Nutraceuticals and Bioactives from Seafood Byproducts

Fereidoon Shahidi
Memorial University of Newfoundland, Department of Biochemistry, St. John's, Newfoundland, Canada

Abstract

Seafood processing byproducts may account for up to 80% of the weight of the harvest and, depending on the species involved, include a variety of constituents with potential for use as nutraceuticals and bioactives. Nutraceuticals, defined as ingredients or extracts with clinically proven health promoting activity, including disease prevention and treatment, may be consumed in the medicinal form, as supplements or as functional food ingredients. Thus, a variety of seafood processing byproducts may render benefits above their nutritional value. As an example, highly unsaturated long-chain omega-3 fatty acids, derived from the liver of white lean fish, flesh of fatty fish, and blubber of marine mammals, exhibit important biological activities. They also serve as the building block fatty acids in the brain, retina, and other organs with electrical activity. Hence, inclusion of oils containing docosahexaenoic acid (DHA) in the diet of pregnant and lactating women as well as infants is encouraged. In addition, chitinous materials, carotenoids, and biopeptides, arising from proteins, may be recovered from processing byproducts of crustaceans, including shrimp, crab, and lobster. The health benefits of chitosan, chitosan oligomers, and glucosamine are related to the multifunctional role of these ingredients, including immunomodulatory activity. Meanwhile, antioxidative peptides with up to 16 amino acids in chain length have been isolated from skin of pollock and their use as nutraceuticals may prove beneficial. Hence, byproduct processed from seafoods may possess multifunctional roles and could serve as important value-added nutraceuticals and functional food ingredients.

Introduction

Seafoods have traditionally been used because of their variety of flavor, color, and texture. More recently, seafoods have been appreciated because of their role in health promotion arising primarily from constituent long-chain omega-3 fatty acids, among others. Nutraceuticals and bioactive components from marine resources and the potential application areas are listed in Table 1. Processing of the catch brings about a considerable amount of discard which may account for 10-80% of the total landed weight, depending on the species under consideration. The components of interest in seafood processing byproducts include lipids, proteins, flavorants, minerals, carotenoids, enzymes, and chitin, among others. The raw material from such resources may be isolated and used in different applications, including functional foods and as nutraceuticals. The importance of omega-3 fatty acids in reducing the incidence of heart disease, certain types of cancer, diabetes, autoimmune disorders, and arthritis has been well recognized. Such lipids are derived primarily from byproducts of the seafood processing industry and originate from the body of fatty fish, liver of white lean fish, and blubber of marine mammals. In addition, the residual protein in seafoods and their byproducts may be separated mechanically or via a hydrolysis process. The bioactive peptides so obtained may be used in a variety of food and non-food applications. The bioactives from marine resources and their application areas are diverse in general. A cursory account of nutraceuticals and bioactives from selected seafood processing byproducts is provided in this contribution.

Nutraceutical lipids

Marine oils

The long-chain omega-3 polyunsaturated fatty acids (PUFA) are of considerable interest because of their proven or perceived health benefits (Simopoulos 1991, Abeywardena and Head 2001, Shahidi and Kim 2002). These fatty acids are found almost exclusively in aquatic resources (algae, fish, marine mammals, etc.) and exist in varying amounts and ratios. While algal sources also provide minerals, such as iodine, as well as carotenoids and xanthophylls, fish body oil contains mainly triacylglycerols and fish liver oils serve as a source of vitamin A, among others. In addition, liver from other aquatic species, such as shark, contain squalene and other bioactives. Another source of long-chain omega-3 fatty acids is the blubber of marine mammals which contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), similar to fish oils, as well as docosapentaenoic acid (DPA). It is worth noting that myristic acid is present in much smaller levels in the blubber oil from marine mammals than in algal or fish oils; this is a definite advantage when considering the atherogenic properties of myristic acid. In humans, DHA accumulates at a relatively high level

Table 1. Nutraceutical and bioactive components from marine resources and their application area.

Component (source)	Application area
Chitin, chitosan, glucosamine	Nutraceuticals, agriculture, food, water purification, juice clarification, etc.
Carotenoids/carotenoproteins	Nutraceuticals, fish feed
/omega-3 fatty acids	Nutraceuticals, foods, baby formula, etc.
Biopeptides	Nutraceuticals, immune-enhancing agents
Minerals/calcium	Food, nutraceuticals
Algae/omega-3, minerals, carotenoids	Nutraceuticals
Chondroitin sulfate	Arthritic pain relief
Squalene	Skin care
Specialty chemicals	Miscellaneous

in organs with electrical activity, such as retinal tissues of the eye and the neural system of the heart. While DHA and other long-chain omega-3 fatty acids may be formed from α-linolenic acid (ALA) (Fig. 1), the conversion efficiency for this transformation is very limited in healthy human adults and is approximately 3-5% (Emken et al. 1994). In infants and in adults with certain ailments, the conversion of ALA to DHA is less than 1% (Salem et al. 1996). Therefore, consumption of seafoods or marine oils, as such, is important. As shown in Fig. 1, DHA may be retroconverted to DPA and EPA. Human feeding trials have indicated a retroconversion of DHA to EPA of about 10% (Coquer and Holub 1996).

The beneficial health effects of marine oils in reducing the incidences of coronary heart disease (CHD) have been attributed to their omega-3 fatty acid constituents (Simopoulos 1991). Omega-3 fatty acids are known to reduce the incidence of CHD by lowering the level of serum triacylglycerols and possibly cholesterol and also to lower the blood pressure in individuals with high blood pressure as well as to decrease the ventricular arrhythmias, among others. In addition, omega-3 fatty acids are known to relieve arthritic swelling and possibly pain, relieve type II diabetes, and to enhance body immunity. However, omega-3 fatty acids may increase fluidity of the blood and hence their consumption by patients on blood thinners such as coumadin and aspirin should be carefully considered in order to avoid any unnecessary complication due to vasodilation and possible rupture of capillaries. The omega-3 fatty acids, especially DHA, are known to dominate the fatty acid spectrum of brain and retina lipids, and play an essential role in the development of the fetus and infants as well as in the health status and body requirements of pregnant and lactating women.

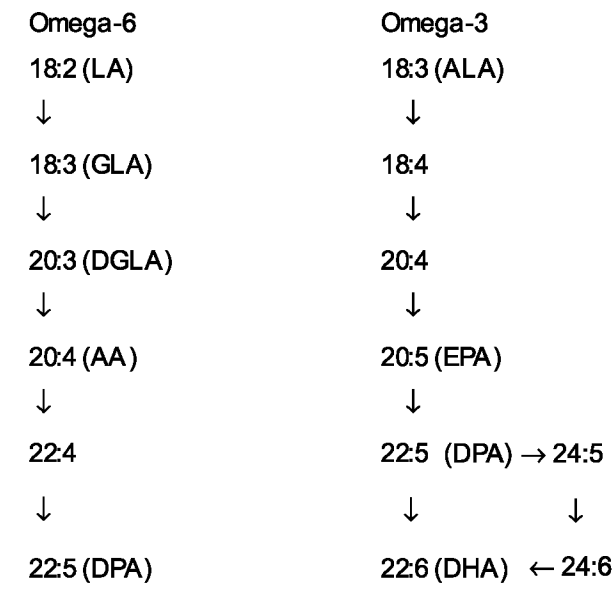

Figure 1. Essential fatty acids of the omega-6 and omega-3 families. Symbols are: LA = linoleic acid; GLA = γ-linolenic acid; DGLA = dihomo-γ-linolenic acid; AA = arachidonic acid; DPA = docosapentaenoic acid; ALA = α-linolenic acid; EPA = eicosapentaenoic acid; and DHA = docosahexaenoic acid.

Consideration of the three dimensional structures of unsaturated fatty acids demonstrates that bending of the molecules increases with an increase in the number of double bonds in their chemical structures, and this is further influenced by the position of the double bonds (i.e., omega-3 versus omega-6). These structural features in the triacylglycerol molecules as well as the location of the fatty acids in the glycerol molecule (i.e., sn-1, sn-2, and sn-3) may have a major effect on the bioavailability of fatty acids involved and their potential health benefits.

Two important sources of omega-3 fatty acids, namely menhaden oil (MO) and seal blubber oil (SBO) were considered in our work. Table 2 summarizes the fatty acids of MO, SBO, cod liver oil, and a commercial algal oil known as DHASCO (docosahexaenoic acid single cell oil). While omega-3 fatty acids, especially DHA, are primarily located in the sn-2 position in menhaden oil, they are mainly in the sn-1 and sn-3 positions of seal blubber oil (Table 3) (Wanasundara and Shahidi 1997a). These differences undoubtedly have a definite influence on their assimilation, absorption, and health benefits as well as reactions in which they are involved.

Table 2. Major fatty acids of omega-3 rich marine and algal oils.

Fatty acid	Seal blubber	Cod liver	Menhaden	Algal (DHASCO)
14:0	3.73	3.33	8.32	14.9
16:0	5.58	11.01	17.4	9.05
16:1ω7	18.0	7.85	11.4	2.20
18:0	0.88	3.89	3.33	0.20
18:1ω9+ω11	26.0	21.2	12.1	18.9
20:1ω9	12.2	10.4	1.44	–
20:5ω3	6.41	11.2	13.2	–
22:1ω11	2.01	9.07	0.12	–
22:5ω3	4.66	1.14	2.40	0.51
22:6ω3	7.58	14.8	10.1	47.4

Units are weight percents of total fatty acids.
DHASCO = docosahexaenoic acid single cell oil.

Table 3. Distribution of long-chain omega-3 fatty acids in menhaden and seal blubber oils.

	Seal blubber			Menhaden		
Fatty acid	sn-1	sn-2	sn-3	sn-1	sn-2	sn-3
EPA	8.36	1.60	11.2	3.12	17.5	16.3
DPA	3.99	0.79	8.21	1.12	3.11	2.31
DHA	10.5	2.27	17.9	4.11	17.2	6.12

Units are weight percents of total fatty acids.
EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; and DHA = docosahexaenoic acid.

Regardless of the source of long-chain omega-3 fatty acids, such oils must undergo appropriate processing. Therefore, refining, bleaching, deodorization, and addition of appropriate antioxidant stabilizers must be practiced in order to allow the use of these oils in food formulations. The type of food in which such omega-3 oils may be incorporated is listed in Table 4. These include foods that could be used within a short period of time and in products that do not develop off-flavors during their expected shelf life.

Table 4. Food application of omega-3 oils.

Food	Country
Bread/hard bread	Australia, France, Germany, Ireland, Denmark
Cereals, crackers, noodles	France, Korea, Taiwan
Bars	U.S.A.
Pasta and cakes	France, U.K.
Infant formula	Australia, Brazil, Japan, New Zealand, Taiwan, U.K.
Milk, fortified	Argentina, Indonesia, Italy, Spain, U.K.
Juices, fortified	Brazil, Germany, Spain
Mayonnaise and salad dressings	Korea
Margarines and spreads	Ireland, Japan, U.K.
Eggs	U.S.A., U.K.
Canned tuna steak and seafood	Japan, U.S.A.
Tuna burger	U.S.A.

Omega-3 concentrates

For therapeutic purposes the natural sources of omega-3 fatty acids, as such, may not provide the necessary amounts of these fatty acids, and hence production and use of concentrates of omega-3 fatty acids may be required (Wanasundara et al. 2002). The omega-3 fatty acid concentrates may be produced in the free fatty acid, simple alkyl ester, and acylglycerol forms. To achieve this, physical, chemical, and enzymatic processes may be employed for concentrate production. The available methods suitable for large-scale production include low-temperature crystallization, fractional or molecular distillation, chromatography, supercritical fluid extraction, urea complexation, and enzymatic splitting (Wanasundara and Shahidi 1997b).

Among the simplest methods for concentrate production is fractional crystallization which takes advantage of the existing differences in the melting points of different fatty acids, as neat compounds or in different solvent systems. The more saturated fatty acids have higher melting points and may crystallize out of the mixtures and hence leave behind, in the liquid form, the more unsaturated fatty acids. Obviously, the free fatty acids and simple alkyl esters are more amenable to provide a higher concentration of omega-3 fatty acids than acylglycerols. This is because the latter mixtures consist of fatty acids with varying chain lengths and degrees of unsaturation in many different combinations in the triacylglycerol molecules.

Fractional distillation is another facile process for separation of mixtures of fatty acid esters under reduced pressure (0.1-1.0 mm Hg) (Brown and Kolb 1955). However, due to sensitivity of more highly unsaturated fatty acids to oxidation, one may use a spinning band column, which does not impose such limitations (Haraldsson 1984). While fractional distillation of menhaden oil ethyl esters increased the content of EPA from 15.9 to 28.4%, and DHA from 9.0 to 43.9%, molecular distillation afforded DHA with 90% parity (Max 1989).

Reverse phase chromatography has been used by Nakahara et al. (1996) to produce a DHA and DPA concentrate from marine microalgae. Teshima et al. (1978) used a silver nitrate–impregnated silica gel column to separate EPA and DHA from squid liver oil fatty acid methyl esters. The yield of the process for the fatty acids was 39% and 48%, respectively, with 85-96% EPA and 95-98% DHA purity. Similar studies on a variety of other oils have recently appeared in the literature using high performance liquid chromatography (Tekiwa et al. 1981, Adlof and Emken 1985, Hayashi and Kishimura 1993, Corley et al. 2000). More recently, centrifugal partition chromatography (CPC) has gained attention for production of omega-3 concentrates (Murayama et al. 1988, Goffic 1997). Wanasundara (2001) used a CPC technique to produce highly concentrated fatty acids such as EPA and DHA with a near quantitative yield.

Supercritical fluid extraction (SFE) is a relatively new process which is desirable for separation of PUFA. Since this method is based on separation of compounds based on their molecular weight and not their degree of unsaturation, a prior concentration step may be required in order to concentrate the omega-3 PUFA. Thus omega-3 fatty acids have been concentrated by SFE from fish oil and seaweed (Choi et al. 1987, Yamagouchi et al. 1986, Mishra et al. 1993). Fish oil esters were fractionated by SFE to obtain an oil with 60-65% DHA (Stout and Spinelli 1987).

Another possibility for concentration of omega-3 fatty acids is urea complexation. The natural acylglycerols are hydrolyzed to their fatty acid constituents in ethanol and the resultant components are allowed to crystallize in the presence of urea. The highly unsaturated fatty acids which deviate more and more from a near linear shape are not included in the urea crystals and remain in the liquid form, referred to as non-urea complexing fraction (NUCF). Meanwhile, saturated fatty acids and, to a lesser extent, mono- and diunsaturated fatty acids may be included in the urea to afford the urea complexing fraction (UCF). In this manner, depending on the variables involved, e.g., the amount of solvent, urea, and time and temperature, optimum conditions may be employed for the preparation of concentrates. If necessary, the urea complexation process may be repeated in order to enhance the concentration of certain fatty acids in the final products. We have used such techniques to prepare concentrates dominated by DHA, EPA, or DPA. The total omega-3 fatty

acids in one such preparation from seal blubber oil was 88.2% and this was dominated by DHA (67 %) (Shahidi and Wanasundara 1999).

Finally, enzymatic procedures may be used to produce concentrates of omega-3 fatty acids. Depending on the type of enzyme, reaction time, temperature, and concentration of the reactants and enzyme, it is possible to produce concentrates in different forms, e.g., as free fatty acids or as acylglycerols. Thus, processes such as transesterification, acidolysis, alcoholysis, and hydrolysis as well as esterification of fatty acids with alcohols or glycerol may be employed.

Wanasundara and Shahidi (1998) have shown that enzymes might be used to selectively hydrolyze saturated and less unsaturated lipids from triacylglycerols, hence concentrating the omega-3 fatty acids in seal blubber and menhaden oils in the acylglycerol form. In this manner, the omega-3 PUFA content was nearly doubled. Furthermore, following urea completion, omega-3 concentrates obtained may be subjected to esterification with glycerol to produce concentrated acylglycerols. Upon glycerolysis of specialty alkyl esters from seal blubber oil, we found that monoacylglycerols (MAG), diacylglycerols (DAG), and triacylglycerols (TAG) were formed simultaneously. The amount of monoacylglycerols decreased continuously while that of triacylglycerols increased (He and Shahidi 1997). Depending on the structural characteristics of final products, the stability of acylglycerols was found to be better than that of their corresponding ethyl esters. Possible loss of natural antioxidants during processing may also affect the stability of products involved. Therefore, it is important to stabilize the modified oils using any of the recommended synthetic antioxidants or preferably natural stabilizers. Thus, TBHQ (tertiary butyl hydroquinone) at 200 ppm was able to inhibit oxidation of menhaden oil at 60°C over a 7-day storage period. Meanwhile, the inhibition effects were 32.5% for mixed tocopherols (500 ppm), 18.0% for α-tocopherol (500 ppm), 39.8% for mixed green tea catechins (200 ppm), 45.1% for EC (epicatechin), 48.2% for ECG (epicatechin gallate), 51.3% for EGC (epigallocatechin), and 50% for EGCG (epigallocatechin-3 gallate) (Shahidi and Wanasundara 2001). For seal blubber oil, the best protection of 56.3% was rendered by TBHQ at 200 ppm and 58.6% by ECG (200 ppm). Alpha-tocopherol inhibited the oxidation by only 14.2%.

Structured lipids

Structured lipids (SL) are triacylglycerols (TAG) containing combinations of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and long-chain fatty acids (LCFA) located in the same glycerol molecule and these may be produced by chemical or enzymatic means (Lee and Akoh 1998, Senanayake and Shahidi 2000). Structured lipids are developed to fully optimize the benefits of their fatty acid varieties in order to affect metabolic parameters such as immune function, nitrogen balance, and lipid clearance from the bloodstream. These specialty lipids may be

produced via direct esterification, acidolysis, and hydrolysis or interesterification.

MCFA are those with 6-12 carbon atoms and are often used for production of structured lipids. As mentioned earlier, MCFA are highly susceptible to β-oxidation (Odle 1997). These fatty acids are not stored in the adipose tissues and are often used in the diet of patients with maldigestion and malabsorption (Willis et al. 1998). They have also been employed in total parenteral nutrition and formulas for preterm infants. Production of structured lipids via acidolysis of blubber oil with capric acid was recently reported (Senanayake and Shahidi 2002). Lipozyme-IM from *Mucor miehei* was used as a biocatalyst at an oil to fatty acid mole ratio of 1:3 in hexane, at 45°C for 24 h and 1% (w/w) water (Senanayake and Shahidi 2002, 2003). Under these conditions, a structured lipid containing 2.3% EPA and 7.6% DHA at 27.1% capric acid (CA) was obtained. In this product, capric acid molecules were primarily located in the sn-1 and sn-3 positions (see Table 5), thus serving as a readily available source of energy to be released upon the action of pancreatic lipase. Incorporation of capric acid into fish oil TAG using immobilized lipase from *Rhizomucor miehei* (IM-60) was also reported (Jennings and Akoh 1999). After a 24 hour incubation in hexane, 43% capric acid was incorporated into fish oil while the content of EPA and DHA in the product was reduced to 27.8 and 23.5%, respectively. Similar results were obtained upon acidolysis of seal blubber with lauric acid (Senanayake and Shahidi 2003).

In an effort to produce specialty lipids containing both omega-3 PUFA and gamma-linolenic acid (GLA), preparation of such products under optimum conditions was reported (Senanayake and Shahidi 2000). GLA is found in relatively large amounts in borage oil (20-25%), evening primrose oil (8-10%), and black currant oil (15-18%). Using borage oil, the urea complexation process afforded a concentrate with 91% GLA under optimum reaction conditions.

Lipase-catalyzed acidolysis of seal blubber oil and menhaden oil with GLA concentrate (Spurvey and Shahidi 2000), under optimum conditions of GLA to TAG mole ratio of 3:1, reaction temperature of 40°C over 24 h and 500 units enzyme per gram oil afforded products with 37.1 and 39.6% GLA incorporation, respectively. Of the two enzymes tested, lipase PS-30 from *Pseudomonas* sp. served better in the acidolysis process than *Mucor miehei* (Spurvey et al. 2001). Incorporation of GLA was in all positions and its content in the sn-2 position of both seal blubber oil and menhaden oil was 22.1 and 25.7%, respectively (Table 6). Thus, PS-30 served in a non-specific manner in the acidolysis process. The structured lipids containing GLA, EPA, and DHA so produced may have health benefits above those exerted by use of their physical mixtures.

Production of structured lipids containing GLA, EPA, and DHA may also be achieved using borage and evening primrose oils as sources of GLA and either EPA or DHA or their combinations (Senanayake and Shahidi

Table 5. Enzymatic modification of seal blubber oil with capric acid.

Fatty acid	Unmodified	Modified	sn-1 & 3[a]
10:0	–	27.1	85.1
14:0	3.4	2.7	48.1
14:1	1.0	0.8	58.3
16:0	5.0	3.7	46.8
16:1ω7	15.1	11.9	55.5
18:1ω9+ω11	26.4	19.3	56.1
18:2ω6	1.3	1.7	66.7
20:ω9	15.0	9.1	72.5
20:5ω3	5.4	2.3	31.9
p22:1ω11	3.6	1.9	52.6
22:5ω3	4.9	3.0	76.7
22:6ω3	7.0	7.6	82.1

[a]Percent of modified fatty acid in sn-1 and 3 positions.
Units are percents of total fatty acids.
The enzyme used was lypozyme-IM from *Mucor miehei.*

Table 6. Fatty acids of seal blubber oil (SBO), menhaden oil (MO) and their acidolysis products with γ-linolenic acid (GLA, 18:3ω6).

	SBO			MO		
Fatty acid	Unmodified	Modified	sn-1 &3[a]	Unmodified	Modified	sn-1 & 3[a]
14:0	3.36	2.40	58.3	8.18	4.55	53.3
16:0	5.14	3.04	51.1	19.89	8.78	53.5
18:1ω9	22.6	14.1	46.6	9.86	4.24	53.7
18:3ω6	0.59	37.1	77.9	0.43	39.6	74.3
20:1ω9	17.3	8.30	55.4	1.62	0.83	20.0
20:5ω3	5.40	3.80	84.6	12.9	11.0	65.9
22:5ω3	5.07	2.99	78.0	2.48	2.07	66.7
22:6ω3	7.73	4.36	79.2	10.0	6.56	77.4

[a]Percent of modified fatty acid in sn-1 and 3 positions.
Units are percents of total fatty acids.

1999a,b). The products so obtained, while similar to those produced by incorporation of GLA into marine oils, differ in the composition and distribution of fatty acids involved.

Bioactive peptides from marine resources

Cleavage of amide linkage in the protein chain leads to the formation of peptides with different numbers of amino acids as well as free amino acids. While enzymes with endopeptidase activity provide peptides with different chain lengths, exopeptidases liberate amino acids from the terminal positions of the protein molecules. Depending on reaction variables as well as the type of enzyme, the degree of hydrolysis of proteins may differ considerably. The peptides produced from the action of a specific enzyme may be subjected to further hydrolysis by other enzymes. Thus, an enzyme mixture or several enzymes in a sequential manner may be advantageous. The peptides so obtained may be subjected to chromatographic separation and then evaluated for their amino acid sequence as well as their antioxidant and other activities.

In a study on capelin protein hydrolysates, four peptide fractions were separated using Sephadex G-10. While one fraction exerted a strong antioxidant activity in a β-carotenelinoleate model system, two fractions possessed a weak antioxidant activity and the fourth one had a prooxidant effect. Two dimensional HPLC separation showed spots with both pro- and antioxidative effects (Amarowicz and Shahidi 1997). Meanwhile, protein hydrolysates prepared from seal meat were found to serve as phosphate alternatives in processed meat applications and reduced the cooking loss considerably (Shahidi and Synowiecki 1997). Furthermore, Alaska pollock skin hydrolysate was prepared using a multienzyme system in a sequential manner. The enzymes used were in the order of Alcalase, Pronase E, and collagenase. The fraction from the second step, which was hydrolyzed by Pronase E, was composed of peptides ranging from 1.5 to 4.5 kDa and showed a high antioxidant activity. Two peptides were isolated, using a combination of chromatographic procedures, and these were composed of 13 and 16 amino acid residues (Kim et al. 2001). The sequence of the peptides involved is given in Table 7 and compared with those of soy 75 protein hydrolysates (Chen et al. 1995). These peptides exert their antioxidant activity via free radical scavenging as well as chelation effects. Recently, proteases from shrimp processing discards were characterized (Heu et al. 2003) and application of salt-fermented shrimp byproduct sauce as a meat tenderizer was reported (J.-S. Kim, F. Shahidi, and M.S. Heu, unpublished results).

Table 7. Antioxidative peptides from gelatin hydrolysate of Alaska pollock skin in comparison with that of soy 75 protein.

Peptide	Amino acid sequence
Alaska pollock skin	
P_1	Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly
P_2	Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly
Soy 75 protein	
P_1	Val-Asn-Pro-His-Asp-His-Glu-Asn
P_2	Leu-Val-Asn-Pro-His-Asp-His-Glu-Asn
P_3	Leu-Leu-Pro-His-His
P_4	Leu-leu-Pro-His-His-Ala-Asp-Ala-Asp-Tyr
P_5	Val-Ile-Pro-Ala-Gly-Tyr-Pro
P_6	Leu-Glu-Ser-Gly-Asp-Ala-Leu-Arg-Val-Pro-Ser-Gly-Thr-Tyr-Tyr

Chitin, chitosan, and related compounds

Chitin is recovered from processing discards of shrimp, crab, lobster, and crayfish following deproteinization and demineralization (Shahidi and Synowiecki 1991, Shahidi et al. 1999). The chitin so obtained may then be deacetylated to afford chitosan (Shahidi and Synowiecki 1991). Depending on the duration of the deacetylation process, the chitosan produced may assume different viscosities and molecular weights. The chitosans produced are soluble in weak acid solutions, thus chitosan ascorbate, chitosan acetate, chitosan lactate, and chitosan malate, among others, may be obtained and these are all soluble in water. Chitosan has a variety of health benefits and may be employed in a number of nutraceutical and health-related applications. Chitosan derivatives may also be produced in order to obtain more effective products for certain applications. However, to have the products solubilized in water without the use of acids, enzymatic processes may be carried out to produce chitosan oligomers. Due to their solubility in water, chitosan oligomers serve best in rendering their benefits under normal physiological conditions and in foods with neutral pH. Furthermore, depending on the type of enzyme employed, chitosan oligomers with specific chain lengths may be produced for certain applications (Jeon et al. 2000).

Chitosans with different viscosities were prepared (Table 8) and used in an experiment designed to protect both raw and cooked fish against oxidation as well as microbial spoilage (Jeon et al. 2002, Kamil et al. 2002, Shahidi et al. 2002). The content of propanal, an indicator of oxidation of omega-3 fatty acids, was decreased when chitosan was used as an edible invisible film in herring. Furthermore, the effects were more pronounced as the molecular weight of the chitosan increased (Table 9). In addition, inhibitory effects of chitosan coatings in the total microbial counts for

Table 8. Characteristics of three different kinds of chitosans prepared from crab shell waste.[a]

	Chitosan		
Properties	I	II	III
Deacetylation time[b]	4 h	10 h	20 h
Moisture (%)	4.50 ± 0.30	3.95 ± 0.34	3.75 ± 0.21
Nitrogen (%)	7.55 ± 0.10	7.70 ± 0.19	7.63 ± 0.08
Ash (%)	0.30 ± 0.03	0.25 ± 0.02	0.30 ± 0.00
AV[c] (cps)[d]	360	57	14
DA[c] (%)	86.3 ± 2.1	91.3 ± 1.2	94.5 ± 1.3
Mv[c] (dalton)	1,816,732	963,884	695,122

[a]Results are expressed as mean ± standard deviation of three determinations.
[b]Deacetylation for chitosan I, II, and III was achieved using 50% NaOH at 100°C.
[c]Mv = viscosity molecular weight; AV = apparent viscosity; and DA = degree of deacetylation.
[d]cps = cycles per second.

Table 9. Content of propanal (milligrams per kilogram of dried fish) in headspace of chitosan-coated herring samples stored at 4°C.[a]

	Storage period (days)					
Chitosan	0	2	4	6	8	10
Uncoated	12.6 ± 3.4[a]	23.7 ± 4.2[b]	29.9 ± 4.2[c]	34.3 ± 1.9[c]	44.1 ± 4.0[c]	46.3 ± 2.4[c]
14 cps	13.8 ± 2.1[a]	18.3 ± 3.0[a]	24.6 ± 1.2[b]	30.9 ± 2.9[bc]	33.0 ± 0.8[b]	39.7 ± 0.9[b]
57 cps	12.6 ± 3.0[a]	15.5 ± 2.1[a]	19.7 ± 2.6[a]	24.9 ± 1.6[a]	22.8 ± 1.9[a]	24.2 ± 1.9[a]
360 cps	14.2 ± 2.4[a]	15.7 ± 2.6[a]	17.6 ± 2.2[a]	20.2 ± 1.4[a]	18.3 ± 2.4[a]	22.7 ± 1.3[a]

[a]Results are expressed as mean ± standard deviation of three determinations. Values with the same superscripts within each column are not significantly different ($P < 0.05$).

cod and herring showed an approximately 1.5 and 2.0 log cycles difference between coated and uncoated samples, respectively, after 10 days of refrigerated storage (results not shown). The monomer of chitin, N-acetylglucosamine (NAG), has been shown to possess anti-inflammatory properties. Meanwhile, glucosamine, the monomer of chitosan, prepared via HCl hydrolysis, is marketed as glucosamine sulfate. This formulation is prepared by addition of ferrous sulfate to the preparation. Glucosamine products may also be sold in formulation containing chondroitin 4- and chondroitin 6-sulfates. While glucosamine helps to form proteoglycans that sit within the space in the cartilage, chondroitin sulfate acts like a liquid magnet. Thus glucosamine and chondroitin work in a complementary manner to improve the health of the joint cartilage.

The byproducts in chitin extraction process from shellfish include carotenoids/carotenoproteins, and enzymes (Shahidi 1995, Shahidi et al. 1998, Shahidi and Kamil 2001). These components may also be isolated for further utilization in a variety of applications.

References

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