Health and nutrition from the sea

Dr. Fereidoon Shahidi

Shahidi reviews the latest developments in the use of seafood in health promotion and disease reduction.

Who should read this paper?

This paper represents a summary review of the state of the art in extracting nutraceuticals (any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease - Foundation for Innovation and Medicine) from the sea. Anyone with an interest in where omega-3 fatty acids and a range of other healthful products (for example, glucosamine) come from, and where new breakthroughs are possible, will be interested in reading this paper.

Why is it important?

The beneficial health effects of fish and other seafood are being increasingly recognized by medical researchers and the general public alike. The benefits of fish oils alone include combating coronary heart disease, and assisting with visual and cognitive development, psychiatric disorders, inflammatory diseases, Crohn's disease and type 2 diabetes, among others. Meanwhile, unexploited resources from the oceans offer many opportunities for future developments. For example, there is a great deal of interest in seaweed because of evidence linking habitual consumption of seaweed to reduced risk of a number of chronic diseases in some Asian populations.

About the author

Dr. Fereidoon Shahidi is a research professor in the Department of Biochemistry at Memorial University. Dr. Shahidi is the author of over 600 research papers and holds 5 patents on nitrite-free curing of meat. His research contributions have led to several industrial developments around the globe. Dr. Shahidi's current research interests include different areas of nutraceuticals and functional foods, as well as marine foods, natural antioxidants and muscle foods. Dr. Shahidi is the editor-in-chief of the Journal of Food Lipids and is currently serving as a member of the Expert Committee for the Natural Health Product Directorate of Health Canada.

Nutraceuticals and Healthful Products from Aquatic Resources

Dr. Fereidoon Shahidi

Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3X9

ABSTRACT

Marine, fresh water and cultured fish, shellfish and other aquatic species provide a rich source of food as well as by-products that could be used for production of a wide range of health promoting compounds. These bioactives include omega-3 fatty acids, proteins and biopeptides, carotenoids and carotenoproteins, enzymes, chitinous materials and glucosamine as well as minerals, among others. The bioactives present in seafoods and aquatic resources are effective in rendering beneficial health effects and reducing the risk of a number of chronic diseases. Thus, such bioactives may serve as important value-added nutraceuticals, natural health products and functional food ingredients that can be used for health promotion and disease risk reduction.

INTRODUCTION

Seafood products such as fish, shellfish and molluscs have traditionally been used because of their variety of flavor, colour, texture and accessibility in the coastal areas. More recently, seafoods are appreciated because of the emergence of new evidence about their role in health promotion and disease risk reduction, primarily arising from their bioactive omega-3 fatty acids, among others. Nutraceuticals and healthful products from aquatic resources belong to several classes of compounds and are used in foods and natural health products (see Table 1). A large proportion of by-products, up to 75%, and low-value fish is also procured as a result of processing and harvesting. Figure 1 provides a

Figure 1a: Landing of fish

Figure 1b: Shellfish used as food

Table 1. Nutraceuticals and natural health products for marine resources

Components	Application Area
Chitin, chitosin, chitosan, oligomer, glucosamine	Food, water and juice clarification, agriculture, supplements
Omega-3 oils	Nutraceuticals, immune enhancement, CVD, others
Chonodostin sulfate	Dietary supplement, arthritic pain
Squalene	Skin care
Biopeptides, collagen and protein	Nutraceuticals, immune enhancement, etc.
Carotenoids and Carotenoprotein	Nutraceuticals, others.
Minerals (calcium)	Nutraceuticals
Enzymes	Food and speciality application, others
Other specialty chemicals	Miscellaneous

graphic representation of harvest of fish and the variety of shellfish that are generally used as food. Figure 2 shows processing of fish for food in an industrial setting. The components of interest that could be extracted or produced from marine resources include lipids, proteins and biopeptides, minerals, flavorants, carotenoids and carotenoproteins, enzymes, chitinous materials and other specialty products. The importance of omega-3 fatty acids, bioactive peptides, glucosamine, chitosan and chitosan oligomers and other bioactives in food and non-food applications as well as for health promotion purposes has been well recognized. This overview provides a cursory account of selected bioactives from aquatic resources.

OMEGA-3 FATTY ACIDS AND NUTRACEUTICAL LIPIDS

The occurrence and health benefits of long-chain omega-3 polyunsaturated fatty acids (PUFA) in aquatic organisms is a well known [Simopoulos, 1991; Abeywardena and Head, 2001; Shahidi and Kim, 2002]. These fatty acids are produced in phytoplanktons in the oceans and are then consumed by fish and other aquatic species directly, or indirectly assimilated through the trophic levels of the food chain, e.g. top predators including marine mammals and tunas. Thus, omega-3 PUFA may be procured from algae, body of fatty fish, liver of white lean fish and the blubber of marine mammals. The constituent fatty acids present in such oils include eicosapentaenoic acid (EPA, C20:5n-3), docosahexaenoic acid (DHA, C22:6n-3; Figure 3), and, to a lesser extent, docosapentaenoic acid (DPA, C22:5n-3) in different proportions, depending on the

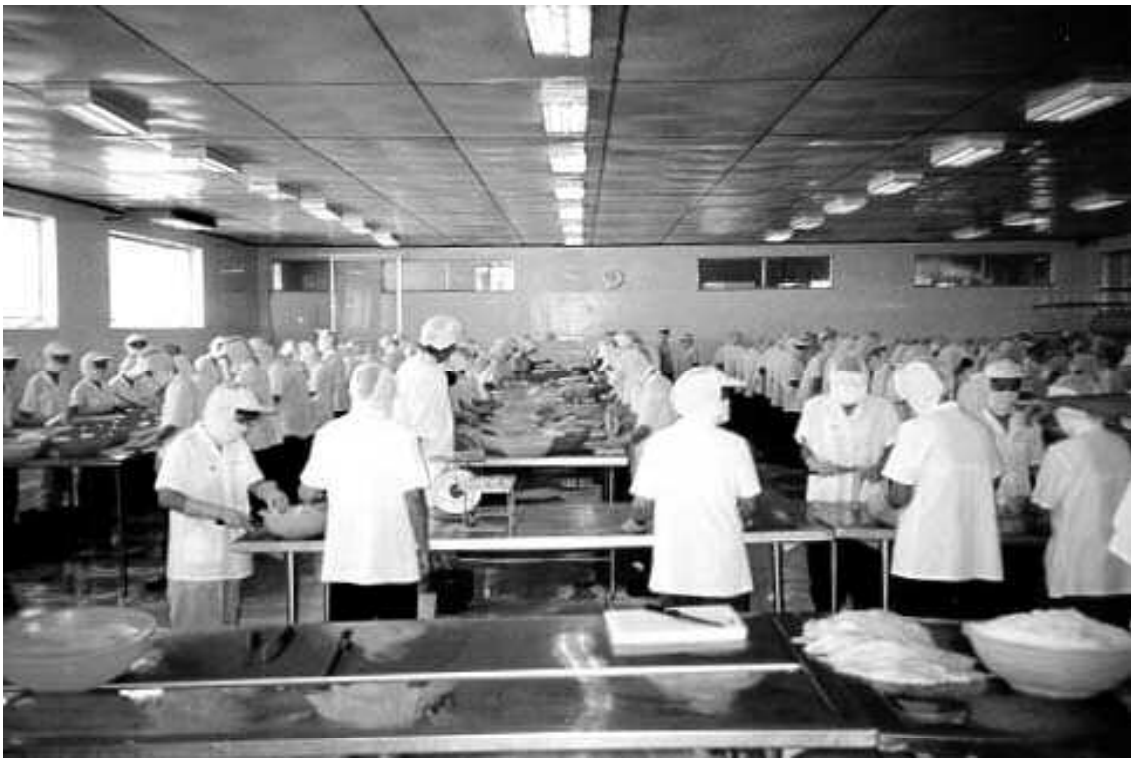

Figure 2: Processing of fish for separation of fillets and other edible parts.

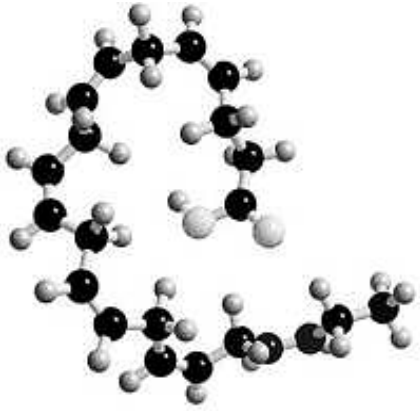

Figure 3: Molecular model representation of docosahexaenoic acid (DHA)

species involved. In addition, liver oil from white lean fish and shark serves as an excellent source of vitamin A and/or squalene and other bioactives.

Table 2 summarizes the fatty acid composition of selected oils from aquatic species and an algal oil produced commercially. As can be seen, the contents of EPA, DHA and DPA in each oil depends on the source material. Thus the ratio of EPA to DHA in menhaden, cod liver and seal blubber oils varies considerably, but the algal oil tested almost exclusively contained DHA. Furthermore, positional distribution of omega-3 fatty acids in such oils, again, depends on source material. The omega-3 fatty acids are primarily located in the sn-2 position of triacylglycerols in fish while they are present mainly in the sn-1 and sn-3 positions of seal blubber oil.

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

Fatty acid	Menhaden	Cod liver	Seal blubber	Algal
14:0	8.32	3.33	3.73	14.9
16:0	17.4	11.0	5.58	9.05
16:1 n-7	11.4	7.85	18.0	2.20
18:0	3.33	3.89	0.88	0.20
18:1 n-9, n-11	12.1	21.2	26.0	18.9
20: n-9	1.44	10.4	12.2	-
20:5 n-3	13.2	11.2	6.41	-
22:1 n-11	0.12	9.07	2.01	-
22:5 n-3	2.40	1.14	4.66	0.51
22:6 n-3	10.1	14.8	7.58	47.4

[a] Units are weight percentage of total fatty acids; algal oil is DHASCO (docosahexaenoic acid single cell oil).

Fish and other marine organism derived oils, similar to other edible oils, are subjected to different processing steps of refining, bleaching and deodorization. As protective components of oils are generally removed to a large extent during processing, it is important to treat the resultant refined, bleached and deodorized (RBD) oils with appropriate antioxidants in order to enhance their oxidative stability. Encapsulation and microencapsulation provide other means for extending the shelf-life of highly unsaturated oils. Regardless, such oils increase the body demand for vitamin E (1). Therefore, addition of vitamin E, usually in the form of mixed tocopherols, to highly unsaturated oils is necessary for enhancing their oxidative stability and also to augment the body's need for vitamin E.

The use of fish oils containing omega-3 fatty acids is recommended for foods that are used within a short period of time in order to avoid possible off-flavor development during their expected shelf-life. While it is possible to mask some of the off-flavors generated due to production of flavor-active secondary oxidation products, presence of primary products of oxidation remains to be a concern. Another way of introducing omega-3 fatty acids into food products is to employ adequately microencapsulated products that may remain intact until they reach the gastrointestinal tract. In this way, there is no flavor effect on the product even if the oil used initially contained some oxidation products. The techniques used for this purpose include multi-layer coaservation employed by Ocean Nutrition Canada. Table 3 summarizes a number of food products that are usually selected to enrich them with omega-3 oils.

Table 3. Food application of omega-3 oils

Bread/bread products	Margarine and spreads
Cereals, crackers, noodles	Eggs
Pasta and cakes	Bars and candies
Milk and dairy products	Infant formula
Juices	Fabricated seafoods, burgers, etc.
Mayonnaise and salad dressings	Others

The beneficial health effects of fish and other highly unsaturated oils are manifold and include

coronary heart disease (CHD), visual and cognitive development, psychiatric disorders inflammatory diseases, Crohn's disease and type 2 diabetes, among others. The omega-3 fatty acids, especially DHA (Figure 3), are known to dominate the fatty acid profile of brain and retina lipids and play a major role in the development of the fetus and infants as well as health status and body requirement of pregnant and lactating women. Both EPA and DHA have been suggested to affect brown fat pad and lead to weight loss. However, more research in this area may be required.

For therapeutic purposes, the natural sources of omega-3 fatty acids as such may not provide the necessary amount of these fatty acids and hence production and use of omega-3 concentrates may be required [Wanasundara et al, 2002]. The omega-3 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 this purpose, on an industrial scale, are low-temperature crystallization, fractional or molecular distillation, urea complexation, chromatography, supercritical fluid extraction, and enzymatic splitting, among others [Wanasundara and Shahidi, 1997]. These procedures have been used, albeit to different extent, by the industry to prepare concentrates that are often sold in the ethyl ester form or re-esterified with glycerol to be offered as triacylglycerols to the market. However, it has been demonstrated that acylglycerols are more stable than their corresponding ethyl esters. Regardless, the modified oils need to be stabilized using synthetic or preferably natural antioxidants.

In preparation of modified lipids containing omega-3 fatty acids, structured lipids (SL) may be produced. SL are triacylglycerols (TAG) or phospholipids (PL) containing combinations of short-chain, medium-chain and long-chain fatty acids (SCFA, MCFA and LCFA, respectively) located in the same molecule and may be produced by chemical or enzymatic means [Lee and Akah, 1998; Senenayake and Shahidi, 2000]. Structured lipids are developed to fully optimize the benefits of their fatty acid constituents 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. We have used the acidolysis process to incorporate capric acid or lauric acid into seal blubber oil [Senenayake and Shahidi, 2002; 2007]. In addition, we produced 91% gamma-linolenic acid (GLA) concentrate from borage oil [Spurvey and Shahidi, 2000] which was subsequently used in acidolysis of menhaden and seal blubber oils [Spurvey et al, 2001]. Such structured lipids that include GLA, EPA and DHA were also prepared using borage and evening primrose as a source of GLA and acidolysis with EPA and/or DHA [Senenayake and Shahidi, 1999a; 1999b]. 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.

PEPTIDES AND PROTEINS FROM AQUATIC RESOURCES

Proteins from aquatic resources are well-balanced in their amino acid composition and hence are quite important dietary sources. Proteins may be

Table 4 . Antioxidative peptides from Alaska pollock skin hydrolyzate and Soy 75 Protein[a]

Peptide	Amino acid sequence
Alaska Pollock	
P_1	Gly-Glu-Hyp (Gly-Pro-Hyp)$_3$-Gly
P_2	(Gly-Pro-Hyp)$_4$-Gly
Soy 75 Protein	
P_1	Val-Asn-Pro-His-Asp-His-Glu-Asn
P_2	Leu-Val-Asn-Pro-His-Asn-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-Gly-Ser-Gly-Asp-Ala-Leu-Arg-Val-Pro-Ser-Gly-Thr-Tyr-Tyr

hydrolyzed to produce 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, use of 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-carotene/ linoleate 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 antioxidant 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 in the order of application were 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 4 and compared with those of soy 7S protein hydrolysates [Chen et al, 1994]. These peptides exert their antioxidant activity via free radical scavenging as well as chelation effects and also possessed ACE (amgiotensin converting enzyme) inhibitory activity. 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 [Kim et al, 2005].

CHITIN, CHITOSAN, CHITOSAN OLIGOMERS AND GLUCOSAMINE

Processing discards of shrimp, crab, lobster, and crayfish contain a sizable proportion of chitin that may be recovered following deproteinization and demineralization [Shahidi and Synowiecki, 1991]. The chitin so obtained may then be deacetylated to afford chitosan [Shahidi and Synowiecki, 1991]. Depending on the duration of the deacylation process, the chitosan produced may assume different viscosities and molecular weights. Chitosans are soluble in weak acid solutions, thus chitosan ascorbate, chitosan acetate, chitosan lactate, and chitosan malate which are all water soluble may be obtained. Chitosan has a variety of health benefits and may be employed in a number of nutraceutical and health-related applications in agriculture, water purification and health promoting commodities [Jeon et al, 2002; Kamil et al, 2002; Shahidi et al, 2002; Jeon et al, 2000]. Chitosan derivatives may also be produced in order to obtain more effective products for certain applications. Chitin and chitosan may be used for juice clarification, water purification and for odor removal, among other industrial applications. Chitosans may also be used for controlled release of nutrients and bioactives in both agricultural and pharmaceutical applications.

Chitosans with different molecular weights and viscosities have been prepared and used 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. In addition, inhibitory effects of chitosan coatings in the total microbial counts for 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). 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].

The low-molecular-weight chitin and chitosan oligomers (also known as chitin/chitosan oligosaccharides (COS's) have received considerable attention as physiologically functional materials having antitumor, immuno-enhancing and antibacterial activities [Jeon and Kim, 2002; Tsukada et al, 1990; Jeon et al, 2001]. Production of COS's via the hydrolysis of chitosan may be achieved chemically or enzymatically. The enzymatic production is preferred, but cost of enzyme may be prohibitive. Therefore, a continuous low cost production method to produce COS's with desired molecular size has been developed [Jeon and Kim, 2000]. The COS's (Table 5) with low-molecular weight and hetero-chitosan oligosaccharides, have been reported to have antibacterial, free radical scavenging, ACE inhibitory and anticoagulant activities [Park et al, 2004]. The

Table 5. Antibacterial Activity of Different Molecular Weight Chitosan Oligosaccharide (COS) Fractions

Bacteria	Antibacterial activity (%)[a]		
	HMWCOs[b]	MMWCOSs[c]	LMWCOSs[d]
Escheria coli[c]	98	62	51
Escheria coli O-157[c]	71	56	60
Salmonella typhi[e]	91	88	89
Pseudomonas aeruginosa[e]	47	35	22
Streptococcus mutans[f]	100	99	99
Straphylococcus aureus[f]	97	95	93
Straphylococcus epidermidis[f]	82	57	23
Bacillus subtilis[f]	63	60	63
Micrococcus luteus[f]	70	67	63

[a]Following the incubation of bacterial culture with 0.1% different COSs fractions, the number of colonies formed on the medium was calculated as a percentage compared to the control. Maximum standard deviation from mean values reported was 10%.
[b]High molecular weight chitosan oligosaccharides (molecular weight range 10-5 kDa).
[c]Medium molecular weight chitosan oligosaccharides (molecular weight range 5-1 kDa).
[d]Low molecular weight chitosan oligosaccharides (molecular weight below than 1 kDa).
[e]Gram-negative.
[f]Gram-positive.

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 formulations containing chondroitin 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 and Kamil, 2001; Simpson, 2007; Simpson et al, 1991; Shahidi et al, 1998]. These components may also be isolated for further utilization in a variety of applications.

ENZYMES

The aquatic environment contains a wide range of genetic material and hence represents exciting potential for discovering different enzymes [Raa, 1990]. Therefore, much effort has been made to recover and characterize enzymes from fish and aquatic invertebrates [Shahidi and Kamil, 2001]. Digestive proteolytic enzymes from stomachless marine fish such as conner, crayfish and puffer appear to inactivate polyphenol oxidase and/or pectin esterase in fruit juices. Successful application of such enzymes has also allowed inactivation of polyphenol oxidase in shrimp processing as an alternate to sulfiting [Simpson, 2007]. Alkaline phosphates from shrimp may be used in different diagnostic kits and some enzymes may be recovered and used in deskinning of fish and squid or cleaning of fish roe for caviar production, among others.

CAROTENOIDS

Carotenoids and carotenoprotiens are present in salmonoid fish as well as in shellfish. These carotenoids may be recovered from shellfish processing by-products and used in a variety of applications [Shahidi et al, 1998]. In addition, certain carotenoids, such as fucoxanthin occur naturally in seaweeds [Czeczuga and Taylor, 1987; Haugen and Liaeen-Jensen, 1994]. Fucoxanthin has been shown to have anti-proliferative activity on tumor cells and has also been implicated in having anti-obesity and anti-inflammatory effects. Fucoxanthinol is a known metabolite of fucoxanthinol.

MINERALS

Among fish processing by-products, fish bone or skeleton (Figure 4) serves as a potential source of calcium which is an essential element for human health. Calcium from fish would be easily absorbed by the body [Larsen et al, 2000]. However, to incorporate fish bone into calcium-fortified foods, it is necessary to first convert it into an edible form by softening its structure. This could be achieved by hot water treatment and heat treatment in an acetic acid solution. Pepsin-assisted degradation of Alaska Pollock bone in acetic acid solution led to highest degree of hydrolysis and dissolution of both mineral and organic parts of fish bone [Jung et al, 2005; Ishikawa, et al, 1990]. As reported by Larsen et al [2000], the intake of small fish with bones could increase calcium bioavailability. Fish bone contains hydroxyapatite, which unlike other calcium phosphates does not break under physiological conditions and takes part in bone bonding. This property has been exploited for rapid bone repair after major trauma or surgery.

Figure 4: Fish bone after removal of proteins.

OTHER BIOACTIVES FROM AQUATIC RESOURCES

Seaweeds are a rich source of iodine, phlorotannins, glutathione, fucoxanthin and also carbohydrates such as alginates. Much interest has also been expressed in algae because of epidemiological evidence has linked habitual consumption of seaweed to reduced risk of a number of chronic diseases in Japanese and Chinese population. Fish skin may also be used for production of collagen as well as gelatin, the latter for special nutritional, religious and nutraceutical purposes. Meanwhile, unexploited aquatic resources from the oceans offer many opportunities for future developments.

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