from waste and in many instances it is identified as leftovers that are not ordinary saleable, but which can be recycled after treatment.

Majority of fisheries byproducts are presently employed to produce fish oil, fishmeal, fertilizer, pet food and fish silage (Choudhury & Bublitz, 1996; Choudhury & Gogoi, 1995). However, most of these recycled products possess low economic value. Recent studies have identified a number of bioactive compounds from remaining fish muscle proteins, collagen and gelatin, fish oil, fish bone, internal organs and shellfish and crustacean shells (Je, Park, Jung, & Kim, 2005; Jeon & Kim, 2002; Kim et al., 2001). Generally, a far better profitability is obtained by producing human consumables and the highest profitability is currently expected from bioactive compounds. These bioactive compounds can be extracted and purified with technologies varying from simple to complex and such compounds may include preparation and isolation of bioactive peptides, oligosaccharides, fatty acids, enzymes, water-soluble minerals and biopolymers for biotechnological and pharmaceutical applications. Furthermore, some of these bioactive compounds have been identified to possess nutraceutical potentials that are beneficial in human health promotion (Defelice, 1995). Therefore, development of new technologies in search of novel bioactive compounds from marine processing byproducts will bring more value out of what is today considered a waste and represents unique challenges and opportunities for the seafood industry.

2. Fish muscle protein

Fish frames and cutoffs result from mechanically deboned fish contain considerable amounts of muscle proteins. These muscle proteins are nutritionally valuable and easily digestible with well-balanced amino acid composition (Venugopal, Chawla, & Nair, 1996). Therefore, fish proteins derived from processing byproducts can be hydrolyzed enzymatically to recover protein biomass otherwise discarded as processing waste. Protein hydrolysates from byproducts of several marine species have been analyzed for their nutritional and functional properties and researches have mainly explored the possibility of obtaining biologically active peptides (Benkajul & Morrissey, 1997). Moreover, with the increasing knowledge on functional properties of fish protein hydrolysates, there are many researches performing on the developments and applications into functional foods and nutraceuticals.

Several hydrolytic enzymes from microbes, plants and animals are employed for the hydrolysis of fish processing byproducts (Simpson, Nayeri, Yaylayan, & Ashie, 1998). Peptides present in enzymatically digested protein hydrolysates have exhibited different physicochemical properties and biological activities depending on their molecular weights and amino acid sequences. It is a well-known fact that molecular weights of peptide fragments are crucial for their biological activities. Therefore, it is a key step to develop methods to separate peptides with different molecular weights. An ultrafiltration membrane system equipped with the appropriate molecular weight cutoff is effective in separating peptides having desired molecular weights from fish protein hydrolysates (Jeon, Byun, & Kim, 2000). In order to obtain functionally active peptides, it is a common method to use a sort of enzymes letting sequential enzymatic digestions. Moreover, as shown in Fig. 1, it has been possible to obtain serial enzymatic digestions in a system using multi-step recycling membrane reactor combined with ultrafiltration membrane system to separate fish protein hydrolysates based on their molecular weights (Byun & Kim, 2001).

2.1. Bioactivities of fish muscle derived peptides

Bioactive peptides isolated from various fish protein hydrolysates have shown a numerous bioactivities such as antihypertensive, antithrombotic, immunomodulatory and antioxidative activities. Kim, Choi, Park, Choi, and Moon (2000) have reported that some peptides derived from fish showed antihypertensive activity inhibiting the action of angiotensin I converting enzyme (ACE) even stronger than that of many other natural peptides. These peptides exhibited in vivo activities by lowering blood pressure in spontaneously hypertensive rats (Je, Park, Kwon, & Kim, 2005; Fujita & Yoshikawa, 1999). Enzymatically hydrolyzed fish muscle peptides also have shown anticoagulant and antiplatelet properties tested in vitro and these results have suggested the capability of fish peptides to inhibit coagulation factors in the intrinsic pathway of coagulation (Rajapakse, Jung, Mendis, Moon, & Kim, 2005).

Peptides derived from fish proteins have shown the ability of exerting potent antioxidative activities in different oxidative systems (Jun, Park, Jung, & Kim, 2004; Kim et al., 2000; Rajapakse, Mendis, Byun, & Kim, 2005). An increasing interest exists currently to explore natural antioxidative substances without side effects and these identified antioxidative activities have a potential to develop

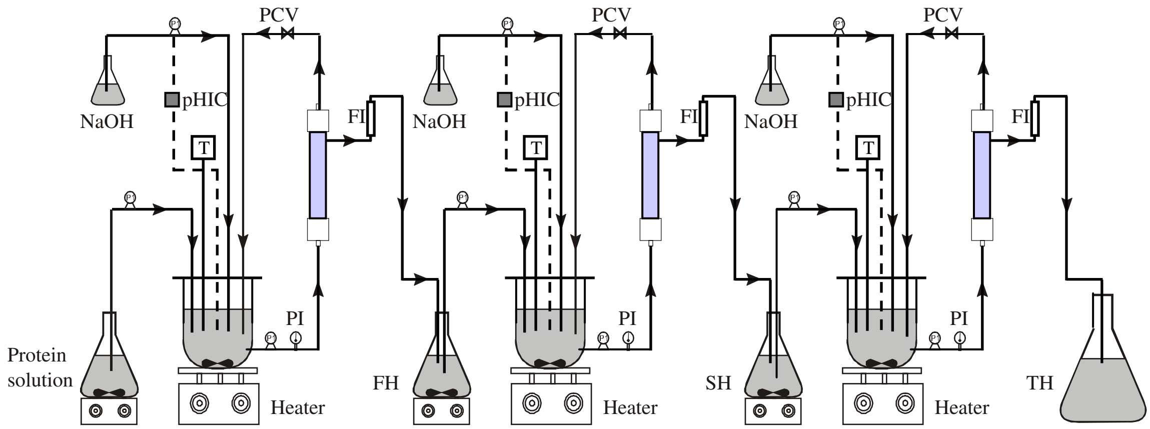

Fig. 1. Schematic diagram of the three-step recycling membrane reactor for production and separation of enzymatic hydrolysates. TI, temperature indicator; PI, pressure indicator; FI, flow indicator; pHIC, pH indicator controller; FH, first hydrolysates; SH, second hydrolysates; TH, third hydrolysate.

safe and non-hazardous natural antioxidants for the complications arose from oxidation of biomolecules.

Development of methods to increase the solubility of calcium compounds can significantly improve the calcium availability in biological systems. Kim et al. (1999) and Jung, Park, Byun, Moon, and Kim (2005) reported that fish peptides are capable of accelerating calcium absorption. Under many conditions dietary calcium becomes unavailable to absorb due to formation of insoluble compounds inside the dietary track and inadequate dietary calcium is associated with a number of common and chronic diseases worldwide including osteoporosis, osteoarthritis, cardiovascular disease (hypertension and stroke), diabetes, obesity and cancer (Anderson & Garner, 1996). Furthermore, researches have identified that fish protein hydrolysates possess hormone-like peptides and growth factors to accelerate calcium absorption (Fouchereau-Peron et al., 1999). These peptides are capable of binding to cell surface receptors on osteoclasts and involved mainly in calcium metabolism by decreasing the number of osteoclasts. Therefore, these peptides could be used in the treatment of osteoporosis and Paget's disease. Further, acidic peptide fractions from Atlantic cod hydrolysate have shown strong immunostimulatory effects and treatment of these peptides have stimulated the oxidative burst of Atlantic salmon leucocytes (Gildberg, Bogwald, Johansen, & Stenberg, 1996). Basically immunomodulators enhance the production of oxygen metabolites in macrophages that are responsible for these oxygen metabolites determine the oxidative burst. Oxidative burst reactions are of major importance for the bactericidal power of phagocytes.

3. Fish skin collagen and gelatin

Fish skin waste could be used as a potential source to isolate collagen and gelatin. Collagen and gelatin are currently used in diverse fields including food, cosmetic, and biomedical industries. Collagen is structurally formed as a triple helix by three extended protein chains that wrap around one another. Collagen and gelatin are different forms of the same macromolecule and gelatin is the partially hydrolyzed form of collagen. Heat denaturation easily converts collagen into gelatin. In addition to fish skin, collagen and gelatin could be isolated from bone and fins of fish processing byproducts. Collagen and gelatin are unique proteins compared to fish muscle proteins and this uniqueness of fish lies in the amino acid content and they are rich in non-polar amino acids (above 80%) such as Gly, Ala, Val and Pro. Even though main industrial sources of collagen and gelatin are bovine and porcine skin, many studies have been conducted to extract collagen and gelatin from fish skin and have used to screen their potential industrial applications (Gomez-Gillen et al., 2002; Kim, Byun, & Lee, 1994). The utilization of fish skin collagen and gelatin is expected to attract the interest of the industry as an alternative source. This may be due to comparative unpopularity of porcine skin collagen and gelatin in relation to some religious reasons. At the same time, use of bovine derived collagen and gelatin are also in active discussion due to the mad cow disease, bovine spongiform encephalopathy (BSE) and the risk they pose in human. In contrast, fish collagen and gelatin have relatively a low risk of possessing unknown pathogens such as BSE.

Collagen is generally extracted with acid treatment and solubilized without altering its triple helix. However, thermal treatment cleaves hydrogen and covalent bonds that stabilizes the triple helix configuration of collagen and converts its helical conformation into coiled conformation resulting a gelatin state (Djabourov, Lechaire, & Gaill, 1993). Therefore as a general procedure, hot water treatment is used to solubilize collagen in skin and extract as gelatin. The properties of extracted gelatin may vary slightly with the extraction conditions such as temperature and pH. Optimum conditions for extraction are dependent on fish species and type of collagen (Kim et al., 1994).

3.1. Biomedical and nutraceutical applications of collagen and gelatin

Collagen is commonly used in medical and pharmaceutical industries as carrier molecules for drugs, proteins and genes (Lee, Singla, & Lee, 2001). Especially, microfibrous collagen sheets are used as promising drug carrier for the treatment of cancer (Sato, Kitazawa, Adachi, & Horikoshi, 1996). Long term maintenance of drug concentration and controlled release at target sites promote the utilization of collagen as efficient drug carriers. In addition, recently collagen film/matrix are used as gene delivery agents promoting bone and cartilage formation (Nakagawa & Tagawa, 2000). Further, collagen has been reported to play a role in formation of tissues and organs and functional expression of cells. Clinical investigations suggest that ingestion of collagen/gelatin hydrolysates reduces pain in patients suffering from osteoarthritis and hydrolyzed collagen have been involved in cartilage matrix synthesis (Moskowitz, 2000). Moreover, collagen/gelatin are currently marketed as supplement for the maintenance of normal bone integrity, treatment for brittle nails and for the nourishment of scalp hair.

3.2. Bioactivities of gelatin peptides

Enzymatically hydrolyzed fish skin gelatin have shown better biological activities compared to the peptides derived from fish muscle protein to act as antioxidants and antihypertensive agents (Byun & Kim, 2001; Kim et al., 2001; Mendis, Rajapakse, Byun, & Kim, 2005; Mendis, Rajapakse, & Kim, 2005). Gelatin peptides have repeated unique Gly–Pro–Ala sequence in their structure and, it is presumed that the observed antioxidative and antihypertensive properties of gelatin peptides can be associated with their unique amino acid compositions. In addition, these peptides have shown to accelerate absorption of dietary calcium in animal models increasing calcium bioavailability (Kim, Jeon, Byun, Lee, & Kim, 1998).

4. Fish oil

Better utilization of marine fish processing byproducts could be achieved by converting these materials into fish oil. In general, the fat content of fish is varied from 2–30% and it basically depends on the type of species, dietary, geographic, environmental, reproductive and seasonal variations. However, with the depleting marine fisheries resources it is not encouraged fishing for their oil. Therefore, a large amount of offal generated from processing, would be a potential source to produce good quality fish oil for human consumption, especially from fatty fish processing byproducts. Composition of fish oil is different from that of other oils and mainly composed of two types of fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are polyunsaturated fatty acids classified as omega-3 fatty acids and predominantly found in many marine animals including cold-water fish species with a higher unsaturated fat content. Compared to saturated fats, polyunsaturated fatty acids in fish oil are readily digested for energy production and have been reported to have various bioactivities.

Methods and conditions of fish oil extraction are vital for quality of fish oil. Presently, several methods such high-speed centrifugation (Hirata et al., 1993), low temperature solvent extraction (Moffat, McGill, Hardy, & Anderson, 1993) and supercritical fluid extraction (Dunford, Temmeli, & LeBlanc, 1997) are utilized to extract fish oil. In addition, wet and steam rendering methods are also utilized to extract fish oil from fish processing byproducts (Chantachum, Benjakul, & Sriwirat, 2000). Separation of omega-3 fatty acids is one of main concerns in the current research. However, due to the presence of complex mixture of triacylglycerols, separation of omega-3 fatty acids becomes complicated. Many separation methods have been developed to isolate them at higher purity including crystallization, distillation, supercritical fluid extraction and chromatography. Especially, column liquid chromatography is frequently used to analyze and separate fatty acids. In addition, combination of chromatographic method with urea crystallization enables successful separation of fatty acids (Hidajat, Ching, & Rao, 1995). Proper separation techniques and advanced processing methods could promote the production of higher quality fish oil and fish oil might play an even more important role in the pharmaceutical and food industry in the near future.

4.1. Health-promoting roles of omega-3 fatty acids

Intake of fish oil, which is an excellent source of omega-3 fatty acids, has been linked to promotion of human health to fight against numerous diseases. Moreover, researchers are convinced that EPA and DHA are the main protective components of fish oil that act against certain types of diseases (Table 1).

The inverse relationship between the amount of omega-3 fatty acid level present in the blood and occurrence of

Table 1
Suggested nutraceutical potentials of omega-3 fatty acids

Health benefits	Reference
Prevention from atherosclerosis	Schacky (2000)
Protection against arrhythmias	Christensen et al. (1997)
Reduce blood pressure	Appel et al. (1993)
Beneficial for diabetic patients	Sheehan et al. (1997)
Fight against manic-depressive illness	Severus et al. (1999)
Reduce symptoms in asthma patients	Broughton et al. (1997)
Protection against chronic obstructive pulmonary diseases	Shahar et al. (1994)
Alliviate symptoms of cystic fibrosis	Lawrence and Sorrell (1993)
Improve the survival of cancer patients	Gogos et al. (1998)
Prevent relapses in patients with Crohn's disease	Belluzzi et al. (1996)

coronary heart disease is the strongest evidence of involvement of these fatty acids in prevention of human diseases. Findings of research revealed that the consumption of fish increase the levels of EPA and DHA in blood which in turn reduces the rate of coronary heart diseases via different actions. Antiathrogenic and antithrombotic effects of omega-3 fatty acids demote the growth of lipid rich atherosclerotic plaques and decrease the risk of thrombosis to stop the formation of thrombus (Schacky, 2000). It has been found that fish oil also exert their protective effect against heart diseases by decreasing serum triglyceride levels, improving vascular endothelial function, lowering blood pressure, and decreasing inflammation (Kris-Etherton, Harris, & Appel, 2003). Christensen et al. (1997) found that dietary $n-3$ fatty acids act to prevent arrhythmias that can lead to sudden cardiac death. It has become one of the most common causes of death in the western world and this is mainly caused by ventricular arrhythmias in heart patients. Moreover, these beneficial effects of omega-3 fatty acids are advantageous for the prevention from other diseases and heart related dysfunctions because they are related to the occurrence of chain of diseases.

Intake of fish oil exerts beneficial effects against diabetes mellitus. Fish oil improves many of the metabolic sequelae of insulin resistance by lowering blood pressure and triacylglycerol concentrations (Sheehan, Wei, Ulchaker, & Tserng, 1997). In addition, dietary intake of fish oil reports to accelerate glucose uptake and maintain normal glucose metabolism (Berry, 1997).

EPA and DHA also exhibit anti-inflammatory action and increase survival for people with autoimmune diseases. Clinical studies have revealed that polyunsaturated fish oil treatments relieve patients who are suffering from rheumatoid arthritis. EPA and DHA are successful at this because they can be converted into natural anti-inflammatory substances called prostaglandins and leukotrienes that help to decrease inflammation and pain (Belch & Muir, 1998). This anti-inflammatory character of fish oil has also been suggested to be advantageous for the treatment of other inflammatory diseases such as Crohn's disease (Belluzzi et al., 1996) and kidney diseases (Donadio, Bergstrahl, & Offord, 1994). Preliminary research suggests that omega-3 fatty acids act against various human carcinomas that include breast, colon, skin, pancreatic, prostate, lung and larynx cancer (Gogos et al., 1998).

Moreover, omega-3 fatty acids of fish oil are reported to associate with the brain development, and important for the vision and the functions of reproductive system. It may be due to the DHA is a component of brain nerve synapses, in the retina of the eye and in the testes and sperms (Rice, 1996). And hence it plays a vital role in the development and functions of these organs and systems. The brain developmental function of omega-3 fatty acids have been linked to their ability to prevent mental health problems (Severus, Ahrens, & Stoll, 1999).

5. Fish bone

Fish bone, which is separated after removal of muscle proteins on the frame, is another valuable source in identifying health-promoting components. The organic component of fish bone, which accounts for 30% of the material, is made out of collagen (Nagai, Izumi, & Ishii, 2004). Therefore, fish bone is considered as a source to isolate collagen and gelatin in addition to fish skin (Nagai & Suzuki, 2000). In contrast, fish bone consists of 60–70% of inorganic substances and mainly composed of calcium phosphate and hydroxyapatite.

5.1. Fish bone as a potential calcium source

Fish bone is considered as a potential source to obtain calcium, which is an essential element for the human health. However, only few studies have been carried out to identify bioavailability of fish bone calcium and its potential applications. Generally, calcium is obtained from the diet and it is severely deficient in most of regular diets. Therefore, to improve calcium intake, several calcium-fortified products are in the market and demand for these products is growing continuously. It is well documented that consumption of whole small fish is nutritionally beneficial providing with a rich source of calcium. Calcium in fish could be absorbed to the body as tested in vivo (Larsen, Thilsted, Konsbak, & Hansen, 2000). However, very little information is available on the beneficial effects of larger fish bone and few attempts have been taken to test their usage for benefits of human health. Fish bone material derived from processing of large fish is a useful calcium source where the quantity of calcium is concerned. In order to incorporate fish bone into calcium-fortified food it should be converted into an edible form by softening its structure. This can be achieved utilizing different methods including hot water treatment and hot acetic acid solutions. In addition, Ishikawa, Kato, Mihori, Watanabe, and Sakai (1990) used superheated steam to reduce the loss of soluble components from fish tissue and that enabled better recovery of bone within a shorter period.

5.2. Fish bone minerals in medicinal science

Recently, hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ has been introduced as a bone graft material in a range of medical and dental applications due to their similar chemical composition. Generally, bone substitution materials such as autografts, allografts and xenografts are used to solve problems related to bone fractures and damages. But, none of these materials provides a perfect bone healing due to mechanical instability and incompatibility. Currently, calcium phosphate bioceramics such as tetracalcium phosphate, amorphous calcium phosphate, tricalcium phosphate and hydroxyapatite are identified as most suitable bone substitution materials to serve the demand. Fish bone material becomes an important source for biomedical applications due to the presence of hydroxyapatite as the major inorganic constituent. Unlike other calcium phosphates, hydroxyapatite does not break under physiological conditions. In fact, it is thermodynamically stable at physiological pH and actively takes part in bone bonding. This property has been exploited for rapid bone repair after major trauma or surgery. Hydroxyapatite is derived from natural materials such as coral and fish bone (Jensen et al., 1996). Attempts have been taken to isolate fish bone derived hydroxyapatite and use them as an alternate for synthetic hydroxyapatite (Kim et al., 1997; Ozawa & Suguru, 2002). Generally, very high heat treatment (~1300 °C) is used for isolation of hydroxyapatite from fish bone and this temperature gives a higher strength to hydroxyapatite structure (Choi, Lee, Jeon, Byun, & Kim, 1999) and results an excellent biocompatible inorganic substance (Kim et al., 1998; Kim & Park, 2000; Kim, Park, & Kim, 2001).

6. Fish internal organs

Fish internal organs represent rich sources of enzymes, and many of them exhibit high catalytic activity at relatively low concentrations (Haard, 1998). Considering the specific characteristics of these enzymes, fish processing byproducts are currently used for enzyme extraction. A range of proteolytic enzymes including pepsin, trypsin, chymotrypsin and collagenases (Byun, Park, Sung, & Kim, 2003; Kim, Park, Kim, & Shahidi, 2002; Park, Lee, Byun, Kim, & Kim, 2002) are commercially extracted from marine fish viscera in large scale.

Marine organisms have adapted excellently to diverse extreme environmental conditions, such as high salt concentration, low or high temperature, high-pressure and low nutrient availability. Therefore, fish proteinases are reported to possess better properties such as higher catalytic efficiency at low temperatures, lower sensitivity to substrate concentrations and greater stability at broader pH range (Haard & Simpson, 1994). These characteristics of fish enzymes have made them suitable for different applications in many food-processing operations.

Even though marine fish derived enzymes do not have direct applications in the field of functional foods or nutraceuticals they can be utilized to produce bioactive components in large scale. Recently, Kim, Jeon, Byun, Kim, and Lee (1997) Kim, Park, Byun, Je, and Moon (2003) have reported several attempts to obtain crude enzyme mixtures from internal organs of some fish species and to utilize them in isolation of bioactive components from fish protein hydrolysates. In addition, chitinases as well as chitosanases can also be isolated from digestive tract and other organs of some marine fish species. These enzymes promote the recovery of chitin and chitosan from marine byproducts necessary for a wide array of biomedical applications. Use of these enzymes would be more economical in the process of isolating bioactive compounds from marine shell wastes.

7. Shellfish and crustacean shells

Another important category of byproducts from marine bioprocessing plants includes crustacean shells and shellfish wastes. Efficient utilization of these marine byproducts also become an environmental priority due to increased quantity of accumulation from processing plants as well as slow natural degradation of these materials. Chitin is one of major structural components of these shell wastes and can be identified as a biologically active polysaccharide and thus valuable for many applications. These shell wastes are potential sources to isolate chitin and are currently utilized for the commercial-scale chitin production as well as production of chitosan and their oligomers (Fig. 2). Chitin is a high-molecular weight linear polymer of *N*-acetyl-D-glucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units and can be easily processed into many other bioactive derivatives. Among them, the most common form is chitosan, which results from the removal of considerable amount of acetyl groups from chitin. According to the chemical structure, chitosan is a positively charged heteropolymer of D-glucosamine (GlcN) and *N*-acetylglucosamine (GlcNAc) units. Generally, chitin and chitosan oligomers are produced either by chemical or microbiological treatments. However, current commercial preparations of chitin and chitosan utilize thermochemical treatments that involve deproteination, demineralization and deacetylation of starting materials (Hirano, 1996). The molecular weight as well as the degree of deacetylation of chitosan is greatly dependent on the conditions utilized during the production process.

Recently much attention has been paid to chitin, chitosan and their oligomers as natural bioactive materials concerning their non-toxicity, biocompatibility and biodegradable nature (Kim, Park, Yang, & Hanm, 2001). These materials have important structural and functional properties that make them attractive for a wide variety of applications in many fields such as food and nutrition, biomedicine, biotechnology, agriculture and environmental protection. Especially, chitosan is highly discussed in relation to biomedical and food applications (Jeon, Shahdi, & Kim, 2000). However, high viscosity and low solubility at neutral pH may limit the application of these materials in functional food and pharmaceutical industries. Therefore currently, there is a growing interest to convert chitin and chitosan into their oligomers that have better functional properties and improved absorption through human digestive track. Production of chitin and chitosan oligomers from the hydrolysis can be done either by chemical or enzymatic methods. Chemical methods involve the use of acid hydrolysis and produce considerable amount of harmful industrial chemicals. In contrast, enzymatic hydrolysis is more preferable for the preparation of oligomers since this method results greater yields of oligomers with higher degree of polymerization (Jeon & Kim, 2000; Uchida, Izume, & Ohtakara, 1989).

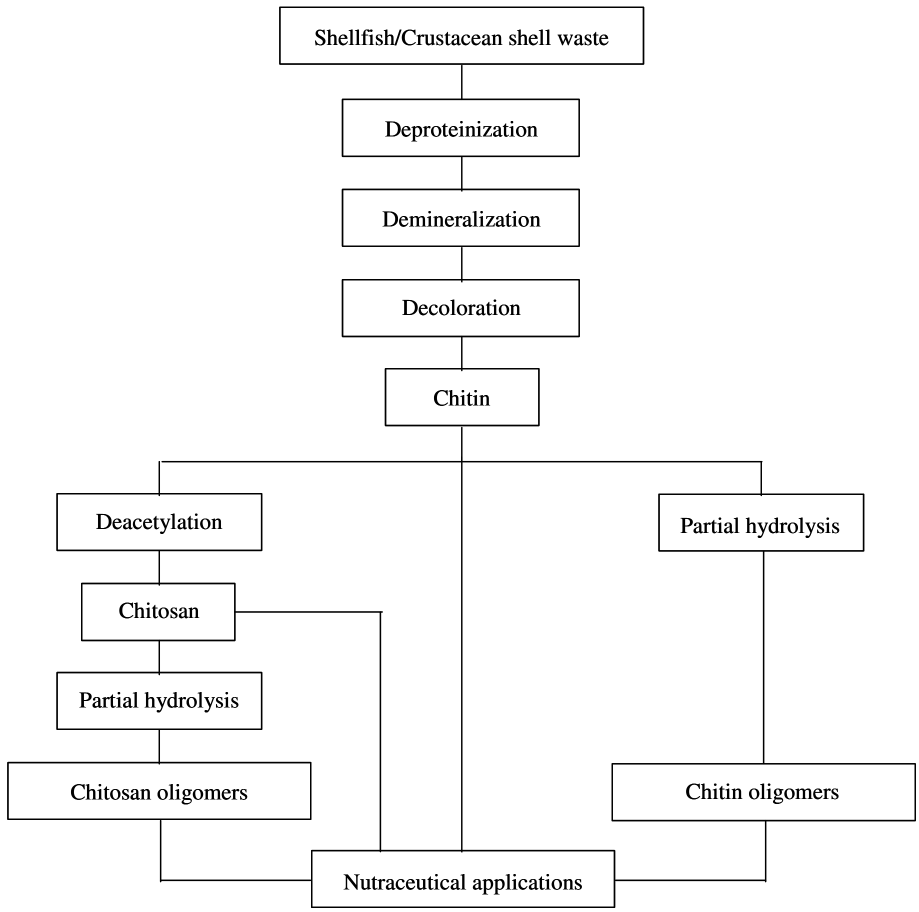

Fig. 2. Schematic diagram for preparation of chitin, chitosan and their oligomers.

7.1. Bioactivities of chitin, chitosan and their oligomers

In a broader sense of explanation the positive-charge nature of chitosan and its oligomers govern most of their biological activities. As identified by many researchers, chitosan and its oligomers are effective in reducing LDL-cholesterol level in liver and blood (Kanauchi, Deuchi, Imasato, Shizukuishi, & Kobayashi, 1995). Even though the exact mechanism is not clearly understood, it is suggested that these molecules act as fat scavengers in the digestive tract and remove fat and cholesterol via excretion (Ikeda et al., 1993). Sugano, Yoshida, Hashimoto, Enomoto, and Hirano (1992) studied the effect of different molecular weights of chitosan oligomers and observed that almost all molecular weights could prevent increase in blood cholesterol level at the 5% dietary level. In another study Ikeda et al. (1993) have mentioned that chitosan hydrolysate having average molecular weight of 10,000 Da could significantly enhance fecal excretion of neutral steroids. In contrast, Enomoto, Hashimoto, Kuramae, and Kanno (1992) argue that relatively low-molecular weight chitosan is beneficial in fat lowering and helpful for the prevention from cardiovascular diseases.

Chitosan oligomers also possess antitumor activities tested both in vitro and in vivo (Jeon & Kim, 2002). Studies carried out using mice ingested with low-molecular weight chitosan revealed significant antimetastatic effects of chitosan against Lewis lung carcinoma. Partially deacetylated chitin as well as chitin with a carboxymethyl group have also been effective to demote tumor progression (Tsukada et al., 1990). The suggested mechanism involves immunostimulating effects of chitin and its carboxymethyl derivatives via stimulation of cytolytic T-lymphocytes. This activity increase with smaller molecular sizes and it is suggested that they have immunostimulating effect that activate peritoneal macrophages and stimulate non-specific host resistance. However, higher molecular weight oligomers also have exhibited antitumor activity. And the same mechanism has been suggested for their activity via increased production of lymphokines by activated lymphocytes (Suzuki et al., 1986). Involvement of chitin and chitosan in wound-healing was found to be associated with their immunostimulating property, which involves higher production of macrophages, that release cytokines necessary for the healing process (Okamoto et al., 2003). Also it is suggested that wound-healing property of chitosan oligomers is due to their ability to stimulate fibroblast production by affecting the fibroblast growth factor. Subsequent collagen production further facilitates the formation of connective tissues (Howling et al., 2002).

Chitosan and their oligomers are capable of inhibiting β-secretase activity and this activity is depend on their molecular weights and degree of deacetylation (Byun, Kim, Park, Lin, & Kim, 2005). Chitosan oligosaccharides having molecular weights below 5 kDa with 90% deacetylation exhibit the highest inhibition. Moreover, chitosan oligomers exhibit non-competitive inhibition to inhibit β-secretase activity. Beta-secretase enzyme plays a major role in the occurrence of Alzheimer's disease by the progressive brain accumulation of β-amyloid peptides into fibrillar aggregates and insoluble plaques resulting severe memory loss and neuronal cell death. Chitosan oligosaccharides are the first reported carbohydrate β-secretase inhibitors and their mechanism of action has not been discussed in detail.

Chitosan and chitosan oligomers act as antioxidants by scavenging oxygen radicals and it is dependent on their molecular weights as well as their degree of deacetylation (Park, Je, & Kim, 2003b). Low-molecular weight of chitosan oligomers are preferred than that of higher molecular weights for the activity. Highly deacetylated (90%) chitosan oligomers are also act as scavengers of hydroxyl, superoxide, alkyl as well as highly stable DPPH radicals tested in vitro (Je, Park, & Kim, 2004a, 2004b). Sun, Xie, and Xu (2003) reported that chitosan and their derivatives act as hydrogen donors to prevent the oxidative sequence. However in-depth on antioxidant and radical scavenging activities of chitin and chitosan derivatives have not been carried out.

Chitin and their derivatives also act as inhibitors of angiotensin converting enzyme, an enzyme which associates with hypertension (Hong, Kim, Oh, Han, & Kim, 1998; Park, Je, & Kim, 2003a). The natural substrates of this enzyme are peptides and the mechanism of inhibition of ACE by chitosan oligomers is not well understood. In addition to their array of direct biomedical applications chitin and their derivatives report to play a role in drug delivery systems to obtain the controlled release (Miyazaki et al., 1990; Vasudev, Chandy, & Sharma, 1997).

8. Other marine processing byproducts

Processing of fish fillets for certain markets require methods to remove scales to obtain scale free superior fish products. Direct bioactivities or functional properties of fish scales have not been studied but it is a potential source to extract collagen (Nagai et al., 2004). Recently, the composition, structure, and properties of fish scales have been analyzed to search more potential applications for their optimum utilization (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003; Jeon, Kim, & Kim, 1998).

Fish eggs resulted from fish processing can be easily processed in to caviar or fish bait. An unrevealed nutraceutical potential exists in fish eggs because they are rich sources of lectins. Lectins are naturally occurring glycoproteins that can bind with carbohydrates to form stable complexes. Reproductive cells, eggs and sperms are thought to be rich sources of lectins because of its role in fertilization. Due to the ability of lectins to bind with carbohydrates it may has a potential to use as a better alternative for antibiotics to make pathogens incapable of causing diseases by making lectin-pathogen complexes. Some recent researches have identified lectins from fish eggs and a great potential exists to development of methods to explore their nutraceutical effects (Bazil & Entlicher, 1999; Jung, Park, & Kim, 2003).

9. Concluding remarks

Marine fish processing byproducts are used in many industries and their commercial applications are expanded every year. However, their applicability as bioactive compounds and their nutraceutical values are not well discussed. Identification of nutraceutical potential of natural compounds is a growing field and use of fish processing byproducts makes a new approach to be able to develop more commercial applications. So far, a limited number of bioactivities have been identified from isolated compounds and further researches are needed to develop methods to apply them for the human health promotion.

Acknowledgement

The authors acknowledge Marine Bioprocess Research Center of Marine Bio 21 Project, funded by the Ministry of Maritime Affairs and Fisheries Republic of Korea, for the support provided through the research grant P-2004-01.

References

Anderson, J. J. B., & Garner, S. C. (1996). Calcium and phosphorous nutrition in health and disease: Introduction. In J. J. B. Anderson & S. C. Garner (Eds.), *Calcium and phosphorous in health and disease* (pp. 1–5). New York: CRC Press.

Appel, L. J., Miller, E. R., Seidler, A. J., & Whelton, P. K. (1993). Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. *Archives of Internal Medicine, 153*, 1429–1438.

Bazil, V., & Entlicher, G. (1999). Complexity of lectins from the hard roe of perch (*Perca fluviatilis* L.). *International Journal of Biochemistry and Cell Biology, 31*, 431–442.

Belch, J. J. F., & Muir, A. (1998). $n-6$ and $n-3$ Essential fatty acids in rheumatoid arthritis and other rheumatic conditions. *Proceedings of the Nutrition Society, 57*, 563–569.

Belluzzi, A., Brignola, C., Campieri, M., Pera, A., Boschi, S., & Miglioli, M. (1996). Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *The New England Journal of Medicine, 334*, 1557–1560.

Benkajul, S., & Morrissey, M. T. (1997). Protein hydrolysates from Pacific whiting solid wastes. *Journal of Agricultural and Food Chemistry, 45*, 3423–3430.

Berry, E. M. (1997). Dietary fatty acids in the management of diabetes mellitus. *American Journal of Clinical Nutrition, 66*, 991–997.

Broughton, K. S., Johnson, C. S., Pace, B. K., Liebman, M., & Kleppinger, K. M. (1997). Reduced asthma symptoms with $n-3$ fatty acid ingestion are related to 5-series leukotriene production. *American Journal of Clinical Nutrition, 65*, 1011–1017.

Byun, H. G., & Kim, S. K. (2001). Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Allaska Pollack (*Theragra chalcogramma*) skin. *Process Biochemistry, 36*, 1155–1162.

Byun, H. G., Kim, Y. T., Park, P. J., Lin, X., & Kim, S. K. (2005). Chitooligosaccharides as a novel ß-secretase inhibitor. *Carbohydrate Polymers, 61*, 198–202.

Byun, H. G., Park, P. J., Sung, N. J., & Kim, S. K. (2003). Purification and characterization of a serine proteinase from the tuna pyloric caeca. *Journal of Food Biochemistry, 26*, 479–494.

Chantachum, S., Benjakul, S., & Sriwirat, N. (2000). Separation and quality of fish oil from precooked and non-precooked tuna heads. *Food Chemistry, 69*, 289–294.

Choi, J. S., Lee, C. K., Jeon, Y. J., Byun, H. G., & Kim, S. K. (1999). Properties of the ceramic composites and glass ceramics prepared by using the natural hydroxyapatite derived from tuna bone. *Journal of Korean Industrial and Engineering Chemistry, 10*, 394–399.

Choudhury, G. S., & Bublitz, C. G. (1996). Computer-based controls in fish processing industry. In G. S. Mittal (Ed.), *Computerized control systems in the food industry* (pp. 513–538). New York: Marcel Dekker Inc.

Choudhury, G. S., & Gogoi, B. K. (1995). Extrusion processing of fish muscle. *Journal of Aquatic Food Product Technology, 4*, 37–67.

Christensen, J. H., Korup, E., Aaroe, J., Toft, E., Moller, J., Rasmussen, K., et al. (1997). Fish consumption, $n-3$ fatty acids in cell membranes, and heart rate variability in survivors of myocardial infraction with left ventricular dysfunction. *American Journal of Cardiology, 79*, 1670–1673.

Defelice, S. L. (1995). The nutritional revolution: Its impact on food industry R&D. *Trends in Food Science and Technology, 6*, 59–61.

Djabourov, M., Lechaire, J. P., & Gaill, F. (1993). Structure and rheology of gelatin and collagen gels. *Biorheology, 30*, 191–205.

Donadio, J. V., Jr., Bergstrahl, E. J., & Offord, K. P. (1994). A controlled trial of fish oil in IgA nephropathy. *New England Journal of Medicine, 331*, 1194–1199.

Dunford, N. T., Temmeli, F., & LeBlanc, E. (1997). Supercritical CO_2 extraction of oil and residual proteins from Atlantic mackerel (*Scomber scombrus*) as affected by moisture content. *Journal of Food Science, 62*, 289–294.

Enomoto, M., Hashimoto, M., Kuramae, T., & Kanno, M. (1992). Low molecular weight chitosan as anticholesterolemic. Japanese Kokai. Tokkyo Koho. JP. 04,108,734. CA. 117:104268.

FAOSTAT, FAO statistical databases, fisheries data (2001). Food and Agriculture Organization of the United Nations, Rome, Italy. Available from interrefhttp://www.fao.orgurlhttp://www.fao.org.

Fouchereau-Peron, M., Duvail, L., Michel, C., Gildberg, A., Batista, I., & Gal, Y. I. (1999). Isolation of an acid fraction from a fish protein hydrolysate with a calcitonin–gene-related-peptide-like biological activity. *Biotechnology and Applied Biochemistry, 29*, 87–92.

Fujita, H., & Yoshikawa, M. (1999). LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *International Journal of Immunopharmacology, 44*, 123–127.

Gildberg, A., Bogwald, J., Johansen, A., & Stenberg, E. (1996). Isolation of acid peptide fractions from a fish protein hydrolysate with strong stimulatory effect on Atlantic salmon (*Salmo salar*) head kidney leucocytes. *Comparative Biochemistry and Physiology, 11*, 97–101.

Gogos, C. A., Ginopoulos, P., Salsa, B., Apostolidou, E., Zoumbos, N. C., & Kalfarentzos, F. (1998). Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy. *Cancer, 82*, 395–402.

Gomez-Gillen, M. C., Turnay, J., Fernandez-Diaz, M. D., Ulmo, N., Lizarbe, M. A., & Montero, P. (2002). Structural and physical properties of gelatin extracted from different marine species: A comparative study. *Food Hydrocolloids, 16*, 25–34.

Haard, N. F. (1998). Specialty enzymes from marine organisms. *Food Technology, 52*, 64–67.

Haard, N. F., & Simpson, B. K. (1994). Protease from aquatic organisms and their uses in the seafood industry. In A. M. Martin (Ed.), *Fisheries processing biotechnological applications* (pp. 132–154). London: Chapman & Hall.

Hidajat, K., Ching, C. B., & Rao, M. S. (1995). Preparative-scale liquid chromatographic separation of ω-3 fatty acids from fish oil sources. *Journal of Chromatography A, 702*, 215–221.

Hirano, S. (1996). Chitin biotechnology applications. *Biotechnology Annual Review, 2*, 237–258.

Hirata, H., Saeki, H., Nonaka, M., Kawasaki, K., Ooizumi, T., & Motoe, K. (1993). Recovery of fish oil from the manufacturing process of highly nutritional fish meat for foodstuffs from sardine. *Nippon Suisan Gakkaishi, 59*, 111–116.

Hong, S. P., Kim, M. H., Oh, S. W., Han, C. H., & Kim, Y. H. (1998). ACE inhibitory and antihypertensive effect of chitosan oligosaccharides in SHR. *Korean Journal of Food Science and Technology, 30*, 1476–1479.

Howling, G. I., Dettmar, P. W., Goddard, P. A., Hampson, F. C., Dornish, M., & Wood, E. J. (2002). The effect of chitin and chitosan on fibroblast-populated collagen lattice contraction. *Biotechnology and Applied Biochemistry, 36*, 247–253.

Ikeda, I., Sugano, M., Yoshida, K., Sasaki, E., Iwamoto, Y., & Hatano, K. (1993). Effects of chitosan hydrolysates on lipid absorption and on serum and liver lipid concentration in rats. *Journal of Agricultural and Food Chemistry, 41*, 431–435.

Ikoma, T., Kobayashi, H., Tanaka, J., Walsh, D., & Mann, S. (2003). Microstructure, mechanical, and biomimetic properties of fish scales from *Pagrus major*. *Journal of Structural Biology, 142*, 327–333.

Ishikawa, M., Kato, M., Mihori, T., Watanabe, H., & Sakai, Y. (1990). Effect of vapor pressure on the rate of softening of fish bone by super-heated steam cooking. *Nippon Suisan Gakkaishi, 56*, 1687–1691.

Je, J. Y., Park, P. J., Jung, W. K., & Kim, S. K. (2005). Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. *Food Chemistry, 90*, 809–814.

Je, J. Y., Park, P. J., & Kim, S. K. (2004a). Free radical scavenging properties of heterochitooligosaccharides using an ESR spectroscopy. *Food and Chemical Toxicology, 42*, 381–387.

Je, J. Y., Park, P. J., & Kim, S. K. (2004b). Radical scavenging activity of heterochitooligosaccharides. *European Food Research and Technology, 219*, 60–65.

Je, J. Y., Park, P. J., Kwon, J. Y., & Kim, S. K. (2005). A novel angiotensin I converting enzyme inhibitory peptide from Allaska Pollack (*Theragra chalcogramma*) frame protein hydrolysate. *Journal of Agricultural and Food Chemistry, 52*, 7842–7845.

Jensen, S. S., Aaboe, M., Pinhold, E. M., Hjrting-Hansen, Z., Melsen, F., & Ruyter, I. E. (1996). Tissue reaction and material characteristics of four bone substitutes. *International Journal of Oral & Maxillofacial Implants, 11*, 55–66.

Jeon, Y. J., Byun, H. G., & Kim, S. K. (2000). Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. *Process Biochemistry, 35*, 471–478.

Jeon, Y. J., & Kim, S. K. (2000). Continuous production of chitooligosaccharides using a dual reactor system. *Process Biochemistry, 35*, 623–632.

Jeon, Y. J., & Kim, S. K. (2002). Antitumor activity of chitosan oligosaccharides produced in an ultra filtration membrane reactor system. *Journal of Microbiology and Biotechnology, 12*, 503–507.

Jeon, Y. J., Kim, Y. T., & Kim, S. K. (1998). Analysis of compositions for effective utilization of fish scales. *Korean Journal of Life Science, 8*, 589–597.

Jeon, Y. J., Shahdi, F., & Kim, S. K. (2000). Preparation of chitin and chitosan oligomers and their application in physiological functional foods. *Food Reviews International, 16*, 159–176.

Jun, S. H., Park, P. J., Jung, W. K., & Kim, S. K. (2004). Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. *European Food Research Technology, 219*, 20–26.

Jung, W. K., Park, P. J., Byun, H. G., Moon, S. H., & Kim, S. K. (2005). Preparation of hoki (Johnius belengerii) boneoligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. *Food Chemistry, 91*, 33–340.

Jung, W. K., Park, P. J., & Kim, S. K. (2003). Purification and characterization of a new lectin from the hard roe of skipjack tuna, *Katsuwonus pelamis*. *International Journal of Biochemistry and Cell Biology, 35*, 255–265.

Kanauchi, O., Deuchi, K., Imasato, Y., Shizukuishi, M., & Kobayashi, E. (1995). Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. *Bioscience Biotechnology and Biochemistry, 59*, 786–790.

Kim, G. H., Jeon, Y. J., Byun, H. G., Lee, Y. S., & Kim, S. K. (1998). Effect of calcium compounds from oyster shell bound fish skin gelatin peptide in calcium deficient rats. *Journal of the Korean Fisheries Society, 31*, 149–159.

Kim, S. K., Byun, H. G., & Lee, E. H. (1994). Optimum extraction conditions of gelatin from fish skins and its physical properties. *Journal of Korean Industrial and Engineering Chemistry, 5*, 547–559.

Kim, S. K., Choi, J. S., Lee, C. K., Byun, H. G., Jeon, Y. J., & Lee, E. H. J. (1997). Synthesis and biocompatibility of the hydroxyapatite ceramic composites from tuna bone (II)—The sintering properties of hydroxyapatite treated with wet milling process. *Journal of Korean Industrial and Engineering Chemistry, 8*, 1000–1005.

Kim, S. K., Choi, J. S., Lee, C. K., Byun, H. G., Jeon, Y. J., & Lee, E. H. (1998). Synthesis and biocompatibility of the hydroxyapatite ceramic composites from tuna bone (III)—SEM photographs of bonding properties hydroxyapatite ceramic composites in the simulated body fluid. *Journal of Korean Industrial and Engineering Chemistry, 9*, 322–329.

Kim, S. K., Choi, Y. R., Park, P. J., Choi, J. H., & Moon, S. H. (2000). Screening of biofunctional peptides from cod processing wastes. *Journal of the Korean Society of Agricultural Chemistry and Biotechnology, 43*, 225–227.

Kim, S. K., Jeon, Y. J., Byun, H. G., Kim, Y. T., & Lee, C. K. (1997). Enzymatic recovery of cod frame proteins with crude proteinase from tuna pyloric caeca. *Fisheries Science, 63*, 421–427.

Kim, S. K., Jeon, Y. J., Byun, H. G., Park, P. J., Kim, G. H., Choi, Y. R., et al. (1999). Calcium absorption acceleration effect on phosphorylated and non-phosphorylated peptides from hoki (*Johnius belengeri*) frame. *Journal of the Korean Fisheries Society, 32*, 713–717.

Kim, S. K., Kim, Y. T., Byun, H. G., Nam, K. S., Joo, D. S., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Allaska Pollack skin. *Journal of Agricultural and Food Chemistry, 49*, 1984–1989.

Kim, S. K., & Park, P. J. (2000). Evaluation of mucous membrane irritation by hydroxyapatite sinter produced from tuna bone in Syrian hamsters. *Korean Journal of Life Science, 10*, 605–609.

Kim, S. K., Park, P. J., Byun, H. G., Je, J. Y., & Moon, S. H. (2003). Recovery of fish bone from hoki (*Johnius belengeri*) frame using a proteolytic enzyme isolated from mackerel intestine. *Journal of Food Biochemistry, 27*, 255–266.

Kim, S. K., Park, P. J., Kim, J. B., & Shahidi, F. (2002). Purification and characterization of a collagenolytic protease from the filefish, Novoden Modestrus. *Journal of Biochemistry and Molecular Biology, 35*, 165–171.

Kim, S. K., Park, P. J., & Kim, Y. T. (2001). Study on acute subcutaneous toxicity of hydroxyapatite sinter produced from tuna bone in Sprague–Dawly rats. *Korean Journal of Life Science, 11*, 97–102.

Kim, S. K., Park, P. J., Yang, H. P., & Hanm, S. S. (2001). Subacute toxicity of chitosan oligosaccharide in Sprague–Dawly rats. Antibiotics. *Arzneim-Forsch. Drug Research, 51*, 769–774.

Kris-Etherton, P. M., Harris, W. S., & Appel, L. J. (2003). Omega-3 fatty acids and cardiovascular disease: New recommendations from the American Heart Association. *Arteriosclerosis Thrombosis and Vascular Biology, 23*, 151–152.

Larsen, T., Thilsted, S. H., Konsbak, K., & Hansen, M. (2000). Whole small fish as a rich calcium source. *British Journal of Nutrition, 83*, 191–196.

Lawrence, R., & Sorrell, T. (1993). Eicosapentaenoic acid in cystic fibrosis: Evidence of a pathogenetic role for leukotriene B4. *Lancet, 342*, 465–469.

Lee, H. C., Singla, A., & Lee, Y. (2001). Biomedical applications of collagen. *International Journal of Pharmaceutics, 221*, 1–22.

Mendis, E., Rajapakse, N., Byun, H. G., & Kim, S. K. (2005). Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects. *Life Sciences, 77*, 2166–2178.

Mendis, E., Rajapakse, N., & Kim, S. K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *Journal of Agricultural and Food Chemistry, 53*, 581–587.

Miyazaki, S., Yamaguchi, H., Takada, M., Hou, W. M., Takeichi, Y., & Yasubuchi, H. (1990). Pharmaceutical application of biomedical polymers. XXIX. Preliminary study on film dosage form prepared from chitosan for oral drug delivery. *Acta Pharmacology Nord, 12*, 401–406.

Moffat, C. F., McGill, A. S., Hardy, R., & Anderson, R. S. (1993). The production of fish oils enriched in polyunsaturated fatty acids containing triglycerides. *Journal of the American Oil Chemists Society, 70*, 133–138.

Moskowitz, R. W. (2000). Role of collagen hydrolysate in bone and joint disease. *Semin Arthritis Rheum, 30*, 87–99.

Nagai, T., Izumi, M., & Ishii, M. (2004). Fish scale collagen. Preparation and partial characterization. *International Journal of Food Science and Technology, 39*, 239–244.

Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste material-skin, bone and fins. *Food Chemistry, 68*, 277–281.

Nakagawa, T., & Tagawa, T. (2000). Ultrastructural study of direct bone formation induced by BMPs-collagen complex implanted into an ectopic site. *Oral Diseases, 6*, 172–179.

Okamoto, Y., Inooue, A., Miyatake, K., Ogihara, K., Shigemasa, Y., & Minami, S. (2003). Effects of chitin/chitosan and their oligomers/monomers on migrations of macrophages. *Macromolecular Bioscience, 3*, 587–590.

Ozawa, M., & Suguru, S. (2002). Microstructural development of natural hydroxyapatite originated from fish-bone waste through heat treatment. *Journal of the American Ceramists Society, 85*, 1315–1317.

Park, P. J., Je, J. Y., & Kim, S. K. (2003a). Angiotensin I converting (ACE) inhibitory activity of hetero-chitooligosacchatides prepared from partially different deacetylated chitosans. *Journal of Agricultural and Food Chemistry, 51*, 4930–4934.

Park, P. J., Je, J. Y., & Kim, S. K. (2003b). Free radical scavenging activity of chitooligosaccharides by electron spin resonance spectrometry. *Journal of Agricultural and Food Chemistry, 51*, 4624–4627.

Park, P. J., Lee, S. H., Byun, H. G., Kim, S. H., & Kim, S. K. (2002). Purification and characterization of a collagenase from the mackerel, *Scomber japonicus*. *Journal of Biochemistry and Molecular Biology, 35*, 576–582.

Rajapakse, N., Jung, W. K., Mendis, E., Moon, S. H., & Kim, S. K. (2005). A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. *Life Sciences, 76*, 2607–2619.

Rajapakse, N., Mendis, E., Byun, H. G., & Kim, S. K. (2005). Purification and in vitro antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. *Journal of Nutritional Biochemistry, 16*, 562–569.

Rice, R. (1996). Fish and healthy pregnancy: more than just a red herring. *Prof Care Mother Child, 6*, 171–173.

Sato, H., Kitazawa, H., Adachi, I., & Horikoshi, I. (1996). Microdialysis assessment of microfibrous collagen containing a *p*-glycoprotein-mediated transport inhibitor, cyclosporine A, for local delivery of etopocide. *Pharmacological Research, 13*, 1565–1569.

Schacky, C. V. (2000). $n-3$ fatty acids and the prevention of coronary atherosclerosis. *American Journal of Clinical Nutrition, 71*, 224–227.

Severus, W. E., Ahrens, B., & Stoll, A. (1999). Omega-3 fatty acids—the missing link? *Archives of General Psychiatry, 56*, 380–381.

Shahar, E., Folsom, A. R., Melnick, S. L., Tockman, M. S., Comstock, G. W., Gennaro, V., et al. (1994). Dietary $n-3$ polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. *New England Journal of Medicine, 331*, 228–233.

Sheehan, J. P., Wei, I. W., Ulchaker, M., & Tserng, K. Y. (1997). Effect of high fiber intake in fish oil-treated patients with non-insulin-dependent diabetes mellitus. *American Journal of Clinical Nutrition, 66*, 1183–1187.

Simpson, B. K., Nayeri, G., Yaylayan, V., & Ashie, I. N. A. (1998). Enzymatic hydrolysis of shrimp meat. *Food Chemistry, 61*, 131–138.

Sugano, M., Yoshida, K., Hashimoto, M., Enomoto, K., & Hirano, S. (1992). Hypocholesterolemic activity of partially hydrolyzed chitosans in rats. In C. Brine, J. P. Zikakis, & P. Sandford (Eds.), *Advances in chitin and chitosan* (pp. 472–478). Amsterdam: Elsevier.

Sun, T., Xie, W., & Xu, P. (2003). Antioxidant activity of graft chitosan derivatives. *Macromolecular Bioscience, 3*, 320–323.

Suzuki, K., Mikami, T., Okawa, Y., Tokoro, A., Suzuki, S., & Suzuki, M. (1986). Antitumor effect of hexa-*N*-aetylchitohexaose and chitohexaose. *Carbohydrate Research, 151*, 403–408.

Tsukada, K., Matsumoto, T., Aizawa, K., Tokoro, A., Naruse, R., Suzuki, S., et al. (1990). Antimetastatic and growth inhibitory effects of *N*-acetyle chitohexaose in mice bearing Lewis lung carcinoma. *Japanese Journal of Cancer Research, 81*, 259–265.

Uchida, Y., Izume, M., & Ohtakara, A. (1989). Preparation of chitosan oligomers with purified chitosanase and its application. In G. Braek, T. Anthonsen, & P. Sandford (Eds.), *Chitin and chitosan: Sources, chemistry, biochemistry, physical properties and applications* (pp. 373–382). London, United Kingdom: Elsevier Applied Science.

Vasudev, S. C., Chandy, T., & Sharma, C. P. (1997). Development of chitosan/polyethylene vinyl acetate co-matrix: Controlled release of aspirin–heparin for preventing cardiovascular thrombosis. *Biomaterials, 18*, 375–381.

Venugopal, V., Chawla, S. P., & Nair, P. M. (1996). Spray dried protein powder from threadfin beam: Preparation, properties and comparison with FPC type B. *Journal of Muscle Foods, 7*, 55–58.