65. Nutraceuticals and Bioactive Compounds from Seafood Processing Waste

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Seafood items are rich in several valuable nutrients and other useful components. The rising global demand for the community is witnessing an increasing quantity of processed seafood entering world markets. Commercial processing of seafood items for diverse products results in significant amounts of wastes consisting of shells, heads, intestines, scales, bones, fins, etc. Moreover, fishing operations aimed at popular species also lead to the capture of substantial amounts of fish that are commercially nonviable and, therefore, regarded as by-catch. Currently, most of these materials are discarded as landfill or converted on a limited scale into products such as animal feed and leather, which leads to serious environmental hazards. With rapid developments in biotechnology there is vast scope to make use of the wastes as sources of valuable nutraceuticals and other ingredients, which encompass proteins including collagen and gelatin, protein hydrolyzates, bioactive peptides, lipids rich in polyunsaturated fatty acids, squalene, carotenoids, polysaccharides such as chitin, chitosan, glycosaminoglycans and their derivatives, mineral-based nutraceuticals, among others. These products, depending upon their characteristics, have potential for various applications such as natural food additives, bioactive compounds, nutraceuticals, medicinal drugs, biodegradable packaging, and as encapsulation materials for diverse nutraceuticals. This chapter highlights the potential benefits of secondary processing of seafood discards, for the isolation of various valuable compounds, such as unsaturated oils, carotenoids, minerals, fine biochemicals, enzymes, proteins, nucleic acids and pharmaceuticals, antimicrobials, antioxidants, enzyme inhibitors, and other bioactive compounds.

The food and nutritional value of fishery products have been well recognized in the last few decades. The diverse species of fish and shellfish from varied marine environments provide opportunities for the development of food products with different flavor, high nutritional value, and hence good consumer acceptance, which has resulted in a rising global demand for the commodity. In the year 2012, capture fisheries and aquaculture supplied the world with about 158 million tons (Mt) of fishery products, of which about 136 Mt was utilized as human food [65.1]. During the last 50 years, the world per capita food fish supply has almost doubled from an average of 9.9 kg (live weight equivalent) in the 1960s to 19.2 kg in 2012. In the same year, aquaculture set an all-time production high of 66 Mt. In 2008, about 39% of global seafood production at a value of US$ 102 billion entered diverse global markets, making fishery products the most internationally traded food commodity [65.2, 3]. This has resulted in huge amounts of processing discards in processing centers all over the world, causing serious environmental hazards.

65.1 Seafood as a Source of Nutraceuticals

Seafood items with their biodiversity are recognized as a functional food due to the presence of several nutraceuticals and biologically active compounds. The term *functional food*, which was coined in Japan in the 1980s, describes foods that contain nutraceutical ingredients that provide nutrition and offer protection against diseases [65.4]. The term *nutraceutical* is defined as 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 diseases. These ingredients are not identified as essential nutrients, but are considered as bioactive substances with one or more health benefits. Public health authorities consider prevention and treatment with nutraceuticals as a powerful instrument to maintain health and to act against nutritionally induced acute and chronic diseases, thereby promoting optimal health, longevity, and quality of life [65.5–7]. Over the past two decades, more than 3000 new compounds have been isolated from various marine organisms, including fishery items, seaweed species, corals, sponges, and microorganisms [65.8]. Nutraceuticals from fishery sources include calcium, unsaturated oils, carotenoids, fine biochemicals, enzymes, proteins, nucleic acids and pharmaceuticals, antimicrobials, antioxidants, enzyme inhibitors, and other specific bioactive compounds [65.9–11]. The US National Oceanic and Atmospheric Administration (NOAA), with support from the Food and Drug Administration in a study titled, *Seafood choices: balancing benefits and risks* concluded that *seafood is a nutrient-rich food that makes a positive contribution to a healthful diet* and, therefore, advises regular consumption of seafood by Americans [65.12].

65.2 Bio-Waste from Processing of Seafood

Several items of seafood such as shrimp, prawns, salmon, tuna, ground fish, flatfish, sea bass, and sea bream are commodities of international trade, involving about 200 countries, and equivalent to US$ 129.8 billion in 2011. Besides marine fish, freshwater and maricultured species such as shrimp, prawns, salmon, mollusks, tilapia, catfish, sea bass, and sea bream also constitute the trade [65.1]. Commercial processing of fish and shellfish for trade generates significant portions of the raw material as waste, which consists of heads, filleting frames, scales, viscera, gills, dark flesh, bone, and skin [65.14]. The various pre-processing operations involve beheading, skinning, gutting, descaling, filleting, etc. Processing discards are as high as 40% of whole shrimp and krill, 50% of crab, and 24% of squid and of crab and consists of heads, exoskeleton, cephalothorax, and carapace. It has been estimated that annually 500 000, 100 000, 490 000, and 60 000 t of wastes are generated from shrimp, squid, crabs, and krill, respectively [65.15]. About 250 000 and 45 000 t of processing discards are generated annually from the processing of Atlantic cod and tuna, respectively. Norwegian fisheries annually produce more than 615 000 t of waste; most of it being converted into fish silage and fish meal, while about 180 000 t of by-catch is dumped into the sea [65.16, 17]. Pre-processing of freshwater fish such as trout, carp, pike-perch, pike, and bream into various products such as gutted, headed, and free or skin on fillets results in 40–60% of the fish as waste. Frames after filleting are a good source of minced meat [65.18]. Scale constitutes ≈ 2% of the weight of large fish. Freshwater fish such as perch, bream, pike-perch, and carp are rich in thick scales. Approximately 49 000 t of scales have been reported to be produced annually [65.1]. Table 65.1 indicates the proportions of waste generated during seafood processing operations. In addition to the tremendous amounts of processing wastes, about 20 Mt of fish consisting of

Table 65.1 Waste (percentage of whole fish) generated during seafood processing operations (after [65.13])

Processing operation	Waste
White fish filleting	Skin, 4–5 Head: 21–25 Bones: 24–34
Oily fish filleting	40–45
Canning	25 (head/entrails)
Scaling of white fish	2–4
Beheading of white fish	27–32 (heads and debris)
Filleting of oily fish	40 (entrails, tails, heads, and frames)
Trimming and cutting white fish	24–34 (bones and cut-off)
Precooking of fish for canning	15 (inedible parts)

various unconventional and underutilized species are landed during commercial fishing operations [65.19]. Processes for better utilization of these underutilized fish have been discussed [65.20].

65.3 Seafood Waste and Discards as Sources of Nutraceuticals

Conventionally, fishery wastes are mostly converted into low market value products, such as fish meal, ensilage, fertilizer, animal feed, and biofuel. Fish skin from larger fishes such as shark, salmon, lingcod, hagfish, tilapia, Nile perch, carp, and sea bass is used as a source of leather for making clothing, shoes, hand bags, etc. In many countries such wastes are discarded as landfill [65.17]. In 2012, about 21 Mt fish was used for fishmeal production [65.1]. In the last two decades, there has been a global awareness of the environmental, economic, and social impacts of fish processing, calling for efficient utilization of the discards [65.10, 13, 23–26]. Because seafood is nutritionally rich, as mentioned above, there is scope for the marine industry to bioprocess seafood discards and also by-catch to extract compounds that are of practical use [65.8]. Many such compounds can possess interesting bioactivities such as antihypertensive, antioxidant, antimicrobial, anticoagulant, antidiabetic, anticancer, immunostimulatory, calcium-binding, and other properties [65.8, 27, 28]. Table 65.2 shows components that can be isolated from seafood waste. For the convenience of the present discussion, these compounds can be classified into four groups (Fig. 65.1) according to their chemical origin, namely:

i) Nitrogenous compounds including proteins and peptides.
ii) Lipid and lipid-derived compounds.
iii) Polysaccharide-based compounds such as chitin, chitosan, glycosaminoglycans, and their derivatives.
iv) Mineral-based compounds.

These aspects are discussed below.

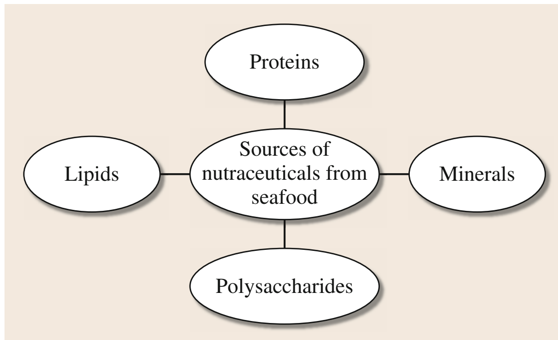

Fig. 65.1 Major sources of nutraceuticals from seafood processing waste

65.4 Nitrogen-Derived Compounds

The interest in protein goes beyond its importance to basic nutrition to specific roles in enhancing the quality of life. A portion of 150 g of fish can provide about 50–60% of an adult's daily protein requirements. In the year 2010, fish accounted for almost 16.7% of the world population's intake of animal protein and 6.5% of all protein consumed [65.1].

65.4.1 Proteins and Protein Hydrolyzates

Fish proteins are rich in all the essential amino acids, particularly methionine and lysine. While there are no significant differences in the amino acid composition of freshwater and marine fish, certain marine fish such as mackerel, tuna, etc., may be exceptionally rich in the amino acid histidine. The nutritive value of marine fish proteins is equal to or better than that of casein and red meat proteins. The protein efficiency ratio (PER) of fish proteins is slightly above that of casein, the major milk protein, and ranges from 3.1 to 3.7. The net protein utilization (NPU) of fish flesh is between 80 and 100, the values for red meat and egg, respectively. Evidently, the protein quality of most fish may exceed that of meat or are equal to that of an ideal protein such as lactalbumin [65.29]. In view of their high nutritive value, recovery of proteins from fish processing wastes for use as food ingredients has received attention. Fish proteins have been isolated by methods such as enzymatic hydrolysis, pH shifting, membrane filtration, and ohmic heating [65.30]. Different methods

Table 65.2 Components from seafood waste and uses (summarized from [65.13, 21, 22])

High-value components and nutraceuticals	Feed, color, flavor, and packaging materials	Nonfood uses
Chitin Chitosan Carotenoid pigments Glucosamine Glycosaminoglycans Chondroitin sulfate Collagen and gelatin Alkaline phosphatase Proteolytic enzymes (pepsin, trypsin, chymotrypsin, collagenases and calpains) Omega-3 fatty acids Deoxyribonucleic acid (DNA) processed as drug in Norway and used in treatment of the human immunodeficiency virus (HIV) Squalene (hydrocarbon found in fish oils useful to treat diabetes and tuberculosis)	Animal feed Crustacean meal Fish/pet food Mollusc shells (used in animal feeds as a calcium source) Encapsulation materials Edible packaging materials Coloring agents (for fish feed) Food flavoring from shrimp heads	Fertilizer Lime Filter media Leather (skin from shark, salmon, ling, cod and hagfish skins) Pearl essence from pelagic fish (iridescent chemical called guanine which gives fresh fish)

Table 65.3 Methods for isolation of proteins and their merits

Methods	Advantages	Disadvantages
Chemical hydrolysis (acid or alkali)	High recovery, inexpensive process	Potential decrease of protein functionality Bitterness Heterogeneous hydrolyzates
Enzymatic hydrolysis	High recovery yields High selectivity Low-salt final product Low contamination of wastes	Bitterness High-cost enzymes (except autolysis) Long-time process Potential decrease of protein functionality
pH shifting	High recovery	Potential decrease of protein functionality
Ultrafiltration	Simultaneous recovery and concentration of soluble proteins	Time-consuming process Expensive membranes
Supercritical fluid extraction with CO_2	Low environmental impact Easy penetration on food systems Mild processing conditions (room temperature and low pressures) Low level of solvent residues in products Inert and nontoxic technique Cost-effective technique	Equipment complexity Necessity of using high pressures Normal use of modifiers, such as ethanol
Ohmic treatment	Rapid process Relatively uniform heating	High-cost installation Possible electrolytic effect

for protein isolation and their merits are pointed out in Table 65.3.

Fish protein hydrolyzates (FPH) have been prepared from several types of fish meat, including processing wastes using various proteolytic enzymes as described in Fig. 65.2. FPHs have obvious nutritional advantages over products like fish protein concentrate or even human grade fish meal. They are useful ingredients to provide functional effects such as whipping, gelling, and texturing properties of foods [65.8, 31, 32]. Techniques for enhancing the storage stability of FPH have been pointed out [65.33]. Proximate compositions of FPHs are moisture, 1–8%, protein, 81–93% fat, 0–5.0%, and ash, 3–8%. Proteins from skin of the blue shark have been isolated using commercial proteinase at an optimal temperature of 51 °C and pH 7.1, with a recovery of 91%. The isolate, which contained all the essential amino acids was rich in low molecular weight (< 6.5 kDa) peptides having high nutritional content and antioxidant activity [65.34]. Proteins were

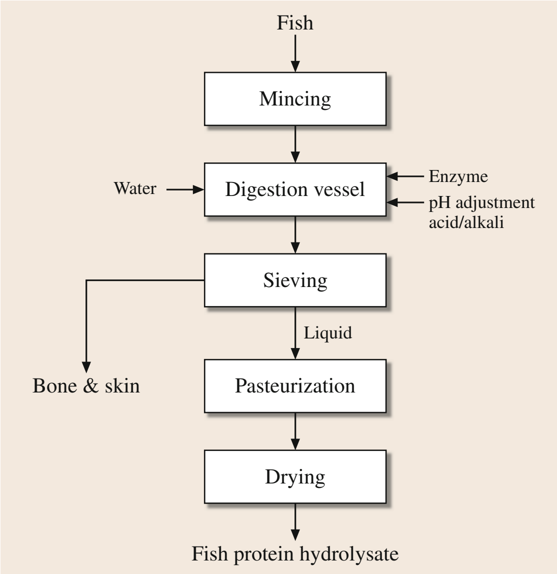

Fig. 65.2 Production of fish protein hydrolysate

recovered from the by-products of silver carp filleting waste using a combination of neutral proteases, giving 82% recovery [65.35].

A novel method is the use of glycation reaction under controlled conditions to effectively isolate functional proteins with gelling and emulsifying capacities [65.36]. Soluble powders prepared from the by-products of the processing of fish and shellfish such as Alaska pollock, herring, salmon, and arrowroot flounder had protein contents ranging between 65–79%. They are good sources of essential amino acids, meeting the requirements of FAO/WHO (FAO: food and agriculture organisation, WHO: world health organization) 1990 recommendations [65.37]. Up to 70% of the protein content of shrimp waste was recovered by boiling water extraction under controlled alkaline conditions [65.38]. Isolates from shrimp head with 12.5% protein, 6.5% chitin, and relatively low ash, can find use as aqua-feed [65.39] weight management, athletic recovery, and maintaining strength and muscle tone for good health in later life. The possible bitter taste of protein hydrolyzates may prevent their use in many products as food additives [65.31]. FPHs are good sources of peptides because they have various bioactive properties, which will be discussed later. In addition, opportunities also exist for isolation of other bioactive substances, including hydroxyapatite, taurine, and creatine [65.23].

Skin and bone waste are rich sources of collagen. Head waste in cod fisheries, yielding ≈ 20% of the whole fish, forms an excellent source of the protein [65.16]. Collagen consists of three identical or different peptide chains, each chain possessing a helical structure, together forming a triple-stranded helix consisting of repeating triplets (glycine-X-Y)$_n$, where X and Y are often proline and hydroxyproline. This basic structural unit of collagen fiber, called tropocollagen, has a molecular weight of ≈ 30 kDa, a length of 280 nm, and a diameter of 1.4–1.5 nm. The process of extraction of collagen from fish bones, which form a major source of the filleting operation, involves initial acetone extraction for about 12 h at room temperature to remove lipids, drying of the treated material, decalcification using a tenfold 0.6 M hydrochloric acid for 1 day to yield collagen [65.40]. Acid-soluble collagen (ASC) and pepsin-solubilized collagen (PSC) were isolated from fish bones and scales. The molecular weights of the collagen subunits were between 110 and 130 kDa. The melting temperatures of ASC and PSC were $> 34\,°C$. Collagen from cod and Pacific whiting surimi processing water showed better functional properties than that from skin and was similar to acid soluble collagen in whiteness, solubility, emulsifying activity, and cooking stability [65.16, 41]. The fish skin collagen was comparable to typical type-I collagens of land-based animals and has potential therapeutic effects in terms of anti-inflammatory activity and inhibition of angiogenesis. However, fish collagen has lower melting temperatures in the range of 25–45 °C compared with mammalian collagens, which melt between 60 and 70 °C.

Collagen from squid waste has also been isolated and characterized [65.42]. Collagen and another connective tissue protein, and elastin have many applications in cosmetics. They provide smoothness to the skin and prevent skin irritation. The proteins are antistatic, film forming, and humectant moisturizers. Collagen combined with the moisturizing properties of chitosan (see Sect. 65.5 on polysaccharide-derived nutraceuticals) is claimed to help restore the elasticity of the skin [65.43, 44].

Collagen is the source of gelatin. The process of isolation of gelatin from collagen involves successive extraction of fish waste at room temperature with dilute alkali and mineral acid [65.45]. Gelatin is a heterogeneous mixture of single or multistranded polypeptides containing 50–1000 amino acid residues. It is translucent, colorless, brittle, and flavorless. Because it is hydrophilic it interacts with water and undergoes changes of its physicomechanical properties depending on the moisture content. It forms transparent elastic thermoreversible gels when heated and then cooled below ≈ 35 °C. The melt-in-the-mouth property is one of the important characteristics responsible for its wide applications in the food and pharmaceutical industries [65.46]. Fish gelatin has a lower melting point of ≈ 10 °C, compared with a melting point of 30 °C of mammalian gelatin, and hence has the potential to replace conventional porcine or bovine gelatin. It can be also used for encapsulation of heat-sensitive compounds. Fish gelatins have a comparable bloom strength (the gel strength equivalent used in the industry), viscosity, and solubility in comparison with mammalian gelatin. Their viscosities range between 15 and 75 mP [65.47–50]; gelatin hydrolyzates are sources of antioxidant and antimicrobial peptides, as will be discussed below. The food uses of gelatin are given in Table 65.4.

65.4.2 Bioactive Peptides

In addition to functioning as sources of essential amino acids, bioactive peptides play significant roles in maintaining health and in preventing diseases of the cardiovascular, nervous, or immune systems. While these peptides are inactive within the sequence of the parent protein, they become active upon release from the parent proteins. They usually contain 3–20 amino acid residues, and their activities are based on their amino acid composition and sequence. Hydrolysis of fish meat by trypsin or other pancreatic enzymes or bacterial and fungal enzymes, either alone or in combination, can give a variety of peptides depending upon the treatment conditions. The prepared hydrolyzates are then subjected to ultrafiltration and/or nanofiltration to fractionate peptides [65.8, 32, 51, 52].

The interesting bioactivities of peptides involve varied functions such as antihypertensive agents, antioxidant, immunomodulatory, antithrombotic, anticancer, and antimicrobial agents. High blood pressure is one of the major risk factors for cardiovascular diseases. The angiotensin-I-converting enzyme (ACE, EC 3.4.15.1) plays a crucial role in the regulation of blood pressure as it promotes the conversion of angiotensin-I to the potent vasoconstrictor angiotensin II. Many peptides, including those from marine sources, can inhibit ACE activity, thereby controlling hypertension [65.32, 53]. The first marine ACE inhibitory peptide was isolated from sardines. Later, ACE-inhibitory peptides were isolated from other fishery items including salmon, sardines, oysters, wakame, yellowfin sole, and dried bonito [65.8, 51, 54, 55].

Some peptides function as excellent antioxidants that can control a wide array of physiological disorders such as cancer, diabetes mellitus, and neurodegenerative and inflammatory diseases. The mechanisms involve binding of metal ions, scavenging of oxygen, converting hydroperoxides to nonradical species, deactivating singlet oxygen and, thereby, suppressing the generation of free radicals. Their antioxidant potency is suggested mostly due to the presence of hydrophobic amino acids and/or their ability to chelate metal ions [65.28, 52]. Antioxidant peptides have been isolated from seafood, including jumbo squid, oysters,

Table 65.4 Food uses of gelatin

Application	Product type
Gelling agent	Jelly confectionery
Whipping agent	Aerated confectionery
Stabilizer	Icings
Emulsifier	Fruit chews, etc.
Adhesive (sticking agent)	Various products
Coating and encapsulation agent	Various nutraceuticals

Table 65.5 Bioactive peptides from various fishery products (after [65.8, 28, 51])

Bioactivity	Fishery source
Antihypertensive action through inhibition of angiotensin-I converting enzyme activity	Sardine, salmon, big-eye tuna, Pacific hake, sea bream, oyster wakame, fish bone collagen, dried bonito digest, shrimp, hard clam, sea cucumber
Antioxidant activity	Alaska pollock, capelin, cod, eel, hake, mackerel, round scad, saithe, sole, sea cucumber, mussel, squid, gelatins from tuna, hoki, squid
Calcium-binding oligophosphopeptide	Bone from the fish hoki
Gastrin and calcitonin gene-related peptides (CGRP)	Atlantic/Greenland cod, sardine, winter flounder, industrial seafood waste
HIV-I protease inhibiting activity	Oyster
Stimulation of nonspecific immune defense system	Chub mackerel, other fish
Antimicrobial activity	Lobster, shrimp and green sea urchin, blue crab, lobster
Anti-coagulant activity	Blue mussel

Table 65.6 Enzymes from seafood wastes

Enzymes	Function	Source	Remark/benefit
Gastric proteases (e.g., pepsins, gastricsins, chymosins)	Cold *renneting* milk, Digestion aid for fish feed	Fish viscera from fishery sources[1]	Catalytic activity at lower temperatures, minimizing unwanted chemical reactions and bacterial growth
Serine and cysteine proteases (e.g., trypsins, chymotrypsins, collagenases, elastases, cathepsin B)	Inactivation of polyphenol oxidase preventing unwanted color changes in foods, low-temperature protein digestion, meat tenderization, fermentation	Pyloric ceca, pancreatic tissues, intestines, hepatopancreas (stomachless bone fish[2])	Catalytic activity at lower temperatures minimizing unwanted chemical reactions and bacterial growth
Lipases	Numerous uses in the fats and oils industry (e.g., production of omega-3-enriched triglycerides)	Various fish items[3]	Higher specificity for omega-3 fatty acids
Polyphenol oxidases (e.g., tyrosinase, polyphenolase, phenolase, catechol oxidase, cresolase, catecholase)	Processing and fermentation of tea, coffee, raisins, and prunes	Crustaceans	Higher activities at lower temperatures, as compared with terrestrial counterparts
Chitinolytic enzymes	Replace HCl for conversion of chitin into oligomeric units	Digestive tracts of fish, shellfish, and shellfish waste, squid liver, octopus saliva	Less harsh than HCl and results in more consistent products
Transglutaminase	Creates protein cross-links to improve rheological properties of gels, i. e., surimi, gelatin	Various fishery items[4]	Strengthens gels with protein cross-linkages

[1] Atlantic cod, carp, harp seals, American smelt, sardine, capelin, salmon, mackerel, orange roughy, tuna; [2] sardine, capelin, cod, cunner, salmon, anchovy. Atlantic white croaker, carp, hybrid tilapia, herring, spiny dogfish, rainbow trout, crustaceans, mollusks, short-finned squid; [3] Atlantic cod, seal, salmon, sardine, Indian mackerel, red sea bream, and others; [4] Red sea bream, rainbow trout, mackerel, walleye, pollock liver, scallop muscles, shrimp, squid

prawns, blue mussels, hoki, tuna, cod, capelin, scads, mackerels, herrings, yellow fin tuna, Alaska pollock, sea cucumbers, and other species. A heptapeptide having a molecular weight of 962 kDa purified from fermented marine blue mussel could scavenge superoxide, hydroxyl, carbon-centered, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Its ability to inhibit lipid peroxidation was higher than α-tocopherol [65.52].

Peptides with antimicrobial activities have been isolated from marine invertebrates, including spider crabs, oysters, American lobsters, shrimp, and green sea urchins [65.28, 30, 51, 54, 56]. Peptides exhibiting high affinity for calcium isolated from pepsin hydrolyzates of Alaska pollack and hoki are able to reduce the risk of osteoporosis. Calcitonin is a hormone (32 amino acid peptide containing a single disulfide bond) known to participate in calcium and phosphorus metabolism. The major source of calcitonin is the thyroid gland, but it is also synthesized in a wide variety of other tissues, including the lung and intestinal tract. The hormone prevents the loss of calcium and phosphorus in urine by reabsorption in the kidney tubules. Calcitonin from salmon can decrease osteoporosis and, hence, is a bone density conservation agent. Salmon calcitonin is about 30 times more potent than that secreted by the human thyroid gland. Nowadays, fish calcitonin is also being made synthetically [65.57]. In addition, the wound healing potential of administering marine collagen peptides (MCP) from chum salmon skin has been demonstrated [65.58]. Some peptides can function as protectants against freezing injury, thereby providing cryostabilization of myofibrils in meat products. Gelatin peptides have been reported to cure osteoporosis. Table 65.5 summarizes various bioactivities of peptides from diverse fishery products.

Marine Enzymes

Seafood processing waste is a rich source of digestive proteolytic enzymes such as gastric, serine, cysteine or thiol proteases, lipases, polyphenol oxidases (PPOs), chitinolytic enzymes, muscle proteases, transglutaminase, and collagenases. Pepsins and gastricins have been isolated from fish gastric mucosa, trypsins and chymotrypsins from pyloric ceca, and trypsin like enzymes from hepatopancreas of marine fish, including cod, mackerel, and salmon, among others. Alkaline phosphatase, hyaluronidase, β-*N*-acetylglucosaminidase, and chitinase have been recovered from shrimp shell waste in good yield. Arctic scallop and clam wastes are good sources of lysozyme, a potential preservative for refrigerated foods [65.8, 59, 60]. A large-scale process for the recovery of enzymes from wastewater of the shrimp processing industry involves flocculation of the water by ferric chloride, concentration by cross-flow ultrafiltration, and then freeze-drying [65.61]. The characteristic properties of marine fish proteinases are a higher catalytic efficiency at low temperatures, lower sensitivity to substrate concentrations, and greater stability at a broader pH range, which are highly useful for their varied applications [65.62].

Collagenase prepared from crab hepatopancreas has been used for skinning of squid (*Loligo* spp.). Protease from mackerel intestines was used for recovery of fish bone from hoki [65.30]. Other applications of endogenous proteases are roe processing, fish sauce, silage, hydrolyzates, and caviar production [65.62, 63]. Because some marine fish and shellfish are inhabitants of extreme low temperatures, their enzymes have significant activities at low temperatures. Further, they may also possess other interesting properties such as salt tolerance and stability to high pressure. Cold-adapted enzymes display a high specific activity associated with relatively high thermosensitivity and lower free energies of activation. Marine enzyme biotechnology can offer novel biocatalysts with properties like high salt tolerance, hyperthermostability, barophilicity, cold adaptivity, and ease in large-scale cultivation. Genes encoding chitinases, proteases, and carbohydrases from microbial and animal sources have been cloned and characterized. Their commercial applications include food processing, biomass conversion, molecular biology, environmental biosensors, bioremediation, and several other processes. Proteases and peroxidases have found industrial applications [65.22, 63–65]. Table 65.6 depicts various enzymes from seafood wastes.

65.5 Lipid-Based Nutraceuticals

65.5.1 Omega-3 Fatty acids

Marine fish generally contain high ($> 7\%$) amounts of lipids, composed of neutral lipids comprised of triacyl glycerols, phospholipids, sterols, wax esters, and some unusual lipids, such as glyceryl esters, glycolipids, sulfolipids, and hydrocarbons. Commercial fish oils are characterized by fatty acids with 12–26 carbon atoms with 0–6 double bonds. The fatty acids are made up of saturated (15–25%), monounsaturated (35–60%) and polyunsatured (25–40%). Marine lipids are rich in long-chain polyunsaturated fatty acids (PUFA) (with more than 14 carbon atoms), particularly, omega-3 (also referred to as ω-3 and or n-3) fatty acids (3 indicating the position of the first double bonds at the third carbon from the methyl end of the fatty acid structure). Other dietary fatty acids include n-6 PUFA, namely, linoleic, $C_{18:2w6}$, γ-linolenic, $C_{18:3w6}$, and arachidonic, $C_{20:4w6}$ acids. The popular ω-3 fatty acids are eicosapentaenoic acid containing five double bonds ($C_{20:5w3}$, cis-5,8,11,14,17-eicosapentaenoic acid, designated as EPA), and the 22-carbon docosahexaenoic acid, containing six double bonds ($C_{22:6w3}$, cis-4,7,10,13,16,19-docosahexaenoic acid, DHA). In contrast with other fats and oils, marine fish oils contain large amounts of EPA and DHA, in the range of 14–19 and 5–8%, respectively. Fish oils such as those from tuna, salmon, etc., may contain more DHA than EPA. Commercial cod liver oil is a complex mixture of more than 50 different fatty acids, forming triacyl glycerols, of which there is usually 8–9% each of EPA and DHA. Marine oils also contain significant amounts of fat-soluble vitamins A and vitamin D. Halibut, shark and cod liver oils are rich sources of vitamin A and D. A 3.5-oz portion of salmon can provide 90% of daily human need of vitamin D. The content of vitamin E (α-tocopherol) is a powerful antioxidant related to its availability from feed. Vitamin E can help protect skin cells and tissues because of its antioxidant activity. The fat soluble vitamin A and carotenes are relatively stable at normal cooking temperatures. Oil from Antarctic krill (*Euphausia suberba*) is rich in omega-3 fatty acids, phospholipids, and also natural pigments and vitamins [65.66, 67].

The beneficial health effects of marine fish oils, because of the rich presence of omega-3 PUFA, have been well documented. A significant body of evidence indicates that intake of the long-chain *n*-3 polyunsaturated fatty acids (omega-3 fatty acids) found in fish is cardio-protective [65.68]. Besides, they can reduce hypertension, lower autoimmune and inflammatory diseases, depression, attention-deficit hyperactivity disorder (ADHD) in children and muscle degeneration in the elderly, arthritis and some types of dermatitis. These fatty acids are also involved in the structure of cell membranes, the development of the nervous system and influence the synthesis of cell mediators (prostaglandins and leukotrienes), which play important roles in coagulation, inflammation and proliferation of certain cells. DHA and arachidonic acid (*n*-6) are also important in visual function. Deficiency of these compounds causes disorders such as restrictive growth, abnormal skin and hair, damage of reproductive system, among others [65.69, 70]. Many psychiatric disorders, particularly schizophrenia and major depressive disorder (MDD), have shown positive results when supplementation has been used as an adjunct to standard pharmacotherapy [65.71, 72]. Cod liver oil has been shown to help slow the destruction of joint cartilage in patients with osteoarthritis [65.73]. The health benefits of PUFA have encouraged regulatory agencies to recommend regular public consumption of fatty fish such as herring, sardine, and mackerel. The WHO recommends consumption of 1−2 servings of fish, containing 200–500 mg of EPA and DHA, per week. The American Heart Association recommends that persons diagnosed with cardiovascular diseases consume 1 g each of the fatty acids per day [65.72, 74]. Table 65.7 summarizes potential health benefits of PUFAs.

Whole or processing wastes (particularly liver) of fish, including anchovies, capelin, Atlantic cod, Atlantic herring, Atlantic mackerel, Atlantic menhaden, cod, saithe, haddock, salmonids, and sardines are rich sources of marine lipids rich in PUFA, particularly EPA and DHA. However, lipid composition in these fish varies with season and also depends upon the tissues being analyzed. The oil contents can be as high as 21% in herring, 22% in tuna, and 18% in sardines in winter. Fish livers are the ideal source of these lipids. Enzymatic methods using proteases are useful to release the bound oil from fish tissues. Depending on the proteolytic enzyme used, oil yield from red salmon heads varied between 4.9−10.6% [65.37]. Figure 65.3 depicts a typical alkaline refining process for fish oil extraction. PUFA contents in different marine fishes and methods for their extraction and fractionation, in terms of fatty acid constituents in the form of methyl esters have also been provided. Methods of isolation consist of molecular and fractional distillation, solvent, and supercritical extraction [65.75, 76]. Because omega-3 fatty acids in oil are highly unsaturated, they are sensitive to oxidation with the formation of hydroperoxides, which depends on exposure to heat, light, and moisture, and the presence of metal ions. Therefore, during their isolation PUFAs must be protected against oxidation by incorporating small amounts of antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and octyl gallate, and the oil must be stored in ampoules with minimum headspace [65.77]. Supercritical fluid extraction (SFE) is a popular technology due to its nontoxic and nonflammable nature. The carbon dioxide atmosphere employed during extraction also protects PUFAs from oxygen-induced oxidation. In the process, PUFAs are usually extracted at an elevated pressure of 10−30 MPa and at 40−80 °C [65.76].

Marine oils have been isolated commercially from several gadoid finfish species [65.3, 77–81]. Tuna fish has a total lipid content of about 22% with up to 20 and 4% of DHA and EPA, respectively Monounsaturated and *n*-6 fatty acids are also present at 23.3 and 3.8%, respectively. The huge discards from the global tuna canning industry, estimated at 450 000 t annually, could be a rich source of oil [65.78]. Total lipid contents in the head, meat, and waste of three commercial varieties of Indian marine fishes, namely, pink perch, Indian mackerel, and Indian oil sardine are in the range of 4.3−13.6, 2.53−10.97, and 2.7−15.1% (wet weight basis), respectively. Neutral lipids were higher in the head (83.2−89.2%). The saturated fatty acid, palmitic acid, was present in all the fishes, irrespective of the body components. EPA and DHA, however, were found in higher concentrations [65.82]. Shark liver is 22–30% of the body weight, with an oil content as high as 90%. Liver oils of some sharks found under a depth

Table 65.7 Potential health benefits of omega-3 fatty acids

Prevention of atherosclerosis
Protection against arrhythmia
Reduction of blood pressure
Beneficial for diabetic patients
Good against manic-depressive illness
Reduction of symptoms in asthma patients
Protection against chronic obstructive pulmonary diseases
Alleviation of symptoms of cystic fibrosis
Prevention of relapse in patients with Crohn's disease
Prevention of various cancers
Good for bone health
Improvement of brain functions in children

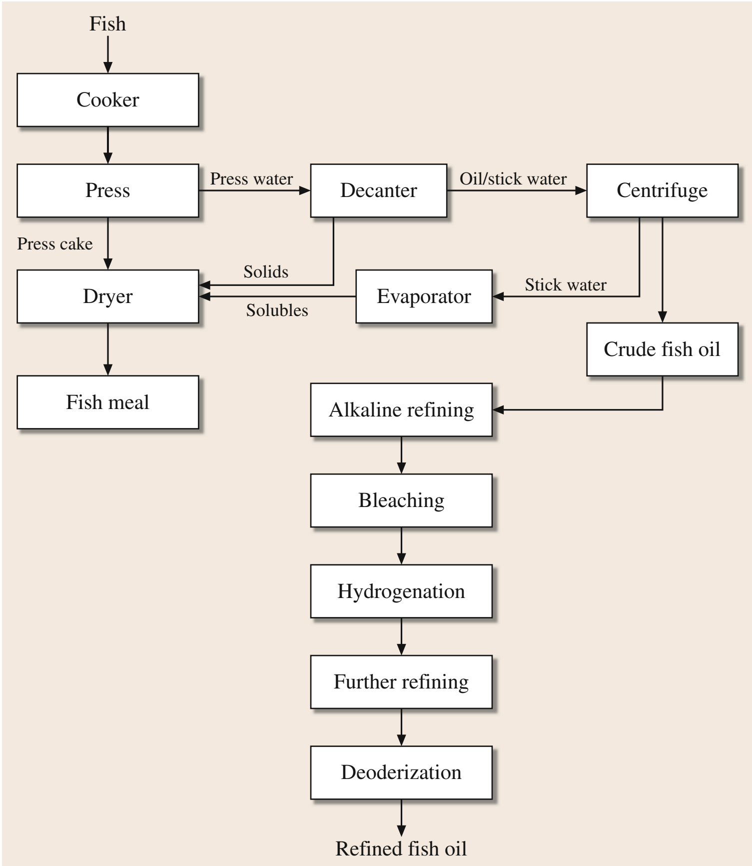

Fig. 65.3 Process for recovery of fish oil

of 300–3000 m in the Pacific, North Atlantic, and Indian Ocean contain ≈ 85–90% unsaponifiable matter, mainly the hydrocarbon, squalene. Shovelnose dogfish liver oil contains 60% hydrocarbons, consisting mainly of squalene and pristane, and 25% diacyl glyceryl ether. Squalene, $C_{30}H_{50}$ is 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) and squalamine, an amino sterol antibiotic, are found in shark liver. The recovery of oil from shark liver consists of natural decomposition, acid ensilage in presence of formic acid, alkali digestion, and steam rendering. The oil recovered is degummed, bleached, and deodorized. The process for isolation of squalene from shark oil consists of heating chopped liver in 2% caustic soda solution for 30–40 min to separate the oil. After removing water by anhydrous sodium sulfate, the oil was subjected to vacuum distillation at 240 °C [65.79]. Herring oil has been extracted from dried fish, giving 41% oil rich in PUFA [65.3]. Enrichment of marine oil to increase the contents of PUFA has been attempted by making use of lipolysis [65.83, 84]. Several industries specialize in fish oil production and purification [65.84–86]. Table 65.8 shows the contents of omega-3 fatty acids in as a percentage of total fatty acids in various commercial fish oils.

65.5.2 Carotenoids

Carotenoids are a family of fat-binding compounds responsible for the red and yellow color of crustaceans and also many plants, algae, and cyanobacteria. The carotenoids found in nature can be classified into two groups, namely, hydrocarbons, such as β-carotene and xanthophylls, and the oxygenated derivatives such as astacene, astaxanthin, canthaxanthin, cryptoxanthin, neoxanthin, violaxanthin, and zeaxanthin, lutein, etc. The color of carotenoids is due to chromophores containing conjugated double bonds. The naturally abundant β-carotene ($C_{40}H_{56}$) is a polyunsaturated hydrocarbon made up of two retinal molecules.

Table 65.8 Contents of omega-3 fatty acids in commercial fish oils (values give the percentage of fatty acids in total fatty acids; after [65.87], courtesy of Wiley-VCH)

Species	18 : 3	18 : 4	20 : 5	22 : 5	22 : 6	EPA + DHA
Anchovy	1	2	22	2	9	31
Atlantic menhaden	1	3	14	2	12	26
Sardine/pilchard	1	3	16	2	9	25
Gulf menhaden	2	3	13	3	8	21
Pollock		2	15		4	20
Capelin	1	3	8		6	14
Sand eel	1	5	11	1	11	22
Mackerel	1	4	7	1	8	15
Blue whiting	1	3	7	1	8	15
Herring	2	3	6	1	6	12
Tuna	1	1	6	2	22	28
Norway pout	1	3	9	1	14	23
Whitefish spp.	1	2	9	2	13	22
Salmon, wild	2	1	8	4	11	19
Salmon, farmed	1	3	9	2	11	18
Sprat	2		6	1	9	15
Tilapia, farmed	2			3	5	5
Catfish, farmed	1		1	1	3	4

Approximately 60 carotenoids possess varying levels of provitamin A activity, β-carotene possessing maximal provitamin A activity. Carotenoids possess remarkable antioxidant properties, which is their primary beneficial role in the diet of humans and animals. Astaxanthin is about ten times stronger in antioxidant activity than other carotenoids (including β-carotene, canthaxanthin, and lutein). Animals, including humans, do not synthesize carotenoids de novo and rely upon diet for these compounds. Oral supplementation of carotenoids appears to increase the photo-protective properties of the epidermis and dermis against environmental stress (e.g., UV radiation, pollution, smoke) by quenching free radicals generated by oxidative stress. Studies have also shown the ability of carotenoids to reduce DNA damage and protect against depletion of Langerhans cells, a key component of immune function. Because of their potent antioxidant properties, carotenoids have been suggested to have a protective role against cancer, aging, ulcers, heart attack, and coronary artery disease [65.88].

Crustacean wastes are abundant sources of β-carotene and astaxanthin (3,3′-dihydroxy-β, β-carotene-4 and 40-dione), the oxidized form of β-carotene. Astaxanthin is responsible for the pink-to-red pigmentation of crustaceans and wild salmonids [65.89–91]. The process of extraction of carotenoids from shell waste consists of initial treatment with proteases to detach the pigments from the bound proteins, followed by extraction in organic solvents. Upon treatment with trypsin at the optimum temperature 45–55 °C for 2 h the carotenoid-rich head wastes of commercially important Indian marine shrimp species yielded significant quantities of astaxanthin, and also minor amounts of β-carotene, canthaxanthin, lutein, zeaxanthin, and crustacyanin [65.74]. Astaxanthin in the form of carotenoprotein can be extracted with a yield of 49% by treating shrimp waste with proteolytic enzymes such as trypsin in the presence of ethylenediamine tetraacetic acid (EDTA) at a pH of 7.7 and 4 °C [65.56].

65.6 Polysaccharide-Derived Nutraceuticals

Chitin is a cationic polysaccharide formed by units of *N*-acetyl-D-glucosamine, joined by (1–4) β-bonds, viz., β-(1-4)-*N*-acetyl-D-glucosamine, which is β-(1-4)-*N*-acetyl-2-amino-2-deoxy-D-glucose.

65.6.1 Chitin and Chitosan

Marine crustaceans, which include shrimp, crabs, squid, cuttlefish, krill, and oysters, are rich in chitin. Chitin forms the outer protective coatings of crustacean shells in a covalently bound network with proteins and dihydroxy phenylalanine, together with some metals and carotenoids. Chitin occurs in three polymorphic forms, α, β, and γ, which differ in the three-dimensional arrangements of the molecular chains. Its most common form is α-chitin, where two units of *N*-acetyl-D-glucosamine are in an anti-parallel arrangement. Chitin is a very light, white or yellowish, powdery/flaky product. It is insoluble in water, almost all common organic solvents, and in acidic and basic aqueous solutions. Chitin may be solubilized in carbon disulfide after treatment with caustic soda and then re-precipitated as a filament or film in the manner of viscose rayon. Chitin swells in cold alkali when deacetylation takes place [65.8, 15, 44, 92–96].

Shellfish waste consisting of crustacean exoskeletons is currently the main source of biomass for chitin production. The isolation process for chitin consists of three-step treatments of shell wastes, and includes demineralization, deproteinization, and bleaching, followed by extraction of chitin using acetone [65.97]. Demineralization is usually carried out by an extraction of 1–3 h with diluted hydrochloric acid, but harsher conditions such as 90% formic acid, 22% HCl, 6 N HCl, or 37% HCl have also been applied. Deproteinization is performed by treating the raw material with either sodium hydroxide or potassium hydroxide at concentrations between 1–10%(w/v) and at temperatures of 65–100 °C for a duration ranging from 0.5–6 h. Since the harsh conditions of alkaline digestion may cause depolymerization and deacetylation of chitin, enzymatic deproteinization by digestion with proteolytic enzymes such as papain, pepsin, trypsin, or pronase has been used. The following step of solvent extraction (e.g., acetone, chloroform, ethanol) helps remove pigments like melanins and carotenoids [65.15, 93–95].

Chitosan is a collective name representing chitin deacetylated to different degrees. Structurally, chitosan is poly-(1-4)-linked-2-amino-2-deoxy-D-glucose. Commercially chitosan is produced by deacetylation of crustacean chitin using 30–60% (w/v) sodium or potassium hydroxide at 80–140 °C. The preparation is purified by dissolving it in dilute acetic acid, reprecipitation with alkali, followed by washing and drying to obtain flakes of chitosan. The resulting molecular weight distribution of deacetylated units along the polysaccharide chain depends on the alkali concentration applied, and the temperature and time of the process. The degree of deacetylation of the polysaccharide can be increased by using high temperatures in the process, but such harsh conditions also cause depolymerization and lead to a reduction in the size of the molecules. To obtain more defined chitosans and to avoid polysaccharide degradation by oxygen it is also recommended to carry out the deacetylation process under nitrogen or to employ thiophenol or sodium borohydride addition as scavengers of oxygen. Alternately, chitin deacetylases derived from different organism sources can be used [65.94]. Shell waste can also be used for fermentation to produce chitinase [65.98]. Figure 65.4 shows a typical process for chitin extraction and its subsequent processing to yield chitosan.

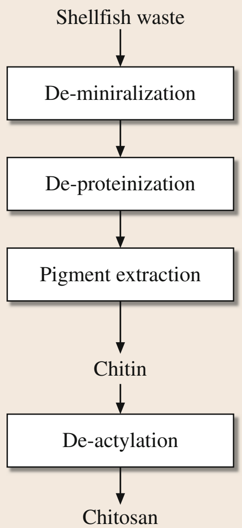

Fig. 65.4 Recovery of chitin and chitosan from shellfish waste

The molecular weight of natural chitosan is generally higher than 10^6 Da. Chitosan is a polycationic, long-chain biopolymer because of the presence of one free amino group and two free hydroxyl groups for each glucose ring. Because of this chemical nature, it has a natural affinity for negatively charged compounds, including biological membranes. Chitosan is insoluble in pure water, but unlike chitin it is soluble in weakly acidic aqueous media. A minimum deacetylation of 70% is required for chitosan to be acceptable for various purposes. The pK_a value for the positively charged ammonium group is ≈ 6.2. When the pH is raised to ≈ 6.5, chitosan precipitates in a gel form. The multidimensional utilization of chitin derivatives, including chitosan, is due to a number of characteristics, including their polyelectrolyte and cationic nature, the presence of reactive groups, the high adsorption capacities, and bacteriostatic and fungistatic influences, which make them very versatile biomolecules [65.99]. Because of its cationic nature, chitosan is incompatible in solution with most anionic water-soluble gums such as alginates, pectate, sulfated carrageenan, as well as carboxymethyl cellulose. On the other hand, acidic solution of chitosan is compatible with nonionic water soluble gums such as starch, dextrins, glucose, polyhydric alcohols, oils, fats, and nonionic emulsifiers.

Chitosan can form films, which are tough, flexible, and transparent. Chitosan is biodegradable by the specific enzyme, chitosanase. By enzymatic treatment, soluble chitosan can also be obtained in oligosaccharide form. Chitosan derivatives in the form of acetate, ascorbate, lactate, malate, and others are water-soluble and have varying functional properties. The role of chitosan as a fiber, however, is challenged by popular fiber products such as oats, soy, and bran. Chitosan film can be extruded from its acidic solution into a 70 °C coagulating bath containing caustic soda and sulfonic acid esters of high molecular weight alcohols. Chitosan in microcrystalline form has several advantages when compared to standard chitosan [65.8, 95, 100, 101].

Polysaccharide-based biomaterials, particularly chitosan, are emerging for applications in several biomedical fields, such as tissue regeneration, particularly for cartilage, drug delivery devices, and gel entrapment systems for the immobilization of cells. Their salient beneficial properties are controllable biological activities, biodegradability and the ability to form hydrogels [65.101]. Recent years have witnessed a marked growth in chitosan and its derivatives, the applications encompassing food and nutrition, biotechnology, material science, drugs and pharmaceuticals, cosmetics, water treatment, cosmetics, agrochemicals, biotechnology, environmental protection, and gene therapy. The medical applications of chitosan cover such diverse fields as hemodialysis membranes, artificial skin, hemostatic agents, hemoperfusion columns, and drug delivery systems. Oral administration of chitosan suppresses the serum cholesterol level and hypertension. The bacteriostatic and fungistatic properties of chitosan mean that it can be used as an ingredient in tropical skin ointments as a wound healing agent [65.43]. The property of chitosan to form gel at slightly acid pH provides antacid and anti-ulcer activities. The antimicrobial activity and film-forming property of chitosan make it a potential source of food preservatives or a coating material of natural origin. Chitosan is also a popular drug carrier. It enhances dissolution properties of poorly soluble drugs and also helps transdermal delivery of drugs [65.43, 95, 102–105]. When intravenously injected, chitin/chitosan oligosaccharides enhance antitumor activity by activating macrophages [65.106]. Chitosan-based scaffolds are useful for tissue regeneration [65.107]. The use of chitin as a source of dietary fiber in chicken feed enhances the growth of bifidobacteria in the guts, which reduce other microorganisms and produce the β-galactosidase necessary for the digestion of feed supplemented by whey or other dairy by-products. The effect has also been noticed in the case of chitosan feed meant for pigs and fish [65.43].

65.6.2 Glucosamine

Glucosamine is natural amino sugar found in large concentrations in certain foods such as milk, eggs, liver, yeast, molasses, and yeast. Glucosamine is obtained by extensive hydrolysis of chitosan with mineral acid, such as hydrochloric acid. If it is produced from chitin, it must be deacetylated. Glucosamine can easily be absorbed into the human intestine [65.93].

65.6.3 Glycosaminoglycans (GAGs)

Glycosaminoglycans are multifunctional polysaccharides composed of repeating disaccharide units. Based on the disaccharide composition, linkage type, and the presence of sulfate groups, GAGs include chondroitin sulfate, dermatan sulfate, heparin sulfate, keratin sulfate, and heparin, and are present in the connective tissues of living systems together with collagen, elastin, fibronectin, and laminin, forming a complex extracellular

Table 65.9 Useful features of chitosan in food applications

Properties	Applications
Renewable resource	Abundantly available from marine sources and hence renewable
Bioactivity	Antimicrobial activity and its use in food packaging as antimicrobial additive, stimulation of immune system, anticholesterolemic activity, use as fiber in foods, obesity control
Biodegradability	Substrate for single cell production, biodegradable packaging material, controlled release of drugs, nutrients, etc.
Reactivity of deacetylated amino groups	Moisture control, thickening action
Chelating capacity	Removal of metals, water treatment, antioxidant activity
Complex formation with other macromolecules	Complexes with proteins (useful to remove hypoallergenic β-lactoglobulin from whey, clarification of wines). Chitosan-alginate removes protein from seafood industry waste water
Biocompatibility	Nontoxic and biological tolerance
Film-forming properties	Useful as edible packaging, encapsulation materials, and delivery of nutraceuticals

Table 65.10 Applications of chitin, chitosan, and some of their derivatives in healthcare (after [65.43, 101, 102, 105, 108])

Compound	Applications
Chitin	Wound dressing In vivo absorbable sutures Drug delivery Dialysis membrane
O-Carboxymethyl chitin and *O*-hydroxypropyl chitin	Cosmetic ingredient
N-Actylchitohexasacharide	Antitumor agent
Chitosan and its various derivatives	Artificial skin Blood anticoagulant and hemostatic materials Chitin and chitosan hydrogels for delivery of nutraceuticals Protein absorbents Drug delivery systems Haemodialysis membranes Immunostimulation, molecular recognition, and entrapment of growth factor, etc. Nanofiber scaffold for nerve tissue regeneration Hypocholesterolemic agents Wound-healing materials Nutrition (dietary fiber, weight reduction, hypocholesterolemic agent, etc.) Water treatment (removal of metals, radioisotopes, pesticides, etc.) Cosmetics (shampoo, skin products)
N-Hexanoylchitosan and *N*-octanoylchitosan	Antithrombogenic material for artificial blood vessels Contact lenses Blood dialysis membranes Artificial organs
N-Carboxybutylchitosan	Wound dressing
5′-Methylpyrrolidinone chitosan	Dentistry
Sulfates of chitin and chitosan	Anticoagulant and lipolytic agents

matrix providing a cushion between bones and joints. Most sulfated GAGs are covalently linked to proteins to form proteoglycans. Chondroitin is composed of a chain of *N*-acetylgalactosamine and glucuronic acid residues linked through alternating β (1–3) and β (1–4) bonds. Chondroitin sulfate (CS) has anti-inflammatory properties, as well as anticancer properties. While chondroitin provides cartilage with strength and resilience, glucosamine inhibits inflammation and stimulates cartilage cell growth [65.109]. Table 65.9 summarizes food applications of chitosan and Table 65.10 shows applications of chitin, chitosan, and some of their derivatives in healthcare.

GAGs in terrestrial vertebrates have been well studied. Cartilage of marine animals such as shark, skate, mussels, and squid can be good sources of these polysaccharides. Shark cartilage, which is rich in chondroitin sulfate, has been found to have several therapeutic effects against diseases such as arthritis and tumors. Shark cartilage along with glucosamine is a highly effective treatment for arthritis and osteoporosis. A process for the preparation of chondroitin sulfate from skate cartilage has been reported [65.84]. Another compound, hyaluronic acid (HA), is a natu-

Table 65.11 Some applications of glycosaminoglycans in medicine (after [65.44, 101, 105])

Glycosaminoglycans	Applications
Chondroitin sulfate and other glycosaminoglycans	Helps rebuild cartilage and joints. Supports body's ability to joint health integrity
Hyaluronic acid	Eye surgery, osteoporosis, tumor marker etc.
Dermatan sulphate	Stimulates cell growth. Role in wound healing, cardiovascular disease, etc.
Heparin	Anticoagulant
Keratan sulfate	Eye treatment (corneal transparency)
Chitin	Chitin and chitosan have wide applications
Chondroitin	Helps rebuild cartilage and joints. Supports body's ability keep joints healthy

rally occurring polysaccharide consisting of glucuronic acid and *N*-acetyl-Dglucosamine units. HA functions as an effective moisturizer due to its strong water holding capacity. With age, the levels of HA in the body decrease, reducing the moisture binding capacity of the skin, resulting in dry skin and wrinkles. Oral consumption of HA can help increase moisture on the surface and within the skin. A combination of gelatin, hyaluronic acid, and chondroitin can have potential in wound healing [65.53]. Table 65.11 shows some medical applications of glycosaminoglycans. The global market for marine-derived drugs was US$ 4.8 billion in 2011 and is expected to reach US$ 8.6 billion in 2016 [65.110].

65.7 Mineral-Based Nutraceuticals

Bone comprises a significant part of seafood processing wastes. Filleting of fish generates a large amount of fish frames. For instance, backbone wastes from the processing of Atlantic cod account for approximately 15% of the wet weight of the fish. Fish bone contains about 40% crude protein and 6% collagen, on a dry weight basis. Inorganic minerals constitute approximately 60% of bones, the major ash components being calcium and phosphorus [65.11, 23, 111]. The mineral constituents can be classified as into three groups, composed mainly of either hydroxyapatite (HAP), tricalcium phosphate, (TCP), or a mixture of HAP and TCP. Sea bream, horse mackerel, carp, and shark have hydroxyapatite type phosphate (as in the case of cattle, swine, and fowl), while Japanese anchovy has TCP type phosphate [65.112].

Fish bone is a potential source of calcium. To isolate the mineral in a bioavailable form, the bone is initially softened by either hot water or hot acetic acid. Superheated steam can reduce the loss of soluble components from fish tissue, which enables better recovery of bone within a shorter period. The treated bones are subjected to saponification, degreasing, and degumming. The preparation is a source of dietary calcium, in addition to some phosphorus [65.113]. Peak bone stone is the bony structure situated near the vertebral column of the dorsal fin base obtained from large fishes like ghol (*Protonibea diacanthus*), koth (*Otolithes biauratusa*), and dara (*Filamanus heptadactyla*). There is potential to use the product as raw material for calcium powder [65.8]. The skeleton discarded from the industrial processing of hoki was digested by a heterogeneous enzyme extracted from the intestine of bluefin tuna, which yielded a fish bone oligophosphopeptide containing 23.6% phosphorus. The peptide (molecular weight of 3.5 kDa) could be a nutraceutical with a potential calcium-binding activity [65.114]. Fish and shellfish are also rich sources of selenium [65.66, 67].

65.8 Novel Marine Organisms and Compounds

Marine invertebrates have been recognized as rich sources of more than 400 bioactive compounds, including hypotensive agents, cardio active substances, muscle relaxants, antibiotics, and antiviral and antitumor agents [65.115]. Sea cucumbers or holothurians are spiky-skinned animals of the phylum Echinodermata (class Holothuroidea). They are commonly found in shallow water areas of the sea to deep ocean floors. Related species are the sea lily, sea urchin, star fish, and sand dollars. The cell wall of the sea cucumber contains large amounts of sulfated glycans. The cell wall polysaccharide is comparable in backbone structure to mammalian chondroitin sulfate, but some of the glucuronic acid residues display sulfated fucose branches. The specific spatial array of the sulfated fucose branches in the fucosylated chondroitin sulfate not only confers high anticoagulant activity to the polysaccharide but also antithrombin activity [65.116].

These glycans also exhibit a wide range of other biological activities, which include recombinant HIV reverse transcriptase activity, anti-inflammatory, antiangiogenic, and antiadhesive properties [65.117]. Green fluorescent protein (GFP) is a novel photoactivatable fluorescent protein (PAFP) compound that was first isolated from jellyfish in 1962. The green color of the protein turns red when exposed to an intense pulse of visible blue light. GFP is an invaluable tool that is useful for in vivo bioprocess monitoring such as protein interactions and drug interaction [65.118].

The ink of squid and cuttlefish has been found to possess some therapeutic activity. Mollusks are another important species that have a wide range of uses in pharmacology. Guanine, (2-amino-6-oxypurin) found as a constituent in scale membranes is responsible for the gleaming effect of fish. Guanine combines with collagen and calcium phosphate, yielding a silvery white thinning substance. The lustrous material can be extracted as crystals from fish scales like those of sardine, herring, ribbonfish, carp, etc. The yield of the recovered material, often called pearl essence because of its luster, is $\approx 0.3\%$ of the body weight in the case of ribbon fish.

65.9 Commercial Aspects

In recent years marine compounds, including those derived from seafood, have been entering commercial markets. Some marine nutraceuticals currently marketed in the US include fish and algal oils that are rich in omega-3 fatty acids, chitin and chitosan, fish and shark liver oil, and marine enzymes and chondroitin from shark cartilage, sea cucumbers, and mussels. Commercially important nitrogen-based seafood nutraceuticals include peptides, fish bone phosphopeptide with calcium binding activity, salmon calcitonin, and squalamine. Salmon calcitonin (CAS No. 47931-85-1) is approved for postmenopausal women who cannot tolerate estrogen.

In Japan foods fortified with nutraceuticals are approved as food for specified health uses (FOSHU). Several FOSHU products with a fish (e.g., sardine) peptide as the functional ingredient have been approved for the control of mild hypertension [65.119]. Enzymes such as trypsin and chymotrypsin, purified from cod viscera, are available commercially. The global market for industrial enzymes was valued at US$ 3.9 billion in 2011 and is supposed to grow to reach $ 6 billion in 2016 [65.110]. Sea cucumber, processed after boiling and sun-drying, marketed as *beche-de-mer* (meaning processed sea slug or sea cucumber) is a highly priced product on the international market. It is said to cure low blood pressure, kidney disorders, and impotence and to prevent ageing [65.120]. The global market for marine-derived drugs was US$ 4.8 billion in 2011 and is expected to reach US$ 8.6 billion in 2016 [65.110].

Omega-3 fatty acids remain one of the most successful and promising functional ingredients in the food and beverage industry. The global market for the product is about 49 000 t annually, worth around US$ 700 million. Marine-based oils used in the food industry include oils from cod, sardine, tuna, and salmon, and also algal oil, many of these oils finding use in margarine and butter. Capsules containing PUFA-rich oils of fish such as cod and salmon, rich in DHA and EPA, as well as vitamins A and D, are being prescribed to reduce blood pressure and to and improve joint mobility. Foods fortified with omega-3 fatty acids are marketed particularly in the US, Europe, Japan, and Southeast Asia. Incorporation of DHA into baby foods is being practiced to enhance memory development. Other fortified products include bakery items, bread spreads, salad dressing, and others [65.85]. The market for these products was propelled when the US Food and Drug Administration (FDA) in 2004 approved a qualified health claim for omega-3 fatty acids (EPA and DHA). About 75% of fortified products on the European market include milk, cake, pasta, cheese, yogurt and chocolate milk, and infant formulae, which contain omega-3 from marine fish and the rest from algae and flax seed oils [65.7]. The demand for omega-3-enriched consumer packaged goods (CPGs) is expected to exceed a value of US$ 13 billion in 2015 [65.49]. Marine oils are also being used to fortify livestock and aquaculture feed to produce omega-3-rich farmed fish, eggs, and milk. The current interests in Japan encompass the potential anticancer properties of deep.sea shark liver oil and tuna body oil as a less expensive source of DHA [65.119].

The worldwide carotenoid demand is growing at an estimated average annual rate of 2.9%. Currently, β-carotene from the alga *Dunaliella salina* is a commercialized high-value product, its major producers being Australia, US, and Israel. β-Carotene is sold mainly as an extract or suspension in vegetable oil or as β-carotene-rich algal powder. Astaxanthin is widely used to pigment salmon and trout, although in recent times, canthaxanthin has become popular in fish pigmentation and also in poultry to give a red color to egg yolks.

Japan is the major producer of chitin and chitosan from the shells of crabs and shrimp (FAO, 2012). The market for chitosan in the US is about US$ 20 million [65.13]. Chitosan is essentially used to assist weight loss. Chitosan-fortified fruit juices and chocolates are marketed in the US. In Europe chitosan is sold as dietary capsules to assist weight loss. Some of the products include *Fat Absorb*, a US product containing 250 mg of chitosan per capsule, the *Seaborne* range of products such as *Essential Sea* and *EssentialSeaPlus*, which contain chitosan together with lecithin, vitamins C and E, garlic, and β-carotene, and the fat trimmer, *Minfat*. In Japan, chitosan is added to noodles, potato crisps, and biscuits. There is potential for supplementing beverages with glucosamine in hydrochloride or sulfate forms prepared from chitosan. It is expected that current clinical trials in Japan and many other countries will result in identifying new functional ingredients beyond the fish oils, chitosan and peptides, that can throw more light on the health protection ability of seafood and other marine products [65.119]. As was pointed out recently [65.121], the refinement of existing technologies with inputs from advances in biotechnology can further help with the isolation of seafood components that are safe and can retain their potential bioactivities for diverse applications.

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