Se-Kwon Kim *Editor*

Seafood Processing By-Products

Trends and Applications

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Preface: Advances in Seafood By-products

A huge amount of underutilized by-products are produced by the seafood processing industry every day. These by-products usually consist of several bioactive materials, such as proteins, enzymes, fatty acids, and biopolymers. Seafood by-products are a food and directly consumable for human health. Fish, shellfish, crustaceans, algae, and some marine-related animals are important sources of seafood by-products. Bioactive substances derived from seafood by-products have been used in various biotechnological, nutritional, pharmaceutical, and biomedical applications. For example, gelatin and collagen are presently used in diverse applications, including food, cosmetics, drug delivery, and tissue engineering. Several studies have reported that products derived from crustaceans and algae show prominent applications similar to collagen and gelatin.

This book contains a total of 27 chapters.

Part I – Chap. 1 provides the general introduction to the topics cover in the present books and deals about seafood derived raw materials, marine products and market sector in detail.

Part II – Status and methods (Chaps. 2, 3, 4 and 5), described the trends the usage of seafood by-products in Europe and Asian countries. In addition, processing methods and characterization of seafood by-products are explained.

Part III – Seafood by-products derived from fish, crustaceans, algae and marine animals (Chaps. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and 21) – explained about isolation and characterization techniques of fish protein, peptides, enzymes, collagen, gelatin, fish oil, biopolymers (Chitin and Chitosan) and bioactive metabolites.

Part IV – Application (Chaps. 22, 23, 24, 25, 26 and 27) the final part of the book deals about nutritional and pharmaceutical value of seafood derived by-products. Application of seafood by-products, which is include fish collagen toward dental and hard tissue regeneration, antidiabetic, and food industry and human nutrition values.

This book provides details about seafood by-products development, isolation, characterization and applications. Hence, seafood by-products are important that show range of functionalities for pharmaceutical, nutraceutical and biomedical industries.

Busan, Republic of Korea Se-Kwon Kim

Short Biography of Professor Se-Kwon Kim

Professor Se-Kwon Kim, Ph.D., currently serves as a distinguished Professor in the Department of Marine-bio Convergence Science in Specialized Graduate School of Convergence Science and Technology and also acts as a director of the Marine Bioprocess Research Center (MBPRC) at Pukyong National University in the Republic of Korea. He received his B.Sc., M.Sc., and Ph.D. from Pukyong National University and joined as a faculty member. He has previously served as a professor in Department of Chemistry at Pukyong National University, Busan, Republic of Korea (1982–2013), scientist at the University of Illinois, Urbana-Champaign, Illinois (1988–1989), and was a visiting scientist at the Memorial University of Newfoundland, Canada (1999–2000).

Professor Se-Kwon Kim was the first president of the Korean Society of Chitin and Chitosan (1986–1990) and the Korean Society of Marine Biotechnology (2006–2007). He was also the chairman for the *7th Asia-Pacific Chitin and Chitosan Symposium,* which was held in South Korea in 2006. He is one of the board members of the International Society of Marine Biotechnology and the International Society for Nutraceuticals and Functional Foods. Moreover, he was the editor in chief of the *Korean Journal of Life Sciences* (1995–1997), the *Korean Journal of Fisheries Science and Technology* (2006–2007), and the *Korean Journal of Marine Bioscience and Biotechnology* (2006–present). His research has been credited with the best paper award from the American Oil Chemist's Society (AOCS) and the Korean Society of Fisheries Science and Technology in 2002.

Professor Se-Kwon Kim's major research interests are the investigation and development of bioactive substances derived from marine organisms and their application in oriental medicine, nutraceuticals, and cosmeceuticals via marine bioprocessing and mass-production technologies. He has also conducted research on the development of bioactive materials from marine organisms for applications in oriental medicine, cosmeceuticals, and nutraceuticals. To date, he has authored over 600 research papers and holds 140 patents. In addition, he has written or edited more than 50 books.

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Chapter 1
Introduction to Seafood Processing By-products

Se-Kwon Kim and Jayachandran Venkatesan

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1 Introduction

Since ancient times, seafood has been considered a healthy food for humans. Seafood by-products also play a major role in the daily lives of humans. Focus has been increased to develop new seafood by-products of marine origin. There could be environmental and economic advantages in utilizing the by-products of marine animals or plants for human consumption, rather than disposing of such materials at sea. Figure 1.1 Gives an overview of the utilization of marine by-products.

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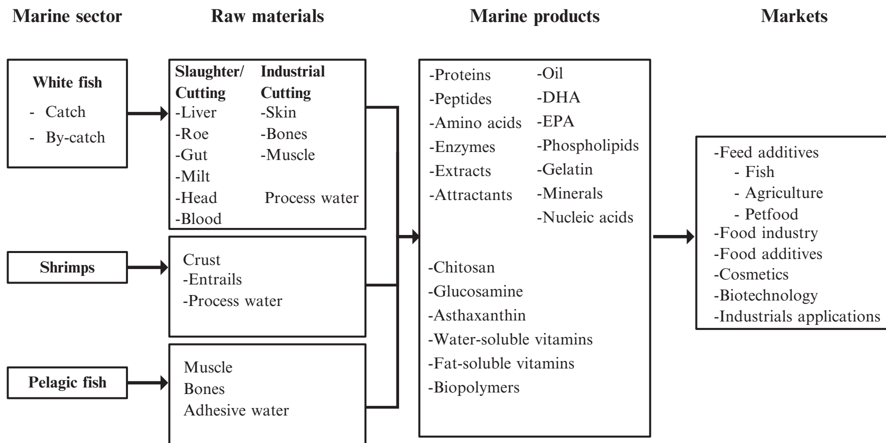

Fig. 1.1 Overview of seafood by-product sources and its utilization

2 Fish By-products

Historically, fish by-products were considered to be of low value and were disposed of in the most convenient way. In many countries, importance is placed on the possibilities for further utilization of by-products, from aquaculture as well as traditional fisheries, rather than the problem of their disposal.

In the past, fish deaths, which can occur for a variety of reasons, have been disposed of in various ways, including by burning, discarding in the land, biogas production, production of fish meal, and incorporation into pet food or food for animals. Finfish and shellfish waste processing is confined to the manufacture of meal and oil. The meal is used mainly for animal feeds, while the oil is used in edible oils and oils for industrial applications. Recent studies have identified a number of biologically active compounds from fish muscle proteins, bones, fins, internal organs, collagen, gelatin, and oyster and crustaceans shells (Je et al. 2005; Jung et al. 2006; Kim and Mendis 2006; Kristinsson and Rasco 2000; Morimura et al. 2002; Nagai and Suzuki 2000; Ozawa and Suzuki 2002; Toppe et al. 2006).

2.1 Protein

A huge amount of protein-rich by-products from the fish processing industries is discarded every day (e.g., skin, bones, and fins). Recovery and alteration of the fish muscle proteins existing in the by-product materials and using them as functional ingredients in food systems is a very exciting and promising alternative. By developing enzyme technologies for protein recovery and modification, the production of a broad spectrum of food ingredients and industrial products will be possible. There are few hydrolyzed fish protein foods, with the exception of East Asian condiments and sauces. Fish protein hydrolysates using acid, base, endogenous

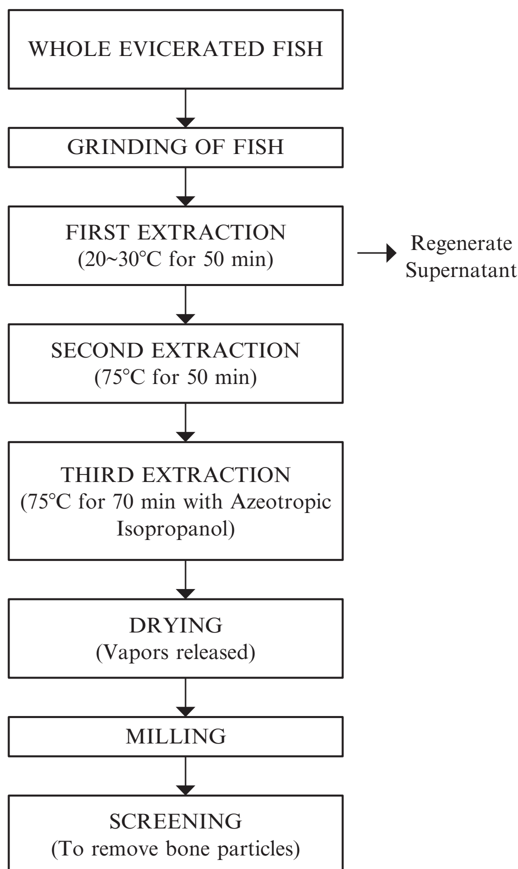

Fig. 1.2 A production scheme for fish protein concentrate

enzymes, and added bacterial or digestive proteases are manufacturing techniques. The functional properties of fish protein hydrolysates are described in this book, including solubility, water-holding capacity, emulsification, and foam-forming ability (Fig. 1.2) (Kristinsson and Rasco 2000).

Discarded wastes from fish usually consist of a considerable amount of protein. These fish wastes are nutritionally important to isolate value-added products, Enzymatic and chemical methods are widely used to isolate the protein (Dyer et al. 1950; Shahidi et al. 1995). Fish hydrolysate is the main form of seafood by-products, which can be used as a fish-based fertilizer, animal food, and human food applications. Fish protein hydrolysates possess a number of biological activities, such as antiproliferative, antioxidant, and inhibitory activity of angiotensin-I-converting enzyme (ACE), as reported in the literature (Amarowicz and Shahidi 1997; Jun et al. 2004; Samaranayaka and Li-Chan 2008; Wu et al. 2003). Fish protein hydrolysates with multiple bioactivities could be useful in formulating functional food products that target the reduction of the symptoms of oxidative stress, hypertension, and possibly dyslipidemia, all of which are common to coronary heart disease (Samaranayaka et al. 2010).

2.2 Peptides

Peptides from marine sources have been proved to have significant antioxidant activity with no cytotoxicity (Kim et al. 2007; Liu et al. 2010; Qian et al. 2008; Rajapakse et al. 2005; Sampath Kumar et al. 2011). The in vitro antioxidant activity of bioactive peptides purified from different body parts of marine fishes have been explored in recent years (Naqash and Nazeer 2010, 2011). Several reviews have been completed related to fish peptides for antioxidant, antihypertensive, antiproliferative, and anticoagulant properties (Kim and Wijesekara 2010; Kim et al. 2007; Najafian and Babji 2012; Rajanbabu and Chen 2011). Fish peptides can be isolated using the following techniques: liquid extraction (Urakova et al. 2012) and microbial fermentation of proteins (Kim and Wijesekara 2010).

2.3 Collagen and Gelatin

Food and pharmaceutical industries all over the world are witnessing an increasing demand for collagen and gelatin. Mammalian gelatins (porcine and bovine) are the most popular and widely used. However, fish gelatin reportedly possesses similar characteristics to porcine gelatin, and may, thus, be considered as an alternative to mammalian gelatin for use in food products. The production and utilization of fish gelatin not only satisfies the needs of consumers, but also serves as a means to utilize some of the by-products of the fishing industry (Karim and Bhat 2009). The major protein constituent of the skin, bone, swim bladder, and scales of fishes resembles, in many ways, the more widely studied collagen of mammals (Eastoe 1957; Giraud-Guille et al. 2000). Type I collagen has also been extracted from the skin, bones, fins, and scales of freshwater and marine fishes.

Marine organisms such as fish and shellfish are rich sources of structurally diverse bioactive nitrogenous components. Based on the emerging evidence of potential health benefits, fish-derived peptides show significant promise as functional food ingredients. Bioactivities including antihypertensive, antioxidant, antimicrobial, anticoagulant, antidiabetic, anticancer, immunostimulatory, calcium binding, hypocholesteremic, and appetite suppression have been reported. Fish and shellfish waste components contain significant levels of high quality protein [10–23 % (w/w)], which represents a source for biofunctional peptide mining (Harnedy and FitzGerald 2012).

2.4 Fish Oil

On the basis of increasing global fish meal and fish oil costs, it is predicted that dietary fish meal and fish oil inclusion levels within compound aquafeeds will decrease in the long term, with fish meal and fish oil usage increasingly being

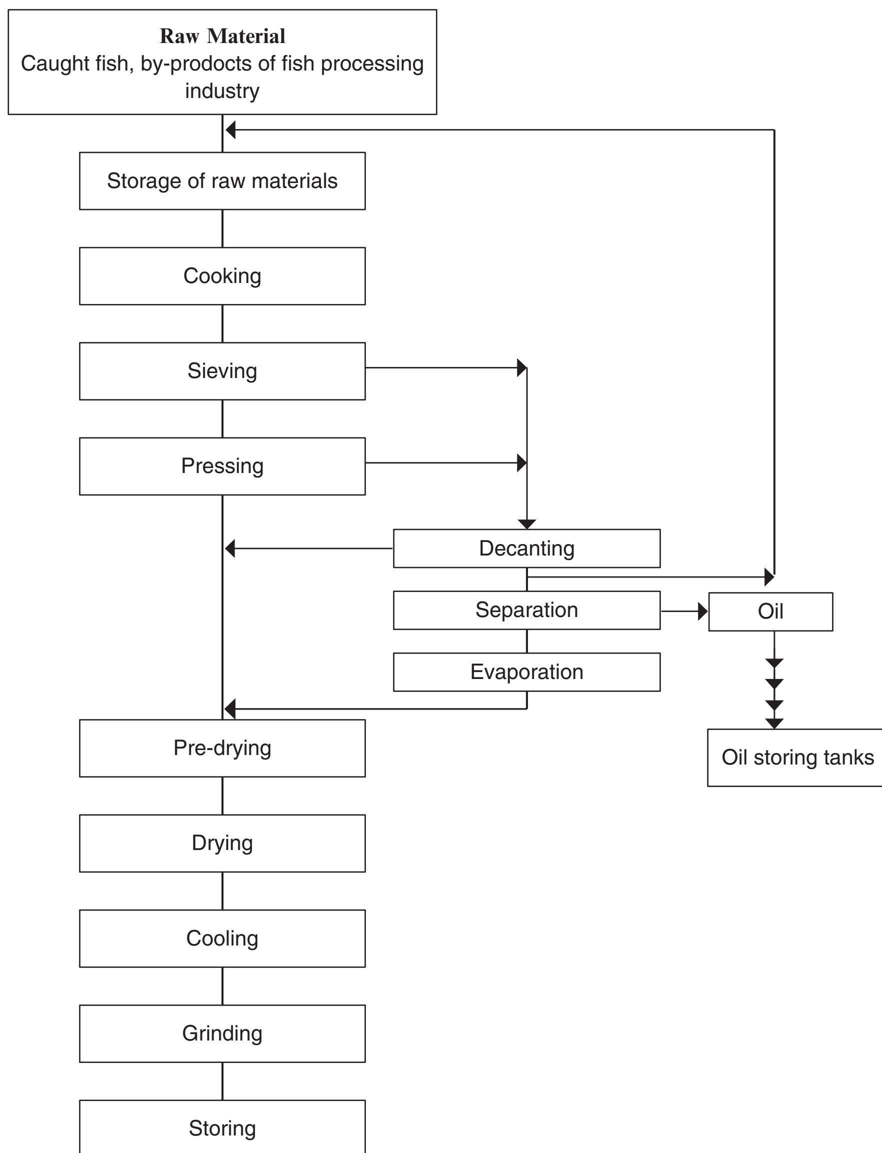

Fig. 1.3 Steps involved in fish oil production

targeted for use as a high value specialty feed ingredient for use within higher value starter, finisher, and broodstock feeds, and by so doing, extending the supply of these much sought after and limited feed ingredient commodities (Fig. 1.3) (Tacon and Metian 2008).

Fish oils are used in a variety of markets, including industrial uses, food, feed, and aquaculture and nutraceutical applications. The aquaculture market requires

oils with low levels of oxidation, low levels of contaminants, and consistent quality. The nutraceutical market wants oils low in oxidation and contaminants, but also with high levels of omega-3 fatty acids. This market pays premiums for high levels of omega-3 and wild salmon oil might be able to command a premium in that market if only because of the name. However, the omega-3 content of salmon oil might present problems. Recently, the nutritional oil companies have revised their definition of omega-3 to include the sum of C18:3, C18:4, C20:4, C20:5, C21:5, C22:5, and C22:6 all n-3 fatty acids.

3 Crustaceans By-products

3.1 Chitin and Chitosan

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. Chitin, poly (β-(1 → 4)-*N*-acetyl-D-glucosamine), is a natural polysaccharide of major importance, first identified in 1884. This biopolymer is synthesized by an enormous number of living organisms, and considering the amount of chitin produced annually over the world, it is the most abundant polymer after cellulose. Chitin occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast (Alishahi and Aïder 2012; Andres et al. 2007; Cheng et al. 2005; Gérente et al. 2010, 2007; Goosey and Kellner 2012; Kim et al. 2008; Krajewska 2005; Kwok and McKay 2010; No et al. 2007; Rinaudo 2006; Rong et al. 2010; Senevirathne and Kim 2012; Shahidi and Abuzaytoun 2005; Wang et al. 2011). Chitin and chitosan produced have also been characterized with Fourier transform infrared spectroscopy (FTIR) (Limam et al. 2011).

3.2 Application of Chitin and Chitosan

Chitin and its deacetylated derivative, chitosan, are non-toxic, antibacterial, biodegradable, and biocompatible biopolymers. Due to these properties, they are widely used for biomedical applications, such as tissue engineering scaffolds, drug delivery, wound dressings, separation membranes and antibacterial coatings, stent coatings, and sensors. Wound dressing is one of the most promising medical applications for chitin and chitosan. The adhesive nature of chitin and chitosan, together with their antifungal and bactericidal character, and their permeability to oxygen, is a very important property associated with the treatment of wounds and burns. Different derivatives of chitin and chitosan have been prepared for this purpose in the form of hydrogels, fibers, membranes, scaffolds, and sponges (Jayakumar et al. 2011). Electrospinning has been found to be a novel technique to produce chitin and chitosan nanofibers (Jayakumar et al. 2010a, b.

4 Seaweed By-products and Their Applications

Seaweeds are consumed by coastal populations, particularly Asians (Japan, China, Korea, Taiwan, Singapore, Thailand, Cambodia, Vietnam, Indonesia, and Malaysia). Seaweeds are also harvested or cultivated for the extraction of alginate, agar, and carrageenan, which are gelatinous substances (hydrocolloids). Alginates are commonly used in wound dressings, tissue engineering, and drug delivery (Li et al. 2005; Paul and Sharma 2004; Tønnesen and Karlsen 2002).

5 Organization of This Book's Chapters

This book exposes knowledge on the view of processing, methods, and application of seafood by-products. The first part of the book includes an introduction to seafood by-products. The second part introduces statistics and methods involved in seafood by-products and illustrates trends in the use of seafood processing by-products in Europe and South Asia. The third part of the book explains in detail fisheries by-products, starting from protein, gelatin, soy sauce, enzymes, and lipids. The fourth part of the book deals with by-products of marine animals, bacteria, crustaceans, and algae. In this part, uses of seafood by-product in the animal seed industry, uses of lactic acid bacteria, chitosan products, chitosan from crustaceans, microalgae for biofuel production, and bioactive compounds from marine algae are explained. The fifth part of the book deals with the biological and biomedical applications, including the application of fish collagen in dental and hard tissue regeneration, food industry and human nutrition, antidiabetic and obesity effects of seafood by-products, bioluminescence, and bioactive marine algal and their therapeutics, and, finally, the functional properties of ascidians by-products for medicinal and nutritional value.

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References

Alishahi A, Aïder M (2012) Applications of chitosan in the seafood industry and aquaculture: a review. Food Bioprocess Technol 5(3):817–830

Amarowicz R, Shahidi F (1997) Antioxidant activity of peptide fractions of capelin protein hydrolysates. Food Chem 58(4):355–359

Andres Y, Giraud L, Gerente C, Le Cloirec P (2007) Antibacterial effects of chitosan powder: mechanisms of action. Environ Technol 28(12):1357–1363

Cheng WP, Chi FH, Yu RF, Lee YC (2005) Using chitosan as a coagulant in recovery of organic matters from the mash and lauter wastewater of brewery. J Polym Environ 13(4):383–388

Dyer WJ, French HV, Snow JM (1950) Proteins in fish muscle. I. Extraction of protein fractions in fresh fish. J Fish Board Can 7(10):585–593

Eastoe JE (1957) The amino acid composition of fish collagen and gelatin. Biochem J 65(2):363

Gerente C, Lee VKC, Le Cloirec P, McKay G (2007) Application of chitosan for the removal of metals from wastewaters by adsorption—mechanisms and models review. Crit Rev Environ Sci Technol 37(1):41–127

Gérente C, Andrès Y, McKay G, Le Cloirec P (2010) Removal of arsenic(V) onto chitosan: from sorption mechanism explanation to dynamic water treatment process. Chem Eng J 158(3): 593–598

Giraud-Guille M-M, Besseau L, Chopin C, Durand P, Herbage D (2000) Structural aspects of fish skin collagen which forms ordered arrays via liquid crystalline states. Biomaterials 21(9):899–906

Goosey M, Kellner R (2012) Recovery of copper from PCB manufacturing effluent using chitin and chitosan. Circ World 38(1):16–20

Harnedy PA, FitzGerald RJ (2012) Bioactive peptides from marine processing waste and shellfish: a review. J Funct Food 4(1):6–24

Jayakumar R, Prabaharan M, Nair SV, Tamura H (2010a) Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv 28(1):142–150

Jayakumar R, Prabaharan M, Nair SV, Tokura S, Tamura H, Selvamurugan N (2010b) Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Prog Mater Sci 55(7):675–709

Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv 29(3):322–337

Je J-Y, Park J-Y, Jung W-K, Park P-J, Kim S-K (2005) Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. Food Chem 90(4):809–814

Jun S-Y, Park P-J, Jung W-K, Kim S-K (2004) Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. Eur Food Res Technol 219(1):20–26

Jung W-K, Lee B-J, Kim S-K (2006) Fish-bone peptide increases calcium solubility and bioavailability in ovariectomised rats. Br J Nutr 95(1):124–128

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23(3):563–576

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts—a review. Food Res Int 39(4):383–393

Kim S-K, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Food 2(1):1–9

Kim S-Y, Je J-Y, Kim S-K (2007) Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame protein by gastrointestinal digestion. J Nutr Biochem 18(1):31–38

Kim I-Y, Seo S-J, Moon H-S, Yoo M-K, Park I-Y, Kim B-C, Cho C-S (2008) Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv 26(1):1–21

Krajewska B (2005) Membrane-based processes performed with use of chitin/chitosan materials. Sep Purif Technol 41(3):305–312

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40(1):43–81

Kwok KCM, McKay G (2010) Novel batch reactor design for the adsorption of arsenate on chitosan. J Chem Technol Biotechnol 85(12):1561–1568

Li Z, Ramay HR, Hauch KD, Xiao D, Zhang M (2005) Chitosan–alginate hybrid scaffolds for bone tissue engineering. Biomaterials 26(18):3919–3928

Limam Z, Selmi S, Sadok S, El Abed A (2011) Extraction and characterization of chitin and chitosan from crustacean by-products: biological and physicochemical properties. Afr J Biotechnol 10(4):640–647

Liu Q, Kong B, Xiong YL, Xia X (2010) Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. Food Chem 118(2):403–410

Morimura S, Nagata H, Uemura Y, Fahmi A, Shigematsu T, Kida K (2002) Development of an effective process for utilization of collagen from livestock and fish waste. Process Biochem 37(12):1403–1412

Nagai T, Suzuki N (2000) Preparation and characterization of several fish bone collagens. J Food Biochem 24(5):427–436

Najafian L, Babji AS (2012) A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications. Peptides 33(1):178–185

Naqash SY, Nazeer RA (2010) Antioxidant activity of hydrolysates and peptide fractions of *Nemipterus japonicus* and *Exocoetus volitans* muscle. J Aquat Food Prod Technol 19(3–4):180–192

Naqash SY, Nazeer RA (2011) Evaluation of bioactive properties of peptide isolated from *Exocoetus volitans* backbone. Int J Food Sci Technol 46(1):37–43

No HK, Meyers SP, Prinyawiwatkul W, Xu Z (2007) Applications of chitosan for improvement of quality and shelf life of foods: a review. J Food Sci 72(5):R87–R100

Ozawa M, Suzuki S (2002) Microstructural development of natural hydroxyapatite originated from fish-bone waste through heat treatment. J Am Ceram Soc 85(5):1315–1317

Paul W, Sharma CP (2004) Chitosan and alginate wound dressings: a short review. Trends Biomater Artif Org 18(1):18–23

Qian Z-J, Jung W-K, Byun H-G, Kim S-K (2008) Protective effect of an antioxidative peptide purified from gastrointestinal digests of oyster, *Crassostrea gigas* against free radical induced DNA damage. Bioresour Technol 99(9):3365–3371

Rajanbabu V, Chen J-Y (2011) Applications of antimicrobial peptides from fish and perspectives for the future. Peptides 32(2):415–420

Rajapakse N, Mendis E, Byun H-G, Kim S-K (2005) Purification and in vitro antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. J Nutr Biochem 16(9):562–569

Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31(7):603–632

Rong C, Qi L, Bang-zhong Y, Lan-lan Z (2010) Combined effect of ozonated water and chitosan on the shelf-life of Pacific oyster (*Crassostrea gigas*). Innov Food Sci Emerg Technol 11(1):108–112

Samaranayaka AGP, Li-Chan ECY (2008) Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (*Merluccius productus*). Food Chem 107(2):768–776

Samaranayaka AGP, Kitts DD, Li-Chan ECY (2010) Antioxidative and angiotensin-I-converting enzyme inhibitory potential of a Pacific hake (*Merluccius productus*) fish protein hydrolysate subjected to simulated gastrointestinal digestion and Caco-2 cell permeation. J Agric Food Chem 58(3):1535–1542

Sampath Kumar NS, Nazeer RA, Jaiganesh R (2011) Purification and biochemical characterization of antioxidant peptide from horse mackerel (*Magalaspis cordyla*) viscera protein. Peptides 32(7):1496–1501

Senevirathne M, Kim SK (2012) Utilization of seafood processing by-products: medicinal applications. Adv Food Nutr Res 65:495–512

Shahidi F, Abuzaytoun R (2005) Chitin, chitosan, and co-products: chemistry, production, applications, and health effects. Adv Food Nutr Res 49:93–135

Shahidi F, Han X-Q, Synowiecki J (1995) Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem 53(3):285–293

Tacon AG, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285(1):146–158

Tønnesen HH, Karlsen J (2002) Alginate in drug delivery systems. Drug Dev Ind Pharm 28(6):621–630

Toppe J, Aksnes A, Hope B, Albrektsen S (2006) Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. Aquaculture 253(1–4):636–645

Urakova IN, Pozharitskaya ON, Demchenko DV, Shikov AN, Makarov VG (2012) The biological activities of fish peptides and methods of their isolation. Russ J Mar Biol 38(6):417–422

Wang SL, Liang TW, Yen YH (2011) Bioconversion of chitin-containing wastes for the production of enzymes and bioactive materials. Carbohydr Polym 84(2):732–742

Wu H-C, Chen H-M, Shiau C-Y (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int 36(9–10):949–957

Chapter 2
Trends in the Use of Seafood Processing By-products in Europe

Fatih Karadeniz and Se-Kwon Kim

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1 Background

1.1 Aquaculture

Aquaculture is the farming of marine organisms, including fish, shellfish, crustaceans, and plants. It is accepted as one of the fastest growing food industries and is a source of a notable amount of seafood consumed by people.

Aquaculture is also progressively improving industry in Europe, which directly has 65,000 employees and provides almost one-fifth of fish production. With the help of the latest technology and research, the European Union (EU) is known for its high quality production methods and high standards of processing units for aquaculture. However, the yield of EU aquaculture industries has been steady for a decade, while worldwide aquaculture production is reported to have grown by 30 % (Eurostat 2009). Hence, for the development of more competitive aquaculture industry, the EU is focusing on new funding and programs through the European Fisheries Fund. All these improvements and other alternatives are supposed to potentially generate a significant amount of waste; however, its utilization is under pressure from commercial and practical difficulties enforced by several European Committee regulations. Except the regulations for obtaining a waste utilization license, the treatment of aquaculture and capture fishery wastes is carried out with methods no different than those used worldwide.

Seafood waste is produced by several different methods in aquaculture, which can be grouped as follows;

Common deaths: Animal deaths can be observed regularly in aquatic farms in relatively low amounts mostly due to chronic diseases, equipment problems, and handling errors. Commonly, these wastes are blended, stored, and later removed according to waste management regulations with a predefined cost, which is around 100 Euros per ton (2007). Removal of these wastes mainly includes incineration, landfill, and export to countries that need it for value addition.

Destructive deaths: These wastes include the animals which are killed en masse from an external effect, such as algal invasion and other animal attacks. These wastes are usually treated directly in waste utilization plants.

Infected animals: Aquaculture disease control is tightly regulated in the EU as it is free of several diseases, such as infectious salmon anemia, viral hemorrhagic septicemia, and infectious hemorrhagic necrosis. In case of any signs of infectious diseases, all suspicious animal ponds are cleaned of organisms by slaughtering, storing, and exporting to countries with capable utilization plants, such as Norway. In addition to infectious diseases, animals which are contaminated with environmental pollution and not suitable for human consumption are also treated the same as infected animals (Anon 2000).

Processing by-products: After successful harvest, fishery animals are processed via several industrial equipments. In addition to main plants that process

harvested seafood from numerous aquatic farms, some small farms have their own processing units, which makes waste production variable and something that needs to be controlled.

1.2 Wild Fisheries

Unlike aquaculture, the amount of waste from wild fisheries varies depending on the species and type of product that is to be obtained. Waste from catch animals mainly comes from on-shore processing, while a small amount of waste is also produced from processing at sea.

Processing on-board: The deep-sea catch is commonly processed at sea. The waste produced, which is mainly viscera and heads, rarely reaches land and is discarded into the sea.

On-shore processing: The remaining catch fisheries are processed mostly in on-shore plants, which results in the main waste production being on-shore as well. Depending on the type of desired product, some parts can either be defined as waste or removed for consumption, such as fillets, flaps, or lugs.

1. Waste Utilization and Disposal Regulation
   The decision of how and whether the waste will be disposed or utilized for value addition is strictly driven by official regulations. In the EU, both the European Committee and national regulations exist to control and regulate waste disposal and utilization in European countries.

The European Communities Disposal, Processing and Placing on the Market of Animal By-products Regulations (SI 257, 1994) have been laid down in order to control the use, sale, and disposal of high- and low-risk animal by-products and tightly limit the use of by-products compared to disposal. Moreover, EC Regulation No. 1774/2002 of the European Parliament (amended by EC No. 808/2003) exists for health concerns regarding the animal by-products which will be used for purposes other than human consumption. All these frameworks provide a regulation over the classification, hence the disposal and utilization mechanism of animal wastes which affect the wild fisheries and aquaculture wastes for the foreseeable future. Waste categorization can be seen in Table 2.1. These regulations put fish wastes mainly in category 2 rather than categories 1 or 3, while processing by-products are considered to be category 3. As the main focus is considered to be on category 2 wastes, it is further regulated by additional regulations to EC No. 1774/2002. The aforementioned regulation, Commission Regulation No. 811/2003, provides further concerns that ban intra-species recycling for fisheries, incineration of by-products, and additional details. Due to these and similarly tight regulations, the processing plants will inevitably be encouraged to develop alternative disposal techniques, such as composting and utilization by further processing.

Table 2.1 Animal-originated by-product categories

Category	Material	Risk and availability
Category 1	Infected suspected infected animal carcasses, toxic compounds, catering waste from international transport, animal material collected from wastewater treatment, contaminated animals, wild animals suspected for human communicable diseases	Very high risk Processing only approved – category 1 plants Incineration
Category 2	Aquaculture mortalities, digestive tract and manure components, animal parts that have been slaughtered for human consumption in case of diseases, animals with veterinary drug residues	High risk Incineration Processing only in approved categories 2 or 1 plants. Biogas production Landfill Feed for zoo and fur animals with prior authorization
Category 3	Parts of animals slaughtered for human consumption, sea animals caught in open sea, fresh fish by-products from processing plants for human consumption	Low risk Processing available in approved plants Raw material for pet food Biogas or biodiesel production Ensilage or composting Animal feed for farming and aquaculture

2 By-product Production by Waste Utilization

2.1 Fish Meal and Fish Oil

Fish meal and fish oil production is the most common way of adding value to fisheries processing waste and turn it into a useful by-product. The most tradition way to produce fish meal starts with breaking down the raw waste by mincing, including whole fish as well as waste parts, such as viscera, heads, and internal organs. Heating of the minced waste in order to overcome any pathogenic or microbial organisms is followed by pressing. The pressing stage separates the solid part of the mixture from the liquid phase, while the liquid phase is subjected to centrifuge and freeze-drying in order to obtain fish oil for further processes. In addition, the remaining pressed solid is processed for the production of fish meal. Air-drying at 60–65 °C is followed by grinding finishes the fish meal manufacturing process. Several countries buy the meal at this stage for further commercial processes; however, commonly, fish meal is bagged and sold after this stage to farms, including aquatic farms, for animal feed purposes after passing the regulations concerning the chemical contents of animal by-products (Tacon and Metian 2008).

The final product of this process results in a fish meal that contains 65–70 % protein, 8–10 % moisture, and 10–12 % oil, as well as varying small amounts of ash, salt, and sand. Although the content and quality of fish meal depend on several points such as plant technology, chemicals, and sterilization techniques used, contamination is unlikely to be preventable. One of the main and suggested preferences is 12 % fat, which prevents combustion.

Throughout the EU, fish meal is mainly used for fish feeds, pig feeds, and poultry feeds, 40 %, 7–12 %, and 2–4 % of the total utilization, respectively. For instance, in the United Kingdom, more than half of 235,000 tons of animal feed were provided by aquaculture by-products annually (Seafish 2001). Recently, the price of fish meal was around 500 Euros per ton in stores; however, the prices changes monthly according to environmental and climate conditions because governmental and EU regulations keep fisheries by-product utilization in a static state and tightly controlled. During the past several decades, fish meal production and use dropped according to the International Fishmeal and Fish Oil Organization (IFFO) annual statistics (IFFO 2003). The ban on fish meal in cattle and sheep feeds by the EU during 2003 is widely accounted for this drop. However, prices tend to remain the same due to high demand worldwide, especially from China and similar countries with improving aquaculture industries (Barlow 2002).

On the other hand, fish oil is also at high demand and is a quite valuable by-product compared to fish meal, which comes from the same source. There can be 20–80 kg of fish oil to be harvested from per ton of fish waste. In Europe, due to economic stability against the dollar and production amounts, fish oil prices stay the same, even after environmental crises, including storms and typhoons. Recently, due to the rising prices of soybean and other vegetable oils used in animal feed, fish oil been becoming more and more demand. However, because of its high free fatty acid content and the 25 % limit of fat in animal feeds due to EU regulations, fish oil is preferred in terrestrial animal feeds rather than aquaculture (Tacon 2004). EU regulations strictly limit the use of these by-products in aquaculture, human health and nutrition, and pet food. The market supply is utilized by other industries, such as leather tanning, lubricants, and supportive materials for the food industry, except for food additives. In this field, Norway leads the production, with 30,000 tons per year, while Chile follows, with a relatively small amount at 8,000 tons.

2.2 Enzymatic Hydrolysate

Enzymatic protein hydrolysate (EPH) is a protein concentrate obtained by the enzymatic breakdown of seafood waste proteins into smaller peptides or amino acids. This method is mostly applied to fresh wastes and, therefore, it is mainly used directly after fresh fish processing in an aquatic environment following oil removal. EPH process protocols differ in every country, and even among plants in the same country. It varies in time, temperature, and pressure used, as well as the

types and amount of enzymes. During the process, any unbreakable parts such as skin and bones are used for other purposes, mainly fish meal production, as mentioned earlier in the chapter. Hydrolysates are obtained in liquid phase, further pasteurized, and dried before final utilization (Kristinsson and Rasco 2000). The drying process can be carried out by either a tunnel drier or a spray drier, which is a method used for obtaining krill hydrolysates without a need for pasteurizing. The hydrolysate method costs more and allocates more time and resources compared to other waste treatment methods; however, the price and benefits of hydrolysates in comparison to storage, incineration, and fish meal production are very favorable. Recently, the production of EPH has been increasing, with new plants and more funding throughout Europe. The hydrolysate plant in Boulogne, France produces 8,000 tons per year as one of the biggest plants in Europe, while Norway's new plants are expected to increase this output in the coming years. EPH is still very expensive at almost triple the price of fish meal, around 1,300 Euros per ton.

EPH can contain different amounts of protein and fat, while 80 % protein is suggested to be the optimum and is the most common. EPH is commonly used in Europe in animal feeds as milk substitute, pig weaning additive, salmon feeds, etc. The main drawback of seafood EPHs is the fishy odor, which makes it attractive for animal feeds, but also means that it is quite unusable for human consumption unless the smell is removed from it with further processes. Recent studies suggest new ways to deodorize the fishy smell from enzymatic hydrolysates and hydrolysate plants are planning to use these scientific developments to pave the way for seafood EPH utilization in human food applications (FAO 2010).

2.3 Alternative By-product Production

In addition to fish meal and protein hydrolysate production, there are several smaller fields that use seafood waste or discards and turn them into by-products, which are mostly on market. On the other hand, limitless options exist and numerous options are promoted by several studies; nevertheless, a couple of them have been considered by several processors.

Surimi: Surimi is a Japanese tradition food where fish fillets are ground, rinsed, flavored, and formed into little cakes and sold steam-cooked. Recent popular waves of East Asian culture increased the Asian-based food consumption in Europe. Surimi is one of them and surimi-based products now cover a significant proportion of European markets (Park 2005).

Fish sauces and flavorings: The production of fish sauce and similar derivatives is an important waste treatment method in Asian countries. As mentioned earlier, the popularity of Asian cuisine using fish sauce and flavoring in the European market has increased rapidly as well. However, these processes are quite new for European

companies and very small numbers of plants are producing fish sauces and flavoring compared to Asian manufacturers. Companies in Norway and Ireland such as Icon Foods, Co Sligo produce these types of foods.

Biodiesel and biogas: Increasing interest in alternative energy sources other than fossil fuels has raised the utilization of biodiesel and biogas from natural wastes. Seafood wastes are also suggested to be available for biodiesel and biogas; however, there was not a suitable plant in Europe for a long time, except for those in Denmark. Recent plants and funding has promoted biodiesel and biogas production in Europe from seafood oil in the coming years.

Chitin and chitosan: One of the most recent research focuses is chitin and its derivative, chitosan, the second most abundant compound on earth after cellulose. Chitin can be extracted from crustacean shells in the seafood industry and is utilized in numerous industries, such as wastewater treatment, surgical equipment production, dietary supplement, and nutraceuticals. Today's markets for chitin and chitosan in Europe is shifting to dietary supplements as glucosamine and cosmetics for skin protection due to the compound's biosafety, high binding capacity, and dense viscosity.

3 Seafood Waste Treatment and By-product Utilization in Some European Countries

3.1 Norway

Norway is the leading country in Europe for its state-of-art seafood processing and waste treatment plants, supported by both national government and the EU. Norway has sufficient resources and technologies for renowned seafood waste treatment, as expected from the leading country in aquaculture and aquaculture-related research. Several EU countries are exporting their wastes to Norway, where wastes are turned into value-added by-products as an income for the country. In this context, the RUBIN foundation needs to be mentioned for an improved and more profitable use of seafood by-products. It works and tries to increase the utilization of seafood by-products from both wild fisheries and aquaculture supported by scientific developments and new technologies. The RUBIN foundation was founded in 1992 by the Norwegian Research Council, Norwegian fisheries and industry, and some ministries. The Norwegian Fishermen's Association and the Norwegian Seafood Association have owned the foundation since 1998. Up to now, no similar extensive operation has been seen in any other country that works with the aquaculture industry from the start to waste utilization.

Norway produces a notable amount of fish meal from wild fisheries waste, unlike other countries that need aquaculture waste for large amounts of fish mean

manufacturing. In 2011, Norway produced 130,000 tons of fish meal from around one million tons of wild fish (USDA, Foreign Agriculture GAIN report 2012). After 2004, followed by Marine Bio Products' involvement in fish protein hydrolysates, Norway started to shift some of its fish meal production into this more profitable and extensive aquaculture waste treatment area dependent on the work of the RUBIN foundation. On the other hand, Silfas, which is the second largest fish meal producer, also developed ways to utilize seafood waste to produce by-products that can be used for human consumption, including protein hydrolysates.

3.2 Spain

Spain is one of the countries that lack a well-established waste treatment plant or industry thereof. Most of the harvested fish are sold to retail shops in unprocessed forms. Therefore, the waste is an individual problem for retailer rather than a large-scale issue for aquafarmers or wild fishers. In Spain, retailers mostly use the waste for direct dumping or bait and feed for further farming or catch. Surprisingly, Spain does not have a planned or funded (national government or EU) seafood waste treatment and by-product solution in spite of the large population and prominent seafood production.

3.3 Ireland

Ireland has a foundation for working at a solution to the disposal of seafood wastes of aquaculture, wild fishers, and final processors named the "National Fish By-products Working Group". In 2003, Ireland's fish waste was declared to be around 65,000 metric tons according to Nautilus Consultants Ltd., including mortalities in aquaculture as well as processing wastes (Anon 2003). Similar to the rest of Europe, Ireland mostly produces fish meal as a by-product of waste. In the light of mass kills and some disciplinary programs in 2003, Ireland's by-product manufacturing tended to be in a recovery state until late 2010, while starting to develop and improve in a steady manner recently.

3.4 Denmark

Denmark hosts two of the largest fish meal companies in all of Europe. Denmark has a role as an intermediate stop for the final processing of Norwegian aquaculture harvest on the way to other parts of Europe. However, Denmark is not usually known to manufacture by-products from aquaculture products. There are other processors in Denmark, such as Lumino for ensilage and composting manufacture and

supplying a broad range of fields, mainly poultry and pig farms, with produced by-products from fisheries wastes. As Denmark focuses on producing fish meal, hence, the use of fish oil, it has recently become leading the biogas and biodiesel producer from fish oil and is paving the way for its wide distribution throughout Europe. In addition, Denmark is in competition with Norway for protein-enriched fish meal and protein hydrolysate production with its newly funded foundations for waste treatment.

References

Anon (2000) A code of practice to avoid and minimise the impact of infectious salmon anaemia. The Crown Estate, Edinburgh

Anon (2003) Disposal and re-utilization of fish and processing waste (including aquaculture wastes). Nautilus Consultants (Ireland) Ltd. DK/01/03

Barlow S (2002) Resources and markets: the world market overview of fish meal and fish oil. Paper presented to the 2nd seafood by-products conference, Alaska, Nov 2002

Eurostat (2009) Statistics in focus: aquaculture statistics; 1–5

FAO (Food and Agriculture Organization) (2010) The state of world fisheries and aquaculture (SOFA). Food and Agriculture Department of United Nations, Rome, pp 1–15

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40(1):43–81

Mittaine JF (ed) (2003) Digest of selected statistics. IFFO, New Orleans

Park JW (2005) Surimi seafood: products, market, and manufacturing. Surimi and Surimi Seafood. In: Park JW (ed) Surimi and surimi seafood. CRC Press, Boca Raton, pp 375–433

Seafish (2001) Fish waste production in the United Kingdom; the quantities produced and opportunities for better utilization. Seafish report number SR537, pp 56–59

Tacon AGJ (2004) Use of fish meal and fish oil in aquaculture: a global perspective. Aqua Resour Cult Dev 1:3–14

Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285(1):146–158

Chapter 3
Prospective Utilization of Fishery By-products in Indonesia

Hari Eko Irianto, Ariyanti Suhita Dewi, and Giyatmi

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1 Introduction

Indonesia is one of the main fish-producing countries in the world, in which the total production volumes of capture and aquaculture fisheries in 2009 were 5,109,980 tons and 4,708,957 tons, respectively. Around 67 % of the total production of capture

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fisheries was marketed as fresh and the remainder was processed into traditional products (19.38 %), frozen products (10.56 %), canned fish (1.53 %), and fish meal (0.76 %) (Ministry of Marine Affairs and Fisheries 2011). Important fisheries commodities coming from aquaculture, such as shrimp, crab, and seaweeds, are mostly exported. Shrimps are exported as frozen products, i.e., head on (HO), headless (HL), peeled tail on (PTO), peeled tail stretch (PTOS), peeled deveined tail on (PDTO), peeled and deveined (PD), peeled undeveined (PUD), and breaded products (Carita 2004).

Fisheries industries including capture, aquaculture, and processing activities produce both by-products and fish wastes. Fishery by-products, produced by modern and traditional industries in Indonesia, have not been utilized optimally, thus leading to the production of waste. Most of them contain organic substances that may cause environmental problems when not treated properly. Fishery by-products should be distinguished from fishery industrial waste due to their economic potential. If by-products are not utilized to produce a product having economical value, they can be classified as waste. Fish waste can be disposed to the environment and landfill, as well as used for direct animal feeds; therefore, sometimes, they can cause environmental problems.

Furthermore, fishery industries are required to optimize their products and performance efficiency, in order to compete in the global market. The government of Indonesia encourages the fishery industries to implement a "zero waste concept" in order to create green industries that are friendlier to the environment. Therefore, by-products should be processed into value-added goods. In the process of the utilization of by-products, consideration needs to be given to the availability of required technology, human resources, and financial support.

2 Fishery By-products

According to the facts in the field, there are five types of fishery by-products, as follows:

1. **By-products from the utilization of fisheries resources**. In marine and freshwater resources, there are various kinds of fish that can be categorized as by-products, mainly from the activities of capture fisheries. The type, size, and the quantity of by-products depend on the types of fishing gear and mesh size. By-products usually consist of different types of fish and whole fish. In aquaculture fisheries, by-products are rarely encountered and there may even be none. So far, there are no by-products produced from the activities of monoculture of shrimp, carp, and Nile tilapia.
2. **By-products from processing industries**. The remaining processing industries of shrimp, frog, and fish fillet for frozen products purposes may reach up to 35 % of the initial weight. Freezing industries in Indonesia have been developing rapidly, especially for tuna, skipjack, red snapper, and shrimp industries. Tuna loin industries usually produce skin and red meat as by-products, whereas red snapper industries produce heads, bones, skin, and tails as by-products.

Additionally, by-products from shrimp industries are heads and skin. The export volume of shrimp and tuna, including skipjack and Eastern little tuna, in 2009 was 150,989 tons and 131,550 tons, respectively (Ministry of Marine Affairs and Fisheries 2011).

Furthermore, canning industries are also developing in Indonesia, although some of them are currently facing raw material supply problems. By-products from canning industries consist of heads, tails, guts, and bones. These by-products are further utilized as fish meal, while liquid by-products consisting of water, oil, and protein from pre-cooking processes are collected for fish oil production.

The processing activities of traditional products, such as dried or smoked rays' meat, also produce skin and bones as by-products. These by-products depend on the raw material and the types of processed products.

3. **By-products as secondary products in addition to the main products**. Some fish processing industries have secondary products in addition to the main products. For instance, fish meal factories produce pressed liquor that can be further processed through centrifugation or other separation methods to obtain fish oil.
4. **By-products from surplus during fishing season**. In the peak fishing season, the number of fish landed is greater than usual, so there is a surplus that can be categorized as a by-product due to limitations of the industry's capacity to absorb as raw material. This usually occurs from the fishing activities of small pelagic fish, such as mackerel, scads, and sardines.
5. **By-products as the remainder of distribution or marketing**. This occurs when handling practices used during the distribution and marketing of fish or processed products are inadequate. So, finally, there are some products that do not deserve to be marketed. For example, dried salted fish in a long period of storage at ambient temperature waiting to be sold bring about quality deterioration, so it is not suitable for human consumption.

3 The Utilization of By-products

The utilization of by-products depends on the types of targeted processed products, such as food, feed, pharmaceutical, cosmetic, and non-food. Moreover, the types of products are determined by the types of by-products and their quality, target market, consumer, and available technology.

3.1 The Utilization of By-products from Shrimp Trawl

Generally, these by-products, which are commonly known as by-catch of shrimp trawl, consist of fish from several species with various sizes and chemical compositions. Previous reports stated that fish by-catch have a length of 6.9–48.7 cm and weight of 8–2,600 g. Meanwhile, their proximate compositions are 15.80–21.62 % protein, 0.22–5.53 % fat, 1.63–5.63 % ash, and 70.68–79.54 % moisture.

With their low fat content, fish by-catch does not cause any processing problems. Furthermore, the proportions of edible and non-edible parts are 51.1 % and 48.9 %, respectively (Nasran and Irianto 1988).

3.1.1 Minced Fish and Surimi

The processing technology and equipment required for processing both products are very simple. However, both products have a wide application, especially for dishes such as meatballs, fishcakes, fish burgers, fish sticks, fish chips, *kamaboko*, *chikuwa*, and *hanpen*, as well as Indonesian traditional products "*otak-otak*" and "*pempek*". Usually, 20–30 % minced fish and surimi are added to processed products. However, there is an exception for fish snacks and chips, in which the proportion of minced fish and surimi should not exceed 20 %, in order to avoid hard-textured products (Fawzya and Irianto 1997).

3.1.2 Food-Grade Fish Flour

Surimi can be processed further by means of steaming, pressing, and drying to produce food-grade fish flour. For example, food-grade fish flour from muraenesox has 9.31 % moisture, 85.68 % protein, 0.93 % fat, and 1.78 % ash (Fawzya et al. 1997). This product has been explored to use as a fortificant in the processing of several products, i.e., 10 % fish flour added to extrusion products and breads; 20 % added to biscuits and crackers, as well as 13 % added to jam.

3.1.3 Fish Meal for Feed

Fish meal is mainly used for the production of fish, shrimp, and livestock feeds. Indonesia's demand of fish meal is about 100,000–120,000 tons per year and 75,000–80,000 tons of that is imported. The processing capacity of Indonesian fishmeal factories is around 175,000 tons per year, but due to raw material supply constraints, they only operate at 25,000–50,000 tons capacity per year (Anonym 2012). The supply problem of raw material can be overcome if by-catch of shrimp trawl can be collected or landed and then used for fish meal processing. Fish meal factories should optimize the surplus of fish during the peak fishing season by properly managing the processing into fish meal. The basic principle of fish meal processing includes boiling, pressing, drying, and grinding (Arifuddin and Murtini 1993).

3.1.4 Fish Silage

Silage is a liquid product that is made from the addition of acids to fish, such as formic acid and propionic acid. Also, it could be produced biologically by using acid-producing bacteria (Kompiang 1977). Silage can be further processed into silage flour by drying (Yunizal 1985) or by adding filler, such as corn meal, prior to drying.

3.1.5 Traditional Products

Several traditional products, such as dried salted fish, moist fermented fish ("peda"), fermented rice-added fish ("bekasam"), fish sauce, and dried spiced fish ("dendeng"), can be produced from the by-catch of shrimp trawl. Those products have a unique flavor, which is able to increase the appetite of the consumer.

3.2 The Utilization of By-products from Fisheries Processing Industries

3.2.1 By-product of Shrimp Processing Industries

The amount of by-product from shrimp processing varies from 40 % to 80 % of raw material, depending on the shrimp species and the types of processed products. Traditionally, this by-product is processed into sticky shrimp extract ("petis"), fermented shrimp paste ("terasi"), and shrimp crackers. Previous research by Suparno and Nurcahya (1982) showed that the potential utilization of shrimp comb is for shrimp paste production. With the addition of 20 % tapioca flour and 30 % fat, consumer-preferred shrimp pastes are yielded.

Protein hydrolysates can be obtained from the hydrolysis of shrimp comb by using strong acid (6N hydrochloric acid) (Suparno and Susana 1984). Ariyani et al. (1986) suggested the use of shrimp comb for shrimp meal processing, while Ariyani and Buckle (1991) introduced the use of shrimp meal in the formula of both fish and shrimp feeds. The proximate compositions of shrimp meal processed using shrimp heads or skin are 35.90 % protein, 4.96 % fat, 9.40 % moisture, 29.7 % ash, and 20.04 % other substances (Basmal 1993).

Other than shrimp meal, Ariyani and Buckle (1991) also proposed the processing of silage from shrimp heads. Furthermore, a stable silage can be produced by using an 8 % mixture of formic and propionic acids at the ratio of 1:1.

Basmal (1993) reported the use of shrimp skin for chitin and chitosan production. Bastaman (1989) outlined the processing procedure of chitosan. Firstly, dried shrimp skin is deproteinated by using 3 % of sodium hydroxide at 80–85 °C for 30 min. After cooling, it is then drained and washed until neutral, followed by demineralization with 1.25N hydrochloric acid at 70–75 °C. Chitin is obtained after drying. Further deacetylation of chitin was carried out by using 50 % sodium hydroxide at 120 °C for 60 min and then drying, leading to the production of chitosan.

Chasanah (2010) offers an idea to produce chitooligosaccharide from chitin using chitonase. Indonesia has an abundance of shrimp waste, which can be used not only for raw material in the production of chitin and its derivates, but also for a source of chitin-degrading enzymes including chitonase. The Research and Development Center for Marine and Fisheries Product Processing and Biotechnology (RDCMFPPB) has a collection of potential chitin-degrading microbes for producing functional chitooligosaccharides for food, pharmaceutical, and biocontrol applications.

Astuti (1996) produced artificial flavoring from shrimp heads by extracting them with water (1:2, w/v) at 121 °C for 2 h to obtain shrimp filtrate. It is then added with sodium proteinate and 40 % maltodextrin as filler, followed by spray drying. Pyrazine compounds are assumed to contribute to the formation of the flavor.

3.2.2 By-product from Fish Canning

Sardine canning yields heads, tails, fins, bones, guts, and rejected sardine as by-product. Meanwhile, by-product produced by tuna and mackerel canning consist of heads, tails, fins, and red meat. They are usually further processed for fish meal, whereas red meat is used for pet food.

Blue crab canning produces used water from the pasteurization process containing 0.67 % protein, 0.12 % fat, 0.33 % ash, 98.76 % moisture, and 52 mg/l total suspended solid. Uju et al. (2009) studied the recovery and concentration of flavorings from the used water by a reverse osmosis membrane. An approximate composition of concentrated flavorings is obtained as 1.2 % protein, 0.21 % fat, 0.33 % ash, and 97.21 % moisture.

3.2.3 By-product from Tuna Loin Processing

Heads, tails, bones, red meat, and skin are produced as by-products in tuna loin industries. Heads, tails, bones, and red meat can be processed into fish meal, while skin can be further processed into tanned skin by using 5 % chrome solution (Herawati 1996). Moreover, this tanned tuna skin can be used to produce bags, shoes, and wallets. Red meat has been developed to be used for making fish spread.

Tazwir et al. (2009) studied the use of tuna bones as raw material in gelatin production. Principally, the processing steps of gelatin are degreasing, washing, soaking in NaOH solution, soaking in HCl solution, extraction, filtration, evaporation, and drying. Gelatin which is processed using 0.4 % NaOH as the soaking solution results in a product with the best properties, i.e., 8.37 % yield, 3.27–3.37 cPs viscosity, 5.03 pH, and 1.57.8 g Bloom gel strength.

Ikasari et al. (2011) utilized trimmed tuna meat from loin processing to produce fermented fish sausage. A mixture of *Lactobacillus plantarum* and *L. fermentum* are added to the dough as a starter culture to accelerate the fermentation process and to improve sausage properties. The approximate composition of the sausage was 54–58 % moisture, 3.54–3.85 % ash, 7.97–12.92 % fat, and 13.65–18.39 % protein.

3.2.4 By-product from Red Snapper Processing

Fresh heads by-product from red snapper processing are usually sold directly to the market, whereas meat still attached to the bones can be processed into fish balls and

fish sausages. Fish meal, on the other hand, is obtained from the processing of heads, tails, bones, fins, guts, and skin. Similar to that of tuna loin, according to Tambunan (1993a), skin from red snapper can also be used to produce a qualified tanned skin by using 10 % chrome tanning solution.

3.2.5 By-product from Frog Leg Processing

By-product from frog leg industries can reach up to 70 % of the whole frog (Ariyani et al. 1984). Research on the use of this by-product has focused on the processing of frog meal and silage. Ariyani (1993) produced frog meal from frog leg by-product through the following processing steps: boiling, pressing, drying I, pelleting, drying II, and grinding. The approximate composition of frog meal was 8.25 % moisture, 61.26 % protein, 11.29 % fat, and 16.37 % ash.

Murtini et al. (1984) produced silage from the by-product of frog leg processing by using lactic acid bacteria from several sources and added molasses as the carbohydrate source. Furthermore, Rabegnatar et al. (1988) used frog silage for shrimp feed. It was shown that no negative effects were found in the use of the silage on the growth of giant freshwater prawn (*Macrobrachium rosenbergii*).

Frog skin has the potential to be processed into tanned skin, especially due to its unique characteristics. It can also be used to make skin crackers, although production is hampered by limitations of the market.

3.2.6 By-product from Freshwater Fish Processing

Freshwater fish is usually marketed as live and fresh fish. However, if the processing industry of freshwater fish is developed, by-products such as heads, scales, guts, bones, and tails are expected to be generated from fish canning, smoking, drying, and other value-added product processing activities. To anticipate this, the processing of by-products from common carp, Nile tilapia, and tilapia can be used to produce fish meal and silage (Ariyani et al. 1986a, b). Both products can be further processed into fish and shrimp feeds. Feeding trials on common carp and giant freshwater prawn showed that the use of feed produced from Nile tilapia meal leads to higher growth rates compared to controls.

3.2.7 By-product from Traditional Products Processing

Traditional industries produce a small quantity of by-products. It is normally buried or disposed into the sea or rivers as waste that contains heads, guts, bones, scales, tails, and gills.

In the North Sulawesi, there is a traditional processing practice, i.e., smoked skipjack called *cakalang fufu*, in which no waste is generated. Prior to smoking, skipjack is eviscerated to remove the guts. Skipjack flesh is then smoked to produce

Table 3.1 Chemical and physical analyses as well as visual observation of fish oil from canned sardine and fish meal processing at Muncar and Negara (Indonesia)

Analysis	Canned fish processing	Fish meal processing	
		Whole fish	Canning by-product
FFA (% oleic acid)	0.06–1.15	0.08–55.68	6.99–25.72
Absorbance	0.22–0.48	1.45–2.56	1.34–2.29
Visual color	Yellow	Orange-blackish brown	Reddish brown-dark brown
Fatty acids (relative %)			
Omega-3	23.7–27.2	20.6–29.5	25.1–26.5
EPA	15.4–17.6	9.2–20.1	15.2–17.2
DHA	4.9–6.0	3.5–12.2	5.8–6.0

Source: Irianto (1992)

cakalang fufu, whereas the guts are fermented with salt addition to prepare *bekasang* and also steamed with spices to make *woku* (Wudianto et al. 1996).

According to Nurhayati and Peranginangin (2009), fish heads, fins, bones, scales, and skins from fish canning and filleting industries can be processed into collagen having high economic value. Collagen can be found in connective tissues in skin, tendon, skeleton, and cartilage. Dissolving non-collagen protein, mineral, and fat is necessary to simplify the process of collagen extraction. Generally, the extraction of collagen is conducted in acidic conditions. However, the use of pepsin in the extraction process may increase the yield.

3.3 *The Utilization of By-products from Secondary Products*

A secondary product that attracts various interests is fish oil from the process of canned fish and fish meal. Fish oil contains omega-3 fatty acids that play significant roles on the development of the human brain and have benefits to reduce the risk of degenerative diseases (Irianto 1992). The quality of fish oil depends on the type of processing practices generating the secondary products and the raw material used, as shown in Table 3.1. Chemical and physical analyses showed that oil from fish meal processing required further quality improvement by reducing the free fatty acids content, as well as improving its color and odor performance.

Degumming, neutralizing, washing, and bleaching by applying the alkali refining method can be carried out to improve the performance of fish oil. Moreover, fish oil is widely applicable for pharmaceutical, feed, food, and non-food industries (Irianto 1995).

3.3.1 Fish Oil in Pharmaceutical Industries

Pharmaceutical industries use fish oil in the form of omega-3 fatty acids concentrate that can be produced by means of the urea crystallization method. Yongmanitchai

and Ward (1989) informed the use of supercritical fluid carbon dioxide to concentrate omega-3 fatty acids. However, this method is cost-inefficient when applied in the industries.

A research on the use of enzyme catalyst has been conducted to produce triglycerides that are rich in omega-3 fatty acids. Basically, fish oil and omega-3 fatty acids are mixed and then lipase enzyme is added to catalyze the acidolysis process.

3.3.2 Fish Oil in Food Industries

In food industries, fish oil can be used mainly for two purposes, i.e., to replace vegetable oil and animal fat, as well as to improve the nutritional value of food products. Research on the use of fish oil in food products has been carried out, especially for the production of sausage (Irianto et al. 1996), canned fish (Irianto 1992), and mayonnaise (Putri 1995).

The hydrogenation process at several degrees of homogenization inducing physical and chemical changes in fish oil may result in the various characteristics of fish oil, thus, a wider application of fish oil can be explored. Other food products which can be processed from fish oil are margarine, table spread, biscuit shortening, pastry fat, bread fat, emulsified shortening, biscuit filling, icing shortening, and salad oil (Bimbo 1989a). Barlow et al. (1990) developed fish spread, peanut butter, coleslaw, yoghurt, and salami using fish oil as one of the ingredients.

3.3.3 Fish Oil in Feed Industries

Fish oil is a good calorie source and growth stimulant for livestock. A homologue of linoleic acid at high concentration is responsible for the growth stimulant characteristic in fish oil. Fish oil added to feed should be fresh, because oxidized fish oil would be toxic. Fish oil has been added into animal and fish feeds, such as feeds for laying hens, broilers, shrimp, and fish. The addition of fish oil in hens feed could enhance the content of omega-3 in eggs and meat, as well as improve their immune system (Bimbo and Crowther 1992).

3.3.4 Fish Oil in Non-food Industries

Fish oil is used in non-food industries to produce elastic and long polymers due to its uniqueness and high unsaturation degrees of fatty acids. This unique composition causes fish oil to have flexible applications. Fish oil-based non-food products that have been developed are fatty acid products and their derivatives, with applications such as detergents, tanning oils, protective coatings in varnish and paint, lubricant oils, plastics, pesticides, fungicides, and polyurethane foam (Bimbo 1989b).

3.4 Shark and Rays Utilization

3.4.1 Shark

Sharks can be captured purposefully or otherwise. Those which are accidentally captured are by-products from other fishing activities. Meanwhile, for those which are purposefully captured, their fins are the main target. The utilization efforts of sharks have been conducted as follows.

Shark meat. Shark meat has been used in the processing of dried salted fish and boiled salted fish ("*pindang*"). The main obstacle of shark utilization is its ammoniac odor and flavor originating from urea decomposition. Priono et al. (1986) has conducted an effort on the reduction of urea content in shark meat by boiling in 2.5 % potassium hydroxide solution for 35 min. This method was able to reduce the urea content by up to 70 %. Washing with cold running water (8–10 °C) may reduce the urea content by up to 58 %. Soaking in 5 % acetic acid solution for 36 h could decrease its urea content by up to 80 %; however, this method may affect the meat structure.

Furthermore, Yunizal et al. (1984) found out that heating shark meat by using high-temperature steam for 90 min could reduce the levels of urea by up to 90 %, whereas Nasran et al. (1986) suggested that washing minced meat with cold water for five times may decrease the urea content of shark meat by up to 95 %. Shark meat has been investigated for use as dried spiced fish (Nasran 1993), shredded fish (Fawzya 1993a), fish balls (Fawzya 1993b), fish sausage (Irianto 1993a), and dried salted fish (Irianto 1993b).

Skin. Research on the tanning process of shark skin has been conducted intensively (Haq 1993). Chrome tanning method with 4 % syntan addition is respected as the best treatment that has been employed to obtain a qualified tanned shark skin. Tanned skins from shark have been introduced to make bags, shoes, and wallets. Furthermore, these products have been disseminated to craftsmen in Jakarta, Bogor, as well as Bandung and received positive feedback. Meanwhile, shark skin crackers have been produced by a small-scale processor in Pelabuhan Ratu, West Java.

Liver. Shark liver oil has been used as a source of squalene, vitamin A, as well as vitamin D and marketed worldwide. Shark liver oil could be extracted by means of boiling, steaming, acid or alkali cooking, rendering, and silage. The last method tends to produce a lower yield of oil but a higher content of vitamin A (Yunizal et al. 1983). Furthermore, alkali cooking produces oil with higher squalene content compared to other methods, i.e., boiling, rendering as well as the chemical and biological silage process (Yunizal and Nasran 1984).

3.4.2 Rays

Rays meat has been used for salted and smoked fish products. The by-products generated from processing activities are skin, bones, and guts. Fishermen at Muara Angke, Jakarta, have used ray's skin for the manufacture of artificial shark fin strings

called as "*hisits*". Tambunan (1993b) has developed the storage and tanning method of rays' skin. The resulting tanned skin has an attractive surface appearance and is used in the production of bags and wallets. Meanwhile, its cartilage is used in wood glue (Embun 1995).

Basmal et al. (1995) processed a mixture of viscera, head, and trimmed meat of rays into silage by the addition of 4.5 % (w/w) formic acid. The silage obtained has a solid and liquid ratio of 40:60 %, while the pH and non-protein nitrogen content of the silage was 3.75 and 2.32 mg% N, respectively.

4 Challenges

According to Irianto and Giyatmi (2004) the use of by-catch of shrimp trawl is a dilemmatic issue because the design and construction of trawl vessels is generally compact. Space inside the vessels is very effectively used for the operational purposes of capturing, handling, and freezing the shrimp. Facilities for catch handling and vessel crew movements are, in fact, specially designed to handle shrimp, not for by-catch.

The great distance between the fishing ground and landing area, as well as unpredictable weather and ocean waves are other challenges that hamper the collection of by-catch for further processing and utilization. Processing industries are usually not paying proper attention to by-products generated from their activities, thus, no preservation efforts, such as freezing, are made. Consequently, these by-products are facing deterioration of freshness or even decomposition. This fact further complicates the challenge to obtain processed products with acceptable quality.

Similar problems also arise when it comes to the processing of fish oils that are categorized as secondary products. Despite the fact that the benefits of fish oil in pharmaceuticals have been widely known, the quality of produced and marketed fish oil is still lower than the expectation. This is mainly caused by the low quality of raw materials used for fishmeal processing generating fish oil as a secondary product. Therefore, in the near future, fish oil needs to be shifted into main products and fish meal as a secondary product. However, a special incentive and market as well as a better fish oil price need to be created in order to implement this idea.

Generally, processing industries are located in remote areas; as a result, it is difficult to collect by-products from those areas. Moreover, due to its characteristics and forms, by-products consisting of visceral, heads, fins, bones, and tails need to be adequately and cautiously transported in order to avoid decomposition as well as disturbance to the surroundings.

References

Anonymous (2012) Limited local fish meal, rampant imports. TROBOS Aqua 1(2):18–21, in Indonesian

Arifuddin R, Murtini JT (1993) Fish silage. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 149–152, in Indonesian

Ariyani F (1993) Frog waste meal. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 160–162, in Indonesian

Ariyani F, Buckle KA (1991) Ensiling of prawn heads. ASEAN Food J 6(2):58–63

Ariyani F, Haq N, Putro S (1984) Utilization of frog waste for feed. Lap Pen Teknol Perik 36:1–7, in Indonesian

Ariyani F, Irianto HE, Asastyasih M (1986a) Fresh water fish waste utilization as meal. J Pen Pasca Panen Perik 53:17–26, in Indonesian

Ariyani F, Murdinah, Putro S (1986b) Feeding trials using feeds supplemented with fish meals and silages. Proceedings of the first ASEAN workshop on fish and fish waste processing and utilization, Jakarta, 22–24 Oct 1986, pp 292–299

Astuti S (1996) Studies on the processing of shrimp flavourings from by products of frozen shrimp industry. Sarjana thesis, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor, in Indonesian

Barlow SM, Young FKV, Duthie IF (1990) Nutritional recommendations for n-3 polyunsaturated fatty acids and the challenge to the food industry. Proc Nutr Soc 49:13–21

Basmal J (1993) Extraction of chitin – chitosan from shrimp shells. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 184–186, in Indonesian

Basmal J, Murtini JT, Tazwir, Indriati N (1995) Effect of formic acid concentration on silage processing from waste of rays (*Trigon* sp.). Jurnal Pen Pasca Panen Perikanan 84:1–7, in Indonesian

Bastaman S (1989) Degradation and extraction of chitin and chitosan from shells of prawn *(Nephrops norvegicus)*. Warta IHP 6(2):1–8, in Indonesian

Bimbo AP (1989a) Fish oils: past and present food uses. J Am Oil Chem Soc 66(12):1717–1726

Bimbo AP (1989b) Technology of production and industrial utilization of marine oils. In: Ackman RG (ed) Marine biogenic lipids, fats and oils. CRC Press, Boca Raton, pp 401–431

Bimbo AP, Crowther IN (1992) Fish meal and oil: current uses. J Am Oil Chem Soc 69(3):221–227

Carita (2004) Studies on yields of various processed tiger shrimp (*Penaeus monodon*) of each size. Sarjana thesis, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, in Indonesian

Chasanah E (2010) Development of enzymatically produced chitooligosachharide from shrimp industrial waste. Squalen 5(2):44–50, in Indonesian

Embun RS (1995) Studies on the effects of acetic acid on yield and quality of fish glue from rays (*Trygon* spp) bones. Faculty of Agricultural Technology, Bogor Agricultural University, Bogor, in Indonesian

Fawzya YN (1993a) Processing of shark meat ball. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 248–249, in Indonesian

Fawzya YN (1993b) Processing of shredded shark meat. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 256–257, in Indonesian

Fawzya YN, Irianto HE (1997) Food fortification by using nutrition source from fish for increasing fish consumption and health. Warta Penelitian Perikanan Indones III 1:2–6, in Indonesian

Fawzya YN, Rufina M, Sugiyono, Irianto HE (1997) Quality of extruded food product made from corn, rice and fish flour mixture. Summary report and papers presented at the tenth session of the working party on fish technology and marketing, FAO fisheries report no. 563, Colombo 4–7 June 1996, pp 265–269

Haq N (1993) Tanning of shark skin. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 240–242, in Indonesian

Herawati SY (1996) Effects of Cr_20_3 concentration on the quality of tanned tuna *(Thunnus albacares)* skin. Sarjana thesis, Faculty of Fisheries, Bogor Agricultural University, Bogor, in Indonesian

Ikasari D, Syamdidi, Suryaningrum TD (2011) The use of lactic acid bacteria and beef fat in the processing of fermented sausage made of tuna waste. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 6(2):101–110, in Indonesian

Irianto HE (1992) Fish oil: refining, stability and its use in canned fish for the Indonesian market. PhD thesis, Massey University, New Zealand

Irianto HE (1993a) Processing of shark meat sausage. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 252–253, in Indonesian

Irianto HE (1993b) Processing of dried salted shark meat. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 258–259, in Indonesian

Irianto HE (1995) Utilization of fish oil for pharmaceutical, food, feed, and non-food. Warta Perikanan Laut 1(2):10–19, in Indonesian

Irianto HE, Giyatmi (2004) Utilization of fisheries by-products. Jurnal Widya Kesehatan dan Lingkungan VI 2:136–147, in Indonesian

Irianto HE, Fawzya YN, Sugiyono, Tentri J, dan Ibrahim B (1996) Utilization of fish oil in chicken and beef sausage production. Paper presented at the tenth session APFIC working party on fish technology and marketing, Colombo, 4–7 June 1996

Kompiang IP (1977) Nutritional value of fish silage processed with the addition of formic acid. J Pen Teknol Perik 2:34–39

Ministry of Marine Affairs and Fisheries (2011) Marine and fisheries statistics 2009. Ministry of Marine Affairs and Fisheries, Jakarta

Murtini JT, Budhiyatni S, Yunizal, Hanafiah TAR (1984) Processing of silage from frog waste using biological method. Lap Pen Teknol Perik 30:1–8, in Indonesian

Nasran S (1993) Processing of dried spiced shark meat. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 254–255, in Indonesian

Nasran S, Irianto HE (1988) Studies on the utilization of by-catch of shrimp trawl in Arafura. Proceeding of seminar on agricultural posh-harvest. Bogor 1–2 Feb 1988, Agency for Agricultural Research and Development, Jakarta, pp 29–37, in Indonesian

Nasran S, Irianto HE, Fawzya YN (1986) Efforts of urea content reduction in shark through mincing and washing for fish ball processing. Bull Limbah Pangan II 2:144–148, in Indonesian

Nurhayati, Peranginangin R (2009) The prospect of fishery waste as collagen source. Squalen 4(3):83–92, in Indonesian

Priono B, Suparno, Sudrajat Y, Haq N (1986) Physical and chemical treatments for urea content reduction in shark meat. Lap Pen Teknol Perik 35:19–23, in Indonesian

Putri JWS (1995) Effects on antioxidant addition and vacuum condition on the quality of sardine (*Sardinella longiceps*) oil as raw material for mayonnaise making. Sarjana thesis, Faculty of Fisheries, Bogor Agricultural University, in Indonesian

Rabegnatar LNS, Hadie LE, Hadie W, Heruwati ES (1988) Effects of frog waste silage as substitute fish meal in feeds on the production and growth of giant shrimp *(Macrobrachium rosenbergii)*. Proceeding of seminar on agricultural posh-harvest, Bogor 1–2 Feb 1988, Agency for Agricultural Research and Development, Jakarta, pp l85–204, in Indonesian

Suparno, Nurcahya SF (1982) Utilization of shrimp waste: I. Shrimp paste processing. Lap Pen Teknol Perik 28:1–7, in Indonesian

Suparno, Susana (1984) Utilization of shrimp waste: II. Processing of protein hydrolysate using acid hydrolysis. Lap Pen Teknol Perik 28:9–16, in Indonesian

Tambunan PR (1993a) Tanning of red snapper skin and similar type of fish. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 230–236, in Indonesian

Tambunan PR (1993b) Tanning of rays skin. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 223–229, in Indonesian

Tazwir, Amiruldin M, Kusumawati R (2009) Effects of tuna (*Thunnus albacares*) bone soaking in sodium hydroxide on the quality of gelatin produced. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 4(1):29–36, in Indonesian

Uju, Ibrahim B, Trilaksani W, Nurhayati T, Purbosari N (2009) Recovery and concentration of flavorings from blue crab processing wastewater using reverse osmosis. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 4(2):177–186, in Indonesian

Wudianto, Naamin N, Susanto K, Irianto HE, Pranowo SA (1996) Survey of fisheries and socio-economic in MCMA Karakelong Manado, North Sulawesi. Agency for Agricultural Research and Development, Jakarta, in Indonesian

Yongmanitchai W, Ward OP (1989) Omega-3 fatty acids: alternative sources of production. Process Biochem 1989(8):117–125

Yunizal (1985) Technology for silage processing. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results on fisheries post-harvest. Puslitbang Perikanan, Jakarta, pp 117–125, in Indonesian

Yunizal, Nasran S (1984) Preliminary studies on squalene content of shark liver oil. Lap Pen Teknol Perik 32:17–21, in Indonesian

Yunizal, Nasran S, Murtini JT (1983) Studies on extraction methods for shark liver oil. Lap Pen Teknol Perik 22:35–37, in Indonesian

Yunizal, Suparno, Nasran S (1984) Efforts to reduce urea content in shark meat using boiling with superheated steam. Lap Pen Teknol Perik 28:27–30, in Indonesian

Chapter 4
Development of Functional Materials from Seafood By-products by Membrane Separation Technology

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1 Introduction

Very few substances in nature are found in pure form. Most require some type of separation before the resulting form can be consumed directly or used as ingredients in various processed food and nutraceutical products. Separation processes are an integral part of today's agricultural, food, and bioproduct industries. This is especially true for the functional food and nutraceuticals industry, where specific bioactive components are targeted for their health benefits. Many of the compounds produced have shown biological activities, including anticoagulation, antioxidant, calcium absorption, antihypertensive, and antimicrobial activities (Rajapakse et al. 2005a, b; Byun et al. 2009; Lee et al. 2011). The overall objective in nutraceuticals processing is to develop a fully integrated system to minimize the degradation of bioactivity throughout raw material handling, processing, packaging, storage, and retail shelf life of the final supplement and food products. Effective separation of bioactive components from biomaterials requires a good understanding of the mechanisms of bioactivity loss due to processing, storage, decomposition, or reactions with other components. The bioactive components must be analyzed accurately prior to the selection of appropriate separation/purification processes, including precipitation, membrane separation, ion exchange chromatography, desalting and bipolar membrane electrodialysis, liquid/liquid separations, solid/liquid separations, supercritical fluid extraction, crystallization, or distillation. One of the key problems in the processing of biomaterials and foods, and indeed in all chemical processes, is the purification of specific components by separating them from a complex, multicomponent matrix. This may be as simple as clarifying a functional beverage by removing small suspended solid impurities and/or precipitated impurities, or as complex as the fractionation of protein fractions from liquid extracts of oilseeds. The cost of these separation and purification steps often determines the economic viability of a product or process.

Industrial membrane technology is an approach that has been successfully applied as a low-cost separation technique for the concentration and purification of bioactives from various feed streams. Membrane filtration processes have gained popularity in the food processing industry over the last 25 years. It is estimated that 20–30 % of the current $350 million turnover of membrane used in the manufacturing industry worldwide was from the food processing industry (Sutherland 2004). The market value of functional foods and nutraceuticals is estimated to be between $180 million and $80 billion, depending on the specific product categories included in the estimate (Wildman and Kelley 2007). Regardless of the current actual real market value, the nutraceutical market is expected to continue its growth as ongoing research establishes solid links between various nutraceuticals and health, and as the public becomes aware of their health benefits. However, as the market grows with new nutraceutical offerings almost daily, there is a growing need to better understand the processing implications for these new products targeting different uses. Therefore, a review of the current state of knowledge is timely and the objectives of this review are to review the basic concepts of membrane separation technology, to assess its applications to different classes of nutraceuticals, including

lipid-, carbohydrate-, and protein-based compounds, and to discuss the challenges and future potential of membrane separation technology for separating functional components from seafood by-products.

2 Membrane Separation Technology

Over the years, many industries have come to accept cross-flow filtration, including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), as standard technologies for clarification or concentration. In some instances, more than one type of membrane process may be used in series to achieve the desired performance. These membrane filtration processes are carried out using the concept of cross-flow, in the sense that the solution to be filtered is flowing across the membrane surface at a certain velocity, while the filtrate is passing through the membrane. The cross-flow technique is used to remove the retentate away from the membrane surface and, thus, ensure reasonable filtration conditions at the membrane surface, contrary to conventional dead-end filtration.

Applications of membrane technology in some conventional processes and separation of bioactives are summarized in Table 4.1 (Akin et al. 2012). For functional beverages, and bioactive extracts, membrane systems are primarily being used for clarification to replace diatomaceous earth filtration. Diatomaceous earth has traditionally been employed for clarifying beverages. However, its use presents a number

Table 4.1 Applications in the nutraceutical and bioactive separation industries

Process	System
Ultrapure water	RO[a]/UF[b]
Water softening	RO/UF
Effluents	RO/NF[c]/UF
Fine chemical processes	NF/UF
Milk/whey bioactives	RO/NF/UF
Extract concentration	RO/NF
Emulsion separation	UF/MF[d]
Fruit juice clarification	MF
Oil/water separation	UF/RO
Alcohol purification	PV[e]
Enzyme recovery	UF/NF
Protein concentration and purification	NF/RO
Wastewater recovery	MF
Wine clarification	MF
Color removal	NF
Phospholipid removal	NF/UF
Bioactivity recovery	NF/RO

[a]Reverse osmosis
[b]Ultrafiltration
[c]Nanofiltration
[d]Microfiltration
[e]Pervaporation

of issues in terms of product quality, disposal, and operator exposure to a potential health hazard. For the processing of bioactives, the main advantage of membrane technology is the elimination of the energy-intensive evaporation process that can reduce their bioactivity. Other advantages of membrane technology include: (1) reduced freshwater requirements due to the re-utilization of wastewater; (2) potential increased profit margins from the creation of new products; (3) reduced waste treatment volume and costs; and (4) relatively low floor space and capital requirements by avoiding the need for steam generation facilities used in evaporation processes. On the other hand, some disadvantages include: (1) expense and time required to document product safety and obtain approval from the regulatory bodies for the use of new membrane materials in food processing; (2) uncertainty about membrane durability, effective operating life, and replacement costs; (3) concerns about chemical inertness and pH sensitivities; (4) operating pressure limitations in certain designs; and (5) fouling problems with certain feed stocks (Jaouen et al. 1999).

2.1 Mechanisms of Membrane Filtration

Membrane processes are generally used to concentrate or fractionate a liquid to yield two liquids that differ in composition. Some other industrial uses of membrane separation processes include the separation of mixtures of gases and vapors, miscible liquids (organic and aqueous/organic mixtures and solid/liquid and liquid/liquid dispersion), and dissolved solids and solutes from liquids (Rosenberg 1995). The feature that differentiates membrane separations from other separation techniques is the provision of another phase, the membrane. This phase, either solid, liquid, or gaseous, introduces an interface between the two bulk phases involved in the separation, and gives the advantages of efficiency and selectivity. The transport of selected species through the membrane is achieved by applying a driving force across the membrane (Scott and Hughes 1996). In the membrane separation process (Fig. 4.1), the feed mixture is separated into a retentate (that part of the feed does not pass through the membrane) and a permeate (that part of the feed passes through the membrane).

2.2 Classification of Membrane Technologies

A membrane is defined as a material that forms a thin wall capable of selectively resisting the transfer of different constituents of a fluid and, thus, effecting a separation of the constituents. Thus, membranes should be produced with a material of reasonable mechanical strength that can maintain a high throughput of a desired permeate with a high degree of selectivity. The optimal physical structure of the membrane material is based on a thin layer of material with a narrow range of pore size and a high surface porosity. This concept is extended to include the separation of dissolved solutes in liquid streams and the separation of gas mixtures by membrane filtration.

Membrane filtration is a pressure-driven technology with membrane pore sizes ranging from 10^{-3} molecular size to 5 µm. The useful ranges of membrane

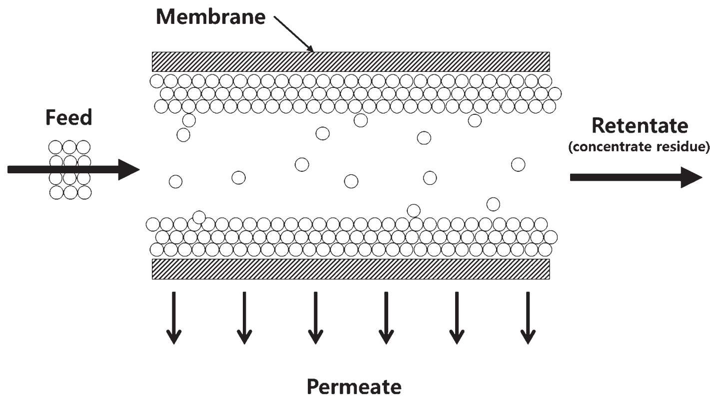

Fig. 4.1 Schematic of a general membrane process

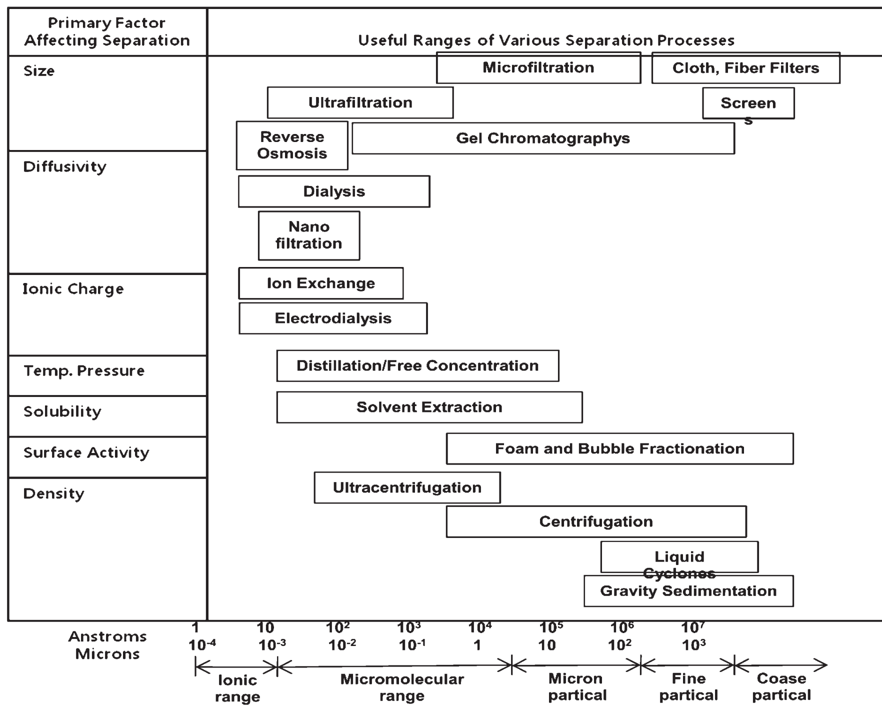

Fig. 4.2 Useful ranges of various separation processes

separation processes in relation to other separation processes are shown Fig. 4.2. The technologies included in membrane filtration are RO, NF, UF, and MF. RO, sometimes called hyperfiltration, describes the tightest of these molecular-level separations. In RO, hydraulic force is applied in excess of the natural osmotic pressure of a solution to provide the driving energy for water molecules to diffuse into

and through the membrane. The typical operating pressure can be in the hundreds to even a thousand pounds per square inch (25~68 bar). RO membranes are generally characterized by their ability to reject sodium chloride (NaCl) under specific conditions of pressure, temperature, and concentration. Typical rejection values can be on the order of 98–99.5 %. NF is the next, more open membrane filtration type. In solutions of mixed ionic species, monovalent ions will tend to permeate (pass through) the membrane, whereas divalent or multivalent species will tend to be highly rejected at the membrane interface. Since some ionic species (the monovalent ions) are transmitted through the membrane, the difference in chemical potential between the two solutions is less and, therefore, lower driving forces are required for NF than for RO. Hence, typical NF operating pressures may be only one to a few hundred pounds per square inch (7~40 bar). NF membranes are generally characterized by their ability to retain a divalent ionic species, such as Mg^{2+} or Ca^{2+} in magnesium sulfate ($MgSO_4$) or calcium chloride ($CaCl_2$). Since more variability in applications exists with NF, the retention of $MgSO_4$ might range from around 80 to 98 %.

In UF, the membranes comprise a discrete porous network. As a mixed solute solution is pumped across the membrane, smaller molecules pass through the pores while larger molecules are retained. This process yields one solution depleted of larger molecules, the permeate stream, and another enriched in larger molecules, the retentate. The open membrane structure means that mass transfer is now more flow-dependent than pressure-dependent, so the operating pressure is further reduced. Typical operating pressures for UF are tens to a hundred or so pounds per square inch (1–10 bar). The convention for UF membrane classification shifts to mass, with the molecular weight cut-off (MWCO) generally expressed in standard Da or kDa units. For example, a 10-kDa membrane would highly retain molecules of that molecular mass or greater, while highly permeating smaller molecules. While this system is inherently flawed since the molecular mass does not describe the actual size or geometry of a molecule, it remains the standard convention for UF membrane classification. Finally, MF is used for clarification and sterile filtration in a wide range of industries, including the food and biochemical industries. Membranes for MF typically have a pore size of 0.1–2 μm and can selectively separate particles with molecular weights greater than 200 kDa. Large and small molecules can, therefore, be separated from very large or complex molecular structures. Typical operating pressures for MF are a few pounds to perhaps one hundred per square inch (0.5–6 bar). Generally, MF has the capability of performing separations equivalent to those obtained by high-speed centrifugation (5,000–10,000 g), while UF is equivalent to ultracentrifugation (10,000–100,000 g). Since centrifugal forces are not capable of separating ions from water, there is no equivalent for RO and NF (Porter 1972).

2.3 Membrane Bioreactor Systems

Membrane bioreactors are reactors that convert or produce materials using functions naturally endowed to living systems. Membrane bioreactor systems essentially consist of the combination of membrane and biological reactor systems (Belfort 1989;

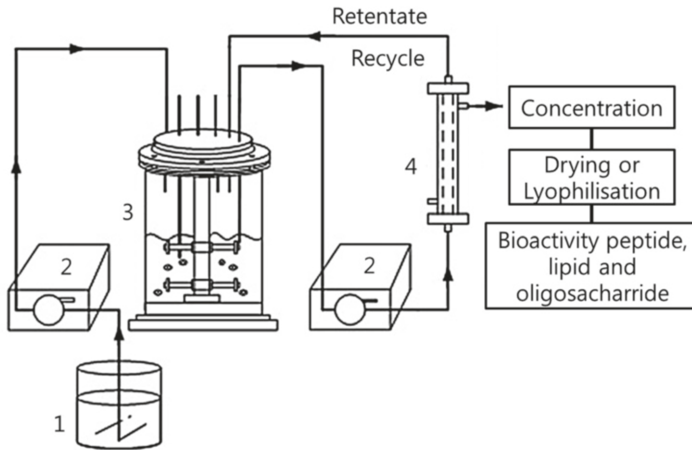

Fig. 4.3 Schematic description of the membrane bioreactor system. (*1*) Supplement tank, (*2*) Pump, (*3*) Bioreactor, (*4*) Membrane

Giorno and Drioli 2000; Giorno et al. 2003). Typical bioreactors include immobilized enzymes, microorganisms, animal or plant cells, and those applying new methodologies such as genetic manipulation or cell fusion. They are alternative approaches to classical methods of immobilizing biocatalysts such as enzymes, microorganisms, and antibodies, which are suspended in solution and compartmentalized by a membrane in a reaction vessel or immobilized within the membrane matrix itself. In the first method, the system might consist of a traditional stirred tank reactor combined with a membrane-separation unit; in the second method, the membrane acts as a support for the catalyst and as a separation unit. The biocatalyst can be flushed along a membrane module, segregated within a membrane module, or immobilized in or on the membrane by entrapment, physical adsorption, ionic binding, covalent binding, or cross-linking. The advantages of immobilizing enzymes are reported to include increased reactor stability and productivity, improved product purity and quality, and reduction in waste (Giorno and Drioli 2000).

Membrane bioreactors differ from conventional reactors as the biocatalysts present in the reactors operate under milder conditions of temperature and pressure. The ranges of operating conditions within bioreactors are usually determined by the biocatalyst (organism) and are usually small. Many studies are oriented to the investigation of operating conditions and optimization of the various properties of these membrane bioreactors. The efficiency of the overall system depends on the biochemical parameters (e.g., catalytic activity, reaction kinetics, concentration, viscosity of substrate and product, immobilization stability), geometric parameters (e.g., membrane configuration, morphology, and pore size distribution), and hydrodynamics parameters (such as transmembrane pressure and flow velocity) (Giorno et al. 2003).

The membrane bioreactor integrates a reaction vessel with a membrane separation system as shown Fig. 4.3. It is composed of two main systems: a bioreactor unit that is responsible for the biodegradation of raw materials and the membrane unit that is responsible for the separation of the desired bioactive molecules

according to molecular size. Various biocatalysts can be used, depending on the desired final products. Compounds with specific molecular weight distributions can be obtained by using membranes with the appropriate MWCO or pore size distribution. Membrane bioreactors equipped with UF membranes or multistep recycling membrane reactors combined with UF membranes system are technologies developed for bioprocessing and efficiently recovering bioactive functional ingredients with the desired molecular size and functional properties. The development of membrane biotechnology has been applied for the separation, fractionation, and recovery of marine ingredients in order to obtain purer compounds with efficient yield ratios. Different approaches have been applied depending on the chemical structure of the target compounds: the potential of membrane processes using MF and UF to obtain food-grade phycocyanin (82 % recovery with 1.0 purity) from *Spirulina* sp. to be applied in the food industry was reported by Chaiklahan et al. (2011). Marine peptides with antiproliferative activity have been fractionated and concentrated using UF and NF membrane systems (Bourseau et al. 2009). A review of membrane applications in functional foods and nutraceuticals has recently been published by Akin et al. (2012).

3 Application of Membrane Separation in Marine Biotechnology

Sources of functional ingredients exist in many different reservoirs that may be found in both terrestrial and marine environments. The terrestrial environment (i.e., fruits, vegetables, cereals, and mushrooms) as a reservoir of bioactive compounds is, by far, more often explored than the marine environment (i.e., fish, sponges, and macro- and microalgae). Although many functional marine ingredients are presently known, it is believed that other marine ingredients remain to be evaluated and new sources yet to be discovered. The marine environment is a major reservoir of bioactive compounds that have potential to be applied in several phases of food processing, storage, and fortification (Rasmussen and Morrissey 2007). The characteristics of marine environments, such as various degrees of salinity, temperature, pressure, and illumination, impart particular interest on compounds derived from marine organisms. In order to increase the availability and chemical diversity of marine functional ingredients, more research is applying biotechnological tools to discover and produce compounds from marine resources (Baerga-Ortiz 2009). In this chapter, we will focus on advances in membrane separation technology to discover, produce, or transform compounds from marine sources to be incorporated as functional ingredients in potential functional materials.

3.1 Devices for the Separation of Bioactive Peptides on Membrane Reactor Processes

One of the most important factors in producing bioactive peptides with the desired functional properties for use as functional materials is their molecular weight

(Deeslie and Cheryan 1982). Therefore, for efficient recovery and in order to obtain bioactive peptides with a desired molecular size and functional property, a UF membrane system can be used. This system's main advantage is that the molecular weight distribution of the desired peptide can be controlled by the adoption of an appropriate UF membrane (Cheryan and Mehaia 1990). In order to obtain functionally active peptides, it is normal to use enzymes in order to allow sequential enzymatic digestion. By applying enzyme technology for effective protein recovery in fish processing plants, it may be possible to produce a broad spectrum of food ingredients or industrial products for a wide range of applications (Kristinsson and Rasco 2000). The enzymatic modification of fish proteins using selected proteolytic enzyme preparation to cleave specific peptide bonds is widely used in the food industry (Mullally et al. 1994). Proteolytic modification of food proteins to improve the palatability and storage stability of the available protein resources is an ancient technology (Adler-Nissen 1986). Hydrolysates can be defined as proteins that are chemically or enzymatically broken down into peptides of varying sizes (Skanderby 1994). The protein hydrolysates are produced for a wide variety of uses in the food industry, including milk replacers, protein supplements, stabilizers in beverages, and flavor enhancers in confectionery products. The benefits of hydrolyzing food proteins to make functional protein ingredients and nutritional supplements are a more recent technology. Bioactive peptides isolated from various fish protein hydrolysates have shown numerous bioactivities, such as antihypertensive, antithrombotic, immunomodulatory, and antioxidative activities. Moreover, it is possible to obtain serial enzymatic digestions in a system using a multistep recycling membrane reactor combined with a UF membrane system to separate marine-derived bioactive peptides (Jeon et al. 1999). This membrane bioreactor technology has recently emerged for the development of bioactive compounds and has potential for the utilization of marine proteins as value-added nutraceuticals with beneficial health effects.

3.1.1 Antihypertensive Peptides

Kim and Mendis (2000) have reported that some peptides derived from fish showed antihypertensive activity inhibiting the action of angiotensin I-converting enzyme (ACE) even stronger than that of many other natural peptides. The renin–angiotensin system (RAS) constitutes one of the most important hormonal systems in the physiological regulation of blood pressure. Indeed, dysregulation of the RAS is considered a major factor in the development of cardiovascular disease, and blockade of this system offers an effective therapeutic regimen. Originally defined as a circulating or endocrine system, multiple tissues express as complete local RAS and compelling evidence factor of action for angiotensin I and angiotensin II, the primary effector peptide of the system. ACE is a dipeptidyl carboxypeptidase that catalyzes the conversion of angiotensin I (decapeptide) to angiotensin II (octapeptide), inactivates the antihypertensive vasodilator bradykinin, and increases blood pressure. Inhibition of ACE activity leads to a decrease in the concentration of angiotensin II and, consequently, reduces blood pressure (Skeggs et al. 1957).

Many studies have confirmed that bioactive peptides separated from various marine organisms and their processing by-products using membrane bioreactors were potent ACE inhibitors. Aleman et al. (2011) and Zeng et al. (2005) suggested that the average molecular weight of peptides for potent ACE inhibitory activity is about 1,500 Da. Apart from their uses in the continuous production of bioactive peptides, UF membranes have also been used to fractionate proteins, peptides, and amino acids contained in hydrolysates (Table 4.2). Byun and Kim (2011) fractionated peptides contained in Atlantic salmon protein concentrate hydrolysate by a third-step UF process, which resulted in a mixture of polypeptides and a fraction rich in small peptides with a molecular mass below 1,000 Da. They applied selective UF membranes (1.9 and 0.9 kDa) to enrich ACE inhibitory peptides purified Gly-Pro-Leu and Gly-Pro-Met. Cinq-Mars and Li-Chan (2007) optimized the ACE inhibitory activity of hydrolysates from Pacific hake (*Merluccius productus*) using response surface methodology to identify the best hydrolysis conditions, followed by ultrafiltration using 10-, 3-, 1-, or 0.5-KDa MWCO membranes. As the majority of peptides in the hydrolysates produced under optimum conditions were smaller than 1,000 Da, there was no significant difference between ultrafiltrates in ACE inhibitory activity, and it was concluded that 10 kDa UF would be the most practical to concentrate the bioactive peptides with the highest recovery of hydrolysate. More recent studies reported the enrichment of ACE inhibitory peptides by UF from an Atlantic salmon protein hydrolysate with a 1-kDa MWCO membrane (Gu et al. 2011) and sea cucumber hydrolysates with UF membranes (10, 5, and 3 kDa). By UF and NF, Amado et al. (2013) purified peptides from cuttlefish which had inhibitory activity against ACE. Peptides with lower molecular weight showed potent ACE inhibitory activity compared to peptides with higher molecular weights. Further, these peptides showed in vivo activities by lowering the blood pressure significantly in spontaneously hypertensive rats following oral administration. This is an advantage of fish protein hydrolysates, since some antihypertensive drugs are reported to have side effects after administration, including abnormal elevation of blood pressure.

3.1.2 Antioxidant Peptides

The increased generation of reactive oxygen species, such as superoxide anion (O_2^-) and hydroxyl (OH^-) radicals, in conjunction with the overpowering of endogenous antioxidant defense mechanisms (enzymatic and non-enzymatic), is another causative factor for the initiation of chronic diseases. These diseases include heart disease, stroke, arteriosclerosis, diabetes, and cancer (Dávalos et al. 2004). Furthermore, lipid peroxidation is a major cause of deterioration in the quality of foods (rancidity and ‘off-flavors’) (Di Bernardini et al. 2011). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate are added to food products to retard lipid oxidation. However, there are safety concerns over the use of synthetic antioxidants as food additives (Shahidi and Zhong 2005). As a result, natural antioxidants with little or no side effects have gained interest in terms of their potential health benefits and food quality prolonging abilities.

Table 4.2 Membrane processes for the separation/concentration of bioactive peptides

Biological activity	Protein hydrolysate source	Process	Sequence or characterization	References
ACE inhibitor	Alaska pollock	Ultrafiltration	Gly-Pro-Leu/Gly-Pro-Met	Byun and Kim (2001)
	Sea cucumber gelatin	Ultrafiltration	Not available/840 Da	Zhao et al. (2009)
	Styela plicata	Ultrafiltration	Met-Leu-Leu-Cys-Ser	Ko et al. (2011)
	Phascolosoma esculenta	Ultrafiltration	Ala-Trp-Leu-His-Pro-Gly-Ala-Pro-Lys-Val-Phe	Du et al. (2013)
	Pacific hake (*Merluccius productus*)	Ultrafiltration	Not available/<1 kDa	Cinq-Mars and Li-Chan (2007)
	Atlantic salmon	Ultrafiltration-nanofiltration	Ala-Pro/Val-Arg	Gu et al. (2011)
	Cuttlefish	Ultrafiltration-nanofiltration	Not available	Amado et al. (2013)
Antioxidant	Bigeye tuna	Ultrafiltration	Leu-Asn-Leu-Pro-Thr-Ala-Val-Tyr-Met-Val-Thr	Je et al. (2008)
	Gastrointestinal digests of *Mytilus coruscus*	Ultrafiltration	Leu-Val-Gly-Asp-Glu-Gln-Ala-Val-Pro-Ala-Val-Cys-Val-Pro	Jung et al. (2007)
	Loach (*Misgurnus anguillicaudatus*)	Ultrafiltration	IC_{50} value = 16.9 ± 0.21 mg/mL	You et al. (2011)
	By-product from cod	Ultrafiltration-nanofiltration	Not available/600~750 Da	Bourseau et al. (2009)
	Cuttlefish	Ultrafiltration-nanofiltration	Not available	Amado et al. (2013)
Antibacterial activity	Snow crab by-product	Electrodialysis-ultrafiltration	Not available	Doyen et al. (2012)
Anticancer	Snow crab by-product	Electrodialysis-ultrafiltration	300~600 Da	Doyen et al. (2011)
Antifatigue	Loach (*Misgurnus anguillicaudatus*)	Ultrafiltration	Not available	You et al. (2012)
DPP-IV inhibitor	Atlantic salmon (*Salmo salar*) skin gelatin	Ultrafiltration	Gly-Pro-Al-Glu, Gly-Pro-Gly-Ala	Li-Chan et al. (2012)
Nitric oxide inhibitor	Sea mussel, *Mytilus coruscus*	Ultrafiltration	Gly-Val-Ser-Leu-Leu-Gln-Gln-Phe-Phe-Leu	Kim et al. (2013)
Neuroprotective	Collagen peptide from chum salmon skin	Ultrafiltration-nanofiltration	Not available	Pei et al. (2010)
β-secretase inhibitor	Chitooligosaccharides	Ultrafiltration	Not available	Byun et al. (2005)

Peptides derived from fish proteins have shown the ability of exerting potent antioxidative activities in different oxidative systems (Samaranayaka and Li-Chan 2011; Jun et al. 2004; Kim and Mendis 2000; Rajapakse et al. 2005a, b). An increasing interest currently exists to explore natural antioxidative substances without side effects and these identified antioxidative activities that offer the potential to develop safe and non-hazardous natural antioxidants to reduce oxidative stress and the risk of disease arising from the oxidation of biomolecules. Marine-derived protein hydrolysates and peptides arising from processing by-products, mollusks, and crustaceans have been shown to exert antioxidant activity in vitro by some of the mechanisms described above. Bigeye tuna protein hydrolysate that had been separated into several fractions based on molecular size showed excellent antioxidant activity based on the thiobarbituric acid assay (Je et al. 2008). A protein hydrolysate obtained from defatted round scads mince containing high amounts of arginine and lysine also exhibited significant antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH), and good radical scavenging, metal chelating, and reducing properties (Thiansilakul et al. 2007). You et al. (2011) optimized the antioxidative activity of papain-derived loach protein hydrolysate using hydroxyl radical scavenging activity. Afterward, they used a 3,000-Da MWCO membrane to separate peptides with a molecular weight less than 1 kDa from loach protein hydrolysate. Jung et al. (2007) reported lipid peroxidation inhibitory and radical scavenging activities of the peptidic fraction obtained from in vitro gastrointestinal digests of *Mytilus coruscus* that had a molecular weight less than 3 kDa. Starting with crude fish protein hydrolysates at a fairly high concentration, Bourseau et al. (2009) used a two-step process involving UF and NF to produce four different fractions with different degrees of hydrolysis. Two chain-breaking low molecular weight antioxidant peptides were obtained from the cod by-product protein hydrolysates (Bourseau et al. 2009). In NF, mechanisms underlying separation selectivity are more complex than just the sieving effect. Indeed, the selectivity is regulated by both size exclusion and electrostatic repulsion mechanisms, which are dependent on the membrane type and the feed composition. To attempt to analyze the mechanisms involved in the separation of biomolecules by NF, several fundamental researches reported the application of NF for peptide fractionation in model mixtures of amino acids and peptides (Tsuru et al. 1994; Martin-Orue et al. 1998). Conventional pressure-driven processes (UF, NF) offer possibilities for a large-scale production of bioactive peptides but seem limited because of fouling and poor selectivity. Indeed, peptides released from protein hydrolysis present small differences in their physicochemical parameters, such as molecular weight, pI, and net charge. For this reason, these processes often have to be used in combination with other technologies to increase their separation potential (Yu et al. 2008).

3.1.3 Other Bioactivities

Shellfish contain significant levels of high-quality protein and are, therefore, a potential source for biofunctional high-value peptides. Enzymatic hydrolysates of *Mytilus coruscus* showed clearly superior nitric oxide inhibitory activity on

lipopolysaccharide (LPS)-stimulated RAW264.7, and were further purified using a UF and consecutive chromatographic methods. The enzymatic hydrolysate of *M. coruscus* was fractionated through UF membranes with a range of MWCO of 30, 10, and 5 kDa. The fraction with 10–30 kDa size molecular weight of *M. coruscus* protein had a higher NO inhibitory activity than the other fractions (Kim et al. 2013). Doyen et al. (2011) separated two anticancer peptides from a snow crab by-products hydrolysate by electrodialysis with a UF membrane. Moreover, a snow crab by-products hydrolysate has demonstrated antibacterial properties due to a peptide with a molecular weight of about 800 Da (Doyen et al. 2012). Two different UF membranes (20 and 50 kDa) and two electrical field strengths (2 and 14 V/cm) were used as the separation parameters. After electrodialysis with UF membrane separation, the 300–600-Da peptide molecular weight range was the most recovered, with an abundance of 94 %. The purified fraction showed antibacterial properties on *Escherichia coli* ATCC 25922 and *Listeria innocua* HPB 13. The antioxidant and antifatigue activities of two peptides of <5 kDa were determined, that is, loach peptide A (LPA, from a papain digestion) and loach peptide B (LPB, from a Flavorzyme digestion) (You et al. 2012). The LPA fraction mainly possessed peptides of $1,000 < MW < 3,000$ Da (65.41 %), whereas LPB mainly possessed peptides of $500 < MW < 1,000$ Da (58.27 %). Compared with LPB, LPA increased the swimming time more effectively and reduced blood urea nitrogen and liver malonaldehyde levels in mice, although both of them had significant antifatigue effects compared to the control mice.

3.2 Production of Fish Oil

Fish oils are readily available sources of n-3 PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play an important role in reducing the risk of a number of diseases, including atherosclerosis, coronary heart disease, hypertension, inflammation, hypotriglyceridemic effect, allergies, and diabetes (Leigh-Firbank et al. 2002). Fish oil intervention resulted in a significant increase in platelet phospholipids EPA content, decrease in arachidonic and γ-linolenic acid levels, and significant decrease in fasting and postprandial triacylglycerol levels (Leigh-Firbank et al. 2002). Long-chain PUFAs are considered as essential for the growth and development of infants, and they have been included in food supplements in the form of concentrated fish oil. Hence, there is a marked demand for marine fatty acids as a health food and for use as a food supplement.

The use of membranes in lipid processing has attracted great interest in recent years, since lipids that are heat-labile can be preserved and concentrated with minimal changes to the lipid (Snape and Nakajima 1996). Most studies in this area have been on the hydrolysis of oils and fats (Prazeres and Cabral 1994), the synthesis of acylglycerols with two-phase membrane reactors (D'Arrigo et al. 2005), and epoxidation of fatty acids (Piazza et al. 2000). Sahashi et al. (1993) combined solvent extraction with membranes to separate fish oil triglycerides from free fatty acids (FFA).

An ethanol solvent (75 % aqueous ethanol) was added to the fish oil, which contained triglycerides with PUFAs and FFA. Several flat-sheet membranes were tested for their ability to separate the oil from the solvent phase, which contained the FFA. A hydrophilic polyimide UF membrane with a 20-kDa molecular cut-off was found to be the most suitable for the separation of the solvent phase as permeate. A rotating disk membrane was effective in a large-scale operation for recovering PUFA-rich glycerides without oil quality deterioration.

In many studies, lipozyme has been used as the catalyst to separate EPA and DHA in fish oil (Haraldsson and Kristinsson 1998). Xu et al. (2000) used a membrane bioreactor to separate medium-chain triacylglycerols and n-3 PUFAs from fish oil. In their study, lipozyme was used as the biocatalyst to accelerate the reaction in the bioreactor. The medium-chain fatty acids were separated by 10-kDa and 2-kDa MWCO membranes. Linder et al. (2002) conducted the enzymatic hydrolysis using a stereospecific *sn-1 sn-3* hydrolytic lipase from *Aspergillus oryzae*, followed by membrane filtration. The substrate was then partially hydrolyzed by the protease Neutrase to obtain most of the oil after centrifugation of the mixture. Immobilized 1,3-specific lipase IM60 (Lipozyme IM) was used for re-esterification. The incubation of the mixture was carried out at 37 °C, pH 7 for 24 h under stirring at 800 rpm. Lipase hydrolysis was carried out in a batch reactor blanketed with nitrogen. Membrane separation increased the PUFAs from 30.4 % in crude oil to 43.3 % in permeate. D'Arrigo et al. (2005) used a membrane bioreactor for hydrolysis as well as transphosphatidylation reactions.

3.2.1 Enzymatic Modification of Fish Oil

Fish oil is a by-product of the use of fishing as a source for fish protein or one of the two main products when whole fish is immediately processed for fish meal and fish oil production. The content of fish oil varies with the fish species. The oil contents of typical fatty fish such as herring, sardine, and pilchard are about 21, 18, and 16 %, respectively, while the average fish oil content is ~3–5 % below these values (Brokisch 1998). Fish oil is rich in PUFAs, the proportion of which depends on the fish species and where they are caught. Fish oil is usually hardened before use because this modification improves the oil's keeping ability and reduces the tendency to become rancid, which is caused by a high degree of unsaturation. Fish oil contains such a large number of fatty acids with equal numbers of carbon atoms but different numbers of double bonds that these fatty acids are usually not individually shown to characterize the oil. More than 90 % of all PUFAs in fish oils are of the ω-3 type, which are low or lacking in land animals and plant oils (Table 4.3) (Fernandez 1986). PUFAs play an essential role in human nutrition, as well as having important biomedical properties. Both the ω-3 and ω-6 families play important roles in human metabolism, but they cannot be interconverted in the body. PUFAs of ω-3 types are α-linolenic acid, EPA and DHA, while ω-6 types are linoleic acid, γ-linolenic acid, and arachidonic acid. Fish oils containing EPA and DHA have been shown to be effective in reducing the risk factors of cardiovascular disease,

Table 4.3 Fatty acid composition of oils from various fish species compared to land animal and plant sources

Fatty acids	Salmon	Herring	Flatfish	Cod	Pig	Beef	Soybean
14:0	4.64	6.14	0.74	0.92	1.4	3.3	–
16:0	18.53	18.90	16.81	22.59	20.4	26.6	11.3
16:1	2.31	6.19	2.14	4.73	3.5	4.1	–
18:0	1.94	1.30	3.69	2.13	10.1	18.2	3.4
18:1	20.85	27.76	49.22	34.39	47.5	41.2	23.1
20:1	0.78	0.53	0.26	0.48	2.7	–	–
22:1	13.52	15.88	5.99	3.75	–	–	–
18:2ω-6	1.12	0.55	0.41	0.47	12.1	3.3	55.8
18:3ω-3	18.10	13.42	7.10	1.30	1.4	–	6.4
20:4ω-6	–	–	6.85	0.12	–	–	–
20:5ω-3	5.97	4.52	1.37	18.67	–	–	–
22:5ω-3	1.08	0.27	0.44	1.36	–	–	–
22:6ω-3	8.37	2.96	1.31	27.98	–	–	–

arteriosclerosis, cancer, diabetes, and allergies in humans (Park et al. 2000a, b; Rose et al. 1995; Hubbard et al. 1998; Pirich et al. 1999; Mukutmoni-Norris et al. 2000). Therefore, many physical methods have been developed for extracting and enriching these fatty acids, including crystallization, distillation, and the use of supercritical carbon dioxide.

The use of lipase biotechnology now offers an alternative method, which has the advantage of mild processing conditions that minimize the degradation/oxidation of these acids. The enrichment processes are based on the principle that, in general, lipases show low reactivity towards long-chain PUFAs compared to more common fatty acids ($<C_{20}$). This can be exploited to enrich long-chain PUFAs from commodity fish oils. Furthermore, many lipases also show low reactivity towards γ-linolenic acid and this technology has been used to enrich this biologically important fatty acid from borage oil (Huang et al. 1997). Lipases can be divided into three classes based on their specificity and selectivity towards position, fatty acid type, and acylglycerol (Diks and Bosley 2000). The catalytic properties of lipase are used in hydrolysis, esterification, and interesterification reactions for the concentration of PUFAs and for the improvement of functional properties of fish oil. The lipase from *Candida rugosa* has been shown to concentrate EPA and DHA from sardine oil (Jonzo et al. 2000), cod liver oil (Hoshino et al. 1990), and tuna oil (Tanaka et al. 1992; McNeill et al. 1996; Moore and McNeill 1996). In these studies, the proportion of long-chain PUFAs containing EPA and DHA was increased from about 27 to 40 %. Tanaka et al. (1992) reported that, when fish oil and tuna oil were employed as the substrate, the lipase from *C. cylindracea* preferentially hydrolyzed EPA residues relative to DHA residues. Several studies are currently engaged in a research effort to develop an efficient and economically feasible method of obtaining polyunsaturated glycerides enriched in EPA and DHA. These studies have applied membrane bioreactor systems for continuous steady-state hydrolysis (Rice et al. 1999; Ceynowa and Adamczak 2001), esterification (Chemseddine and Audinos 1995;

Zhu et al. 1996; Isono et al. 1998), and transesterification (Ceynowa and Rauchfleisz 2001; Basheer et al. 1995) reactions of oil by various lipases. Membrane materials ranging from hydrophilic to hydrophobic in nature were reported in the literature. The former include cellulose (Guit et al. 1991; Van der Padt et al. 1990), polyamide (Molinari et al. 1994; Giorno et al. 1995), polyacrylonitrile (Lopez et al. 1990; Wu et al. 1993), polytetrafluoroethylene (Goto et al. 1992), polyvinylchloride (Rucka and Turkiewicz 1990), and polysulfone (Zbigniew 1994). Recently, a membrane used for the bioconversion of oil and fatty acid derivatives utilized a microporous hydrophobic membrane, either in a flat or in a hollow fiber module.

In the typical membrane reactor for fish oil processing, the effluent from the bioreactor is fed to the membrane module. Products such as FFA, mono-, di-, and triglycerides, and solvents are sufficiently small to permeate the membrane. The flow of non-permeable material and retentate is recycled directly back into the bioreactor. In addition to continuous operation, a second advantage of this system is the removal of FFA, a competitive inhibitor of lipase, facilitating an increase in the rate and extent of reaction (Prazeres and Cabral 1994). However, a major problem of all bioreactor systems is the significant fouling of oil–water emulsions (Kajitvichyanukul et al. 2008). This occurrence leads to complications in downstream product recovery and the need to replenish the emulsion supply in the bioreactor to maintain a steady state. In addition, a significant amount of the lipase and surfactant is adsorbed to the membrane surface, leading to a decreased permeation flux (Prazeres et al. 1993; Nakamura and Hakoda 1995). Moreover, emulsion systems have difficulty in not only controlling the reaction, but also in the reuse and stability of lipases (Sakaki et al. 2001). To overcome this difficulty, some types of bioreactor using immobilized lipases have been proposed (Warmuth et al. 1995; Lye et al. 1996; Giorno et al. 2007). Multiphase enzyme membrane reactors show remarkable advantages over emulsion systems (Giorno et al. 1997; Lopez and Matson 1997). In the multiphase membrane reactor, hydrophobic substrate molecules come in contact with enzyme molecules on/in the membrane. The membrane provides reaction sites and fixes the interface, therefore promising more stable operation.

3.2.2 Fish Oil O/W Emulsions Produced by Membrane Emulsification

Fish oils are in high demand by the food industry because they contain omega-3 PUFAs which have numerous health benefits (Larsson et al. 2004; Simopoulos et al. 1999). However, omega-3 fatty acids, including the long-chain PUFAs such as EPA and DHA, in fish oil have a very strong odor and their constituent highly unsaturated long-chain fatty acids can easily become oxidized (Augustin et al. 2006). To minimize or control these negative attributes, fish oil can be formulated as solid microcapsules. Microencapsulation can provide controlled release of encapsulated compounds, helps in masking the odor and/or taste of encapsulated materials, and simplifies the handling, storage, and delivery of the powder-like materials produced. Microencapsulation technology is widely used in the pharmaceutical and food industries (Madene et al. 2006) and provides a means of converting fish oil into a dry powder.

In the food industry, the most widely employed technologies for encapsulating lipophilic compounds are based on the production of an oil-in-water (O/W) emulsion, which is then followed by either spray drying (Gharsallaoui et al. 2012; Aghbashlo et al. 2013), freeze drying (Heinzelmann et al. 2000), molecular inclusion (Yoshii et al. 1996), or enzymatic gelation (Cho et al. 2003). Of these techniques, the most common method of producing encapsulated fish oil is by spray drying the emulsion because it is a very efficient and flexible process that quickly removes water by vaporization and can be carried out with readily available equipment.

Fish oil microcapsules were prepared by combining a low-energy emulsification method (premix membrane emulsification) with spray drying. O/W emulsions were prepared by a two-step emulsification method that used a rotor–stator homogenizer followed by membrane emulsification (Ramakrishnan et al. 2013). The influence of the emulsification method (mechanical stirring or membrane emulsification), the emulsification conditions (membrane and emulsifier type), and the amount of wall material on the physicochemical characteristics of the microcapsules was studied (Ramakrishnan et al. 2013). The results show that the emulsification method as well as the type and amount of emulsifier and wall material affect the final amount of encapsulated oil. Microcapsules produced by membrane emulsification and stabilized with 2 % Tween-20 or 10 % whey protein presented the highest values (higher than 50 %) of oil encapsulation efficiency. It was found that the oil encapsulation efficiency increases with decreasing droplet size of the emulsions, as well as with the increase of the amount of wall material employed during drying. Morphology analysis showed that the microcapsules obtained from O/W emulsions produced by premix membrane emulsification were rounder in shape, without visible cracks on the surface, and no vacuoles on the inside. Oxidation stability tests performed on some selected samples indicate that the microcapsules with higher stability are the ones produced with a higher amount of wall material and have less surface oil.

3.3 β-Secretase Inhibitory Activity of Chitosan and Its Derivatives

The production of chitooligosaccharides (COS) from chitosan is one of the premium applications of membrane bioreactors in seafood applications. Crustacean shells and shellfish wastes from seafood processing plants are an attractive source of bioactive compounds which could potentially be used in the food and pharmaceutical industries (Jayakumar et al. 2010; Wang et al. 2005). Shells are currently utilized for the commercial-scale production of chitin, as well as for the production of chitosan and COS. There are two methods for the production of COS: enzymatic hydrolysis using chitosanase and chemical hydrolysis using acids (Jeon et al. 2000). Chemical hydrolysis is relatively harsh and non-selective and, thus, the product contains relatively large amounts of impurities, including D-glucosamine and chitosan monomers (Horowitz et al. 1957). In contrast, enzymatic hydrolysis in a membrane bioreactor has several advantages with regard to product specificity.

Further, the size of the final products can be controlled by the pore size of the membranes used in the membrane bioreactor system. Their final product concentration of pentamers and hexamers was 2.6 g/L. The membrane bioreactor was more effective compared to other systems for the production of COS by the hydrolysis of chitosan. Jeon and Kim (2000) introduced a new system using dual reactors for producing COS by combining a column reactor packed with immobilized enzymes with a UF membrane bioreactor.

A promising application of COS is for treatment of the most common neurodegenerative disorder, Alzheimer's disease (AD), which generally occurs after 65 years of age, with a prevalence of around 2–4 % at age 70 years and ~30–50 % by age 90 years (LaFerla et al. 2007). The pathological hallmark of AD is the deposition of senile plaques and neurofibrillary tangles (Agdeppa et al. 2001). Senile plaques are composed of the β-amyloid (Aβ) peptides, which are cleaved from amyloid precursor proteins (APPs) by proteolytic enzymes such as β- and γ-secretase (Okamura et al. 2004). In APP proteolysis, it seems that the key enzyme is β-secretase, which is also known as β-amyloid cleavage enzyme (BACE-1), since it initiates the formation of Aβ peptides (Hampel and Shen 2009). Moreover, the necessity of proteolytic cleavage for Aβ generation suggests the potential therapeutic intervention targets using standard protease inhibition approaches (John et al. 2003).

The inhibition of β-secretase is an attractive target for the treatment of AD. There have been some studies on BACE-1 inhibition activities and Aβ formation inhibition activities of chitosan and its derivatives in the past decade. An investigation of the BACE-1 inhibitory activity of COS was done by Byun et al. (2005). Nine kinds of hetero-COS with different degrees of deacetylation and molecular weight were prepared by using a UF membrane reactor. The 90, 75, and 50 % deacetylated chitosans were hydrolyzed and fractionated by passing them through three UF membranes of MWCO 10, 5, and 1 kDa. The hetero-COSs were named 90-HMWCOSs, 75-HMWCOSs, 50-HMWCOSs, 90-MMWCOSs, 75-MMWCOSs, 50-MMWCOSs, 90-LMWCOSs, 75-LMWCOSs, and 50-LMWCOSs, respectively. The 90-MMWCOSs, which are 90 % deacetylated COS with molecular weight 3–5 kDa, exhibited the highest BACE-1 inhibitory activity (25–42 mM) compared to the others. The inhibitor was found to be non-competitive by the Dixon plot, and the Ki of 90-MMWCOSs was 3.87–6.47 mM. The study of Byun et al. (2005) and his colleagues indicated that the degree of deacetylation and sulfation at the C-2 position of COS has an effect on the BACE-1 inhibitory activity. A further amine group at the C-2 position was shown to be beneficial for the BACE-1 inhibitory activity.

The BACE-1 inhibition activity of chitosan derivatives was also reported by Je and Kim (2005). They prepared chitosan with two degrees of deacetylation (90 % and 50 %) and grafted amino functionality into chitosan to improve the solubility and bioactivity. Chitosan derivatives were designated as aminoethyl (AE-chitosan) (90 %), dimethylaminoethyl (DMAE-chitosan) (90 %), and diethylaminoethyl (DEAE-chitosan) (90 %) prepared from 90 % deacetylated chitosan, and AE-chitosan (50 %), DMAE-chitosan (50 %), and DEAE-chitosan (50 %) prepared from 50 % deacetylated chitosan. The potencies of chitosan derivatives are expressed as an IC_{50}

value, which is the BACE-1 inhibitor concentration leading to 50 % inhibition of the BACE-1 activity. AE-chitosan (90 %) shows the strongest inhibitory activity compared to the other derivatives. COS and its derivatives are promising neuroprotective agents, as they showed neuroprotective properties such as, suppression of β-amyloid formation, antineuroinflammatory activity, apoptosis inhibitors, etc. Up until now, most neuroprotective activities of chitosan and its derivatives have been observed in vitro. Therefore, further studies are needed in order to investigate their activity in mouse model systems and/or human subjects. In conclusion, these results reveal the potential of chitosan and its derivatives as potential therapeutic candidates for neurodegenerative disorders and their involvement in future pharmaceuticals is promising.

3.4 Fisheries Processing Wastewater

3.4.1 Concentration of Wastewater by Membranes

In the fish processing industry, UF is mainly used for fractionation and wastewater recovery processes. Chabeaud et al. (2009) used a UF membrane to improve the bioactivity of a pollack protein hydrolysate containing peptides having a size smaller than 7 kDa by fractionating or concentrating some specific molecular weight peptide classes. The wastewaters generated in fish processing industries contain a large amount of organic load, including typically more than 20 % of fish protein, and present a pollution problem due to the high biochemical oxygen demand and chemical oxygen demand (Chowdhury et al. 2010). In the past, these wastewaters were usually discharged into the sea without any treatment, but stricter regulations are now in place to protect the environment.

Wastewater produced from fish processing plants is traditionally treated with agglutinating or flocculating materials, but recently, it has also been treated with MF, UF, and RO membrane processes. Furthermore, the discovery of potentially valuable proteins in the wastewaters in recent years has drawn much attention from several researchers to recover the proteins by membrane filtration. Concentration carried out using a ceramic tubular UF membrane (MWCO = 15 kDa) showed that UF reduces the organic load from the fish meal wastewaters and allows the recovery of valuable raw materials comprising proteins (Afonso and Bórquez 2002). Afonso et al. (2004) assessed the technical and economical feasibility of protein recovery from fish meal effluents using membrane UF and NF. They concluded that the integrated process comprising MF pretreatment and UF would enable 69 % recovery of proteins, allowing for productivity and revenue increases, in addition to a significant reduction of environmental burdens. Therefore, the application of UF in fish meal effluents is technically and economically feasible for protein recovery and pollution reduction. On the other hand, Lo et a. (2005) investigated the feasibility of recovering protein from poultry processing wastewater using UF and the optimization of processing parameters. The result indicated that almost all crude proteins in poultry

processing wastewater were retained, subsequently reducing the chemical oxygen demand in the effluent to less than 200 mg/L.

Boiled extracts containing various useful materials are produced from crabs, shrimps, squids, fishes, and shellfishes in fisheries processing plants. The boiled extracts recovered and concentrated using centrifugation and evaporation in the fisheries processing contain high salt concentration. The technology for producing boiled extracts by UF and RO membranes has been developed for natural seasoning products (Kim et al. 1999a). In producing the low-salt boiled extract from shellfishes by UF and RO membranes, permeate flux of boiled extract of shellfishes by a UF membrane is decreased, but that of the boiled extracts treated with enzymes could be increased. If boiled extract permeated by a UF membrane is concentrated by an RO membrane, the permeate flux is decreased with the concentrate ratio, but the gel and concentration fraction layer do not appear due to the removal of polymer materials. Boiled extract of oyster contains 4 % amino acids and 6 % inorganic salts, and is used as a seasoning material because it possesses good flavor and taste. It is also used as a health food due to its contents of taurine, glycogen, various amino acids, and nucleic acid. Moisture, protein, ash, and fat as proximate compositions of tuna boiled extracts were 59.8, 27.7, 11.6, and 1.0 %, respectively, and its salinity was 15.7 %. To improve the nutritional composition of tuna boiled extracts, Kim et al. (1999b) removed salts by electrodialysis. Factors studied for their effects on the desalting of the tuna boiled extract were concentration, desalting times, pH, and volumes of the boiled extract. The removal of salt was affected by concentration and pH, but not by the volume of tuna boiled extract. Park et al. (2000a, b) also reported that oyster boiled extract containing 5 % salt was desalted more than 95 % by electrodialysis.

3.4.2 Desalination of Wastewater by Electrodialysis Membranes

The most widely applied and commercially proven desalination technologies fall into two categories of thermal (evaporative) and membrane-based methods. Membrane methods are less energy intensive than thermal methods, and since energy consumption directly affects the cost-effectiveness and feasibility of using desalination technologies, membrane methods such as RO and electrodialysis (ED) have attracted great attention lately. Desalination is a process that removes dissolved minerals from seawater, brackish water, or treated wastewater. About 71 % of the earth's surface is covered by water, which is in the form of the oceans, seas, and ice in the poles. However, only about 3 % of water is fresh and suitable for drinking. The water of the oceans and seas is salty and, thus, not directly utilizable. Therefore, some special processes are needed in order to desalinate these waters (Mohammadi and Kaviani 2003). Suitable desalinating methods for the treatment of seawater can be effective to overcome the water shortage. ED is one of the methods which have been used for many years. ED is an electrochemical process for the separation of ions across charged membranes from one solution to another under the influence of an electrical potential difference used as a driving force. This process has been widely used for the production of drinking water and to process wastewater from

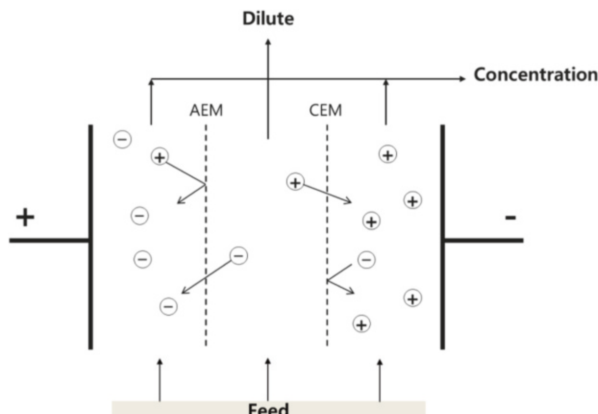

Fig. 4.4 Schematic view of an electrodialysis cell. *AEM* anion exchange membrane, *CEM* cation exchange membrane

brackish water and seawater, treatment of industrial effluents, recovery of useful materials from effluents, and salt production. The basic principles of ED have been reviewed in the literature (Ho and Sirkar 1992). In a typical ED cell, a series of anion and cation exchange membranes are arranged in an alternating pattern between an anode and a cathode to form individual cells. When a DC (rectifier, DC-01, RST SPASTELL TRF LSF 0.1) potential is applied between two electrodes, positively charged cations move toward the cathode, pass through the negatively charged cation exchange membrane, and are retained by the positively charged anion exchange membrane. On the other hand, negatively charged anions move toward the anode, pass through the positively charged anion exchange membrane, and are retained by the negatively charged cation exchange membrane. This causes ion concentration increases in alternate compartments, with a simultaneous decrease of the ion concentration in the other compartments. A schematic view of an ED cell is presented in Fig. 4.4. Sadrzadeh and Mohammadi (2007) suggest that the maximum percentage of desalination was obtained at the lowest feed concentration and flow rate levels (10,000 ppm and 0.07 mL/s, respectively) and the highest voltage and temperature levels (9 V and 55 °C, respectively). It has been found that the simultaneous increase in the voltage and temperature as well as decrease in feed concentration and flow rate optimizes the separation percent. ED was found to be very effective for wastewater and seawater desalination, specifically at lower concentrations.

4 Conclusion

Seafood processing by-products and waste materials, as well as underutilized species of fish, are potential sources of functional and bioactive compounds. A large number of bioactive substances can be produced through enzyme-mediated hydrolysis. Suitable enzymes and an appropriate bioreactor system are needed in order to incubate and transform the raw materials to value-added functional products.

Membrane technology provides a useful approach to extract, concentrate, separate, or fractionate the compounds. The use of membrane bioreactors to integrate a reaction vessel with a membrane separation unit is emerging as a beneficial method for producing bioactive materials such as peptides, chitooligosaccharides, and PUFAs from diverse seafood-related by-products. These bioactive compounds from membrane bioreactor technology show diverse biological activities such as antihypertensive, antimicrobial, antitumor, anticoagulant, antioxidant, and radical scavenging properties. This review discusses the application of membrane bioreactor technology for the production of value-added functional materials from seafood processing wastes and their biological activities in relation to health benefits.

References

Adler-Nissen J (1986) Enzymic hydrolysis of food proteins. Elsevier Applied Science Publishers, Barking

Afonso MD, Bórquez R (2002) Review of the treatment of seafood processing wastewaters and recovery of proteins therein by membrane separation processes—prospects of the ultrafiltration of wastewaters from the fish meal industry. Desalination 142:29–45

Afonso MD, Ferrer J, Bórquez R (2004) An economic assessment of proteins recovery from fish meal effluents by ultrafiltration. Trends Food Sci Technol 15:506–512

Agdeppa ED, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, Cole GM, Small GW, Huang SC, Barrio JR (2001) Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer's disease. J Neurosci 21:RC189

Aghbashlo M, Mobli H, Madadlou A, Rafiee S (2013) Influence of wall material and inlet drying air temperature on the microencapsulation of fish oil by spray drying. Food Bioprocess Technol 6:1561–1569

Akin O, Temelli F, Köseoğlu S (2012) Membrane applications in functional foods and nutraceuticals. Crit Rev Food Sci Nutr 52:347–371

Alemán A, Giménez B, Pérez–Santin E, Gómez-Guillén MC, Montero P (2011) Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chem 125:334–341

Amado IR, Vázquez JA, González MP, Murado MA (2013) Production of antihypertensive and antioxidant activities by enzymatic hydrolysis of protein concentrates recovered by ultrafiltration from cuttlefish processing wastewaters. Biochem Eng J 76:43–54

Augustin MA, Sanguansri L, Bode O (2006) Maillard reaction products as encapsulants for fish oil powders. J Food Sci 71:E25–E32

Baerga-Ortiz A (2009) Biotechnology and biochemistry of marine natural products. P R Health Sci J 28:251–257

Basheer S, Mogi KI, Nakajima M (1995) Development of a novel hollow-fibre membrane reactor for the interesterification of triglycerides and fatty acids using modified lipase. Process Biochem 30:531–536

Belfort G (1989) Membranes and bioreactors: a technical challenge in biotechnology. Biotechnol Bioeng 33:1047–1066

Bourseau P, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, Masse A, Guérard F, Chabeaud A, Fouchereau-Péron M, Le Gal Y, Ravallec-Plé R, Bergé J-P, Picot L, Piot J-M, Batista I, Thorkelsson G, Delannoy C, Jakobsen G, Johansson I (2009) Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: impact on peptidic populations. Desalination 244:303–320

Brokisch M (1998) Fats and oils handbook, chapter 3, animal fats and oils. AOAC Press, Champaign, pp 121–173

Byun H-G, Kim S-K (2001) Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (*Theragra chalcogramma*) skin. Process Biochem 36:1155–1162

Byun H-G, Kim Y-T, Park P-J, Lin X, Kim S-K (2005) Chitooligosaccharides as a novel β-secretase inhibitor. Carbohydr Polym 61:198–202

Byun H-G, Lee JK, Park HG, Jeon J-K, Kim S-K (2009) Antioxidant peptides isolated from the marine rotifer, *Brachionus rotundiformis*. Process Biochem 44:842–846

Ceynowa J, Adamczak P (2001) Analysis of the bond graph network model of membrane reactor for olive oil hydrolysis. Sep Purif Technol 22:443–449

Ceynowa J, Rauchfleisz M (2001) Resolution of racemic *trans*-2-methyl-1-cyclohexanol by lipase-catalysed transesterification in a membrane reactor. J Mol Catal B Enzym 15:71–80

Chabeaud A, Vandanjon L, Bourseau P, Jaouen P, Chaplain-Derouiniot M, Guérard F (2009) Performances of ultrafiltration membranes for fractionating a fish protein hydrolysate: application to the refining of bioactive peptidic fractions. Sep Purif Technol 66:463–471

Chaiklahan R, Chirasuwan N, Loha V, Tia S, Bunnag B (2011) Separation and purification of phycocyanin from *Spirulina* sp. using a membrane process. Bioresour Technol 102:7159–7164

Chemseddine B, Audinos R (1995) An ion-exchange membrane reactor for esterification of oleic acid and methanol: REMI. Catal Today 25:417–422

Cheryan M, Mehaia MA (1990) Membrane bioreactors: enzyme processes. In: Schwartzberg H, Rao MA (eds) Biotechnology and food process engineering. Marcel Dekker, New York

Cho YH, Shim HK, Park J (2003) Encapsulation of fish oil by an enzymatic gelation process using transglutaminase cross-linked proteins. J Food Sci 68:2717–2723

Chowdhury P, Viraraghavan T, Srinivasan A (2010) Biological treatment processes for fish processing wastewater – a review. Bioresour Technol 101:439–449

Cinq-Mars CD, Li-Chan ECY (2007) Optimizing angiotensin I-converting enzyme inhibitory activity of Pacific hake (*Merluccius productus*) fillet hydrolysate using response surface methodology and ultrafiltration. J Agric Food Chem 55:9380–9388

D'Arrigo P, Fasoli E, Pedrocchi-Fantoni G, Servi S, Tessaro D (2005) Membrane assisted coupled enzyme system for phospholipid modification. Enzyme Microb Technol 37:435–440

Dávalos A, Miguel M, Bartolomé B, López–Fandiño R (2004) Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. J Food Prot 67:1939–1944

Deeslie WD, Cheryan M (1982) A CSTR-hollow-fiber system for continuous hydrolysis of proteins. Factors affecting long-term stability of the reactor. Biotechnol Bioeng 24:69–82

Di Bernardini R, Harnedy P, Bolton D, Kerry J, O'Neill E, Mullen AM, Hayes M (2011) Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. Food Chem 124:1296–1307

Diks RMM, Bosley JA (2000) The exploitation of lipase selectivities for the production of acylglycerols. In: Bornscheuer UT (ed) Enzymes in lipid modification. Wiley-VCH, Germany, pp 3–22

Doyen A, Beaulieu L, Saucier L, Pouliot Y, Bazinet L (2011) Demonstration of *in vitro* anticancer properties of peptide fractions from a snow crab by–products hydrolysate after separation by electrodialysis with ultrafiltration membranes. Sep Purif Technol 78:321–329

Doyen A, Saucier L, Beaulieu L, Pouliot Y, Bazinet L (2012) Electroseparation of an antibacterial peptide fraction from snow crab by-products hydrolysate by electrodialysis with ultrafiltration membranes. Food Chem 132:1177–1184

Du L, Fang M, Wu H, Xie J, Wu Y, Li P, Zhang D, Huang Z, Xia Y, Zhou L, Wei D (2013) A novel angiotensin I-converting enzyme inhibitory peptide from *Phascolosoma esculenta* water-soluble protein hydrolysate. J Funct Foods 5:475–483

Fernandez CC (1986) Refinement of fish oil for human consumption: engineering investigations. UMI Dissertation, University of Washington, 342 pp

Gharsallaoui A, Saurel R, Chambin O, Voilley A (2012) Pea (*Pisum sativum*, L.) protein isolate stabilized emulsions: a novel system for microencapsulation of lipophilic ingredients by spray drying. Food Bioprocess Technol 5:2211–2221

Giorno L, Drioli E (2000) Biocatalytic membrane reactors: applications and perspectives. Trends Biotechnol 18:339–349
Giorno L, Molinari R, Drioli E, Bianchi D, Cestic P (1995) Performance of a biphasic organic/aqueous hollow fibre reactor using immobilized lipase. J Chem Tech Biotechnol 64:345–352
Giorno L, Molinari R, Natoli M, Drioli E (1997) Hydrolysis and regioselective transesterification catalyzed by immobilized lipases in membrane bioreactors. J Membr Sci 125:177–187
Giorno L, De Bartolo L, Drioli E (2003) Chapter 9: Membrane bioreactors for biotechnology and medical applications. In: Bhattacharyya D, Butterfield DA (eds) New insights into membrane science and technology: polymeric and biofunctional membranes. Elsevier, Amsterdam
Giorno L, D'Amore E, Mazzei R, Piacentini E, Zhang J, Drioli E, Cassano R, Picci N (2007) An innovative approach to improve the performance of a two separate phase enzyme membrane reactor by immobilizing lipase in presence of emulsion. J Membr Sci 295:95–101
Goto M, Goto M, Nakashio F, Yoshizuka K, Inoue K (1992) Hydrolysis of triolein by lipase in a hollow fiber reactor. J Membr Sci 74:207–214
Gu R-Z, Li C-Y, Liu W-Y, Yi W-X, Cai M-Y (2011) Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (*Salmo salar* L.) skin. Food Res Int 44:1536–1540
Guit RPM, Kloosterman M, Meindersma MM, Mayer M, Meijer EM (1991) Lipase kinetics: hydrolysis of triacetin by lipase from *Candida cylindracea* in a hollow-fiber membrane reactor. Biotechnol Bioeng 38:727–732
Hampel H, Shen Y (2009) Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a biological candidate marker of Alzheimer's disease. Scand J Clin Lab Invest 69:8–12
Haraldsson GG, Kristinsson B (1998) Separation of eicosapentaenoic acid and docosahexaenoic acid in fish oil by kinetic resolution using lipase. J Am Oil Chem Soc 75:1551–1556
Heinzelmann K, Franke K, Velasco J, Márquez–Ruiz G (2000) Microencapsulation of fish oil by freeze-drying techniques and influence of process parameters on oxidative stability during storage. Eur Food Res Technol 211:234–239
Ho WSW, Sirkar KK (1992) Membrane handbook. Chapman & Hall, New York
Horowitz ST, Roseman S, Blumenthal HJ (1957) The preparation of glucosamine oligosaccharides. I. Separation. J Am Chem Soc 79:5046–5049
Hoshino T, Yamane T, Shimizu S (1990) Selective hydrolysis of fish oil by lipase to concentrate n-3 polyunsaturated fatty acids. Agric Biol Chem 54:1459–1467
Huang FC, Ju YH, Huang CW (1997) Enrichment of γ-linolenic acid from borage oil via lipase-catalyzed reactions. J Am Oil Chem Soc 74:977–981
Hubbard NE, Lim D, Erickson KL (1998) Alteration of murine mammary tumorigenesis by dietary enrichment with n-3 fatty acids in fish oil. Cancer Lett 124:1–7
Isono Y, Nakajima M, Nabetani H (1998) Solvent-free esterification of oleic acid and oleyl alcohol using membrane reactor and lipase-surfactant complex. J Ferment Bioeng 86:138–140
Jaouen P, Lépine B, Rossignol N, Royer R, Quéméneur F (1999) Clarification and concentration with membrane technology of a phycocyanin solution extracted from *Spirulina platensis*. Biotechnol Tech 13:877–881
Jayakumar R, Prabaharan M, Nair SV, Tamura H (2010) Novel chitin and chitosan nanofibers in biomedical applications. Biotechnol Adv 28:142–150
Je JY, Kim SK (2005) Water-soluble chitosan derivatives as a BACE1 inhibitor. Bioorg Med Chem 13:6551–6555
Je JY, Qian ZY, Lee SH, Byun HG, Kim SK (2008) Purification and antioxidant properties of bigeye tuna (*Thunnus obesus*) dark muscle peptide on free radical-mediated oxidative systems. J Med Food 11:629–637
Jeon Y-J, Kim S-K (2000) Continuous production of chitooligosaccharides using a dual reactor system. Process Biochem 35:623–632
Jeon Y-J, Byun H-G, Kim S-K (1999) Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochem 35:471–478
Jeon YJ, Shahidi F, Kim S-K (2000) Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. Food Rev Int 16:159–176

John V, Beck JP, Bienkowski MJ, Sinha S, Heinrikson RL (2003) Human β-secretase (BACE) and BACE inhibitors. J Med Chem 46:4625–4630

Jonzo MD, Hiol A, Zagol I, Druet D, Comeau L-C (2000) Concentrates of DHA from fish oil by selective esterification of cholesterol by immobilized isoforms of lipase from C*andida rugosa*. Enzyme Microb Technol 27:443–450

Jun S-Y, Park P-J, Jung W-K, Kim S-K (2004) Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. Eur Food Res Technol 219:20–26

Jung W-K, Qian Z-J, Lee S-H, Choi SY, Sung NJ, Byun H-G, Kim S-K (2007) Free radical scavenging activity of a novel antioxidative peptide isolated from *in vitro* gastrointestinal digests of *Mytilus coruscus*. J Med Food 10:197–202

Kajitvichyanukul P, Hung Y-T, Wang LK (2008) Membrane technologies for oil–water separation. Membr Desalination Technol 13:639–668

Kim S-K, Mendis E (2000) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39:383–393

Kim S-K, Byun H-G, Jeon Y-J (1999a) Desalination of tuna boiled extract by electrodialysis. J Korean Fish Soc 32:68–74

Kim S-K, Byun H-G, Jeon Y-J, Joo D-S, Kim J-B (1999b) Development of natural seasoning using desalinated tuna boiled extract. J Korean Fish Soc 32:75–82

Kim S-K, Kim Y-T, Byun H-G, Nam K-S, Joo D-S, Shahidi F (2001) Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. J Agr Food Chem 49:1984–1989

Kim E-K, Kim Y-S, Hwang J-W, Kang S-H, Choi D-K, Lee K-H, Lee JS, Moon S-H, Jeon B-T, Park P-J (2013) Purification of a novel nitric oxide inhibitory peptide derived from enzymatic hydrolysates of *Mytilus coruscus*. Fish Shellfish Immunol 34:1416–1420

Ko S-C, Kang MC, Lee J-K, Byun H-G, Kim S-K, Lee S-C, Jeon B-T, Park P-J, Jung W-K, Jeon Y-J (2011) Effect of angiotensin I-converting enzyme (ACE) inhibitory peptide purified from enzymatic hydrolysates of *Styela plicata*. Eur Food Res Technol 233:915–922

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40:43–81

LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-β in Alzheimer's disease. Nat Rev Neurosci 8:499–509

Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A (2004) Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 79:935–945

Lee JK, Jeon J-K, Byun H-G (2011) Effect of angiotensin I converting enzyme inhibitory peptide purified from skate skin hydrolysate. Food Chem 125:495–499

Leigh-Firbank EC, Minihane AM, Leake DS, Wright JW, Murphy MC, Griffin BA, Williams CM (2002) Eicosapentaenoic acid and docosahexaenoic acid from fish oils: differential associations with lipid responses. Br J Nutr 87:435–445

Li-Chan ECY, Hunag S-L, Jao C-L, Ho K-P, Hsu K-C (2012) Peptides derived from Atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. J Agr Food Chem 60:973–978

Linder M, Matouba E, Fanni J, Parmentier M (2002) Enrichment of salmon oil with n-3 PUFA by lipolysis, filtration and enzymatic re-esterification. Eur Lipid Sci Technol 104:455–462

Lo YM, Cao D, Argin-Soysal S, Wang J, Hahm T-S (2005) Recovery of protein from poultry processing wastewater using membrane ultrafiltration. Bioresour Technol 96:687–698

Lopez JL, Matson SL (1997) A multiphase/extractive enzyme membrane reactor for production of diltiazem chiral intermediate. J Membr Sci 125:189–211

Lopez JL, Wald SA, Matson SL, Quinn JA (1990) Multiphase membrane reactors for separating stereoisomers. Ann N Y Acad Sci 613:155–166

Lye GJ, Pavlou OP, Rosjidi M, Stuckey DC (1996) Immobilization of *Candida cylindracea* lipase on colloidal liquid aphrons (CLAs) and development of a continuous CLA-membrane reactor. Biotechnol Bioeng 51:69–78

Madene A, Jacquot M, Scher J, Desobry S (2006) Flavour encapsulation and controlled release—a review. Int J Food Sci Technol 41:1–21

Martin-Orue C, Bouhallab S, Garem A (1998) Nanofiltration of amino acid and peptide solutions: mechanisms of separation. J Membr Sci 142:225–233

McNeill GP, Ackman RG, Moore SR (1996) Lipase-catalyzed enrichment of long-chain polyunsaturated fatty acids. J Am Oil Chem Soc 73:1403–1407

Mohammadi T, Kaviani A (2003) Water shortage and seawater desalination by electrodialysis. Desalination 158:267–270

Molinari R, Santoro ME, Drioli E (1994) Study and comparison of two enzyme membrane reactors for fatty acids and glycerol production. Ind Eng Chem Res 33:2591–2599

Moore SR, McNeill GP (1996) Production of triglycerides enriched in long-chain n-3 polyunsaturated fatty acids from fish oil. J Am Oil Chem Soc 73:1409–1414

Mukutmoni-Norris M, Hubbard NE, Erickson KL (2000) Modulation of murine mammary tumor vasculature by dietary n-3 fatty acids in fish oil. Cancer Lett 150:101–109

Mullally MM, O'Callaghan DM, FitzGerald RJ, Donnelly WJ, Dalton JP (1994) Proteolytic and peptidolytic activities in commercial pancreatic protease preparations and their relationship to some whey protein hydrolyzate characteristics. J Agric Food Chem 42:2973–2981

Nakamura K, Hakoda M (1995) Ultrafiltration of reverse micelles containing enzyme. J Chem Eng Jpn 28:14–18

Okamura N, Suemoto T, Shiomitsu T, Suzuki M, Shimadzu H, Akatsu H, Yamamoto T, Arai H, Sasaki H, Yanai K, Staufenbiel M, Kudo Y, Sawada T (2004) A novel imaging probe for *in vivo* detection of neuritic and diffuse amyloid plaques in the brain. J Mol Neurosci 24:247–255

Park HS, Choi JS, Kim KH (2000a) Docosahexaenoic acid-rich fish oil and pectin have a hypolipidemic effect, but pectin increases risk factor for colon cancer in rats. Nutr Res 20:1783–1794

Park P-J, Lee S-H, Kim S-K (2000b) Desalination of boiled oyster extract by electrodialysis. Korean J Biotechnol Bioeng 15:167–173

Pei X, Yang R, Zhang Z, Gao L, Wang J, Xu Y, Zhao M, Han X, Liu Z, Li Y (2010) Marine collagen peptide isolated from Chum Salmon (*Oncorhynchus keta*) skin facilitates learning and memory in aged C57BL/6J mice. Food Chem 118:333–340

Piazza GJ, Foglia TA, Nuñez A (2000) Epoxidation of fatty acids with membrane-supported peroxygenase. Biotechnol Lett 22:217–221

Pirich C, Gaszo A, Granegger S, Sinzinger H (1999) Effects of fish oil supplementation on platelet survival and ex vivo platelet function in hypercholesterolemic patients. Thromb Res 96:219–227

Porter MC (1972) Concentration polarization with membrane ultrafiltration. Ind Eng Chem Prod Res Dev 11(3):234–248

Prazeres DMF, Cabral JMS (1994) Enzymatic membrane bioreactors and their applications. Enzyme Microb Technol 16:738–750

Prazers DMF, Garcia FAP, Cabral JMS (1993) An ultrafiltration membrane bioreactor for the lipolysis of olive oil in reversed micellar media. Biotechnol Bioeng 41:761–770

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

Rajapakse N, Mendis E, Byun H-G, Kim S-K (2005b) Purification and in vitro antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. J Nutr Biochem 16:562–569

Ramakrishnan S, Ferrando M, Aceña-Muñoz L, De Lamo-Castellví S, Güell C (2013) Fish oil microcapsules from O/W emulsions produced by premix membrane emulsification. Food Bioprocess Technol. doi:10.1007/s11947-012-0950-2

Rasmussen RS, Morrissey MT (2007) Marine biotechnology for production of food ingredients. Adv Food Nutr Res 52:237–292

Rice KE, Watkins J, Hill CG Jr (1999) Hydrolysis of menhaden oil by a *Candida cylindracea* lipase immobilized in a hollow-fiber reactor. Biotechnol Bioeng 63:33–45

Rose DP, Connolly JM, Rayburn J, Coleman M (1995) Influence of diets containing eicosapentaenoic or docosahexaenoic acid on growth and metastasis of breast cancer cells in nude mice. J Nat Cancer Inst 87:587–592

Rosenberg M (1995) Current and future applications for membrane processes in the dairy industry. Trends Food Sci Technol 6:12–19

Rucka M, Turkiewicz B (1990) Ultrafiltration membranes as carriers for lipase immobilization. Enzyme Microb Technol 12:52–55

Sadrzadeh M, Mohammadi T (2008) Sea water desalination using electrodialysis. Desalination 221:440–447

Sahashi Y, Ishizuka H, Koike S, Suzuki K (1993) Purification of polyunsaturated fatty acids from fish oil hydrolyzate by membrane/extraction system. Prog Bioseparation Eng 33:79–83

Sakaki K, Giorno L, Drioli E (2001) Lipase-catalyzed optical resolution of racemic naproxen in biphasic enzyme membrane reactors. J Membr Sci 184:27–38

Samaranayaka AGP, Li-Chan ECY (2011) Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications. J Funct Foods 3:229–254

Scott K, Hughes R (1996) Industrial Membrane Separation Technology; Membrane classification. Chapman & Hall, Wester Cleddens Road, Bishopbriggs, Glasgow, UK, pp 3–5

Shahidi F, Zhong Y (2005) Antioxidants: regulatory status. In: Shahidi F (ed) Bailey's industrial oil and fat products, vol I, 6th edn. John Wiley & Sons, New York, pp 491–512

Simopoulos AP, Leaf A, Salem N Jr (1999) Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann Nutr Metab 43:127–130

Skanderby M (1994) Protein hydrolysates: their functionality and applications. Food Technol Int Eur 10:141–144

Skeggs LT, Kahn JE, Shumway NP (1957) The preparation and function of the angiotensin-converting enzyme. J Exp Med 103:295–299

Snape JB, Nakajima M (1996) Processing of agricultural fats and oils using membrane technology. J Food Eng 30:1–41

Sroka Z (1994) The activity of lipase from *Rhizopus* sp. in native form and after immobilization on hollow-fiber membranes. J Membr Sci 97:209–214

Sutherland K (2004) Profile of the international membrane industry: market prospects to 2008, 3rd edn. Elsevier, Oxford

Tanaka Y, Hirano J, Funada T (1992) Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with *Candida cylindracea* lipase. J Am Oil Chem Soc 69:1210–1214

Thiansilakul Y, Benjakul S, Shahidi F (2007) Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem 103:1385–1394

Tsuru T, Shutou T, Nakao SI, Kimura S (1994) Peptide and amino acid separation with nanofiltration membranes. Sep Sci Technol 29:971–984

Van der Padt A, Edema MJ, Sewalt JJW, Vant't Riet K (1990) Enzymatic acylglycerol synthesis in a membrane bioreactor. J Am Oil Chem Soc 67:347–352

Wang S-L, Yen Y-H, Tzeng G-C, Hsieh C (2005) Production of antifungal materials by bioconversion of shellfish chitin wastes fermented by *Pseudomonas fluorescens* K-188. Enzyme Microb Tech 36;49–56

Warmuth W, Wenzig E, Mersmann A (1995) Selection of a support for immobilization of a microbial lipase for the hydrolysis of triglycerides. Bioprocess Eng 12:87–93

Wildman REC, Kelley M (2007) Handbook of nutraceuticals and functional foods; second edition. CRC Press, New York

Wu DR, Cramer SM, Belfort G (1993) Kinetic resolution of racemic glycidyl butyrate using a multiphase membrane enzyme reactor: experiments and model verification. Biotechnol Bioeng 41:979–990

Xu X, Skands A, Jonsson G, Adler-Nissen J (2000) Production of structured lipids by lipase-catalysed interesterification in an ultrafiltration membrane reactor. Biotechnol Lett 22:1667–1671

Yoshii H, Furuta T, Yasunishi A, Linko Y-Y, Linko P (1996) Oxidation stability of eicosapentaenoic and docosahexaenoic acid included in cyclodextrins. J Inclus Phenom Mol Recogn Chem 25:217–220

You L, Zhao M, Liu RH, Regenstein JM (2011) Antioxidant and antiproliferative activities of Loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. J Agric Food Chem 59:7948–7953

You L, Ren J, Yang B, Regenstein J, Zhao M (2012) Antifatigue activities of loach protein hydrolysates with different antioxidant activities. J Agric Food Chem 60:12324–12331

Yu D, McLean MD, Hall JC, Ghosh R (2008) Purification of monoclonal antibody from tobacco extract using membrane-based bioseparation techniques. J Membr Sci 323:159–166

Zeng MY, Li BF, Chen SJ, Li LH, Wu YY (2005) Study on enzymic hydrolytic conditions of skin collagen from *Oreochromis niloticus*. Chin J Mar Drugs 24:24–29

Zhao Y, Li B, Dong S, Liu Z, Zhao X, Wang J, Zeng M (2009) A novel ACE inhibitory peptide isolated from *Acaudina molpadioidea* hydrolysate. Peptides 30:1028–1033

Zhu Y, Minet RG, Tsotsis TT (1996) A continuous pervaporation membrane reactor for the study of esterification reactions using a composite polymeric/ceramic membrane. Chem Eng Sci 51:4103–4113

Chapter 5
Processing and Characterization of Salt-Fermented Fish (Jeotgal) Using Seafood By-products in Korea

Jin-Soo Kim and Ki Hyun Kim

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1 Introduction

Annually, over 7.55 million metric tons (M/T) of aquatic products are harvested and imported in Korea (Statistics Korea 2012), and they are used as raw material for preparing various seafood products, such as frozen foods, dried foods, smoked foods, salted foods, canned foods, seasoned foods, surimi seafoods, and salt-fermented fish (jeotgal). During the processing of these seafood products, seafood processing by-products, such as head, gill, fish frame, viscera, scale, and skin, are

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generated in a large quantity (approximately more than 50 % based on the whole fish) (Je et al. 2007; Kim and Park 2004). These by-products could serve as an excellent food resource for extracting taste components (Han et al. 2007; Heu et al. 2007), muscle (Wendel 1999), minerals (Kim et al. 2003a), enzymes (Ezquerra-Brauer et al. 2002; Kishimura et al. 2001), lipids (Kim et al. 1997), and collagen (Muyong et al. 2004; Ogawa et al. 2004; Park et al. 2007). However, most seafood processing by-products are conventionally used to produce fish meal and fertilizer or are directly discharged into estuaries, resulting in environmental pollution (Ciarlo et al. 1997). Various challenges must be faced in order to find a way to upgrade the processing of by-products to food-grade ingredients.

Jeotgal, the Korean traditional salt-fermented fishery, is usually prepared by the addition of salt to freshwater or marine fish, shellfish, crustaceans, and its by-products before fermentation (Guan et al. 2011). Among these jeotgal, a part is prepared by using seafood processing by-products, such as roe, viscera containing stomach and intestine, and gill.

The favorable taste and flavor of the jeotgal using seafood by-products may also develop gradually during fermentation by several enzymatic reactions and microbial degradation processes. Thus, the jeotgal products are popular for consumption not only as side dishes, but also as ingredients in preparing kimchi, because they contain relatively high amounts of amino acids, the degradation products of fish protein. If the use of seafood processing by-products as resources of jeotgal could be increased, it will become a path for the effective use of the processing of by-products.

This chapter will discuss the kinds of seafood by-products as resources of jeotgal, classification of jeotgal using seafood by-products (by-product jeotgal), manufacturing methods of by-product jeotgal, its microbiological and biochemical characterizations, and safety.

2 Classification and Output of Jeotgal

According to the major ingredients and regional preparation methods, more than 160 kinds of jeotgal are known to exist in Korea, of which about 31 kinds are sold commercially (Mah et al. 2002).

2.1 Classification

2.1.1 Classification by Parts and Kinds of Fishery Used

As shown in Table 5.1, the commercial jeotgal can be subdivided into four groups of whole-fish jeotgal (17 kinds: anchovy jeotgal, sardine jeotgal, yellow croaker jeotgal, bighead croaker jeotgal, squid jeotgal, pearl-spot chromis jeotgal, konoshiro gizzard shad jeotgal, dark-banded rockfish jeotgal, scaly hairfin anchovy jeotgal, jack mackerel jeotgal, slime flounder jeotgal, big-eyed herring jeotgal, largehead hairtail

Table 5.1 Classification of salt-fermented fish (jeotgal) by parts and kinds of fishery used

Raw material			
Whole fish	By-products	Mollusks	Crustaceans
Anchovy jeotgal	Abalone viscera jeotgal	Surf clam jeotgal	Shrimp jeotgal
Dark-banded rockfish jeotgal	Changran jeotgal	Japanese littleneck jeotgal	Fresh shrimp jeotgal
Japanese sardinella jeotgal	Chub mackerel viscera jeotgal	Hard clam jeotgal	Crab pickle in soy sauce
Konoshiro gizzard shad jeotgal	Konoshiro gizzard shad stomach jeotgal	Oyster jeotgal	
Largehead hairtail jeotgal	Largehead hairtail viscera jeotgal	Variously colored abalone jeotgal	
Big-eyed herring jeotgal	Myungran jeotgal	Jacknife clam jeotgal	
Pearl-spot chromis jeotgal	Pacific cod gill jeotgal	Squid jeotgal	
Bighead croaker jeotgal	Sea cucumber viscera jeotgal	Spiny top shell jeotgal	
Scaly hairfin anchovy jeotgal	Sea urchin egg jeotgal	Whiparm octopus jeotgal	
Slime flounder jeotgal		Beka squid jeotgal	
Yellow croaker jeotgal		Mitre squid jeotgal	
Jack mackerel jeotgal			

From Lee et al. (1986a), with permission

jeotgal, Japanese sardinella jeotgal, beka squid jeotgal, mitre squid jeotgal, and whiparm octopus jeotgal), shellfish jeotgal (six kinds: surf clam jeotgal, hard clam jeotgal, oyster jeotgal, Japanese littleneck jeotgal, spiny top shell jeotgal, and variously colored abalone jeotgal), crustacean jeotgal (three kinds: shrimp jeotgal, fresh shrimp jeotgal, and crab pickle in soy sauce), and by-product jeotgal [nine kinds: changran jeotgal (salt-fermented intestine and stomach of Alaska pollock), myungran jeotgal (low-salt fermented roe of Alaska pollock), Pacific cod gill jeotgal, sea cucumber viscera jeotgal, chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal, abalone viscera jeotgal, konoshiro gizzard shad stomach jeotgal (jenoe bam jeotgal in Korean), and sea urchin egg jeotgal] according to the type, such as fish, shellfish, and crustaceans, and part of fishery used, such as whole fish and its processing by-products. Shellfishes are fermented after deshelling, while crustaceans, fish, and by-products are fermented without removing the shell or the skin.

2.1.2 Classification by Salinity of Jeotgal

According to the salinity of jeotgal, by-product jeotgal can be subdivided into three groups of traditional jeotgal: high-salt jeotgal, of more than 18 % salinity, medium-salt jeotgal, with salinity in the range 12–18 %, and low-salt jeotgal of less than 12 % salinity (Table 5.2). Among the nine kinds of commercial by-product jeotgal, four kinds (Pacific cod gill jeotgal, chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal, and konoshiro gizzard shad stomach jeotgal)

Table 5.2 Classification of commercial by-product jeotgal by salinity

Salinity of jeotgal		
High (more than 18 %)	Medium (12–18 %)	Low (less than 12 %)
Chub mackerel viscera jeotgal	Sea cucumber viscera jeotgal	Myungran jeotgal
Largehead hairtail viscera jeotgal	Abalone viscera jeotgal	Changran jeotgal
Konoshiro gizzard shad stomach jeotgal		Sea urchin egg jeotgal
Pacific cod gill jeotgal		

belong to the domain of high-salt jeotgal, two kinds (abalone viscera jeotgal and sea cucumber viscera jeotgal) belong to that of medium-salt jeotgal, and three kinds (changran jeotgal, myungran jeotgal, and sea urchin jeotgal) belong to that of low-salt jeotgal.

Sodium chloride is an essential ingredient for preparing jeotgal, contributing not only to the flavor, but also to the microbiological stability (Phelps et al. 2006). However, changes in consumers' life styles, who are concerned about the harmful effects of a high level of sodium chloride in their diet, motivate the tendency to reduce the amount of sodium in food (Llorente Holgado et al. 2007; Lynch 1987). In that sense, the consumption of jeotgal, including by-product jeotgal, is limited due to the high concentration of salt. Therefore, more research on the development of low-salt jeotgal with similar taste and shelf life compared to high-salt jeotgal is needed for the wide consumption of jeotgal.

2.2 Output of Jeotgal

In 2011, 27,228 M/T of jeotgal in Korea was produced, which was 2.0 % based on the output of seafood products (Fig. 5.1) (Fisheries Information Service 2012). Among these commercial jeotgal, the output was highest in anchovy jeotgal (10,999 M/T), followed by shrimp jeotgal (4,864 M/T) and those of myungran jeotgal (4,643 M/T) and changran jeotgal (522 M/T), which are jeotgal produced using seafood processing by-products.

These anchovy and shrimp jeotgal are mainly used for subingredients of kimchi, while myungran jeotgal and changran jeotgal are mainly used as side dishes.

3 Kinds of Seafood Processing By-products

As shown in Table 5.3, the seafood processing industry is still producing a large quantity of by-products and discards in various processing procedures. These include heads, skin, fish frames, viscera, gills, and fish roe. Most seafood processing by-products are used to produce animal and aquaculture feed, food ingredients, and

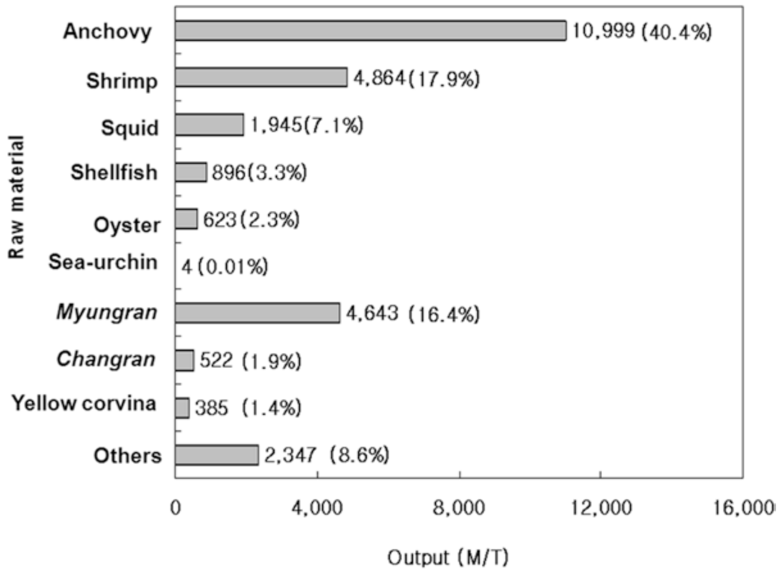


Fig. 5.1 Output of jeotgal in Korea in 2011

Table 5.3 Flow chart for processing Alaska pollock surimi and the mass balance. Processing flow of surimi and waste (From Kim and Park (2007), with permission)

Product			Surimi processing		Byproduct	
Yield	Product				Byproduct	Ratio
100%	Whole fish	←	Whole fish			0%
80~85%	Gutted fish	←	Gutting	→	Viscera	15~20%
60~71%	Headed and gutted	←	Deheading	→	Heads	14~20%
43~57%	Fillet skin -on	←	Filleting	→	Frames	14~17%
33~49%	Mince	←	Mincing	→	Skin/Bones	8~10%
17~35%	Washed mince	←	Washing	→	Soluble proteins	14~16%
12~30%	Refined mince	←	Refining	→	Connective tissue	4~6%
			Mixing	←	Cryoprotectans	0%
18~36%	Surimi	←	Surimi			

novel and value-added products for the nutraceutical, pharmaceutical, and fine chemical industries (Shahidi 2007).

However, among these seafood processing by-products, a part of gills, viscera, and fish roe is used as raw material for preparing various jeotgal, such as Pacific cod gill jeotgal, changran jeotgal, chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal,

konoshiro gizzard shad stomach jeotgal, sea cucumber viscera jeotgal, abalone viscera jeotgal, myungran jeotgal, and sea urchin roe jeotgal, in Korea, as shown in Table 5.4.

Therefore, one of the efficient uses of seafood by-products will be wide and continuous consumption by upgrading the quality of the by-product jeotgal.

4 Processing of By-product Jeotgal

The favorable taste and flavor of by-product jeotgal may also develop gradually during fermentation by several enzymatic reactions and microbial degradation processes (Lee 1993). There are also differences in the sensory properties on the taste, flavor, and color of jeotgal according to the source of the raw materials and the processing methods used in the manufacturing process (Kim et al. 1993).

In this section, the processing methods for nine kinds of by-product jeotgal will be discussed in detail.

4.1 Viscera Jeotgal

4.1.1 Low-Salt Fermented Intestine and Stomach of Alaska Pollock (Changran Jeotgal)

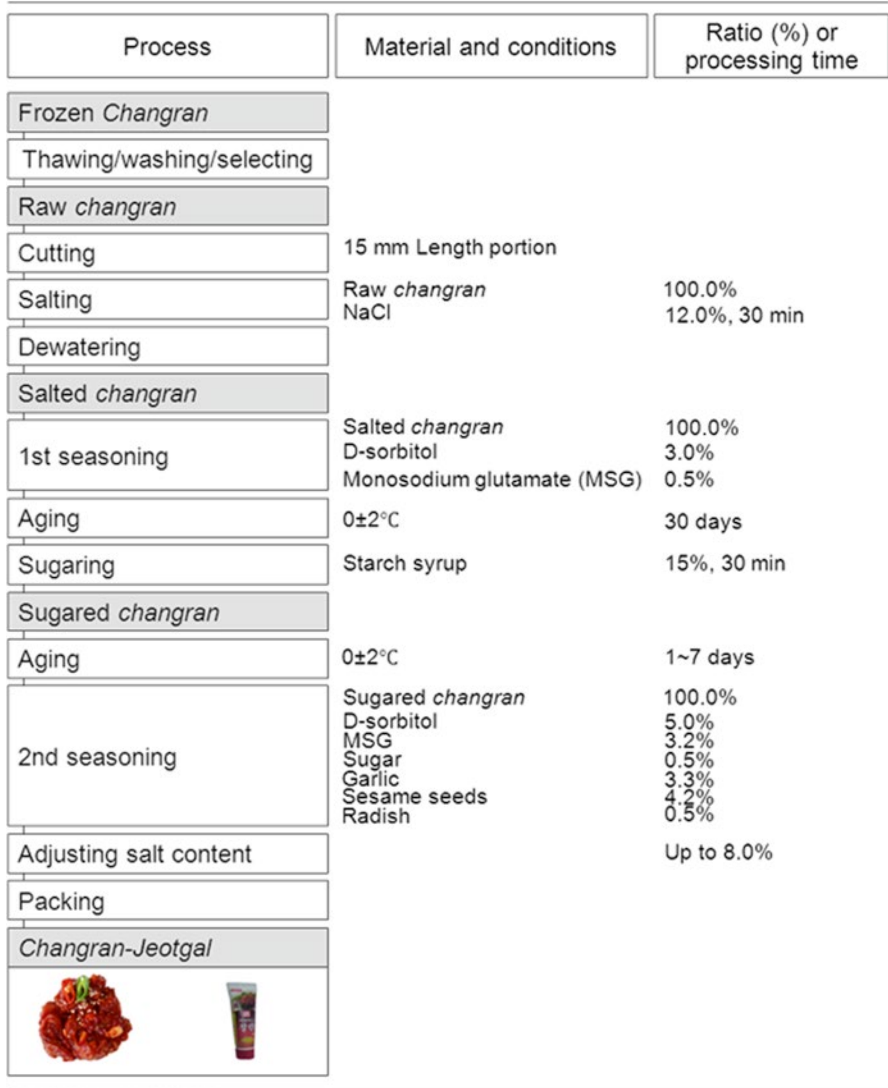
Changran jeotgal is the representative low-salt jeotgal (Jo et al. 2004a, b). It is very popular in Japan and other Eastern countries, as well as Korea.

The general processing procedure of changran jeotgal is shown in Fig. 5.2. Unlike most processing by-products, changran, which is stomach and intestine of Alaska pollock, has been effectively used to produce changran jeotgal. The ratio of changran is about 18 % based on the viscera weight (Lee et al. 1997).

For processing changran jeotgal, frozen changran is thawed, washed three times with tap water, selected after removing any odd substances, such as bones and parasites, and cut into 15 mm length portions. To adjust the final salt content to 8 %, the first seasoning is conducted and mixed for 30 min after adding 12 % salt based on the weight of the material. The drip water is removed and mixed again for 30 min with the addition of sorbitol and monosodium glutamate (MSG).

Table 5.4 Kinds of commercial by-product jeotgal

Raw material		
Viscera	Roe	Gill
Changran jeotgal	Myungran jeotgal	Pacific cod gill jeotgal
Chub mackerel viscera jeotgal	Sea urchin roe jeotgal	
Largehead hairtail viscera jeotgal		
Konoshiro gizzard shad stomach jeotgal		
Sea cucumber viscera jeotgal		
Abalone viscera jeotgal		



Process	Material and conditions	Ratio (%) or processing time
Frozen *Changran*		
Thawing/washing/selecting		
Raw *changran*		
Cutting	15 mm Length portion	
Salting	Raw *changran* NaCl	100.0% 12.0%, 30 min
Dewatering		
Salted *changran*		
1st seasoning	Salted *changran* D-sorbitol Monosodium glutamate (MSG)	100.0% 3.0% 0.5%
Aging	0±2℃	30 days
Sugaring	Starch syrup	15%, 30 min
Sugared *changran*		
Aging	0±2℃	1~7 days
2nd seasoning	Sugared *changran* D-sorbitol MSG Sugar Garlic Sesame seeds Radish	100.0% 5.0% 3.2% 0.5% 3.3% 4.2% 0.5%
Adjusting salt content		Up to 8.0%
Packing		
Changran-Jeotgal		

Fig. 5.2 General processing procedure of changran jeotgal

The seasoned changran is fermented at 0 ± 2°C for 30 days. After aging, starch syrup is added and mixed for 30 min, and then stored for 24 h before adding the second seasoning (Jo et al. 2003). After 30 min, the final salt content of 8 % is re-adjusted. The seasoned changran jeotgal is packed into glass bottles.

The changran jeotgal is characterized by its specific flavor, chewy texture, and grayish red color.

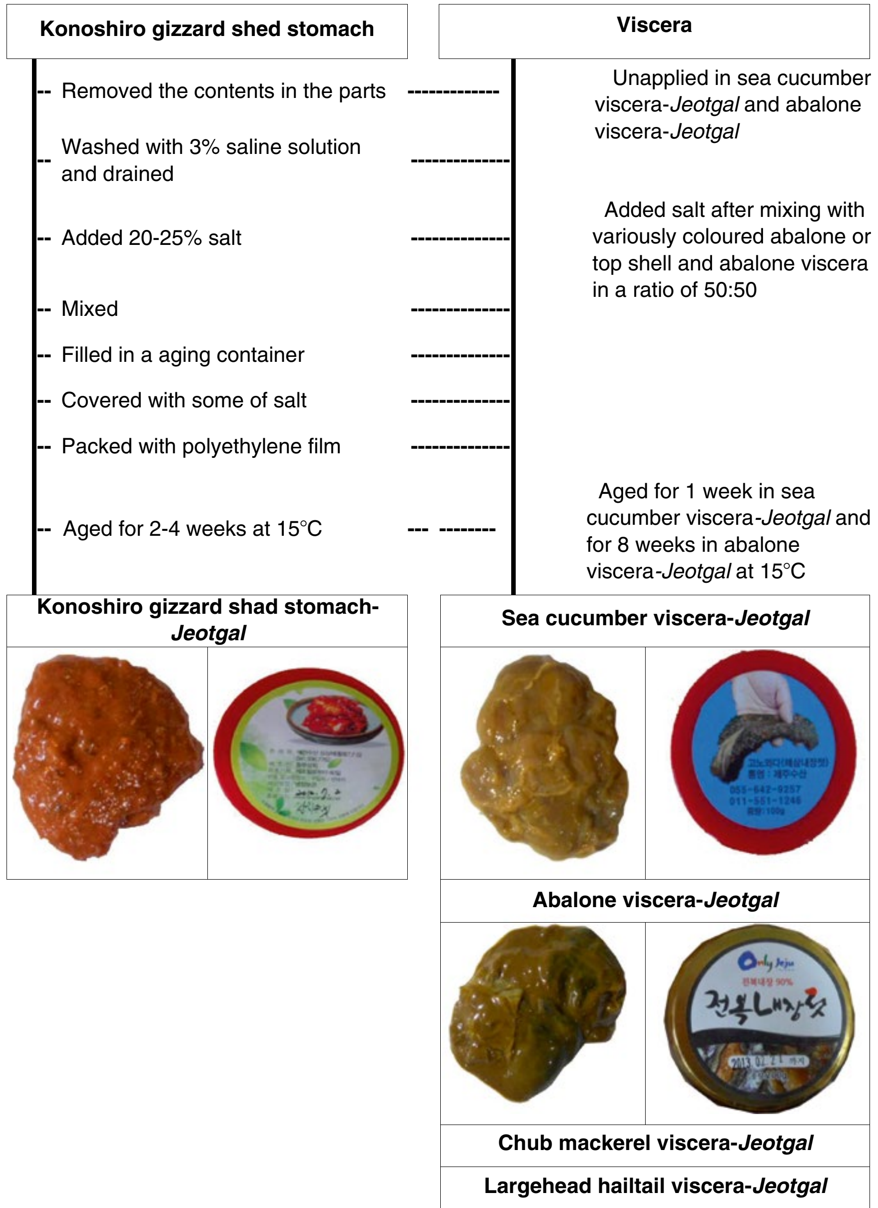


Fig. 5.3 General processing procedure of the other viscera jeotgal

4.1.2 Other Viscera Jeotgal

The general processing procedure of the other viscera jeotgal, such as chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal, konoshiro gizzard shad

Table 5.5 Sensory characterizations of the other viscera jeotgal

	Sensory item		
Jeotgal	Color	Texture	Taste
Chub mackerel viscera jeotgal	Grayish-white	Chewy texture	Specific flavor and salty smell
Largehead hairtail viscera jeotgal	Gray	Soft texture	Specific flavor and salty smell
Konoshiro gizzard shad stomach jeotgal	Dark-brown	Chewy texture	Salty smell
Sea cucumber viscera jeotgal	Yellow-brown	Very soft texture	Sour and fishy taste
Abalone viscera jeotgal	Grayish-brown	Soft texture	Specific flavor and low salty taste

stomach jeotgal, sea cucumber viscera jeotgal, and abalone viscera jeotgal, except for changran jeotgal are shown in Fig. 5.3. Intestine is used as raw material for preparing chub mackerel viscera jeotgal and largehead hairtail viscera jeotgal, stomach is used as raw material for preparing konoshiro gizzard shad stomach jeotgal, and viscera is used as raw material for preparing sea cucumber viscera jeotgal and abalone viscera jeotgal. Therefore, unlike other by-products, fishery intestine, stomach, and viscera are effectively used as resources for preparing various jeotgal.

Except for changran jeotgal, for preparing the other viscera jeotgal, konoshiro gizzard shad stomach, chub mackerel viscera, and largehead hairtail viscera are pretreated by cutting into pieces and removing the contents in the parts. These internal organs of sea cucumber viscera and abalone viscera are washed three times with tap water and drained before mixing with 25 % salt. But the drained abalone is salted after mixing with a same weight of variously colored abalone or spiny top shell based on the drained abalone. The top of the internal organs filled in an aging container is covered with some quantity of salt and packed with polyethylene film to prevent entry by insects before aging for 2–4 weeks in the shade.

The sensory characterizations of the other viscera jeotgal are shown in Table 5.5.

4.2 Roe Jeotgal

4.2.1 Low-Salt Fermented and Seasoned Roe of Alaska Pollock (Myungran Jeotgal)

Myeongran jeotgal is a low-salt fermented and seasoned roe of Alaska pollock, which is called mentaiko in Japanese. Myeongran jeotgal is used as a common ingredient in Japanese and Korean cuisines.

For preparing myeongran jeotgal, the roe is extracted from Alaska pollock and separated from the other viscera after heading. It is gently spray washed, sorted, and graded. The resultant roe is packed and quickly frozen in a contact plate freezer.

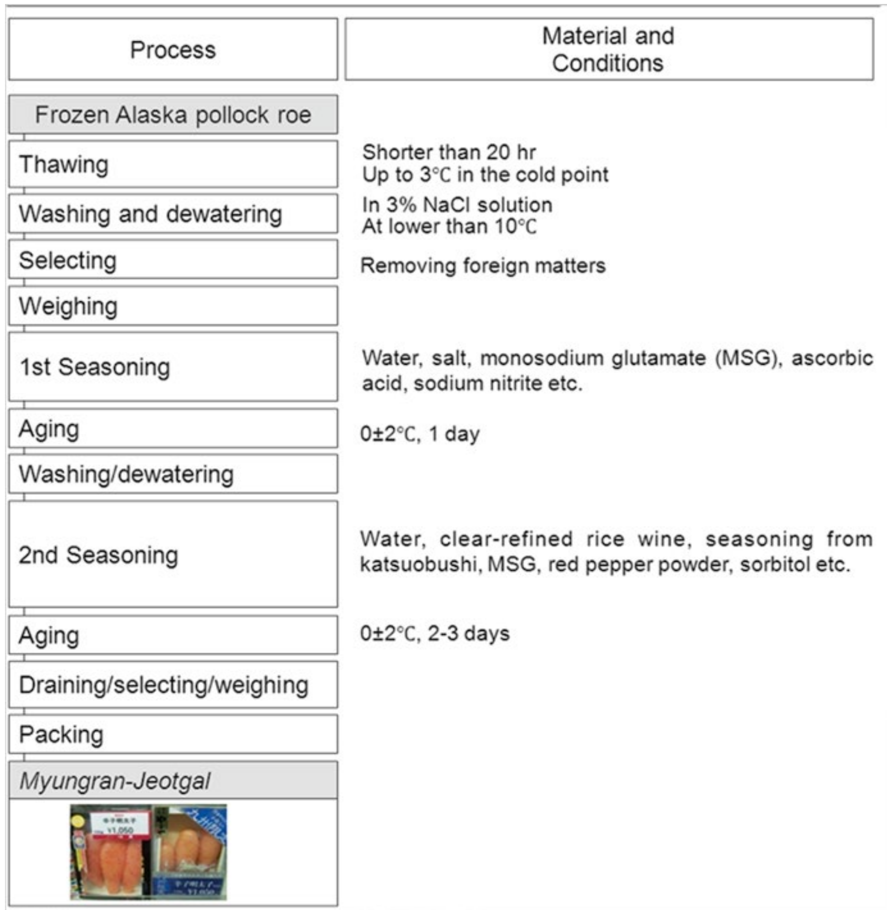


Fig. 5.4 General processing procedure of myungran jeotgal

The most valuable form of myungran jeotgal is whole, matched pairs of skeins, defect free, and preferably with the oviduct intact.

The general processing procedure of myungran jeotgal is shown in Fig. 5.4. For preparing myeongran jeotgal, frozen myungran (Alaska pollock roe) is thawed, washed with 3 % saline, and selected after removing foreign materials. The first seasoning is conducted and aged at 0 ± 2°C for 20 ± 5 days before washing and separating. The aged myungran jeotgal is reseasoned with water, clear-refined rice wine, seasoning from katsuobushi, MSG, red pepper powder, sorbitol, and so on, and packed before shipment. The resultant myungran jeotgal may be dyed (mostly red) and/or flavored. There are literally dozens of grades of myungran jeotgal, depending on the type and degree of the defect.

Myungran jeotgal is characterized by its low salty and sweet taste, and reddish-yellow color.

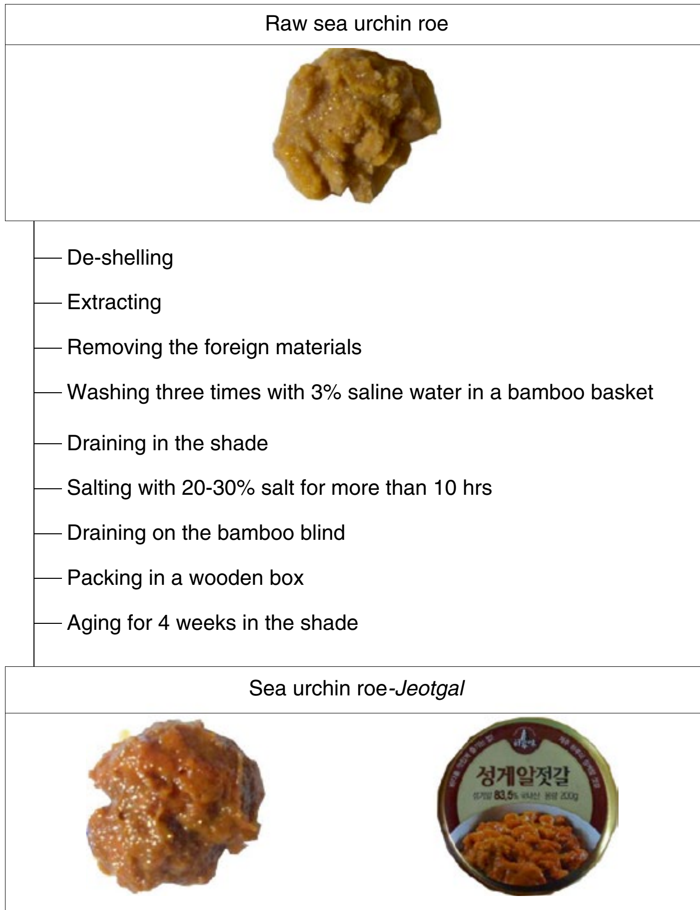


Fig. 5.5 General processing procedure of sea urchin roe jeotgal

4.2.2 Salt-Fermented Sea Urchin Roe (Sea Urchin Roe Jeotgal)

For the preparation of sea urchin roe jeotgal, as shown in Fig. 5.5, the roe is immediately extracted from the sea urchin and removed from the foreign material after deshelling. It is gently washed three times with 3 % saline water in a bamboo basket and drained in the shade. The resultant roe is salted with 20–30 % salt for more than 10 h and drained on a bamboo blind before packing in a wooden box. The packed roe is aged for 4 weeks in the shade.

Sea urchin roe jeotgal is characterized by its specific flavor, low salty smell, and brownish-yellow color.

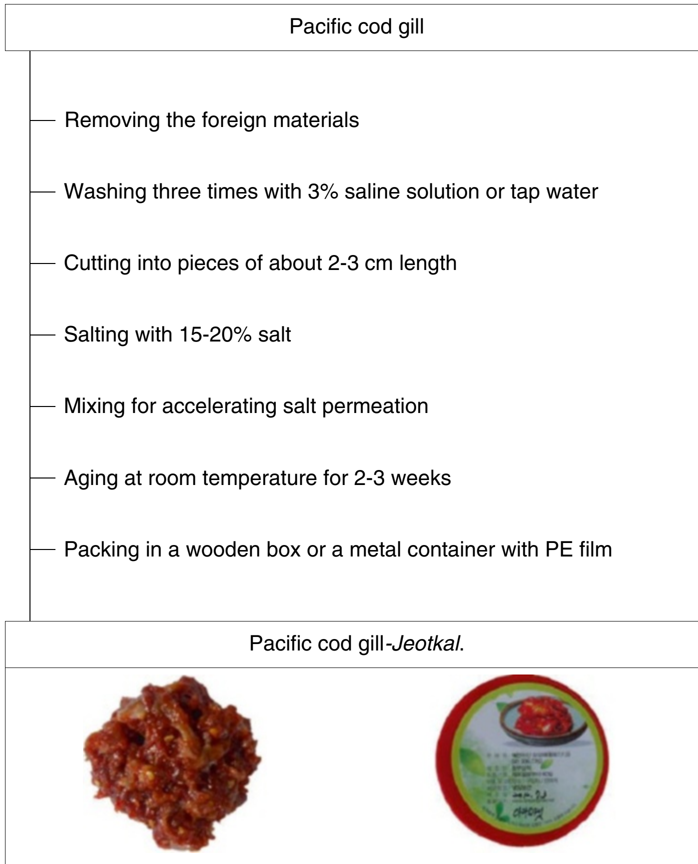


Fig. 5.6 General processing procedure of Pacific cod gill jeotgal

4.3 Salt-Fermented Pacific Cod Gill (Pacific Cod Gill Jeotgal)

The Pacific cod gill is generated in large quantities from Pacific cod processing factories. It can be used as a raw material for the preparation of Pacific cod gill jeotgal.

For the preparation of Pacific cod gill jeotgal as shown in Fig. 5.6, the Pacific cod gill generated during Pacific cod processing is thoroughly washed with saline solution or tap water and cut into 2–3 cm length portions before adding ingredients, as shown in Table 5.6. The permeation of salt into the Pacific cod gill is relatively slow. To improve this, Pacific cod gill should be mixed well with the subingredients. The salted Pacific cod gill is aged at room temperature for 2–3 weeks and packed in a wooden box or a metal container with PE film.

The Pacific cod gill jeotgal is characterized by its chewy texture, salty and sweet taste, and grayish-red color.

Table 5.6 Ratios of the subingredients for preparing Pacific cod gill jeotgal

Ingredient	Ratio (%)	Ingredient	Ratio (%)
Pacific cod gill	80.0	Sugar	2.0
Salt	8.0	D-sorbitol	1.5
Powdered red pepper	4.5	MSG[a]	0.5
Garlic	2.0	Lactic acid	0.2

[a]*MSG* monosodium glutamate

5 Microbiological Characterization of By-product Jeotgal

Seafood products containing jeotgal are fermented by bacteria better than by molds and yeasts, unlike in most Asian countries, where fermented agricultural products are mainly fermented by yeasts or molds (Lee 1993; Shinano et al. 1975). These reports suggest that, regardless of salinity, jeotgal are significantly affected by proteolytic enzymes produced by the microorganisms distributed in the fishery muscle, viscera, and roe, because fish, shellfish, and crustaceans contain more than 70 % based on the dry weight (Lee 1993).

The number of total viable cells of by-product jeotgal varies depending on the raw materials, salt concentration, temperature, and fermentation period.

The total cell numbers reached 10^3–10^5 CFU/g in high-salt jeotgal, while it reached 10^7 CFU/g in low-salt jeotgal (Mheen 1993).

Cha et al. (1983) studied the changes in the viable counts of high-salt traditional jeotgal with 20 % salt and low-salt jeotgal with 8 % salt, which was prepared with the addition of 0.02 % butylated hydroxyanisole (BHA), 0.5 % lactic acid, 6 % sorbitol, and 6 % ethyl alcohol. In their results, the numbers of viable cells in the traditional jeotgal and low-salt jeotgal after a 60-day fermentation reached 5.0×10^6 (CFU/g) and 8.0×10^3 (CFU/g), respectively. These results suggest that the growth of microorganisms was restrained by alcohol, which was added to improve the flavor (Uno 1974). Kochi and Hata (1964) also reported that the total viable count in sea urchin egg jeotgal added with alcohol, which is a kind of low-salt jeotgal, was 8.0×10^2 (CFU/g) after a 60-day fermentation and alcohol used could restrain the growth of yeasts, which could diminish the quality of these jeotgal (Lee 1993). Cha et al.(1983) also reported that, during the fermentation of sardine jeotgal, 46 representative bacteria from seven species and two yeasts from one species were isolated, and most of them were identified to be facultative anaerobes, being catalase- and oxidase-positive (Lee 1993). Mheen (1993) also reported that ten genera of bacteria, *Achromobacter*, *Bacillus*, *Brevibacterium*, *Flavobacterium*, *Halobacterium*, *Leuconostoc*, *Micrococcus*, *Pediococcus*, *Pseudomonas*, and *Sarcina*, and two genera of yeast, *Saccharomyces* and *Torulopsis*, were isolated and identified from seven kinds of jeotgal, shrimp jeotgal, anchovy jeotgal, sardine jeotgal, clam jeotgal, yellow corvenia jeotgal, cuttlefish jeotgal, and oyster jeotgal.

These results suggest that high salt tolerance bacteria are dominant in the traditional jeotgal, while halophile bacteria (which grow well in 3–7 % salt) are predominant in the low-salt jeotgal.

6 Biochemical Characterization and Shelf Life of By-product Jeotgal

6.1 Viscera Jeotgal

6.1.1 Low-Salt Fermented Intestine and Stomach of Alaska Pollock (Changran Jeotgal)

- *Nutritional characterization*: The moisture content of changran jeotgal is 64.3 %, which is lower than that of raw changran (80.9 %) (Table 5.7). The other proximate compositions of changran jeotgal are 12.9 % crude protein, 3.2 % crude lipid, 11.4 % ash, and 8.2 % carbohydrate, which are higher than those of raw changran (11.5 % crude protein, 2.2 % crude lipid, 5.2 % ash, and 0.2 % carbohydrate). These changes in the proximate composition between raw changran and its jeotgal may be due to the effect of salt, sorbitol, and sugar used for the seasoning of the final product (Chae 2011; Park et al. 1998).
- *Taste characterization*: The trichloroacetic acid (TCA)-soluble nitrogen components, on which specific tastes of seafoods relied, are defined as water-soluble, low molecular weight components, with the exception of minerals, vitamins, and pigments, and classified into two groups; that is, nitrogenous compounds, such as free and combined amino acids, ATP and its related compounds, organic bases, and so on, and non-nitrogenous compounds, such as sugars and organic acids (Fuke 1994).

The free amino acid nitrogen (FAA-N), ATP and its related compound-N, total creatinine-N, betaine-N, trimethylamine oxide nitrogen (TMAO-N), and trimethylamine nitrogen (TMA-N) contents of changran jeotgal are 1,436.2 mg/100 g, 45.2 mg/100 g, 62.0 mg/100 g, 26.0 mg/100 g, 129.0 mg/100 g, and 6.0 mg/100 g, respectively (Fig. 5.7). According to the results of the nitrogenous compound content of changran jeotgal, FAA-N is a major nitrogenous component of changran jeotgal.

In general, FAAs are the most important components influencing the taste of jeotgal. The total content of FAAs of changran jeotgal is 1,436.23 (mg/100 g of jeotgal) (Table 5.8). From the concentration, more than 9 % of FAAs in changran jeotgal are of two kinds, arginine (142.09 mg/100 g, 9.9 %) and glutamic acid (281.60 mg/100 g, 19.6 %).

The total taste value of changran jeotgal is 104.12. Among FAA, aspartic acid (35.03) and glutamic acid (56.32) have the highest taste values calculated from the ratio of amino acid content to the taste threshold (Table 5.8) (Kato et al. 1989). According to these results, one may conclude that the taste of changran jeotgal is influenced by glutamic acid for umami and by aspartic acid for sweetness.

- *Shelf life*: These changran jeotgal can be stored for more than 6 months at 5°C.

Table 5.7 Proximate composition of raw changran and changran jeotgal

	Proximate composition (g/100 g)				
Changran	Moisture	Crude protein	Crude lipid	Carbohydrate	Ash
Raw[a]	80.9	11.5	2.2	0.2	5.2
Jeotgal[b]	64.3	12.9	3.2	8.2	11.4

[a]Source: data from Kim and Kim (1990), with permission
[b]Source: data from the National Rural Resources Development Institute (2007)

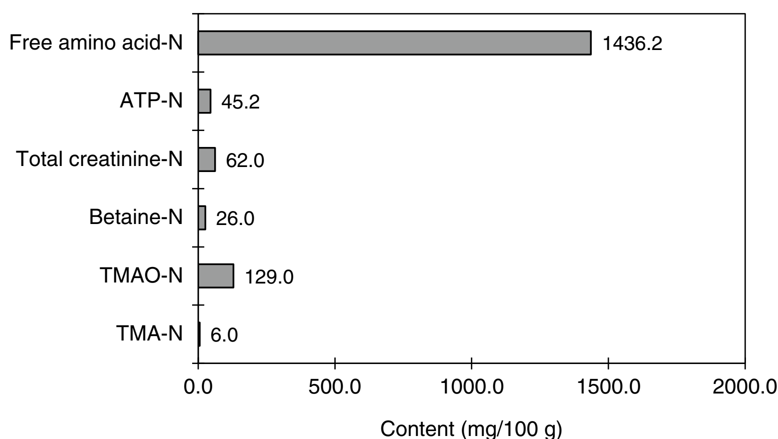


Fig. 5.7 Nitrogenous compound content of changran jeotgal

Table 5.8 Free amino acid (FAA) content and taste value of changran jeotgal

		Changran jeotgal		
Amino acid	Taste threshold (g/dL)[a]	FAA content (mg/100 g)[d]		Taste value[c]
Lys	0.050	86.18	(6.0)[b]	1.72
His	0.020	20.85	(1.5)	1.04
Arg	0.050	142.09	(9.9)	2.84
Asp	0.003	105.09	(7.3)	35.03
Thr	0.260	61.11	(4.3)	0.24
Ser	0.150	66.59	(4.6)	0.44
Glu	0.005	281.60	(19.6)	56.32
Pro	0.300	61.88	(4.3)	0.21
Gly	0.130	41.17	(2.9)	0.32
Ala	0.060	85.94	(6.0)	1.43
Cys	–	8.86	(0.6)	–
Val	0.140	72.69	(5.1)	0.52
Met	0.030	51.45	(3.6)	1.72
Ile	0.090	67.10	(4.7)	0.75
Leu	0.190	116.62	(8.1)	0.61
Tyr	–	83.27	(5.8)	–
Phe	0.090	83.74	(5.8)	0.93
Total	–	1,436.23	(100.1)	104.12

[a]Source: data from Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
[d]Source: data from Kang et al. (2000), with permission

6.1.2 Salt-Fermented Chub Mackerel Viscera (Chub Mackerel Viscera Jeotgal)

- *Nutritional characterization*: In a study on the salt-fermented fish in Korea, Lee et al. (1987) reported that the proximate composition of chub mackerel viscera jeotgal is 60.9 % moisture, 14.8 % protein, 5.3 % lipid, and 18.9 % ash.

The salinity is 20 %, which is similar to that of other fish jeotgal, such as myungran jeotgal, changran jeotgal, anchovy jeotgal, and shrimp jeotgal, which are popular salt-fermented fish in Korea.

- *Shelf life*: These chub mackerel viscera jeotgal can be stored for more than 8 months at 5°C.

6.1.3 Salt-Fermented Largehead Hairtail Viscera (Largehead Hairtail Viscera Jeotgal)

- *Nutritional characterization*: The moisture content of largehead hairtail viscera jeotgal is 64.8 %, which is lower than that of raw largehead hairtail (75.4 %) (Table 5.9). The other proximate compositions of largehead hairtail viscera jeotgal are 12.8 % crude protein, 1.2 % crude lipid, and 20.1 % ash, which are higher in ash (1.4 %) and lower in crude protein (18.6 %) and crude lipid (2.4 %) than those of raw largehead hairtail. The differences in the proximate compositions between raw material and salt-fermented largehead hairtail viscera jeotgal may be due to the effect of the high salt content used for salting (Lee et al. 1987).

The salinity of largehead hairtail viscera jeotgal is 19.5 %, which is similar to that of whole-fish jeotgal, such as anchovy jeotgal and shrimp jeotgal, which are popular salt-fermented fish in Korea.

Twenty-two different types of fatty acids are detected in largehead hairtail viscera jeotgal (Table 5.10). Monoenoic acid showed the highest level (38.8 %), followed by polyenoic acid (31.8 %) and saturated acid (29.6 %), in that order. Prominent fatty acids of largehead hairtail viscera jeotgal are 16:0 (17.1 %), 18:1 (28.8 %), and 22:6 (14.2 %), accounting for approximately 60.1 % of the total fatty acids. These prominent fatty acids of largehead hairtail viscera jeotgal are similar to those of fish (Statistics Korea 2012). By these results, the lipids in largehead hairtail viscera jeotgal have significantly higher levels of n-3 long-chain polyunsaturated fatty acids, such as docosahexaenoic acid (DHA, 22:6n-3), which are associated with reduced risk of geriatric and cardiovascular disorders and certain forms of cancer. In the sense of fatty acid composition, largehead hairtail viscera jeotgal is considered to be a form of healthy food.

- *Taste characterization*: In general, FAAs are the most important components influencing the taste of jeotgal. The total content of FAA (expressed by moisture and salt-free basis) in the largehead hairtail viscera jeotgal is 3,284.9 mg/100 g, which is 3.15 times higher than that of raw material (1,041.3 mg/100 g)

Table 5.9 Proximate composition of raw material and product of largehead hairtail viscera jeotgal

	Proximate composition (%)				
Largehead hairtail viscera	Moisture	Crude protein	Crude lipid	Crude ash	Salinity (%)
Raw	75.4	18.6	2.4	1.4	0.7
Jeotgal	64.8	12.8	1.2	20.1	19.5

Source: data from Kim and Park (1984), with permission

Table 5.10 Fatty acid composition of largehead hairtail viscera jeotgal

Saturated acid	Area %	Monoenoic acid	Area %	Polyenoic acid	Area %
12:0	0.1	16:1	8.8	18:2	2.9
14:0	4.3	18:1	28.8	18:3	2.3
15:0	0.9	20:1	0.9	18:4	0.2
16:0	17.1	22:1	0.3	20:2	0.2
17:0	1.3	Total	38.8	20:4	2.4
18:0	4.6			20:5	5.2
20:0	0.5			22:2	1.5
22:0	0.8			22:4	0.8
Total	29.6			22:5	2.1
				22:6	14.2
				Total	31.8

Source: data from Lee et al. (1986b)

(Table 5.11). From the concentration, more than 10 % of FAA in the largehead hairtail viscera jeotgal are of two kinds, proline (366.4 mg/100 g, 12.4 %) and alanine (321.1 mg/100 g, 11.6 %) and leucine (329.3 mg/100 g, 11.5 %), which are different from those of raw material [lysine (120.7 mg/100 g, 11.6 %), aspartic acid (120.3 mg/100 g, 11.6 %), and alanine (150.3 mg/100 g, 14.4 %)].

The total taste value, which is calculated from the ratio of amino acid content to the taste threshold, of the largehead hairtail viscera jeotgal is 120.78, which is 2.03 times higher than that of raw largehead hairtail viscera (59.37) (Table 5.11) (Kato et al. 1989). Among FAAs of the largehead hairtail viscera jeotgal, the taste value is higher in aspartic acid (40.10) and glutamic acid (48.06) than in the other amino acids.

ATP and its related compound contents, expressed as μmole/g dry weight, of the largehead hairtail viscera jeotgal and its raw materials are 0.17 μmole/g and 0.24 μmole/g, respectively, for ATP, 0.66 μmole/g and 1.14 μmole/g, respectively, for ADP, 0.82 μmole/g and 1.02 μmole/g, respectively, for AMP, 1.34 μmole/g and 1.19 μmole/g, respectively, for IMP, 0.04 μmole/g and 0.08 μmole/g, respectively, for inosine, and 5.00 μmole/g and 1.19 μmole/g, respectively, for hypoxanthine (Fig. 5.8).

According to the results, the largehead hairtail viscera jeotgal is the highest in hypoxanthine. These results are probably because of the ATP decomposition pathway, which is ATP → ADP → AMP → IMP → inosine → hypoxanthine, and the accumulation of hypoxanthine (Lee et al. 1987).

- *Shelf life*: The largehead hairtail viscera jeotgal can be stored for more than 8 months at 15°C.

Table 5.11 Free amino acid content and taste value of largehead hairtail viscera jeotgal (moisture and salt-free basis)

Amino acid	Taste threshold (g/dL)[a]	FAA content (mg/100 g)		Taste value[c]	
		Raw material	Salted-fermented[d]	Raw material	Salted-fermented
Lys	0.050	120.7 (11.6)[b]	210.18 (9.3)	2.41	4.20
His	0.020	24.2 (2.32)	98.2 (3.0)	1.21	4.91
Arg	0.050	1.2 (0.1)	21.3 (1.6)	0.02	0.43
Asp	0.003	120.3 (11.6)	120.3 (3.6)	40.10	40.10
Thr	0.260	60.1 (5.8)	180.3 (4.8)	0.23	0.69
Ser	0.150	40.9 (3.9)	159.3 (4.7)	0.27	1.06
Glu	0.005	40.9 (3.9)	240.3 (6.9)	8.18	48.06
Pro	0.300	60.3 (5.8)	366.4 (12.4)	0.20	1.22
Gly	0.130	55.1 (5.3)	293.2 (9.0)	0.42	2.26
Ala	0.060	150.3 (14.4)	321.1 (11.6)	2.51	5.35
Cys	–	Trace (trace)	30.41 (1.8)		
Val	0.140	69.9 (5.8)	270.41 (8.6)	0.50	1.93
Met	0.030	42.4 (4.1)	121.3 (3.6)	1.41	4.04
Ile	0.090	50.7 (4.9)	170.3 (5.6)	0.56	1.89
Leu	0.190	95.1 (9.1)	329.3 (11.5)	0.50	1.73
Tyr	–	42.8 (4.1)	92.3 (3.7)		
Phe	0.090	75.3 (7.2)	260.3 (7.2)	0.84	2.89
Total	–	1,041.3 (100.0)	3,284.9 (100.0)	59.37	120.78

[a]Source: data from Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
[d]Source: data from Kim and Park (1984), with permission

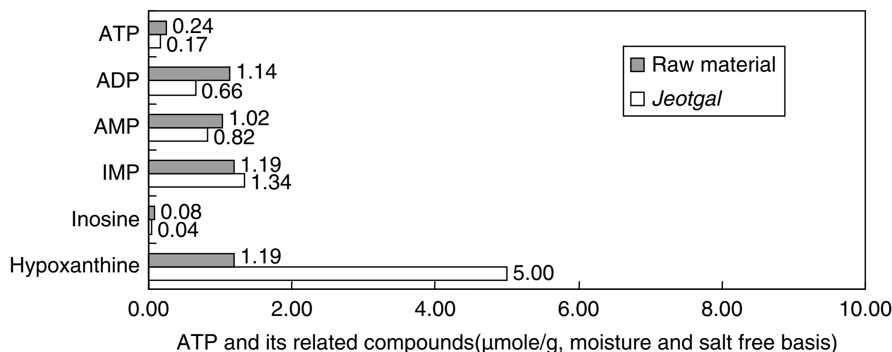


Fig. 5.8 ATP and its related compounds of raw material and product of longhead hairtail viscera jeotgal

Table 5.12 Proximate composition of konoshiro gizzard shad stomach jeotgal

Konoshiro gizzard shad stomach	Proximate composition (%)				Salinity (%)
	Moisture	Crude protein	Crude lipid	Ash	
Raw	76.0	15.1	2.2	2.1	1.1
Jeotgal	59.1	12.6	1.3	21.2	22.4

Source: data from Kim and Kim (1990), with permission

Table 5.13 Fatty acid composition of konoshiro gizzard shad stomach jeotgal

Saturated acid	Area %	Monoenoic acid	Area %	Polyenoic acid	Area %
12:0	–	16:1	9.2	18:2	2.2
14:0	6.6	17:1	1.5	18:3	2.1
15:0	0.7	18:1	23.4	18:4	0.1
16:0	20.5	20:1	2.1	20:2	0.2
17:0	1.0	22:1	Trace	20:4	1.9
18:0	6.1	Total	36.2	20:5	10.1
20:0	0.5			22:2	1.0
22:0	0.6			22:4	0.3
Total	36.0			22:5	1.7
				22:6	8.3
				Total	27.9

Source: data from Lee et al. (1986b), with permission

6.1.4 Salt-Fermented Konoshiro Gizzard Shad Stomach (Konoshiro Gizzard Shad Stomach jeotgal)

- *Nutritional characterization*: The moisture content of konoshiro gizzard shad stomach jeotgal is 59.1 %, which is lower than that of raw material (76.0 %) (Table 5.12).

The other proximate compositions of konoshiro gizzard shad stomach jeotgal are 12.6 % crude protein, 1.3 % crude lipid, and 21.2 % ash, which are lower than crude protein (11.5 %) and crude lipid,(2.2 %) of raw material, but higher than ash (5.2 %). The results may be due to the effect of salt added for prolonging shelf life. The salinity of konoshiro gizzard shad stomach jeotgal is 22.4 %.

Twenty-two kinds of fatty acids were detected in konoshiro gizzard shad stomach jeotgal (Table 5.13). The monoenoic acid showed the highest level (36.2 %), followed by saturated acid (36.0 %) and polyenoic acid (27.9 %), in that order. Prominent fatty acids of konoshiro gizzard shad stomach jeotgal are 16:0 (20.5 %), 16:1 (9.2 %), 18:1 (23.4 %), 20:5 (10.1 %), and 22:6 (8.3 %), accounting for approximately 71.5 % of the total fatty acids. These prominent fatty acids of konoshiro gizzard shad stomach jeotgal are similar to those of fish (Statistics Korea 2012). By these results, the lipids in konoshiro gizzard shad stomach jeotgal have significantly higher levels of n-3 long-chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6n-3), which are associated with reduced risk of geriatric and cardiovascular disorders and certain forms of cancer.

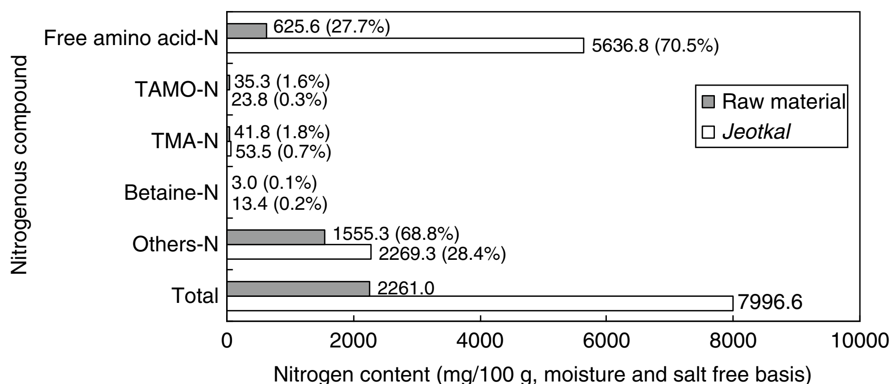


Fig. 5.9 Nitrogenous compound content of raw material and product of konoshiro gizzard shad stomach jeotgal

In the sense of fatty acid composition, konoshiro gizzard shad stomach j*eotgal* is considered to be a form of healthy food.

- *Taste characterization*: The TCA-soluble nitrogen content of konoshiro gizzard shad stomach jeotgal is 7,996.6 mg/100 g, which is 3.54 times higher than that (2,261.0 mg/100 g) of raw material (Fig. 5.9). The contents of FAA-N, TMAO-N, TMA-N, and betaine-N, which are composed of TCA-soluble nitrogen, konoshiro gizzard shad stomach jeotgal, are 5,636.8 mg/100 g, 23.8 mg/100 g, 53.5 mg/100 g, and 13.4 mg/100 g, respectively. Among the TCA-soluble nitrogen components, the FAA-N, TMA-N, and betaine-N contents of konoshiro gizzard shad stomach jeotgal are higher than those (625.6 mg/100 g, 41.8 mg/100 g and 3.0 mg/100 g) of raw material, while the TMAO-N content of konoshiro gizzard shad stomach jeotgal is lower. The low content of TMAO-N in konoshiro gizzard shad stomach jeotgal is probably because TMA-N is mainly formed through the bacterial decomposition of TMAO-N (Arason 1994). These results suggest that the taste of konoshiro gizzard shad stomach jeotgal enriches by fermentation.

Adenosine triphosphate (ATP) and its related compound contents, expressed as µmole/g dry weight, of konoshiro gizzard shad stomach jeotgal are 0.12 µmole/g for ATP, 0.73 µmole/g for adenosine diphosphate (ADP), 1.16 µmole/g for adenosine monophosphate (AMP), 0.88 µmole/g for IMP, 0.52 µmole/g for inosine, and 7.17 µmole/g for hypoxanthine (Fig. 5.10). Among ATP and its related compounds, which are major taste compounds, konoshiro gizzard shad stomach jeotgal is higher in inosine and hypoxanthine contents than raw material (0.18 µmole/g for ATP, 1.10 µmole/g for ADP, 1.38 µmole/g for AMP, 1.07 µmole/g for IMP, 0.27 µmole/g for inosine, and 3.57 µmole/g for hypoxanthine), while it is lower in the other ATP and its related compounds. These results are probably because of the ATP decomposition pathway, which is ATP → ADP → AMP → IMP → inosine → hypoxanthine, and the accumulation of hypoxanthine (Lee et al. 1987).

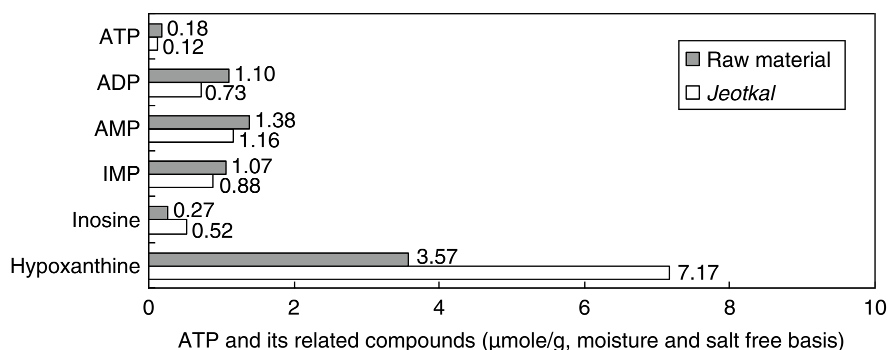


Fig. 5.10 ATP and its related compounds content of raw material and product of konoshiro gizzard shad stomach jeotgal

Table 5.14 Free amino acid content and taste value of raw material and product of konoshiro gizzard shad stomach jeotgal

Amino acid	Taste threshold (g/dL)[a]	Raw material FAA content (mg/100 g)		Raw material Taste value[c]	Jeotgal FAA content (mg/100 g)		Jeotgal Taste value
Lys	0.050	826.5	(18.7)[b]	16.53	6,457.6	(16.1)	129.15
His	0.020	124.7	(2.8)	6.24	904.3	(2.3)	45.22
Arg	0.050	28.3	(0.6)	0.57	1,962.4	(4.9)	39.25
Asp	0.003	161.3	(3.6)	53.77	654.4	(1.6)	218.13
Thr	0.260	329.3	(7.4)	1.27	2,278.0	(5.7)	8.76
Ser	0.150	327.6	(7.4)	2.18	2,296.2	(5.7)	15.31
Glu	0.005	617.0	(13.9)	123.40	3,613.2	(9.0)	722.64
Pro	0.300	Trace	–	–	724.3	(1.8)	2.41
Gly	0.130	294.4	(6.7)	2.26	1,406.0	(3.5)	10.82
Ala	0.060	474.0	(10.7)	7.90	2,346.7	(5.9)	39.11
Cys	–	Trace	–	–	242.8	(0.6)	–
Val	0.140	497.3	(11.2)	3.55	2,985.4	(10.0)	21.32
Met	0.030	169.6	(3.8)	5.65	3,010.3	(7.5)	100.34
Ile	0.090	116.4	(2.6)	1.29	3,799.3	(9.5)	42.21
Leu	0.190	304.3	(6.9)	1.60	3,463.5	(8.7)	18.23
Tyr	–	74.8	(1.7)	–	1,420.2	(3.6)	–
Phe	0.090	88.1	(2.0)	0.98	1,448.5	(3.6)	16.09
Total	–	4,433.6	(100)	227.19	39,013.1	(100)	1,428.99

[a]Source: data from Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
Source: data from Kim and Kim (1990), with permission

In general, FAAs are the most important components influencing the taste of jeotgal. The total content of FAA of konoshiro gizzard shad stomach jeotgal is 39,013.1 (mg/100 g of jeotgal), which is 8.80 times higher than that of raw material (4,433.6 mg/100 g) (Table 5.14).

These results suggest that combined amino acids convert into FAAs by the action of various enzymes. From the concentration, more than 10 % of FAA in konoshiro gizzard shad stomach jeotgal are of two kinds, lysine (6,457.6 mg/100 g, 16.1 %) and valine (2,985.4 mg/100 g, 10.0 %), which are different from those of raw material [lysine (826.5 mg/100 g, 18.7 %), glutamic acid (617.0 mg/100 g, 13.9 %), alanine (474.0 mg/100 g, 10.7 %), and valine (497.3 mg/100 g, 11.2 %)].

The other FAA contents of konoshiro gizzard shad stomach jeotgal as well as the major FAAs are also higher than those of raw material.

The total taste value of the konoshiro gizzard shad stomach jeotgal is 1,428.99, which is 6.29 times higher than that of 227.19. Among FAAs, lysine (129.15), aspartic acid (218.13), glutamic acid (722.64), and methionine (100.34) have the highest taste values calculated from the ratio of amino acid content to the taste threshold (Table 5.14) (Kato et al. 1989). According to these results, one may conclude that the taste of konoshiro gizzard shad stomach jeotgal is influenced by glutamic acid for umami and by aspartic acid for sweetness.

- *Shelf life*: The konoshiro gizzard shad stomach jeotgal can be stored for more than 6 months at 5°C.

6.1.5 Salt-Fermented Sea Cucumber Viscera (Sea Cucumber Viscera Jeotgal)

- *Nutritional characterization*: The proximate composition of sea cucumber viscera jeotgal is 76.5 % moisture, 9.3 % protein, 1.3 % lipid, and 12.4 % ash (Table 5.15). Among these proximate compositions of sea cucumber viscera jeotgal, the moisture content is higher than that of other jeotgal, such as myungran jeotgal, changran jeotgal, anchovy jeotgal, and shrimp jeotgal, which are popular jeotgal in Korea, while the other proximate compositions are lower. These results are probably because of the difference in the ratio of salt added for preparing sea cucumber viscera jeotgal.

Calcium and phosphorus concentrations of sea cucumber viscera jeotgal are 75 mg/100 g and 112 mg/100 g, respectively, which is higher in calcium and lower in phosphorus than that of pork rib (12.0 mg/100 g and 196.0 mg/100 g, respectively) (Table 5.15) (Guan et al. 2011). The ratio of calcium to phosphorus of sea cucumber viscera jeotgal is 1:1.49. In general, the absorption of calcium is limited (10–40 %) in adults. High amounts of phosphorus are thought to decrease calcium availability. Although the recommend ratio of dietary calcium to phosphorus is 1:2–2:1, most people consume considerably more phosphorus. In that sense, sea cucumber viscera jeotgal is thought to be an excellent source for calcium in the diet. The dietary reference intakes for Korean adults for calcium and phosphorus are in the ranges of 650–750 mg/day and 700 mg/day, respectively (Guan et al. 2011).

- *Taste characterization*: The TCA-soluble nitrogen content of sea cucumber viscera jeotgal, expressed as moisture and salt-free basis, is 7,184 mg/100 g, which

Table 5.15 Proximate composition and mineral content of sea cucumber viscera jeotgal

Proximate composition (%)				Mineral (mg/100 g)	
Moisture	Crude protein	Crude lipid	Ash	Ca	P
76.5	9.3	1.3	12.4	75	112

Source: data from Kim and Kim (1990), with permission

Table 5.16 Nitrogenous compound content of raw material and product of sea cucumber viscera jeotgal

	Nitrogenous compound (mg/100 g, moisture and salt-free base)				
Sea cucumber viscera	Trichloroacetic acid-soluble-N	Free amino acid-N	TMAO-N	TMA-N	Betaine-N
Raw	770.0	773.8	40.1	25.4	545.0
Jeotgal	7,184.0	5,146.0	35.7	32.6	34.2

Source: data from Kim and Kim (1990), with permission

is 9.33 times higher than that (770 mg/100 g) of raw material (Table 5.16). These results suggest that the taste of sea cucumber viscera jeotgal enriches by fermentation process. The FAA-N, TMAO-N, TMA-N, and betaine-N contents, expressed as moisture and salt-free basis, of sea cucumber viscera jeotgal are 5,146 mg/100 g, 35.7 mg/100 g, 32.6 mg/100 g, and 34.2 mg/100 g, respectively. Among the TCA-soluble nitrogen components, the FAA-N and TMA-N contents of sea cucumber viscera jeotgal are higher than those (773.8 mg/100 g and 25.4 mg/100 g) of raw material, while TMAO-N and betaine-N of sea cucumber viscera jeotgal is lower. The low content of TMAO-N in sea cucumber viscera jeotgal is probably because TMA-N is mainly formed through the bacterial decomposition of TMAO-N (Arason 1994).

Adenosine triphosphate (ATP) and its related compound contents, expressed as μmole/g dry weight, of sea cucumber viscera jeotgal are 3.4 μmole/g for CMP, 25.3 μmole/g for UMP, 3.3 μmole/g for IMP, 62.5 μmole/g for hypoxanthine, 10.1 μmole/g for uridine, and trace for AMP (Fig. 5.11). Regardless of the kinds of ATP and its related compounds, the ATP and its related compounds contents of sea cucumber viscera jeotgal are higher than those of raw material (1.1 μmole/g for CMP, 6.4 μmole/g for UMP, 1.6 μmole/g for IMP, 7.6 μmole/g for hypoxanthine, 1.0 μmole/g for uridine, and 3.6 μmole/g for AMP). Among ATP and its related compounds of sea cucumber viscera jeotgal, hypoxanthine is the dominant one.

In general, FAAs are the most important components influencing the taste of jeotgal. The total content of FAA of sea cucumber viscera jeotgal, expressed as mg/100 g, moisture, and salt-free basis, is 22,537.5 mg/100 g, which is 3.97 times higher than that of raw material (5,680.6 mg/100 g) (Table 5.17). These results suggest that combined TCA-soluble compounds convert into free TCA-soluble compounds by the action of various enzymes. From the concentration of FAA in sea cucumber viscera jeotgal, the major FAAs are of two kinds, lysine (2,825.8 mg/100 g, 12.5 %) and glutamic acid (3,886.5 mg/100 g, 17.2 %), which are different from

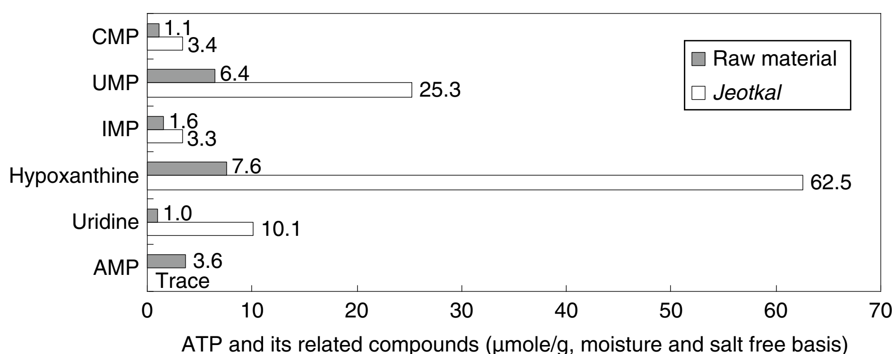


Fig. 5.11 ATP and its related compounds content of raw material and product of sea cucumber viscera jeotgal

Table 5.17 Free amino acid (FAA) content and taste value of raw material and product of sea cucumber viscera jeotgal (moisture and salt-free basis)

Amino acid	Taste threshold (g/dL)[a]	Raw material FAA content (mg/100 g)		Raw material Taste value[c]	Jeotgal FAA content (mg/100 g)		Jeotgal Taste value
Lys	0.050	236.4	(4.2)[b]	4.73	2,825.8	(12.5)	56.52
His	0.020	53.5	(0.9)	2.68	824.5	(3.7)	41.23
Arg	0.050	240.9	(4.2)	4.82	2,069.3	(9.2)	41.39
Asp	0.003	–	–	–	–	–	–
Thr	0.260	245.3	(4.3)	0.94	1,420.9	(6.3)	5.47
Ser	0.150	1,829.0	(32.2)	12.19	1,408.9	(6.3)	9.39
Glu	0.005	1,721.6	(30.3)	344.32	3,886.5	(17.2)	777.3
Pro	0.300	–	–	–	–	–	–
Gly	0.130	–	–	–	–	–	–
Ala	0.060	874.2	(15.4)	14.57	2,157.4	(9.6)	35.96
Cys	–	Trace	–	–	64.0	(0.3)	–
Val	0.140	111.5	(2.0)	0.80	1,172.8	(5.2)	8.38
Met	0.030	84.7	(1.5)	2.82	860.6	(3.8)	28.69
Ile	0.090	89.2	(1.6)	0.99	1,323.9	(5.9)	14.71
Leu	0.190	120.4	(2.1)	0.63	2,129.4	(9.4)	11.21
Tyr	–	62.4	(1.1)	–	1,140.7	(5.1)	–
Phe	0.090	11.5	(0.2)	0.13	1,252.8	(5.6)	13.92
Total	–	5,680.6	(100)	389.62	22,537.5	(100.1)	1,044.17

[a]Source: data from Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
Source: data from Kim and Kim (1990), with permission

those of raw material (serine 1,829.0 mg/100 g and 32.2 %, glutamic acid 1,721.6 mg/100 g and 30.3 %, and alanine 874.2 mg/100 g and 15.4 %). Except for serine, the other FAA contents of jeotgal as well as the major amino acids are also higher than those of raw material.

Table 5.18 Free sugar content of raw material and product of sea cucumber viscera jeotgal

	Free sugar (mg/100 g, moisture and salt-free basis)				
Sea cucumber viscera	Xylose	Arabinose	Fructose	Glucose	Galactose
Raw	41.7	38.6	Trace	Trace	238.3
Jeotgal	77.1	78.6	27.5	Trace	988.0

Source: data from Kim and Kim (1990), with permission

The total taste value of sea cucumber viscera jeotgal is 1,044.17, which is 2.68 times higher than that of 389.62. Among the various FAAs of sea cucumber viscera jeotgal, glutamic acid (777.3) has the highest taste values calculated from the ratio of amino acid content to the taste threshold (Table 5.17) (Kato et al. 1989). According to these results, one may conclude that the taste of sea cucumber viscera jeotgal is influenced by glutamic acid for umami (Heu et al. 2003; Kim et al. 2003b).

Free sugar of sea cucumber viscera jeotgal is detected as five kinds: xylose, arabinose, fructose, glucose, and galactose, and their contents are 77.1 mg%, 78.6 mg%, 27.5 mg%, trace, and 988.0 mg% (Table 5.18). Regardless of the kinds of free sugar detected, their contents of sea cucumber viscera jeotgal are higher than those of raw material (41.7 mg% for xylose, 38.6 mg% for arabinose, trace for fructose, trace for glucose, and 238.3 mg% for galactose). According to the results of free sugar, galactose is the dominant free sugar in sea cucumber viscera jeotgal.

6.1.6 Salt-Fermented Abalone Viscera (Abalone Viscera Jeotgal)

- *Nutritional characterization*: The proximate composition of abalone viscera jeotgal is 66.0 % moisture, 12.2 % protein, 4.8 % lipid, and 16.0 % ash (Table 5.19). The moisture content of abalone viscera jeotgal is higher than that of other salt-fermented fish, such as changran jeotgal (64.3 %), shrimp jeotgal (58.4 %) (National Rural Resources Development Institute 2007), and anchovy jeotgal (54.4 %) (National Rural Resources Development Institute 2007), which are popular jeotgal, while the other proximate compositions are lower. These results are probably because of the difference in the kinds of raw material and the ratio of salt added for preparing abalone viscera jeotgal.

Calcium and phosphorus concentrations of abalone viscera jeotgal are 223 mg/100 g and 91 mg/100 g, respectively. The calcium concentration is 18.58 times higher, while the phosphorus concentration is lower than that of pork rib (12.0 mg/100 g and 196.0 mg/100 g, respectively) (Table 5.19) (Guan et al. 2011). The ratio of calcium to phosphorus of abalone viscera jeotgal is 1:0.41. In general, the absorption of calcium is limited (10–40 %) in adults. High amounts of phosphorus are thought to decrease calcium availability. Although the recommend ratio of dietary calcium to phosphorus is 1:2–2:1, most people consume considerably more phosphorus. In that sense, abalone viscera jeotgal is thought to be an excellent source for calcium in the diet. The dietary reference intakes for Korean adults for

Table 5.19 Proximate composition and mineral content of raw material and product of abalone viscera jeotgal

	Proximate composition (%)				Mineral (mg/100 g)	
Abalone viscera	Moisture	Crude protein	Crude lipid	Ash	Ca	P
Raw	70.4	18.5	7.6	3.5	27	299
Jeotgal	66.0	12.2	4.8	16.0	223	91

Source: data from the National Rural Resources Development Institute (2007)

Table 5.20 Fatty acid composition (area %) of raw material and product of abalone viscera jeotgal

Saturated acid	Raw	Jeotgal	Monoenoic acid	Raw	Jeotgal	Polyenoic acid	Raw	Jeotgal
14:0	9.9	10.1	14:1	0.6	0.5	18:2	3.2	3.5
15:0	0.9	0.7	16:1	5.8	6.0	18:3	5.2	5.5
16:0	16.7	17.0	18:1	10.7	11.4	18:4	2.1	1.7
17:0	0.7	0.8	20:1	3.7	3.1	20:2	5.5	5.6
18:0	1.9	1.9	22:1	1.7	2.0	20:4	12.0	12.1
20:0	0.5	0.3	Total	22.5	23.0	20:5	7.7	7.6
22:0	1.3	1.0				22:2	2.3	2.2
Total	31.9	31.8				22:4	3.2	3.2
						22:5	4.4	3.8
						22:6	0.1	0.1
						Total	45.6	45.2

Source: data from Kim and Kim (1990), with permission

calcium and phosphorus are in the ranges of 650–750 mg/day and 700 mg/day, respectively (Guan et al. 2011).

Twenty-two kinds of fatty acids were detected in abalone viscera jeotgal (Table 5.20). In abalone viscera jeotgal, polyenoic acid showed the highest level (45.2 %), followed by saturated acid (31.8 %) and monoenoic acid (23.0 %), in that order. These patterns of fatty acid composition in abalone viscera j*eotgal* are similar to that of raw abalone viscera. Prominent fatty acids of abalone viscera jeotgal are 16:0 (17.0 %), 18:1 (11.4 %) and 20:4 (12.1 %), accounting for approximately 40.5 % of the total fatty acids. No difference in the kinds of prominent fatty acids between raw material and product of abalone viscera jeotgal are found.

- *Taste characterization*: In general, FAAs are the most important component influencing the taste of jeotgal. The total content of FAA of abalone viscera jeotgal, expressed as mg/100 g dry weight, is 3,110.4 mg/100 g (Table 5.21). From the concentration of FAA in abalone viscera jeotgal, the major FAAs are of four kinds: glutamic acid (538.8 mg/100 g, 17.3 %), glycine (388.6 mg/100 g, 12.5 %), alanine (309.2 mg/100 g, 9.9 %), and arginine (298.1 mg/100 g, 9.6 %).

Table 5.21 Free amino acid content and taste value of abalone viscera jeotgal (based on dry weight)

Amino acid	Taste threshold (g/dL)[a]	Salt-fermented abalone viscera		
		FAA content (mg/100 g)		Taste value[c]
Lys	0.050	171.9	(5.5)[b]	3.44
His	0.020	125.5	(4.0)	6.28
Arg	0.050	298.1	(9.6)	5.96
Asp	0.003	230.0	(7.4)	76.67
Thr	0.260	178.7	(5.8)	0.69
Ser	0.150	194.1	(6.2)	1.29
Glu	0.005	538.8	(17.3)	107.76
Pro	0.300	153.0	(4.9)	0.51
Gly	0.130	388.6	(12.5)	2.99
Ala	0.060	309.2	(9.9)	5.15
Cys	–	–	–	–
Val	0.140	149.0	(4.8)	1.06
Met	0.030	61.6	(2.0)	2.05
Ile	0.090	83.4	(2.7)	0.93
Leu	0.190	77.3	(2.5)	0.41
Tyr	–	65.2	(2.1)	–
Phe	0.090	86.0	(2.8)	0.96
Total	–	3,110.4	(100.0)	216.15

[a]Source: data from Kato et al. (1989) with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
Source: data from Kim and Kim (1990) with permission

The total taste value of abalone viscera jeotgal is 216.15. Among the various FAAs of abalone viscera jeotgal, glutamic acid (107.76) has the highest taste values calculated from the ratio of amino acid content to the taste threshold (Table 5.17) (Kato et al. 1989), followed by aspartic acid (76.67). According to these results, one may conclude that the taste of abalone viscera jeotgal is influenced by glutamic acid for umami (Heu et al. 2003; Kim et al. 2003b) and by aspartic acid for sweetness.

ATP and its related compound contents, expressed as μmole/g dry weight, of abalone viscera jeotgal are trace for ATP, 0.1 μmole/g for both ADP and AMP, 0.3 μmole/g for IMP, 0.7 μmole/g for inosine, and 11.8 μmole/g for hypoxanthine (Fig. 5.12).

According to the results, abalone viscera jeotgal is highest in hypoxanthine. These results are probably because of the ATP decomposition pathway, which is ATP → ADP → AMP → IMP → inosine → hypoxanthine, and the accumulation of hypoxanthine (Lee et al. 1987).

- *Shelf life*: Abalone viscera jeotgal can be stored for more than 6 months at 15°C.

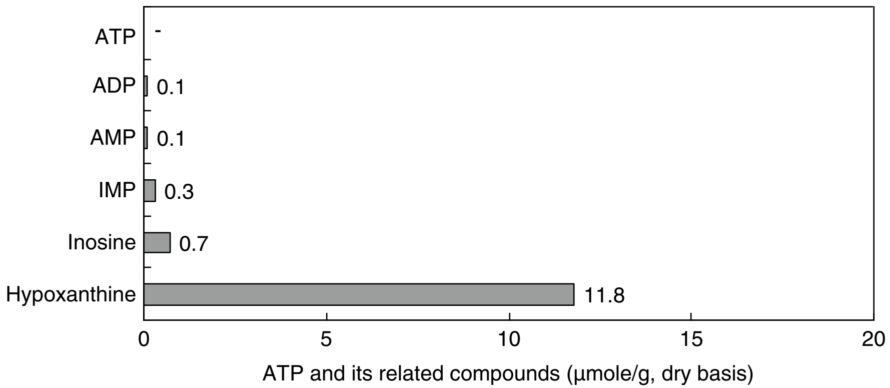


Fig. 5.12 ATP and its related compounds content of abalone viscera-jeotgal

Table 5.22 Proximate composition of raw roe and product of myungran jeotgal

	Proximate composition (g/100 g)				
Alaska pollock roe	Moisture	Crude protein	Crude lipid	Carbohydrate	Ash
Raw roe	73.5	22.4	1.5	1.5	1.1
Jeotgal	66.0	20.5	3.0	2.7	7.8

Source: data from the National Rural Resources Development Institute (2007)

6.2 *Roe Jeotgal*

6.2.1 Salt-Fermented Alaska Pollock Roe (Myungran Jeotgal)

- *Nutritional characterization*: The moisture and crude protein contents of myungran jeotgal are 66.0 % and 20.5 %, respectively (Table 5.22), which are lower than that of raw roe of Alaska pollock (73.5 % and 22.4 %). The other components of myungran jeotgal are 3.0 % crude lipid 7.8 % ash, and 2.7 % carbohydrate, respectively, which are higher than those of raw roe of Alaska pollock (1.5 % crude lipid, 1.1 % ash, and 1.5 % carbohydrate, respectively). The changes may be due to the effect of various additives, such as salt, sorbitol, and sugar used for seasoning of jeotgal.
- *Taste characterization*: In general, FAAs are the most important component influencing the taste of jeotgal. The total content of FAAs of myungran jeotgal, expressed as mg/100 g of wet weight, is 2,211.9 mg/100 g (Table 5.23). From the concentration of FAAs in myungran jeotgal, the major FAAs are of two kinds, glutamic acid (999.4 mg/100 g, 45.2 %) and arginine (162.8 mg/100 g, 7.4 %). These results are probably because of MSG used for seasoning myungran jeotgal (Lee et al. 1997).

The total taste value of myungran jeotgal is 211.88. Among the various FAAs of myungran jeotgal, glutamic acid (199.80) has the highest taste values calculated

Table 5.23 FAA content and taste value of myungran jeotgal

Amino acid	Taste threshold (g/dL)[a]	Myungran jeotgal		
		FAA content (mg/100 g)[d]		Taste value[c]
Taurine	–	90.6	(4.1)[b]	–
Phosphoethanolamine	–	6.4	(0.3)	–
Aspartic acid	0.003	0.5	(Trace)	0.17
Threonine	0.260	85.0	(3.8)	0.33
Serine	0.150	44.2	(2.0)	0.29
Glutamic acid	0.005	999.4	(45.2)	199.80
Sarcosine	–	11.9	(0.5)	–
α-Aminoadipic acid	–	4.8	(0.2)	–
Proline	0.300	87.7	(4.0)	0.29
Glycine	0.130	38.7	(1.7)	0.30
Alanine	0.060	76.0	(3.4)	1.27
Amino-iso-n-butyric acid	–	5.7	(0.3)	–
Valine	0.140	72.5	(3.3)	0.52
Cystine	–	88.2	(4.0)	–
Methionine	0.030	24.4	(1.1)	0.81
Isoleucine	0.090	79.5	(3.6)	0.88
Leucine	0.190	93.7	(4.2)	0.49
Tyrosine	–	48.0	(2.2)	–
β-Alanine	–	11.2	(0.5)	–
Phenylalanine	0.090	44.2	(2.0)	0.49
Ethanolamine	–	19.9	(0.9)	–
Lysine	0.050	95.3	(4.3)	1.91
Histidine	0.020	21.3	(1.0)	1.07
Arginine	0.050	162.8	(7.4)	3.26
Total	–	2,211.9	(100.0)	211.88

[a]From Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total free amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
[d]From Nam (2009), with permission

from the ratio of amino acid content to the taste threshold (Table 5.23) (Kato et al. 1989), followed by arginine (3.26). According to these results, one may conclude that the taste of myungran jeotgal is influenced by glutamic acid for umami (Heu et al. 2003; Kim et al. 2003b).

- *Shelf life*: Myungran jeotgal can be stored for more than 6 months at 5°C.

6.2.2 Salt-Fermented Sea Urchin Roe (Sea Urchin Roe Jeotgal)

- *Nutritional characterization*: The proximate composition of sea urchin roe jeotgal is 55.6 % moisture, 21.4 % protein, 2.8 % lipid, and 15.8 % ash (Table 5.24). The moisture content of sea urchin roe jeotgal is higher than that of other jeotgal, such as changran jeotgal (64.3 %), shrimp jeotgal (58.4 %) (National Rural

Table 5.24 Proximate composition and mineral content of sea urchin roe jeotgal

Proximate composition (%)				Mineral (mg/100 g)	
Moisture	Crude protein	Crude lipid	Ash	Ca	P
55.6	21.4	2.8	15.8	32	127

Source: data from the National Rural Resources Development Institute (2007)

Table 5.25 Fatty acid composition of sea urchin roe jeotgal

Saturated acid	Area %	Monoenoic acid	Area %	Polyenoic acid	Area %
14:0	8.5	16:1	5.7	18:2	2.1
15:0	1.1	17:1	0.2	18:3	11.7
16:0	17.6	18:1	7.3	18:4	0.8
17:0	0.3	20:1	10.1	20:2	3.0
18:0	2.5	22:1	Trace	20:4	11.9
20:0	0.9	Total	23.3	20:5	10.8
22:0	2.3			22:2	1.0
Total	33.2			22:4	0.1
				22:5	0.5
				22:6	1.6
				Total	43.5

Source: data from Lee et al. (1986b), with permission

Resources Development Institute 2007), and anchovy jeotgal (54.4 %) (National Rural Resources Development Institute 2007), which are the popular in Korea, while the other proximate compositions are lower. These results are probably because of the difference in the kinds of raw material and the ratio of salt added for preparing sea urchin roe jeotgal.

Calcium and phosphorus concentrations of sea urchin roe jeotgal are 32 mg/100 g and 127 mg/100 g, respectively. The calcium concentration is 2.67 times higher, while the phosphorus concentration is lower than that of pork rib (12.0 mg/100 g and 196.0 mg/100 g, respectively) (Table 5.24) (Guan et al. 2011). The ratio of calcium to phosphorus of sea urchin roe jeotgal is 1:7.09. In general, the absorption of calcium is limited (10–40 %) in adults. High amounts of phosphorus are thought to decrease calcium availability. In that sense, sea urchin roe jeotgal is thought to be a fair source for calcium in the diet. The dietary reference intakes for Korean adults for calcium and phosphorus are in the ranges of 650–750 mg/day and 700 mg/day, respectively (Guan et al. 2011).

Twenty-two types of fatty acids were detected in sea urchin roe jeotgal (Table 5.25).

The fatty acid composition of the sea urchin roe jeotgal is the highest for the total polyenoic acid (43.5 %), followed by saturated acid (33.2 %) and monoenoic acid (23.3 %), in that order. Prominent fatty acids of sea urchin roe jeotgal are 16:0 (17.6 %), 20:1 (10.1 %), 18:3 (11.7 %), 20:4 (11.9 %), and 20:5 (10.8 %), which account for 62.1 % of the total fatty acids.

- *Shelf life*: The sea urchin roe jeotgal can be stored for 12 months at 5°C.

Table 5.26 Proximate composition and mineral content of Pacific cod gill jeotgal

Proximate composition (%)				Mineral (mg/100 g)	
Moisture	Crude protein	Crude lipid	Ash	Ca	P
64.1	14.4	1.3	19.6	28	75

Source: data from the National Rural Resources Development Institute (2007)

Table 5.27 Fatty acid composition of Pacific cod gill jeotgal

Saturated acid	Area %	Monoenoic acid	Area %	Polyenoic acid	Area %
14:0	3.5	16:1	5.6	18:2	2.2
15:0	0.5	17:1	0.7	18:3	7.6
16:0	19.0	18:1	18.6	18:4	0.1
17:0	1.1	20:1	0.9	20:2	–
18:0	5.5	22:1	–	20:4	5.5
20:0	1.7	Total	25.8	20:5	9.5
22:0	0.6			22:2	1.5
Total	31.9			22:4	–
				22:5	1.8
				22:6	14.0
				Total	42.2

Source: data from Lee et al. (1986b), with permission

6.2.3 Salt-Fermented Pacific Cod Gill (Pacific Cod Gill Jeotgal)

- *Nutritional characterization*: The proximate composition of Pacific cod gill jeotgal is 64.1 % moisture, 14.4 % protein, 1.3 % lipid, and 19.6 % ash (Table 5.26). The moisture content of Pacific cod gill jeotgal is similar to or higher than that of other jeotgal, such as changran jeotgal (64.3 %), shrimp jeotgal (58.4 %) (National Rural Resources Development Institute 2007), and anchovy jeotgal (54.4 %) (National Rural Resources Development Institute 2007), which are popular jeotgal in Korea, while the ash content is similar or lower.

These results are probably because of the difference in the kinds of raw material and the ratio of salt added for preparing Pacific cod gill jeotgal.

The calcium and phosphorus concentrations of Pacific cod gill jeotgal are 28 mg/100 g and 75 mg/100 g, respectively. The calcium concentration is 2.33 times higher, while the phosphorus concentration is lower than that of pork rib (12.0 mg/100 g and 196.0 mg/100 g, respectively) (Table 5.26) (Guan et al. 2011). The ratio of calcium to phosphorus of Pacific cod gill jeotgal is 1:2.68. The dietary reference intakes for Korean adults for calcium and phosphorus are in the ranges of 650–750 mg/day and 700 mg/day, respectively (Guan et al. 2011).

Twenty-four types of fatty acids were detected in Pacific cod gill jeotgal (Table 5.27).

The fatty acid composition of Pacific cod gill jeotgal is the highest for the total polyenoic acid (42.2 %), followed by saturated acid (31.9 %) and monoenoic acid

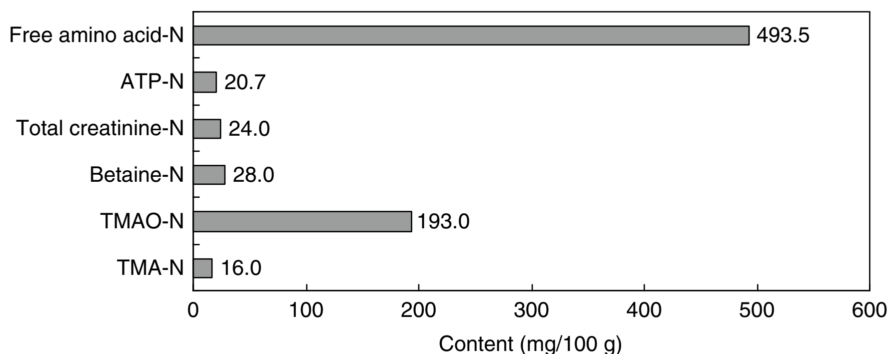


Fig. 5.13 Nitrogenous compound content of Pacific cod gill jeotgal

(25.8 %), in that order. Prominent fatty acids of Pacific cod gill jeotgal are 16:0 (19.0 %), 18:1 (18.6 %), 20:5n-3 (9.5 %), and 22:6n-3 (14.0 %), which account for approximately 61.1 % of the total fatty acids. These prominent fatty acids of Pacific cod gill jeotgal are observed to be typical of fish lipids, containing substantial quantities of some highly unsaturated long-chain fatty acids, such as 20:5n-3 (9.5 %) and 22:6n-3 (14.0 %) (Lee et al. 1986). By the results of the fatty acid composition, the lipids in the Pacific cod gill jeotgal have significantly higher levels of n-3 long-chain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are associated with reduced risk of geriatric and cardiovascular disorders and certain forms of cancer. In that sense, the Pacific cod gill jeotgal is considered to be a form of healthy food.

- *Taste characterization*: The TCA soluble-N content of Pacific cod gill j*eotgal* is 493.5 mg/100 g for FAA-N, 20.7 mg/100 g for ATP and its related compounds-N, 24.0 mg/100 g for total creatinine-N, 28.0 mg/100 g for betaine-N, 193.0 mg/100 g for TMAO-N, and 16.0 mg/100 g for TMA-N (Fig. 5.13). According to these results, the major components of TCA soluble-N are FAA-N and TMAO-N.

In general, FAAs are the most important components influencing the taste of jeotgal. The total content of FAAs of Pacific cod gill jeotgal, expressed as mg/100 g of wet weight, is 346.08 mg/100 g (Table 5.28). From the concentration of FAAs in Pacific cod gill jeotgal, the major FAAs are of two kinds, glutamic acid (162.34 mg/100 g, 46.9 %) and aspartic acid (36.72 mg/100 g, 10.6 %). These results are probably because of the MSG used for seasoning Pacific cod gill jeotgal (Kang et al. 2000).

The total taste value of Pacific cod gill jeotgal is 46.25. Among the various FAAs of Pacific cod gill jeotgal, glutamic acid (32.47) has the highest taste values calculated from the ratio of amino acid content to taste threshold (Table 5.28) (Kato et al. 1989), followed by aspartic acid (10.6). According to these results, one may

Table 5.28 FAA content and taste value of Pacific cod gill jeotgal

		Pacific cod gill jeotgal		
Amino acid	Taste threshold (g/dL)[a]	FAA content (mg/100 g)[d]		Taste value[c]
Lys	0.050	9.73	(2.8)[b]	0.19
His	0.020	2.59	(0.7)	0.13
Arg	0.050	18.58	(5.4)	0.37
Asp	0.003	36.72	(10.6)	12.24
Thr	0.260	12.85	(3.7)	0.05
Ser	0.150	13.07	(3.8)	0.09
Glu	0.005	162.34	(46.9)	32.47
Pro	0.300	23.15	(6.7)	0.07
Gly	0.130	6.65	(1.9)	0.05
Ala	0.060	8.23	(2.4)	0.14
Cys	–	1.51	(0.4)	–
Val	0.140	11.13	(3.2)	0.08
Met	0.030	3.15	(0.9)	0.11
Ile	0.090	7.73	(2.2)	0.09
Leu	0.190	11.67	(3.4)	0.06
Tyr	–	6.89	(2.0)	–
Phe	0.090	10.09	(2.9)	0.11
Total	–	346.08	(99.9)	46.25

[a]Source: data from Kato et al. (1989), with permission
[b]Values in parentheses represent FAA composition (g/100 g of the total amino acid)
[c]Taste values are calculated as FAA concentration/taste threshold
[d]Source: data from Kang et al. (2000), with permission

conclude that taste of Pacific cod gill jeotgal is influenced by glutamic acid for umami (45.46) and by aspartic acid for sweetness.

- *Shelf life*: The Pacific cod gill jeotgal can be stored for 3 months at 5°C.

7 Safety of By-product Jeotgal

Biogenic amine, including tyramine, histamine, putrescine, cadaverine, spermine, and spermidine, can be formed and degraded as a result of normal metabolic activity in animals, plants, and microorganisms (Arena and Manca de Nadra 2001). The amines are usually produced in a wide variety of foods by the decarboxylation of amino acids (Halasz et al. 1994). Typical symptoms of biogenic amine intoxication in human can be nausea, respiratory distress, hot flushes, sweating, heart palpitations, headaches, bright red rash, oral burning, and hypertension, as well as hypotension (Mah et al. 2002; Rice et al. 1976).

Putrescine and cadaverine inhibit intestinal diamine oxidase and histamine N-methyltransferase, which metabolize histamine, resulting in an increase of histamine toxicity (Mah et al. 2002; Stratton et al. 1991). Furthermore, putrescine,

Table 5.29 Biogenic amine contents (mg/kg) of Korean commercial jeotgal

Biogenic amine	Raw material			
	Changran	Myungran	Pacific cod gill	Anchovy
Putrescine	ND-20	15–136	ND-46	92–241
Cadaverine	ND	ND-85	ND	ND-665
Histamine	ND	ND	ND	155–579
Tyramine	ND	22–171	ND-14	63–244
Spermidine	ND	ND	ND	ND-43
Spermine	ND-51	26–58	ND-43	ND-77

Source: data from Mah et al. (2002)
ND not detected

cadaverine, spermidine, spermine, and agmatine are reported to be potentially carcinogenic by converting to nitrosamine (Mah et al. 2002). Tyramine has also been identified as the major mutagenic precursor in animals (Ochiai et al. 1984).

Two (putrescine and spermine) and three (putrescine, tyramine, and spermine) different types in biogenic amines are detected in changran jeotgal and Pacific cod gill jeotgal, by-product jeotgal, and the contents are ND-20 mg/kg and ND-46 mg/kg, respectively, in putrescine, ND and ND-14 mg/kg, respectively, in tyramine, ND-51 mg/kg and ND-43 mg/kg, respectively, in spermine (Table 5.29). Four different types of biogenic amine are also detected in myungran jeotgal, and the contents are ND-136 mg/kg in putrescine, ND-85 mg/kg in cadaverine, 22–171 mg/kg in tyramine, and 26–58 mg/kg in spermine. The results indicate that biogenic amine of *myungran jeotgal* is higher in content and more often found in types than the other by-product jeotgal. However, the biogenic amine contents of myungran jeotgal have lower levels than those of anchovy jeotgal (92–231 mg/kg in putrescine, ND-665 mg/kg in cadaverine, 155–579 mg/kg in histamine, 63–244 mg/kg in tyramine, ND-43 mg/kg in spermidine, and ND-77 mg/kg in spermine).

The amounts of biogenic amines in the tested by-product jeotgal are within the safe levels for human health, although certain samples which have been stored poorly need to be monitored carefully to ensure their safety for human consumption.

8 Conclusion

Jeotgal, the Korean traditional salt-fermented fishery, is usually prepared by the addition of salt to freshwater or marine fish, shellfish, crustaceans, and its by-products before fermentation and used popularly not only as side dishes, but also as ingredients in preparing kimchi in Korea, because it contains relatively high amounts of amino acids, the degradation products of fish protein.

During seafood processing, seafood processing by-products, such as head, gill, fish frame, viscera, scale, and skin, are generated in large quantities (approximately

more than 50 % based on the whole fish). Most seafood processing by-products are conventionally used to produce fish meal and fertilizer or are directly discharged into estuaries, resulting in environmental pollution. However, among these seafood by-products, viscera, roe, and gill can be used as good resources for preparing jeotgal.

There are nine kinds of commercial jeotgal by-products: changran jeotgal (salt-fermented intestine and stomach of Alaska pollock), myungran jeotgal (low-salt fermented roe of Alaska pollock), Pacific cod gill jeotgal, sea cucumber viscera jeotgal, chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal, abalone viscera jeotgal, konoshiro gizzard shad stomach jeotgal (called jenoe bam jeotgal in Korea), and sea urchin roe jeotgal.

The number of total viable cells of by-product jeotgal varied depending on the raw materials, salt concentration, temperature, and fermentation period. The total cell numbers reached 10^3–10^5 CFU/g in high-salt jeotgal, such as Pacific cod gill jeotgal, chub mackerel viscera jeotgal, largehead hairtail viscera jeotgal, and konoshiro gizzard shad stomach jeotgal, while it reached 10^7 CFU/g in low-salt jeotgal, such as changran jeotgal, myungran jeotgal, and sea urchin roe jeotgal.

Changes in consumers' life styles, who are concerned about the harmful effects of a high level of sodium chloride in their diet, motivate the tendency to reduce the amount of sodium in food. In that sense, the consumption of jeotgal, including by-product jeotgal, is limited due to the high concentration of salt. Therefore, more research on the development of low-salt jeotgal with similar taste and shelf life compared to high-salt jeotgal is needed for the wide consumption of jeotgal.

The amounts of biogenic amines in the tested by-product jeotgal are within the safe levels for human health, although certain samples which have been stored poorly need to be monitored carefully to ensure their safety for human consumption.

References

Arason S (1994) Production of fish silage. In: Martin AM (ed) Fisheries processing. Chapman & Hall, London/Glasgow/Weinheim/New York/Tokyo/Melbourne/Madras, pp 244–272

Arena ME, Manca de Nadra MC (2001) Biogenic amine production by *Lactobacillus*. J Appl Microbiol 90:158–162

Cha YJ, Cho SY, Oh KS, Lee EH (1983) The taste compounds of low salt fermented sardine. Bull Korean Fish Soc 16:140–146

Chae S-K (2011) Studies on microbial and enzymatic actions during the ripening process of salted Alaska pollack tripe. Korean J Food Nutr 24:340–349

Ciarlo AS, Paredi ME, Fraga AN (1997) Isolation of soluble collagen from hake skin (*Merluccius hubbsi*). J Aquat Food Prod Technol 6:65–77

Ezquerra-Brauer JM, Haard NF, Ramirez-Olivas R, Olivas-Burrola H, Velazquez-Sanchez CJ (2002) Influence of harvest season on the proteolytic activity of hepatopancreas and mantle tissues from jumbo squid (*Doswicus gigas*). J Food Biochem 26:459–475

Fisheries Information Service (2012) http://www.fips.go.kr/

Fuke S (1994) Taste-active components of seafoods with special reference to umami substances. In: Shahidi F, Botta JR (eds) Seafoods: chemistry, processing technology and quality. Chapman & Hall, London/Glasgow/Weinheim/New York/Tokyo/Melbourne/Madras, pp 115–139

Guan L, Cho KH, Lee J-H (2011) Analysis of the cultivable bacterial community in Jeotgal, a Korean salted and fermented seafood, and identification of its dominant bacteria. Food Microbiol 28:101–113

Halász A, Baráth A, Simon-Sarkadi L, Holzapfel W (1994) Biogenic amines and their production by microorganisms in food. Trends Food Sci Technol 5:42–49

Han BW, Kim HS, Jee SJ, Lee JH, Kim HJ, Park SH, Ji SG, Heu MS, Kim JS (2007) Characteristics of hot-water extracts from salmon frame as basic ingredients for *Gomtang*-like products. J Korean Soc Food Sci Nutr 36:1326–1333

Heu MS, Kim JS, Shahidi F (2003) Components and nutritional quality of shrimp processing by-products. Food Chem 82:235–242

Heu M-S, Kang K-T, Kim H-S, Yeum D-M, Lee T-G, Park T-B, Kim J-S (2007) Preparation and characteristics of functional sauce from shrimp byproducts. J Korean Soc Food Sci Nutr 36:209–215

Je J-Y, Qian Z-J, Byun H-G, Kim S-K (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42:840–846

Jo CH, Kim DH, Byun MW, Lee WD, Lee JJ (2003) Application of gamma irradiation on manufacturing changran jeotgal (aged and seasoned intestine of Alaska pollack): microbiological and sensory characteristics. J Korean Soc Food Sci Nutr 32:673–678

Jo C, Kim DH, Kim HY, Lee WD, Lee HK, Byun MW (2004a) Studies on the development of low-salted, fermented, and seasoned *Changran Jeotkal* using the intestines of *Therage chalcogramma*. Radiat Phys Chem 71:123–126

Jo C, Lee WD, Kim DH, Kim JH, Ahn HJ, Byun MW (2004b) Quality attributes of low salt Changran Jeotkal (aged and seasoned intestine of Alaska pollock, *Theragra chalcogramma*) developed using gamma irradiation. Food Control 15:435–440

Kang YS, You DW, Park KB, Kang NJ, Kim JS, Kim HY, Cho YH, Lee KN, Byun JH, Ha JH, Lee YH, Kim HR, Koo JG, Jeon JG (2000) A comprehensive bibliography on the fishery special commodity in Korea. Suhyup Publishing, Seoul, pp 94–95, 130–131

Kato H, Rheu MR, Nishimura T (1989) Role of free amino acids and peptides in food taste. In: Teranishi R, Buttery RG, Shahidi F (eds) Flavour chemistry: trends and development. ACS Symposium Series/American Chemical Society, Washington, DC, pp 158–174

Kim YM, Kim DS (1990) Salted-fermented fish of Korea-the raw material and product. Korea Food Research Institute, Seoul, pp 269–279

Kim HJ, Park JO (1984) Compositions of amino acids and nucleotides in fermented salted gut of hairtail. J Korean Home Econ Assoc 50:39–47

Kim J-S, Park JW (2004) Characterization of acid-soluble collagen from Pacific whiting surimi processing byproducts. J Food Sci 69:C637–C642

Kim JS, Park JW (2007) Mince from seafood processing by-product and surimi as food ingredients. In: Shahidi F (ed) Maximising the value of marine by-products. Woodhead Publishing/CRC Press LLC, Boca Raton/New York/Washington, DC, pp 196–228

Kim YM, Jeong YM, Hong JH (1993) Processing conditions for low-salted squid *Jeotkal*. Bull Korean Fish Soc 26:312–320

Kim E-U, Jo J-H, Oh S-W, Kim Y-M (1997) Characteristics of squid viscera oil. J Korean Fish Soc 30:595–600

Kim JS, Cho ML, Heu MS (2003a) Quality improvement of heat-induced surimi gel using calcium powder of cuttlefish, *Sepia esculenta*, bone treated with acetic acid. J Korean Fish Soc 36:198–203

Kim J-S, Shahidi F, Heu M-S (2003b) Characteristics of salt-fermented sauces from shrimp processing byproducts. J Agric Food Chem 51:784–792

Kishimura H, Saeki H, Hayashi K (2001) Isolation and characteristics of trypsin inhibitor from the hepatopancreas of a squid (*Todarodes pacificus*). Comp Biochem Physiol B 130:117–123

Kochi N, Hata Y (1964) Changes in chemical composition of mass products of *Uni-shiokara* during the storage. Bull Shimonoseki Univ Fish 13:23–28

Lee CH (1993) Fish fermentation technology in Korea. In: Lee CH, Steinkraus KH, Alan Reilly PJ (eds) Fish fermentation technology. United Nations University Press, Seoul, pp 259–279

Lee CH, Lee EH, Lim MH, Kim SH, Chae SK, Lee KW, Ko KH (1986a) Characteristics of Korean fish fermentation technology. Korean J Diet Cult 3:267–278

Lee EH, Oh KS, Lee TH, Ahn CB, Cha YJ (1986b) Fatty acid composition of salted and fermented sea foods on the market. Korean J Food Sci Technol 18:42–47

Lee CH, Lee EH, Lim MH, Kim SH, Chae SG, Lee GW, Ko KH (1987) Fermented fish products in Korea. Youlim Publishing, Seoul

Lee W-D, Chang D-S, Koh B-H, Lee M-S, Jeong E-T (1997) Quality analysis of viscera of Alaska pollock treated on vessel for raw materials of *Changran-Jeotgal*. J Korean Fish Soc 30:271–276

Llorente Holgado R, Altonaga Zubiaga M, Peral Díez I, Ibargüen Salaverri IM, Gartzia Palacios I (2007) Salting dynamics for anchovy (*Engraulis encrasicholus*) with salt replacers. Proceedings of the European congress of chemical engineering (ECCE-6), Copenhagen, 16–20 Sept 2007

Lynch NM (1987) In search of the salty taste. Food Technol 41:82–86

Mah J-H, Han H-K, Oh Y-J, Kim M-G, Hwang H-J (2002) Biogenic amine in *Jeotkal*s, Korean salted and fermented fish products. Food Chem 79:239–243

Mheen TI (1993) Microbiology of salt-fermented fishery products in Korea. In: Lee C-H, Steinkraus KH, Alan Reilly PJ (eds) Fish fermentation technology. United Nations University Press, Seoul, pp 231–247

Muyonga JH, Cole CGB, Duodu KG (2004) Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chem 85:81–89

Nam GH (2009) Quality characteristics and shelf-life of Myungran-Jeotgal by gamma-irradiation. MSci thesis, Gyeongsang National University, Tongyeong

National Rural Resources Development Institute (2007) Food composition table (7th revision). Hyoil Publishing, Seoul

Ochiai M, Wakabayashi K, Nagao M, Sugimura T (1984) Tyramine is a major mutagen precursor in soy sauce, being convertible to a mutagen by nitrite. Gann 75:1–3

Ogawa M, Portier JR, Moody MW, Bell J, Schexnayder MA, Losso JN (2004) Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). Food Chem 88:495–501

Park SM, Park CK, Lee KT, Kim SM (1998) Changes in taste compound of low salt fermented pollack tripe during controlled freezing point aging. Korean J Food Sci Technol 30:49–53

Park C-H, Lee J-H, Kang K-T, Park J-W, Kim J-S (2007) Characterization of acid-soluble collagen from Alaska pollock surimi processing by-products (refiner discharge). Food Sci Biotechnol 16:549–556

Phelps T, Angus F, Clegg S, Kilcast D, Narain C, Den Ridder C (2006) Sensory issues in salt reduction. Food Qual Prefer 17:629–634

Rice SL, Eitenmiller RR, Koehler PE (1976) Biologically active amines in food: a review. J Milk Food Technol 39:353–358

Shahidi F (2007) Maximizing the value of marine by-products: an overview. In: Shahidi F (ed) Maximizing the value of marine by-products. Woodhead Publishing/CRC Press LLC, Boca Raton/Boston/New York/Washington, DC, pp 3–21

Shinano H, Sato M, Akiba M (1975) Studies on the microorganisms in foods. Distribution of yeasts in fermented and salted foods. Bull Fac Fish Hokkaido Univ 26:207–215

Statistics Korea (2012) http://www.index.go.kr/egams/stts/jsp/potal/stts/PO_STTS _IdxMain.jsp?idx_cd=2748&bbs=INDX_001&clas_div=C&rootKey=1.48.0

Stratton JE, Hutkins RW, Taylor SL (1991) Biogenic amines in cheese and other fermented foods: a review. J Food Prot 54:460–470

Uno T (1974) Studies on the marine fermented foods. 5. On the preservative effects of lactic acid added to squid shiokara. J Hokkaido Fish Exp Sta 31:23–33

Wendel AP (1999) Recovery and utilization of Pacific whiting frame meat for surimi production. MSci thesis, Oregon State University, Corvallis

Chapter 6
Isoelectric Solubilization/Precipitation as a Means to Recover Protein and Lipids from Seafood By-products

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1 Introduction

In traditional fisheries, most of the aquatic food resources were almost completely utilized for human consumption, animal feed, or plant fertilizer. The more recent technological advancements and, subsequently, economy-driven industrialization of fisheries have brought about tremendous development. At the same time, however, the amount of by-products generated during processing has increased accordingly (Gehring et al. 2011).

While global fish capture and aquaculture production data are available from the Food and Agriculture Organization (FAO) Fisheries Department, the amounts of by-products can only be estimated (Anonymous 2007). There are different estimates as to how much by-products are available. The FAO estimates postharvest losses to be 25 % of the catch. The amount of by-products varies depending on species, size, season, and the fishing grounds (Falch et al. 2006). However, up to 50 % of the fish is commonly discarded when preparing seafood industrially (Guérard et al. 2005). Others claim that seafood processing discards and by-products make up around 75 % of the total weight of the catch (Shahidi 1994; Pastoriza et al. 2004; Torres et al. 2007). Typical examples are commercial fish filleting, shrimp trawling, or krill processing. Recovery yields of meat from Antarctic krill in commercial processing are extremely low, being between 10 % and 15 % by weight of the whole animal. Krill are small, shrimp-like crustaceans with the largest biomass of any multicellular animal species on earth, yet are significantly underutilized for human consumption (Nicol and Foster 2003). It is not unusual in shrimp trawling to have 90 % of the total catch volume as by-catch. The by-catch is usually discarded.

Some of the aquatic species are not utilized for human consumption for various reasons. One example is Atlantic menhaden, which is solely used by the reduction fisheries to manufacture fish meal and fish oil. However, menhaden is not processed for human consumption, due to its bony and oily carcass characteristics. This is why menhaden is considered a low-value fish; yet, if muscle proteins were recovered from menhaden and subsequently used in human food products, likely, a significant increase in value would result. Similarly, typical mechanical means of meat recovery on a commercial scale from carp is impractical due to the bony nature of the carp carcass. According to the FAO, carp provides the greatest and lowest cost biomass of aquatic foods in the world (Anonymous 2007; Lowther 2005).

The annual harvest of Pacific salmon (*Oncorhynchus* sp.) in Alaska is around 300,000 metric tons, generating around 50,000 metric tons of heads and 30,000 metric tons of viscera (Sathivel et al. 2005). Solid by-products generated from seafood factories range from 30 to 85 % of the weight of the landed fish. With a grand total of over 90 million metric tons of wild-caught fish and a global aquaculture production of over 70 million metric tons (FAO 2011), the amount of aquatic by-products is huge and there is huge potential for making more value-added products from this raw material (Rustad et al. 2011).

The by-products and low-value species can be used as a basis to derive three major groups of products: (1) plant fertilizers, (2) livestock feeds, and (3) human value-added foods and specialty foods. In general, conversion of the by-products

to fertilizers results in the least value addition to the by-products; while the value addition is the highest when human foods or, particularly, specialty foods are developed. It has been estimated that, if some components are recovered from fish processing by-products and used in the development of human food products, their value increases five times (Gildberg 2002).

2 Definitions of Seafood By-products

There is some misunderstanding as to how the aquatic food processing "by-products" are defined. Three terms are frequently and interchangeably used in the fishing industry and scientific literature to describe the same materials: (1) "offal"; (2) "waste"; and (3) "by-products". The first two terms imply that those materials cannot be used for any application and should be disposed of, and, therefore, are often misleading and trigger a negative connotation. The third term suggests that there may be some valuable components to be recovered if treated properly, and, therefore, it is a positive term. Since offal, waste, and by-products refer to the same material generated, the term "by-products" should be used, as this communicates more accurately the type of the material generated.

Currently, the most common definition of "by-products" is all of the edible or inedible materials left over following processing of the main product. A typical example is fish filleting to recover boneless and skinless marketable fillets. The fillets would be considered the main product and the frames, heads, and viscera would be typical "by-products". As mentioned above, the amount of filleting by-products is about two times higher than that of the fillets. It would be misleading to name these by-products as "waste", since, at the time of filleting, the quality of fish meat left on the frame (i.e., fish bones) and in the heads is not compromised (Strøm and Eggum 1981). If proper meat recovery technology is successfully applied, the recovered meat can result in added revenue for a processor as well as reduce environmental stress associated with the disposal of the processing by-products.

3 Opportunities for the Development of Protein and Lipid Recovery Technology from Seafood By-products

Fish oil is highly polyunsaturated and, therefore, very susceptible to lipid oxidation. If fish oil were to be efficiently recovered from the fish processing by-products, the use of fat-free fish by-products for animal feed and pet food could be expanded. The protein fraction is easily digestible and can be used for the production of hydrolysates, surimi, thermostable protein dispersions, gelatin, and collagen, as well as protamine. By-products are a very complex group of fractions, and the composition as well as the stability may, therefore, vary over a wide range. The composition of marine by-products has been extensively reviewed (Falch et al. 2006; Kerry and

Murphy 2007; Rustad 2007). In order to develop efficient recovery technologies, it is necessary to understand some fundamental properties of the raw materials. By understanding these properties, it may be possible to manipulate the behavior of biomolecules such as fish muscle proteins and lipids, and, consequently, increase the recovery yields.

4 Fundamental Properties of Seafood Lipids

The fat content in fish muscle is highly variable. It depends on species, age, spawning season, fish diet, and muscle type. While the protein content in fish muscle is relatively constant, the fat content is typically inversely correlated to the moisture content. Light muscle is considered anaerobic and provides energy quickly via glycogen hydrolysis and subsequent glycolysis. In contrast, dark muscle functions to provide long-term energy and relies primarily on the β-oxidation of lipids for energy (Hultin and Kelleher 2000a). Fish dark muscle contains more fish oil (i.e., lipids) than light muscle. Not only does the dark muscle have more oxygen supplied by the blood to deteriorate the oxidation-sensitive fish oils, but the dark muscle also has more pro-oxidative hemoglobin and mitochondria, and, therefore, is more prone to lipid oxidation and development of rancidity (Hultin et al. 2005). Consequently, light-fleshed fish are preferred by consumers, and, thus, processed by the industry. However, it would be economically beneficial to develop a technology capable of fish oil removal from dark fish meat in order to add value to this low-value or currently undesirable raw material.

The intramuscular lipids (i.e., triglycerides) of importance to food technologists are primarily composed of a glycerol backbone covalently bonded via ester bonds to three fatty acid (FA) chains of various lengths and degree of saturation (Fig. 6.1). The functional properties of fish oil depend on the composition of FAs attached to glycerol. Lipids are hydrophobic and have a lower specific density than water; therefore, when fish oils are in water, they tend to coalesce and form globules, floating to the top of the solution if enough time is allowed or sufficiently high *g* force is applied. Unlike fish muscle proteins, fish oil is apolar and does not have surface charges to allow hydrogen bonding with water. Conversely, fish oil can interact with hydrophobic side chains of amino acids (AAs) in fish muscle proteins, and, therefore, create weak lipid–protein hydrophobic bonds.

Another type of lipid that is probably more important to fish technologists than triglycerides is phospholipids. Phospholipids are an integral component of cell membranes. Like triglycerides, phospholipids contain a glycerol backbone; however, instead of three FAs, only two are covalently attached via ester bonds to the glycerol backbone. The third place on the glycerol backbone is filled with a positively charged phosphate group linked with some other charged moiety (often choline) (Fig. 6.2). Due to the positive charge, phospholipids are amphiphilic and have both hydrophobic and hydrophilic properties. Phospholipids can bond hydrophobically with other

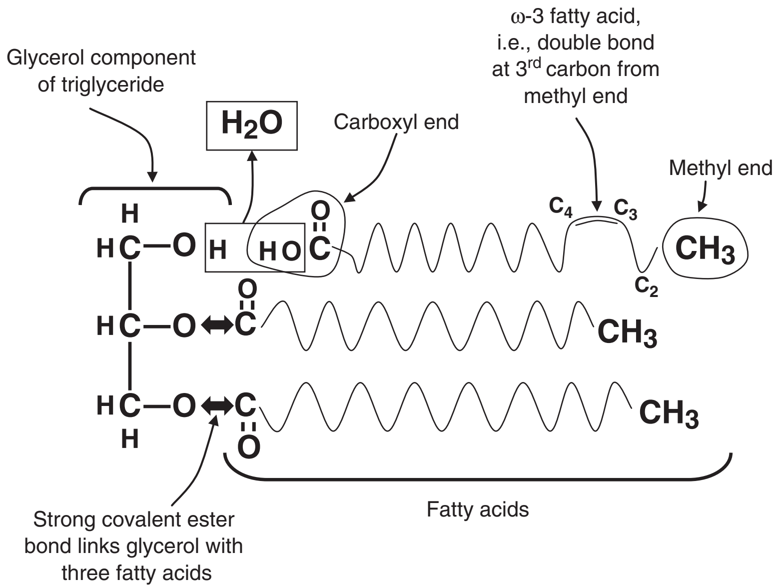

Fig. 6.1 Triglycerides are a major component of meat lipids (Adopted from Torres et al. 2007)

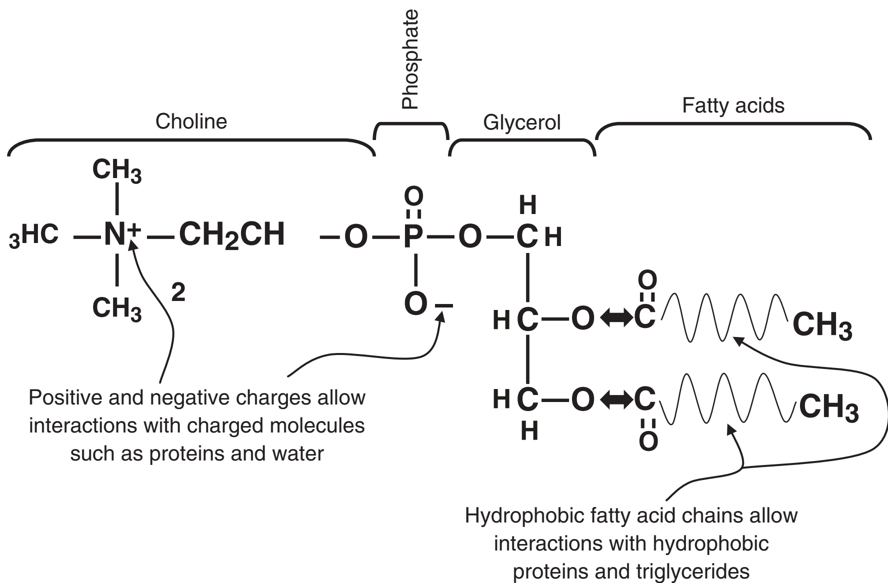

Fig. 6.2 Phospholipids, another major component of meat lipids, are water soluble and can interact with charged proteins and water dipoles (Adopted from Torres et al. 2007)

apolar substances (e.g., fish oil) and, at the same time, they can interact with water and/or charged proteins (e. g., the polar side chains of fish muscle proteins) (Fig. 6.2). Thus, phospholipids are typical emulsifiers. When phospholipids are present in solution during the processing of aquatic foods (e. g., minced fish meat), the separation of fish oil from water and proteins is more difficult. The membrane phospholipids tend to have a higher level of unsaturation as well as a greater surface area than muscle triglycerides. In addition, membrane phospholipids are also often in a close association with pro-oxidative processes, such as those in mitochondria. Therefore, membrane phospholipids are more susceptible to oxidation than intramuscular triglycerides. Although the content of phospholipids is lower than the content of triglycerides in fish muscle, the phospholipids contribute to rancidity more than triglycerides (Hultin et al. 2005). The problem is exacerbated because phospholipids are difficult to separate from minced fish due to their amphiphilic nature.

Fish oils have a higher level of unsaturation than their terrestrial counterparts. Although the polyunsaturated fatty acids (PUFAs) have been correlated with improved cardiovascular health in humans, they are highly susceptible to lipid oxidation, leading to rancidity (Chen et al. 2006, 2007). Most of the human health benefits of fish oil PUFAs have been ascribed to the omega-3 (ω-3) FAs, eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3). While DHA is commonly used to fortify infant formula in the USA, fish and fish products are excellent natural sources of this "heart-friendly" FA for the adult diet. The ω-3 FAs are considered "essential" (EFAs) from a human nutrition stand point, because, similarly to the essential amino acids, the EFAs cannot be synthesized by the human body. Therefore, the EFAs must be provided in the diet for proper development and function.

The ω-3 nomenclature refers to the third carbon atom in the FA chain where the first double bond (C=C) occurs counting from the methyl end (CH_3) of the FA (Fig. 6.1). In general, fish oils are highly valued by consumers, as demonstrated by their frequent inclusion in dietary supplements and functional foods. However, if fish are not properly processed and stored, the oxidation of fish lipids is rapid, leading to the onset of rancidity. Many customers find fish rancidity offensive. Since lipid oxidation is a chemical reaction, a thorough understanding of the oxidation events that fish oils undergo allows the development of proper strategies to prevent this detrimental reaction. Common strategies include vacuum packaging (lack of oxygen), antioxidants (for example, tocopherols, vitamin E), oxygen scavengers, freezing, etc. (Chen et al. 2007). Fish lipids are commonly referred to as fish oils due to their low melting point. The melting point depends primarily on the composition of FAs that are attached to the glycerol backbone. Due to a high concentration of long-chain (LC) PUFAs, fish oils tend to have lower melting points than their counterparts derived from terrestrial animals, such as lard or suet. The melting point of fish oil is below room temperature, leaving the lipids in a liquid state. However, fish are often processed at cold temperatures, and, therefore, fish oil becomes more viscous. In order to facilitate the separation of fish oil from minced fish meat, a processor may increase the temperature slightly, just enough to make the oil less viscous, and, therefore, per Stoke's law, facilitate their separation under *g* force by centrifugation.

5 Fundamental Properties of Seafood Proteins

Proteins are composed of approximately 20 AAs. While all of the AAs are indispensable for normal growth and sustaining metabolic processes, nine of them cannot be synthesized by adult humans. Thus, the nine AAs are referred to as "essential" (EAAs). The EAAs must be obtained through the diet. Although a certain protein may have a favorable AAs profile and be rich in EAAs, proteins are typically not 100 % bioavailable. The biological value (BV) of a protein measures its efficiency in supporting the human body's needs. Egg proteins are regarded as a reference protein and have a BV of 100, meaning that 100 % of the nitrogen from egg protein is absorbed and retained. Milk, beef, fish, corn, and rice proteins have BVs of 93, 75, 75, 72, and 59, respectively (Whitney and Rolfes 2005; Murano 2003). The BV of krill proteins has been reported as being slightly higher than the BV of milk proteins (Suzuki and Shibata 1990).

As in their name, all of the AAs contain amino and acid (carboxyl) groups that are bonded to a central carbon atom (Fig. 6.3). The condensation of α-amino and α-carboxyl groups on adjacent AAs can form a strong covalent bond, known as a peptide bond. A polypeptide consists of several AAs joined together by peptide bonds. Peptide bonds are much stronger than the bonds that hold the water dipoles together or result in protein–water interactions. Amino and carboxyl groups that are not involved in peptide bonds can be electrostatically charged in solutions, and, therefore, participate in protein–water interactions via weak hydrogen bonds (i.e., water solubility) (Fig. 6.3). The bonding energy of a hydrogen bond is low (2–40 kJ/mol); however, when present in high numbers, these bonds are primarily responsible for the stabilization of the complex secondary, tertiary, and quaternary structures of proteins. AAs share a common amino and carboxyl group, but differ in their functional groups, commonly referred to as side chains (Fig. 6.3). The different side chains, unique to each AA, give rise to the vastly diverse characteristics of different food proteins.

Muscle of aquatic animals is composed mainly of water, protein, and lipid. Two muscle types are present in aquatic foods: striated muscle characterized by transverse stripes and smooth muscle that lacks them. Striated muscle is the main component in fish meat, while smooth muscle is typical of meat from molluscans. Fish muscle is divided further into light and dark muscle. The dark muscle lies alongside the fish body and under the skin. The proteins that comprise light muscle can be further classified into three major groups according to their water solubility characteristics; myofibrillar proteins, sarcoplasmic proteins, and stroma proteins (Tahergorabi et al. 2011).

5.1 Myofibrillar Proteins

Myofibrillar proteins are the proteins that form myofibrils. They are soluble in concentrated saline solutions (ionic strength above 0.6), as well as having extremely low ionic strength, but are water insoluble in typical physiological ionic strength in

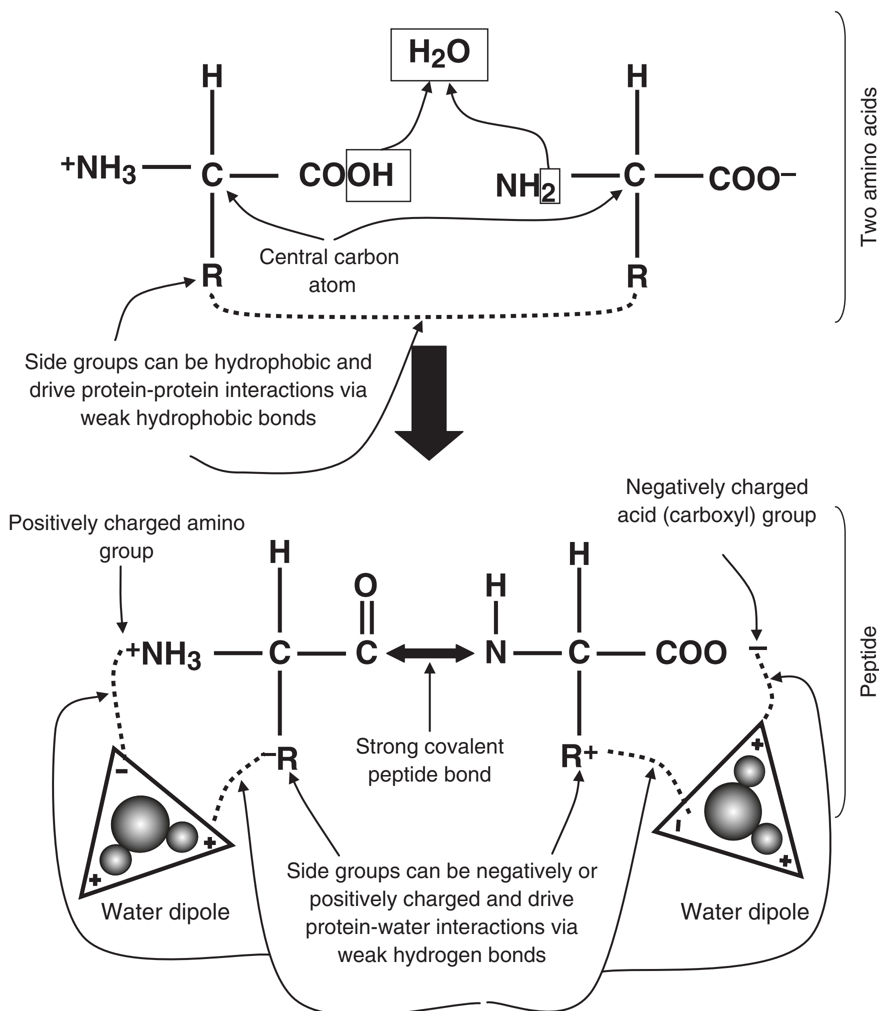

Fig. 6.3 Proteins are chains of amino acids containing an amino ($^{+}NH_3$) group, an acid (COO^{-}) group, and a side chain (R) all bonded to a central carbon atom. The side chain allows proteins to participate in protein–protein hydrophobic interactions and can also form protein–water hydrogen bonds (Adopted from Torres et al. 2007)

the fish muscle (ionic strength approximately 0.05 for rainbow trout). Myofibrillar proteins are composed of myosin, actin, and regulatory proteins such as tropomyosin, troponin, and actinin. Myofibrillar proteins comprise 66–77 % of the total proteins in fish muscle and provide several functional properties that are useful in food products. Generally, seafood myofibrillar proteins are less thermally stable than the proteins isolated from terrestrial animals. The pH and ionic strength affect the thermal stability of seafood myofibrillar proteins, and, hence, heat-induced denaturation. Myofibrillar proteins isolated from cold water species are typically less

thermally stable than warm water species. This property translates into different requirements for the handling and freezing of seafood from cold and warm waters (Jaczynski et al. 2011). Protein gelation and rheological properties responsible for texture development, and, therefore, consumer acceptability mainly depend on the quality of myofibrillar proteins. The quality is affected by seafood species, age, seasonality, freshness, and processing parameters, such as protein concentration, pH, ionic strength, and temperature (Suzuki 1981).

5.2 Sarcoplasmic Proteins

Sarcoplasmic proteins contain several individual types of water-soluble proteins called myogen. Since sarcoplasmic proteins are water soluble, they are isolated from fish muscle by simply pressing the fish muscle tissue or by extraction with low ionic strength saline solution. Pelagic fish such as sardine and mackerel have generally higher contents of sarcoplasmic proteins compared to demersal fish like plaice and snapper. These proteins may interfere with myosin cross-linking during gel matrix formation because they do not gel and have poor water-holding capacity (Sikorski et al. 1994). However, the contribution of sarcoplasmic proteins to the overall gelation properties of fish muscle has recently been debated.

5.3 Stroma Proteins

Stroma proteins form connective tissue in the muscle structure. These proteins are completely water insoluble. They cannot be extracted in acid or alkaline solution, or physiological saline solution. Stroma proteins are composed of collagen and elastin. Elastin is very resistant to wet heat and cooking does not affect elastin in the connective tissue. Dark fish meat contains more stroma proteins and less sarcoplasmic proteins than light fish meat (Tahergorabi 2012).

5.4 Isoelectric Behavior of Seafood Muscle Proteins

ISP of fish muscle proteins with concurrent separation of lipids was proposed by Hultin and Kelleher (1999, 2000b, 2001, 2002). Following these pioneering developments along with earlier works by Meinke et al. (1972) as well as Meinke and Mattil (1973), several food science laboratories began active research with ISP. In fish muscle homogenates, myofibrillar proteins are present as aggregates that are held by weak protein–protein hydrophobic interactions (Undeland et al. 2003). However, the protein side chains can assume different electrostatic charges, depending on the conditions to which the fish muscle proteins are subjected (Fig. 6.4).

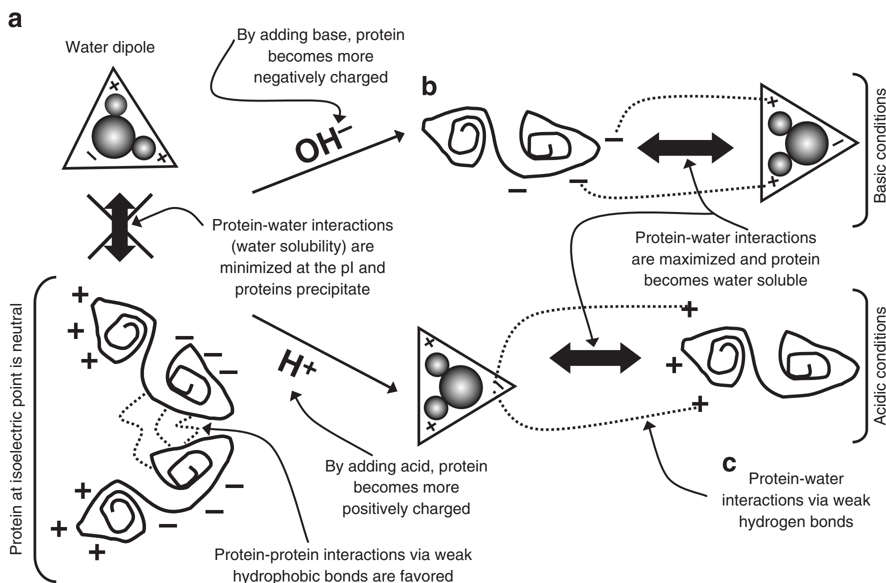

Fig. 6.4 A protein at its isoelectric point (pI) has a zero net electrostatic charge (adopted from Gehring et al. 2011). (**a**) At its pI, protein–water interactions are at their minimum, while protein–protein interactions via weak hydrophobic bonds are at their maximum, causing protein precipitation. (**b**) Protein–water interactions prevail under acidic or basic conditions far from the pI, resulting in protein solubility

This means that the solubility of fish muscle proteins can be turned "on" or "off" by providing conditions which either favor or disfavor protein solubility, respectively. When an acid is added to a solution, it dissociates, yielding hydronium ions (H_3O^+). The protonation of negatively charged side chains on glutamyl or aspartyl residues results in an increased net positive surface charge. Similarly, when a base (OH^-) is added to a solution, deprotonation of the side chains on tyrosyl, cysteinyl, or lysyl residues contributes to an increased net negative surface charge (Fig. 6.4). Consequently, the solubilization of fish muscle proteins is ascribed to the protonation of aspartyl and glutamyl (pKa = 3.8 and 4.2, respectively) residues at acidic pH and deprotonation of lysyl, tyrosyl, and cysteinyl (pKa = 9.5–10.5, 9.1–10.8, and 9.1–10.8, respectively) residues at basic pH. When the charge equilibrium is reached and a protein solution attains homeostasis, the final status of a protein surface electrostatic charge at a given pH is referred to as the net charge. The accumulation of a net positive or negative charge induces protein–protein electrostatic repulsion and an increased hydrodynamic volume due to expansion and swelling (Kristinsson et al. 2005; Undeland et al. 2003). As proteins assume a more positive or negative net charge, they gradually start electrostatic interactions with water (i.e., protein–water interactions). Due to increased protein–water interactions, the protein–protein hydrophobic interactions decrease. Therefore, as the protein molecules become more polar (charged), more water associates on and around the protein surface and the proteins become water soluble. However, it is possible to adjust the pH of a

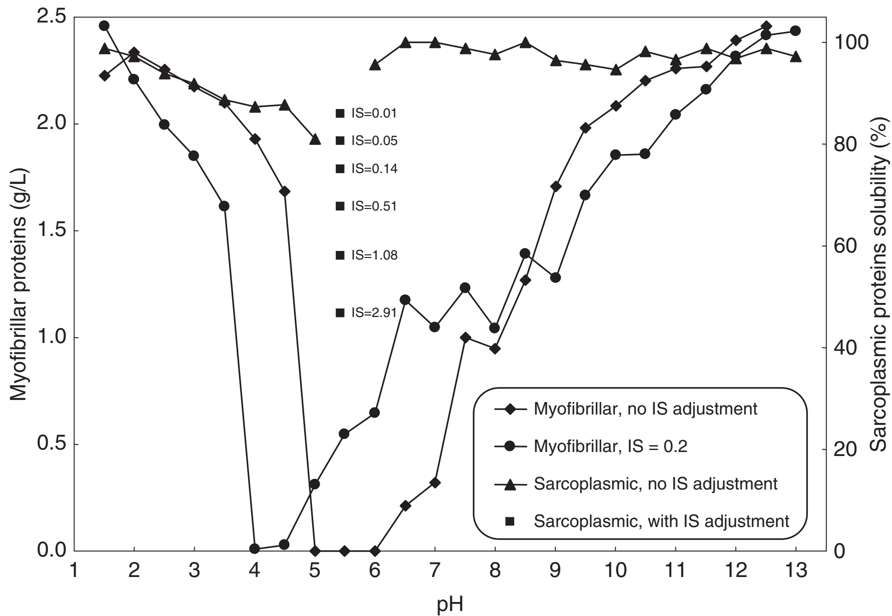

Fig. 6.5 Solubility of myofibrillar and sarcoplasmic fish proteins as a function of pH and ionic strength (IS) (Adopted from Chen and Jaczynski 2007b)

protein solution so that the number of negative charges on a protein's surface is equal to the number of positive charges, and, therefore, the protein molecule assumes a zero net electrostatic charge. The pH at which the net electrostatic charge of a protein is equal to zero is called the isoelectric point (pI) (Fig. 6.4).

The pI is very specific for different proteins and isoelectric focusing is often used to pinpoint it. The pI of fish muscle proteins is an important parameter because as the charges on a protein's surface diminish, so do the protein–water interactions, and hence, protein water solubility and water-holding capacity (WHC). In addition, proteins gel poorly at their pI. However, the hydrophobicity-driven protein–protein interactions are favored at the pI, and, therefore, proteins at their pI achieve minimum solubility and typically precipitate. This pH-mediated, protein isoelectric behavior allows the modification of protein solubility/precipitation by proper pH adjustment. Protein solubility curves (or profiles) as a function of the pH are often experimentally constructed in order to determine protein water solubility characteristics (Fig. 6.5).

The ionic strength (IS) influences the pH-mediated ISP (Chen and Jaczynski 2007a; Stefansson and Hultin 1994; Thawornchinsombut and Park 2004). Torres et al. (2007) proposed a continuous bioreactor system using ISP for protein and lipid recovery at a pilot/industrial scale. One of the advantages of using such a system is water recycling. However, due to water recycling, salt accumulation occurs, and, therefore, the IS increases, affecting the pI of fish muscle proteins. As a consequence of the increased IS, the myofibrillar proteins precipitate at a lower pH, and, therefore, the pH must reflect the changing pI in order to achieve maximum protein precipitation (i.e., recovery efficiency) during continuous ISP processing.

The increased IS causes a shift in the pI to a lower pH because Cl^- binds positively charged AAs to a greater extent than Na^+. Na^+ has a greater affinity for negatively charged AAs than Cl^- (Ockerman 1996). Thus, more H_3O^+ must be introduced into the system to neutralize the increased negative charges so that the net electrostatic charge becomes zero again and the pI is reached. The second major group of fish muscle proteins, sarcoplasmic proteins, is water soluble at both acidic and basic pH. However, sarcoplasmic proteins do not precipitate demonstrably between pH 5.0 and 6.0 as myofibrillar proteins (Fig. 6.5). As the IS increases, more sarcoplasmic proteins precipitate at pH 5.5 (Chen and Jaczynski 2007b). Therefore, in a continuous system, more sarcoplasmic proteins are recovered as salt accumulation increases.

The isoelectric behavior of fish muscle proteins can be used to recover functional proteins from aquatic animals processing by-products as well as low-value aquatic species. While muscle proteins are in a soluble form (protein–water interactions are favored), the insoluble components (bone, skin, scale, etc.) can be removed from the solution by, for example, centrifugation, followed by protein precipitation and recovery at the pI (protein–protein interactions are favored). Functional muscle proteins from fish have, thus far, been recovered at the laboratory scale using a batch mode (Choi and Park 2002; Kim et al. 2003; Kristinsson and Hultin 2003; Undeland et al. 2002) and pilot plant scale (Mireles DeWitt et al. 2007). The ISP processing in a continuous mode has also been applied to fish processing by-products (Chen and Jaczynski 2007b; Chen et al. 2007), krill (Chen and Jaczynski 2007a; Chen et al. 2009), and whole fish (Taskaya et al. 2009a, b).

5.5 *Protein Recovery Yield and Fat Recovery Yield from Seafood By-products*

The protein recovery yield is a critical parameter to initiate the determination of the economical feasibility of a new technology. As the ISP also allows the recovery of fish oil (fat), determination of the fat recovery yield may aid in a more comprehensive understanding of the economical feasibility of this technology. Fish oil is often perceived by consumers as beneficial for human health, particularly cardiovascular health associated with the abundance of ω-3 fatty acids. At the same time, fish oil that is retained with the recovered protein may contribute to the development of rancidity. Therefore, the determination of fat reduction in the recovered protein is also desirable. This is why one of the objectives in surimi processing is the removal of fish oil and, typically, the fat content in surimi is greatly reduced to <1 %. Surimi processing is a commercial technology that allows the recovery of muscle proteins (mainly myofibrillar) from headed-and-gutted fish; therefore, in this regard, it is similar to ISP. However, heading-and-gutting generates a significant amount of processing by-products with unrecovered fish meat and oil attached to bones, skin, etc. The processing of 100 kg of live fish using surimi technology recovers about 19.5 kg of proteins (i.e., surimi) (Lee 1999). This relatively low protein recovery with surimi technology means that the unrecovered proteins (water-soluble sarcoplasmic proteins)

end up in the process water. Surimi processing requires tremendous amounts of process water, about 20 times the weight of the deboned meat (Lee 1999). The process water is high in biological oxygen demand, and, therefore, requires treatment before it can be discharged. Unlike surimi technology, ISP allows the processing of by-products such as frames and heads or whole fish such as krill or carp. If ISP is designed as a continuous bioreactor system with recycling of process water, much less water is needed (Torres et al. 2007).

ISP results in relatively high recovery of proteins from the starting materials. Protein recovery yields for ISP reported in the literature range between 42 % and 90 % (Chen and Jaczynski 2007b; Chen et al. 2009; Kristinsson and Liang 2006; Nolsøe and Undeland 2009; Taskaya et al. 2009b). The differences may be attributed to the different methods used to determine the protein concentration and their recovery yield, fish species, centrifugation force used during ISP, and a relative concentration of water-soluble sarcoplasmic proteins in the starting material. Sarcoplasmic proteins are only partly recovered with ISP (Chen and Jaczynski 2007b). However, the application of flocculants during ISP improves the recovery of water-soluble proteins, and, consequently, increases the protein recovery yield (Taskaya and Jaczynski 2009). Sathivel et al. (2004) developed freeze-dried protein powders from various fish processing by-products by heating them at 85 °C for 60 min, followed by protein separation via centrifugation at 2,560 × *g* and subsequent freeze-drying. The protein recovery yields were in the range 14–31 %, depending on the starting material.

Fat recovery is more efficient when ISP is conducted at basic rather than acidic conditions. Liang and Hultin (2005a, b) demonstrated enhanced fat recovery when citric acid and its Ca salts were used during ISP. However, these reports did not list fat recovery yields. Okada and Morrissey (2007) extracted oil from skin-on sardine fillets by adjusting the pH of the mince to the pI, followed by centrifugation. Although there was no protein solubilization step, the pH adjustment to the pI increased oil recovery when compared to the conventional heat-aided oil recovery. Taskaya et al. (2009b) reported fat recovery yield in the range 80–95 % for whole gutted carp processed with ISP. Kristinsson et al. (2005) reported similar fat recoveries and fat reduction in the recovered proteins for catfish fillets. Kristinsson and Liang (2006) reported fat reduction in the proteins recovered from Atlantic croaker fillets using surimi processing and ISP. ISP using basic pH resulted in the highest fat reduction, followed by acidic pH, while surimi processing was the least efficient at fat reduction in the recovered proteins.

5.6 Recovery of Functional Proteins and Lipids from Seafood Processing By-products by Isoelectric Solubilization/Precipitation at the Pilot Scale

The principle of the pI has been used in cheese making and the manufacture of soy protein isolates. Following acidification of milk to pH 4.6 by the action of rennet

and/or lactic acid bacteria, casein, the major milk protein, precipitates at its pI. A casein curd is formed with subsequent draining of water-soluble protein, whey. The same principle may be applied to muscle proteins with the precipitated fraction composed of myofibril and stromal proteins (the casein fraction of cheese) and the soluble fraction composed of sarcoplasmic proteins (the whey fraction of cheese). The pI of fish muscle proteins is 5.5. Therefore, the fish muscle proteins precipitate at pH 5.5 and become water-soluble gradually as the pH becomes more acidic or alkaline.

In general, there are five steps in recovering proteins and lipids from fish processing by-products using ISP (Fig. 6.6). The first step is to homogenize a 1:6 (wt/wt) solution of by-products in water in order to provide reaction medium and increase the available surface area for the subsequent protein solubilization reaction. In the second step, the fish muscle proteins are solubilized under either acidic or alkaline conditions. As the pH moves further away from the pI, the fish muscle proteins assume a more uniform negative or positive surface charge for alkaline or acidic conditions, respectively (Fig. 6.4). The charge shift results in weaker protein–protein hydrophobic interactions, while protein–protein electrostatic repulsion becomes more predominant and results in protein–water interaction (i.e., water solubility). When proteins begin interaction with water, a drastic increase of viscosity occurs. In muscle homogenates, myofibrillar proteins are present as aggregates, induced by hydrophobic forces (Undeland et al. 2003). The solubilization of fish muscle proteins is ascribed to the protonation of aspartyl and glutamyl ($pK_a = 3.8$ and 4.2, respectively) residues at acidic pH and deprotonation of lysyl, tyrosyl, and cysteinyl ($pK_a = 9.5–10.5$, 9.1–10.8, and 9.1–10.8, respectively) residues at alkaline pH. Undeland et al. (2002, 2003) found that the viscosity of herring light muscle homogenate increased drastically around pH 3.5 and 10–10.5. In contrast, muscle protein homogenates from skinless catfish fillets and tilapia white muscle had much higher viscosity peaks at alkaline versus acidic pH (Kristinsson et al. 2005). The viscosity decreases sharply as soon as the proteins become water soluble. Undeland et al. (2003) as well as Kristinsson and Ingadottir (2006) attributed this fact to the break-up of existing aggregates/myofibrillar assemblies or deprotonation of the ε-amino groups of lysyl residues. The viscosity increase is an important processing parameter that may result in mixing issues (i.e., pH and protein solubility gradient), foam formation, etc. One way to compensate for this is to maintain the solution continuously at the desired pH; for example, in a continuous protein and lipid recovery system (Torres et al. 2007).

While the muscle proteins are in full interaction with water (i.e., solubilized), the third step, separation, is applied. Typically, centrifugation separates the solution for light, medium, and heavy fractions containing fish oil, solubilized muscle proteins, and impurities (bones, scale, skin, insoluble proteins, etc.), respectively. While the hydrophobic triglycerides (Fig. 6.1) are fairly easy to separate from the solution, the membrane phospholipids are relatively persistent because they are amphiphilic (Fig. 6.2). Greater than 50 % of the membrane phospholipids are retained with the solubilized proteins after step 3 (Undeland et al. 2002; Liang and Hultin 2005a, b). Although membrane phospholipids are present in smaller

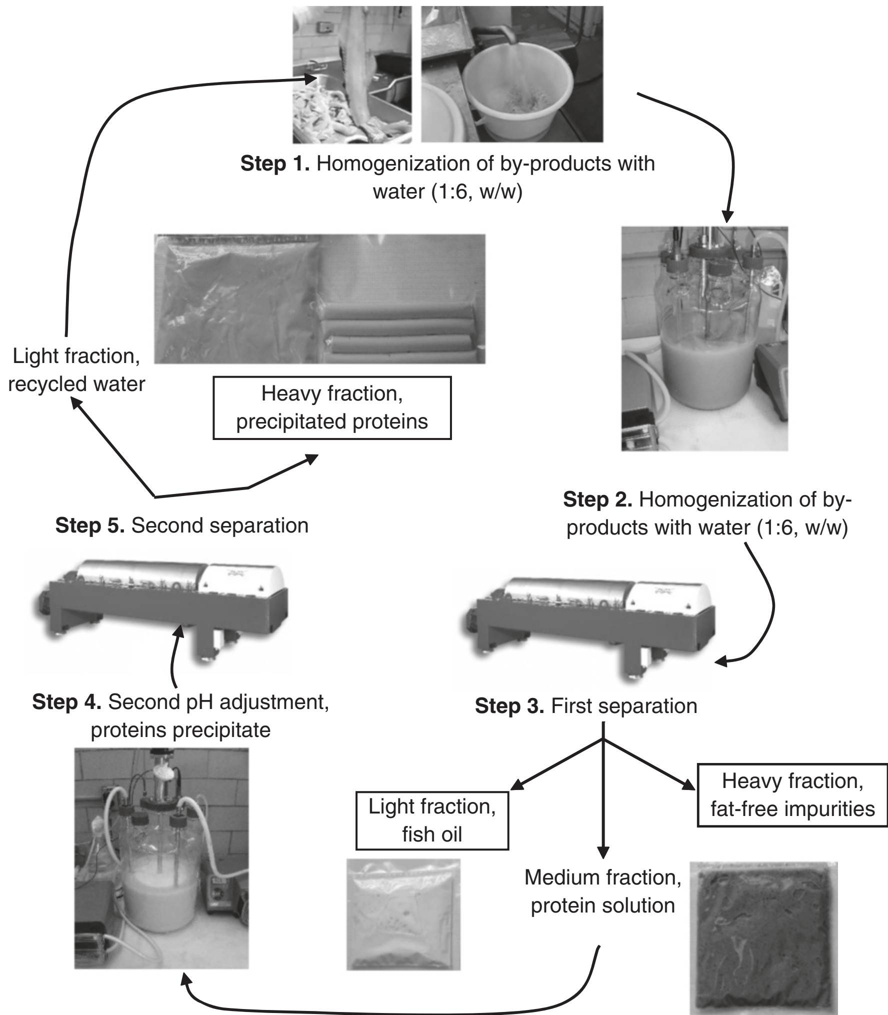

Fig. 6.6 Diagram of the isoelectric precipitation and solubilization technology with concurrent oil separation proposed for processing fish by-products. Materials in boxes are fractions to be further processed into food and other applications (Adopted from Torres et al. 2007)

amounts in the fish muscle than triglycerides, the membrane phospholipids have been demonstrated to contribute more to rancidity (Vannuccini 2004). Therefore, it is desirable to remove as much lipid as possible during the separation step. The third step results in the separation of crude fish oil that is rich in ω-3 PUFAs and can be further processed for numerous food and non-food applications (Chen et al. 2007). The heavy fraction is rich in minerals such as Ca, Mg, and P, and, therefore, can be a main ingredient in the development of animal feeds and value-added pet foods (Chen et al. 2007).

The medium fraction, containing the water-soluble fish muscle proteins, is recovered and subjected to the second pH adjustment in step 4. The pH is adjusted to the average pI of the fish muscle proteins (pH 5.5). At pH 5.5, fish muscle proteins precipitate due to increased protein–protein hydrophobic interactions and decreased protein–water interactions, as well as decreased protein–protein electrostatic repulsion. Similar to the first pH adjustment in the second step, as the proteins gradually stop interacting with water dipoles, the viscosity increases significantly. This viscosity issue can be overcome by maintaining the pH at 5.5 continuously. The precipitated fish muscle proteins are separated from the process water typically by centrifugation. The muscle proteins retain their gel-forming ability, and, therefore, can be used as a functional and major ingredient in human food products such as surimi seafood (commonly referred to as crab-flavored seafood). Processors should keep in mind that, like in surimi and other muscle foods products, in order to preserve protein functionality, protein isolates obtained using ISP must include cryoprotectants (Thawornchinsombut and Park 2006) for frozen storage. Following ISP, the process water can be reused in a continuous system, reducing processing costs. However, the purity of the process water and, thus, its reuse greatly depend on the processing parameters.

6 Continuous Bioreactor System for Seafood Processing By-products

Torres et al. (2007) proposed a continuous bioreactor system for fish processing by-products and whole fish. This system was based on ISP principles. The bioreactors were equipped with built-in pumps for various processing additives, such as antifoams, coagulants, flocculants, etc. The flocculants enabled more efficient separation of the precipitated fish muscle proteins from the process water and, consequently, could facilitate process scale-up from a laboratory to pilot/industrial scale (Taskaya and Jaczynski 2009). Following homogenization (Fig. 6.6, Step 1), the homogenate is pumped to the first bioreactor for a 10-min solubilization reaction (Step 2). The bioreactor continuously controls and maintains the pH. Because the pH of the incoming homogenate is close to neutral (~6.6–7.0), a base is rapidly pumped into the vessel to adjust the pH to 11. Bioreactors are also equipped with mixing baffles to prevent pH gradients and excessive foaming. A refrigerant is used to maintain constant temperature, while small pumps are used to inject food-grade emulsion breakers and antifoam agents. The experimental recovery system works at 300 L/h and can process ~43 kg/h of fish by-products (Fig. 6.7). Although these small-scale bioreactors are manufactured from glass and stainless steel components, industrial-strength, high-density polyethylene can be used in a fish processing plant. Based on the experimental system (Fig. 6.7), a modular 600-L bioreactor system has been designed to process 12 tons/day of fish processing by-products.

Following the pH adjustment in Step 2, the solution is pumped to a decanter centrifuge (Fig. 6.8), working typically below $4{,}000 \times g$. Decanters are commonly

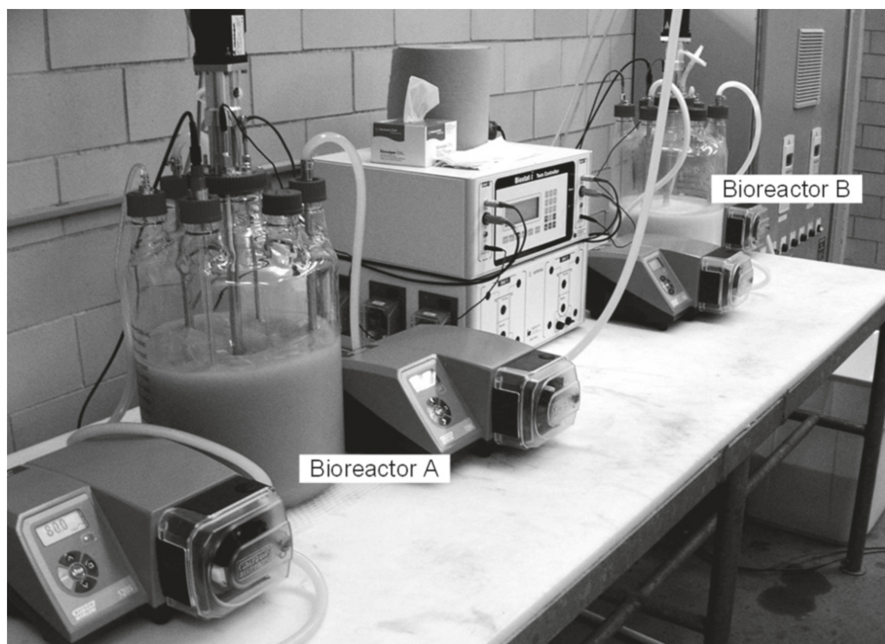

Fig. 6.7 Bioreactors equipped with automatic pH and temperature controls, continuous pumping of feed and treated stream, and dosing of food-grade additives such as emulsion breakers, protein flocculants, and antifoaming agents. Bioreactor A is used for protein solubilization (Step 2), while Bioreactor B is used for isoelectric precipitation (Step 4). A control box is placed between both bioreactors. This configuration is working in a continuous mode at a flow rate of 300 L/h (Adopted from Torres et al. 2007)

used in surimi processing plants. However, surimi technology does not work under acidic or basic pH; therefore, the assessment of an available decanter should be performed prior to use with ISP. There are no pH issues when separating proteins (Step 5); however, this can be relatively slow unless the particle size of the precipitated muscle proteins is increased by promoting protein–protein hydrophobic interactions using an extended precipitation time (~24 h) in Step 4.

The particle settling velocity under the centrifugal force (*g*) depends on the density differential between phases (Δρ), viscosity (μ), and particle size expressed as the equivalent diameter (D):

$$S = \frac{\Delta\rho * g * D^2}{\mu} \tag{6.1}$$

D is the only variable that a processor can modify in ISP. Using a precipitation time of only 10 min in Step 4, the protein particle size can be increased by adding commercially available flocculants.

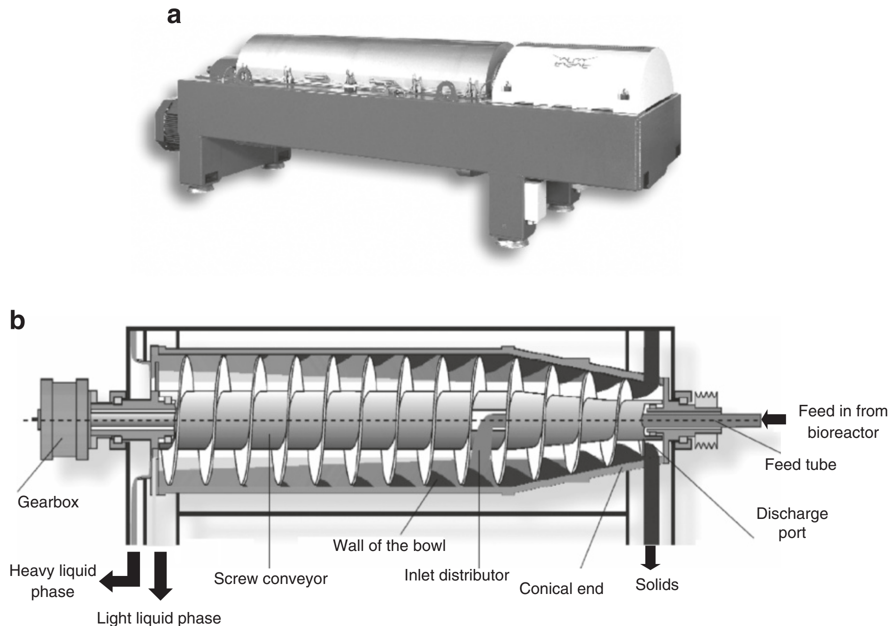

Fig. 6.8 Decanter centrifuges, typical separating equipment used in fish processing, could be utilized in the new isoelectric solubilization/precipitation technology (Adopted from Torres et al. 2007). (**a**) Commercial unit shown by courtesy of Alfa Laval. (**b**) Cross-sectional view of the decanter centrifuge bowl

7 Alternative Applications of Recovered Proteins

Most studies have focused on using proteins recovered via ISP directly or as the main ingredient for restructured human food products similar to surimi. For instance, Tahergorabi et al. (2012a) developed functional food products using ISP fish protein isolate, ω-3-rich oils, and salt substitute. Tahergorabi et al. (2012b) also found that the substitution of NaOH with KOH may decrease the Na content in the ISP fish protein isolate, and, thus, allow the development of reduced-Na seafood products. There are several laboratories actively researching alternative uses for the ISP-recovered protein isolates, including the development of hydrolysates (Theodore and Kristinsson 2007). Hydrolysates have been previously shown to inhibit angiotensin-converting enzyme (ACE), a biocatalyst that converts angiotensin I into the bioactive protein angiotensin II (Bordenave et al. 2002; Jung et al. 2006; Fahmi et al. 2004). Angiotensin II is responsible for arteriolar vasoconstriction and the degradation of bradykinin, an effective vasodilator (Theodore and Kristinsson 2007). Thus, ACE functions to increase blood pressure. Theodore and Kristinsson (2007) produced hydrolysates from ISP protein isolate using catfish as the starting material. Hydrolysates show great promise as natural food-based alternatives to ACE-inhibiting drugs.

Hydrolysates also have antioxidant potential (Bishov and Henick 1972). Alkaline-solubilized tilapia protein hydrolysate inhibited the development of hydroperoxides in a washed muscle system (Raghavan and Kristinsson 2008).

The injection of ISP protein isolates into whole muscle to increase textural and sensory properties has also been evaluated. ISP protein isolates from tilapia were injected as a 1–5 % solution in tilapia filets, improving their drip loss (Hussain 2007). Vann and Mireles DeWitt (2007) evaluated the effect of ISP protein isolate as an injection alternative to phosphates for the enhancement of select beef strip loins.

The recovered proteins can be chemically modified to make a biodegradable superabsorbent hydrogel (SAH) (Damodaran 2004). The modified proteins increase the carboxyl content due to alteration of the amino groups of lysine. The proteins are incorporated into a matrix of an anionic polysaccharide, such as carboxymethyl cellulose (Damodaran 2004). The SAH is capable of trapping up to 400 g water/g SAH. The SAH may be a viable replacement for non-biodegradable hydrocarbon-based hydrogels used in diapers and paper towels, biodegradable tray liners for meat packaging, or unconventional non-food applications, such as fire retardant. Another potential non-human food application for ISP protein isolates is as a nutritive binder in domestic animal feeds. Gehring et al. (2011) demonstrated improvement of pellet quality of broiler grower diets with the addition of ISP protein isolate.

8 Future Market Trends

Compared with milk and soy proteins, the market for marine proteins and peptides is not large and they have, so far, mainly been used as seafood flavors (Thorkelsson and Kristinsson 2009). The sale of marine proteins for more advanced applications is still low and development has been slow. Alta Vida AS has carried out a survey of the American market for protein ingredients. The potential has been estimated to be US$ 45–60 million. Marine protein ingredients may be an alternative to milk- and soy-based products. Several commercially available products with bioactive peptides (Thorkelsson and Kristinsson 2009), including products from bonito (http://www.nippon-sapuri.com/english, http://www.metagenics.com), sardines, collagen, and hydrolyzed whitefish (http://www.propernutrition.com, http://www.copalis.fr, http://www.nutrimarine.com) are available. A new protein ingredient needs to have a competitive price, good flavor (little or no flavor), documentation of health benefits, and an adequate shelf life. There is a market for water-retaining proteins of marine origin that can reduce drip loss, increase shelf life, and improve juiciness. The protein isolate NutriLean produced by Proteus Industries (http://www.proteusindustries.com) reduces fat uptake in fried foods and increases juiciness. The market for sports nutrition products is growing by 5–7 % per year. The main ingredients are amino acids and proteins. Sports nutrition is, therefore, an area that is well suited for marine proteins. Marine proteins have very good amino acid composition and a high digestibility.

9 Conclusions

The amount of seafood by-products is huge and there is great potential for making more value-added products from this raw material. It has been estimated that, if some components are recovered from seafood processing by-products and used in the development of human food products, their value increases five times. Isoelectric solubilization/precipitation (ISP) allows selective, pH-induced water solubility of muscle proteins with concurrent separation of lipids and removal of materials not intended for human consumption, such as bones, scales, skin, etc. Muscle proteins from seafood processing by-products have, thus far, been recovered using batch-mode ISP at the laboratory and pilot scales. A continuous bioreactor system for seafood processing by-products and whole fish using the ISP principles has been proposed. Currently, there is an increasing interest in different applications of ISP-recovered protein isolates. Thus, ISP processing may represent a more responsible use of natural resources and provides a cost-effective production of novel and value-added consumer food products.

References

Anonymous (2007) The state of world fisheries and aquaculture (SOFIA). Food and Agriculture Organization of the United Nations, Rome

Bishov SJ, Henick AS (1972) Antioxidant effect of protein hydrolysates in a freeze-dried model system. J Food Sci 37:873–875

Bordenave S, Fruitier I, Ballandier I, Sannier F, Gildberg A, Batista I, Piot JM (2002) HPLC preparation of fish waste hydrolysate fractions. Effect on guinea pig ileum and ACE activity. Prep Biochem Biotechnol 32:65–77

Chen YC, Jaczynski J (2007a) Gelation of protein recovered from whole Antarctic krill (*Euphausia superba*) by isoelectric solubilization/precipitation as affected by functional additives. J Agr Food Chem 55(5):1814–1822

Chen YC, Jaczynski J (2007b) Protein recovery from rainbow trout (*Oncorhynchus mykiss*) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. J Agr Food Chem 55(22):9079–9088

Chen Y-C, Nguyen J, Semmens K, Beamer S, Jaczynski J (2006) Enhancement of omega-3 fatty acid content in rainbow trout (*Oncorhynchus mykiss*) fillets. J Food Sci 71:C383–C389

Chen Y-C, Tou JC, Jaczynski J (2007) Amino acid, fatty acid, and mineral profiles of materials recovered from rainbow trout (*Oncorhynchus mykiss*) processing by-products using isoelectric solubilization/precipitation. J Food Sci 72(9):C527–C535

Chen Y-C, Tou JC, Jaczynski J (2009) Amino acid and mineral composition of protein and other components and their recovery yields from whole Antarctic krill (*Euphausia superba*) using isoelectric solubilization/precipitation. J Food Sci 74(2):H31–H39

Choi YJ, Park JW (2002) Acid-aided protein recovery from enzyme-rich Pacific whiting. J Food Sci 67:2962–2967

Damodaran S (2004) Protein-polysaccharide hybrid hydrogels. U.S. patent no. 6,821,331

Fahmi A, Morimura S, Guo HC, Shigematsu T, Kida A, Uemura Y (2004) Production of angiotensin I converting enzyme inhibitory peptides from sea bream scales. Process Biochem 39:1195–2000

Falch E, Rustad T, Jonsdottir R, Shaw NB, Dumay J, Berge JP, Arason S, Kerry JP, Sandbakk M, Aursand M (2006) Geographical and seasonal differences in lipid composition and relative weight of by-products from gadiform species. J Food Compos Anal 19:727–736

FAO (2011) Global aquaculture production. URL available at: http://www.fao.org/fishery/statistics/globala-quaculture-production/en. Accessed 10 July 2012

Gehring CK, Gigliotti JC, Moritz JS, Tou JC, Jaczynski J (2011) Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: a review. Food Chem 124:422–431

Gildberg A (2002) Enhancing returns from greater utilisation. In: Bremner HA (ed) Safety and quality issues in fish processing. Woodhead Publishing, Cambridge, pp 425–449

Guérard F, Sellos D, Le Gal Y (2005) Fish and shellfish upgrading, traceability. In: Ulber R, Le Gal Y (eds) Advances in biochemical engineering/biotechnology. Springer, Berlin/Heidelberg, pp 127–163

Hultin HO, Kelleher SD (1999) Process for isolating a protein composition from a muscle source and protein composition. U.S. patent no. 6,005,073

Hultin HO, Kelleher SD (2000a) High efficiency alkaline protein extraction. U.S. patent no. 6,136,959

Hultin HO, Kelleher SD (2000b) Surimi processing from dark muscle fish. In: Park JW (ed) Surimi and surimi seafood. Marcel Dekker, New York

Hultin HO, Kelleher SD (2001) Process for isolating a protein composition from a muscle source and protein composition. U.S. patent no. 6,288,216

Hultin HO, Kelleher SD (2002) Protein composition and process for isolating a protein composition from a muscle source. U.S. patent no. 6,451,975

Hultin HO, Kristinsson HG, Lanier TC, Park JW (2005) Process for recovery of functional proteins by pH shifts. In: Park JW (ed) Surimi and surimi seafood, 2nd edn. CRC Press, Boca Raton

Hussain S (2007) Effect of tilapia protein isolate on water-holding capacity and quality of tilapia fish muscle. Thesis, University of Florida

Jaczynski J, Tahergorabi R, Hunt AL, Park JW (2011) Safety and quality of frozen aquatic food products. In: Sun D-W (ed) Handbook of frozen food processing and packaging, 2nd edn. CRC Press/Taylor and Francis Group, Boca Raton, pp 343–385

Jung W-K, Mendis E, Je J-Y, Park P-J, Son BW, Kim HC, Choi YK, Kim S-K (2006) Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (*Limanda aspera*) frame protein and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 94:26–32

Kerry JP, Murphy SC (2007) Physical and chemical properties of lipid by-products from seafood waste. In: Shahidi F (ed) Maximising the value of marine by-products. Woodhead Publishing, Cambridge, pp 22–46

Kim YS, Park JW, Choi YJ (2003) New approaches for the effective recovery of fish proteins and their physicochemical characteristics. Fish Sci 69(6):1231–1239

Kristinsson HG, Hultin HO (2003) Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J Agr Food Chem 51(24):7187–7196

Kristinsson HG, Ingadottir B (2006) Recovery and properties of muscle proteins extracted from tilapia (*Oreochromis niloticus*) light muscle by pH shift processing. J Food Sci 71:E132–E141

Kristinsson HG, Liang Y (2006) Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. J Food Sci 71:C304–C312

Kristinsson HG, Theodore AE, Demir N, Ingadottir B (2005) A comparative study between acid- and alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. J Food Sci 70:C298–C306

Lee CM (1999) Surimi: science and technology. In: Francis FJ (ed) Wiley encyclopedia of food science and technology, 2nd edn. Wiley, Hoboken, pp 2229–2239

Liang Y, Hultin HO (2005a) Separation of muscle membrane from alkali solubilized fish muscle proteins. J Agr Food Chem 53:10012–10017

Liang Y, Hultin HO (2005b) Separation of membranes from acid-solubilized fish muscle proteins with the aid of calcium ions and organic acids. J Agr Food Chem 53:3008–3016

Lowther A (2005) Highlights from the FAO database on aquaculture production statistics – fishery information. Data and Statistics Unit FAO Fisheries Department. Food and Agriculture Organization of the United Nations, Rome
Meinke WW, Mattil KF (1973) Autolysis as a factor in the production of protein isolates from whole fish. J Food Sci 38:864–867
Meinke WW, Rahman MA, Mattil KF (1972) Some factors influencing the production of protein isolates from whole fish. J Food Sci 37:195–198
Mireles DeWitt CA, Nabors RL, Kleinholz CW (2007) Pilot plant scale production of protein from catfish treated by acid solubilization/isoelectric precipitation. J Food Sci 72:E351–E355
Murano PS (2003) Understanding food science and technology. Wadsworth/Thomson Learning, Belmont
Nicol S, Foster J (2003) Recent trends in the fishery for Antarctic krill. Aquat Living Resour 16:42–45
Nolsøe H, Undeland I (2009) The acid and alkaline solubilization process for the isolation of muscle proteins: state of the art. Food Bioprocess Technol 2:1–27
Ockerman HW (1996) Chemistry of meat tissue. The Ohio State University, Columbus
Okada T, Morrissey MT (2007) Recovery and characterization of sardine oil extracted by pH adjustment. J Agr Food Chem 55:1808–1813
Pastoriza L, Sampedro G, Cabo ML, Herrera JJR, Bernárdez M (2004) Solubilisation of proteins from rayfish residues by endogenous and commercial enzymes. J Sci Food Agr 84:83–88
Raghavan S, Kristinsson HG (2008) Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: a comparative study of five enzymes. J Agr Food Chem 56:1434–1441
Rustad T (2007) Physical and chemical properties of protein seafood by-products. In: Shahidi F (ed) Maximising the value of marine by-products. Woodhead Publishing, Cambridge, pp 3–21
Rustad T, Storrø I, Slizyte R (2011) Possibilities for the utilisation of marine by-products. Int J Food Sci Technol 46(10):2001–2014
Sathivel S, Bechtel PJ, Babbitt J, Prinyawiwatkul W, Negulescu II, Reppond KD (2004) Properties of protein powders from arrowtooth flounder (*Atheresthes stomias*) and herring (*Clupea harengus*) byproducts. J Agr Food Chem 52:5040–5046
Sathivel S, Smiley S, Prinyawiwatkul W, Bechtel PJ (2005) Functional and nutritional properties of red salmon (*Oncorhynchus nerka*) enzymatic hydrolysates. J Food Sci 70:C401–C406
Shahidi F (1994) Proteins from seafood processing discards. In: Sikorski ZE, Pan BS, Shahidi F (eds) Seafood proteins. Chapman and Hall, New York, pp 171–193
Sikorski Z, Pan BS, Shahidi F (1994) Seafood proteins. Chapman and Hall, New York
Stefansson G, Hultin HO (1994) On the solubility of cod muscle proteins in water. J Agr Food Chem 42:2656–2664
Strøm T, Eggum BO (1981) Nutritional value of fish viscera silage. J Sci Food Agr 32:115–120
Suzuki T (1981) Fish and krill protein: processing technology. Applied Science Publishers, London
Suzuki T, Shibata N (1990) The utilization of Antarctic krill for human food. Food Rev Int 6:119–147
Tahergorabi R (2012) Development of value-added food products from isoelectrically recovered proteins. PhD. Dissertation, West Virginia University, Morgantown
Tahergorabi R, Hosseini SV, Jaczynski J (2011) Seafood proteins. In: Philips GO, Williams PA (eds) Handbook of food proteins. Woodhead Publishing, Cambridge, UK, pp 116–143
Tahergorabi R, Beamer SK, Matak KE, Jaczynski J (2012a) Functional food products made from fish protein isolate recovered with isoelectric solubilization/precipitation. LWT- Food Sci Technol 48(1):89–95
Tahergorabi R, Beamer SK, Matak KE, Jaczynski J (2012b) Isoelectric solubilization/precipitation as a means to recover protein isolate from striped bass (*Morone saxatilis*) and its physicochemical properties in a nutraceutical seafood product. J Agr Food Chem 60(23):5979–5987
Taskaya L, Jaczynski J (2009) Flocculation-enhanced protein recovery from fish processing by-products by isoelectric solubilization/precipitation. LWT- Food Sci Technol 42(2):570–575

Taskaya L, Chen Y-C, Jaczynski J (2009a) Functional properties of proteins recovered from silver carp (*Hypophthalmichthys molitrix*) by isoelectric solubilization/precipitation. LWT- Food Sci Technol 42(6):1082–1089

Taskaya L, Chen Y-C, Beamer S, Jaczynski J (2009b) Texture and colour properties of proteins recovered from whole gutted silver carp (*Hypophthalmichthys molitrix*) using isoelectric solubilisation/precipitation. J Sci Food Agr 89(2):349–358

Thawornchinsombut S, Park JW (2004) Role of pH in solubility and conformational changes of pacific whiting muscle proteins. J Food Biochem 28:135–154

Thawornchinsombut S, Park JW (2006) Frozen stability of fish protein isolate under various storage conditions. J Food Sci 71:C227–C232

Theodore AE, Kristinsson HG (2007) Angiotensin converting enzyme inhibition of fish protein hydrolysates prepared from alkaline-aided channel catfish protein isolate. J Sci Food Agr 87:2353–2357

Thorkelsson G, Kristinsson HG (2009) Bioactive peptides from marine sources. State of art. Report to the NORA fund, Skýrsla Matís 14–09

Torres JA, Chen YC, Rodrigo-Garcia J, Jaczynski J (2007) Recovery of by-products from seafood processing streams. In: Shahidi F (ed) Maximising the value of marine by-products. Woodhead Publishing, Cambridge, pp 65–90

Undeland I, Kelleher SD, Hultin HO (2002) Recovery of functional proteins from herring (*Clupea harengus*) light muscle by an acid or alkaline solubilization process. J Agr Food Chem 50:7371–7379

Undeland I, Kelleher SD, Hultin HO, McClements J, Thongraung C (2003) Consistency and solubility changes in herring (*Clupea harengus*) light muscle homogenates as a function of pH. J Agr Food Chem 51:3992–3998

Vann DG, Mireles DeWitt CA (2007) Evaluation of solubilized proteins as an alternative to phosphates for meat enhancement. J Food Sci 72:C072–C077

Vannuccini S (2004) Overview of fish production, utilization, consumption and trade. Food and Agriculture Organization Fisheries Information: Data and Statistics Unit, Rome

Whitney EN, Rolfes SR (2005) Understanding nutrition, 10th edn. Wadsworth/Thomson Learning, Belmont

Chapter 7
Advances in the Processing of Marine Discard and By-products

Maria Hayes and Kevin McKeon

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1 Introduction

By-products from fish and shellfisheries processing represent a serious environmental and economic problem due to inadequate disposal options and/or costs associated with disposal in landfills. Processing leftovers including trimmings, fins, frames, heads, shells, skin, viscera, and stickwater/effluent are currently used in Ireland for the production of fish meal, fish oil, fertilizer, and animal feeds. However, these by-products may be viewed as valuable sources of bioactive compounds, including functional and technologically relevant peptides, as well as lipids, minerals, carbohydrates, and small molecules, with associated applications in human, carnivorous, or omnivorous fish and shellfish diets and animal feedstuffs. The function of these bioactive compounds is usually health maintenance in nutritional products. Marine processing by-products may be considered as coproducts and should be viewed as a potential resource for new, value-added product generation. Fish species without current commercial value, such as *Capros aper* (Boarfish) (Fig. 7.1) (White et al. 2011), blue whiting, and herring, may also be viewed as a novel resource for new ingredient and product development, and this resource is often overlooked and wasted at present. Whitefish (Blue whiting and Herring), Boarfish, Crabs, and Prawns are plentiful around the Irish coastline. Over the last several years, Ireland has seen an increase in Boarfish numbers and it currently has a catch quota of 56,000 boarfish per annum. The 2012 season produced some of the highest numbers of Boarfish on record.

Furthermore, the cooking juices of fish and shellfish products contain two kinds of flavoring compounds. These include low molecular weight compounds such as the N- and S-containing compounds that provide pleasant cucumber/green, almond/nutty, potato-type aromas which characterize seafood products (Kubota et al. 1986, 1996). Water-soluble flavor compounds, including low molecular weight free amino acids (Taurine, Glutamic acid, Glycine), peptides, organic acids, and inorganic salts, are also found in the cooking juices of fish and shellfish by-products (Hayes 2013). These flavors can be targeted for use in functional "taste/flavor imparting"

Fig. 7.1 *Capros aper* (Boarfish)

meat/cereal/beverages for consumers. Fish processing by-products are also a source of bioactive peptides, with heart health, antimicrobial, antioxidant, anti-inflammatory, and other potential health benefits (Morrissey et al. 2011). Indeed, bioactive peptides were isolated from marine processing by-products as part of the NutraMara program (http://www.nutramara.ie).

2 Reasons for Discarding Fish

Fishermen catch fish, shellfish, and crustaceans, and also untargeted fish and marine life, such as jellyfish, starfish, and coral species. Discarding fish takes place after sorting the catch on board the vessel. Fish thrown overboard on economic grounds may include: fish with no local market, crushed or damaged fish, or fish subject to premarket selection. Fishermen keep only the most valuable fish sizes in order to maximize returns due to low quota or storage limitations. However, since 2008, the EU parliament has stressed that discarding should be eliminated, as it is an unsustainable and immoral practice. In February 2013, the Common Fisheries Policy (CFP) adopted its first reading position on the future CFP regulation and a phased-in discard ban in the EU will start from January 2014 (Weissenberger 2013). Apart from unwanted catch at sea, discard can also include processing waste or by-products. New solutions for discard/by-products are required due to stringent environmental regulations issued by many Governments and because it makes sense that fish processing should be a sustainable practice, both environmentally and economically. Indeed, the utilization of by-products from the fish and shellfisheries processing industries can be a lucrative practice if costs associated with the processing of by-products are kept low and if functional ingredients/products can be obtained using easily accessible technologies and sustainable scientific practices. The EU fisheries "discard ban" may assist fish oil and fish meal producers to source new raw materials for Omega-3 production. At present, the main fisheries for Omega-3 production in Peru and Morocco are near capacity and a reduction in unsustainable waste will help to keep the Omega-3 production industry vibrant. A large quantity of money has been invested in the area of by-product utilization, as illustrated in Table 7.1.

3 By-product Categories

A report by the FAO Fisheries and Aquaculture Department stated that, in 2005, about 75 % of the estimated world fish production was used for direct human consumption and the remaining 25 % was destined for non-food use (Sanmartin et al. 2009). With the exception of China, approximately 90 % of the world fish production destined for non-food use is used in the manufacture of fish meal and oil, and 10 % is used in aquaculture (FAO 2009). Delgado et al. predicted that an increasing

Table 7.1 Recently funded European and international projects concerning the utilization of marine by-products

Project title	Coordinator and collaborators	Funding body	Short description
The Marine Functional Foods Research Initiative NutraMara program	Teagasc Food Research Centre, Ashtown, Dublin 15	The Irish Department of Agriculture, Food and the Marine (DAFM)	The proposal aims to develop infrastructure and human capital regarding the isolation and characterization of marine-origin bioactive compounds for use in functional food products
Maximum resource utilisation – Value-added fish by-products Nordic Innovation Centre project number: 04275 **(2006–2009)**	Matis (The University of Iceland, SINTEF, NTNU, Mills DA, Fisheries Research Project, Hojmarklaboratoriet A.S.)	Norwegian Government and Norden – The Nordic Innovation Centre	The aim of the project was to improve the competitiveness of the fish industry by industry-driven research. Both existing and improved ingredients from rest raw materials in the fish processing industry were evaluated for utilization in processing lines and emulsion-based foods
MARIFUNC – Nordic Network for Marine Functional Food **(July 2006- July 2009)**	Nofima, Norwegian College of Fishery Science, University of Tromsø, SINTEF Fisheries and Aquaculture, National Institute of Aquatic Resources, Denmark; Marinova, Denmark; Matis, Iceland; University of Iceland, Landspitali University Hospital, Iceland; Chalmers University of Technology, Sweden; Sahlgrenska University Hospital, Sweden; University of Turku, Finland	Private companies and the Norwegian Government under the Nordic Innovation Centre	The main activity was to create an overview of the role of fish- and seafood-derived components in health and the quality of functional seafood components
Biotecmar – Biotechnological valorisation of marine products and by-products project number 2008-1/032 **(2008–2012)**	Université Europénne de Bretagne – Université de Bretagne Occidentale, Brest, Quimper, Lorient, Vannes, France; Consejo Superior de Investigaciones Científicas (CSIC); Muséum National d'Histoire, France; National Institute for Fisheries and Sea Research, Portugal; Université de La Rochelle, France; Irish Seaweed Centre, Galway Ireland; Université de Nantes, France; Indigo Rock Marine Research Centre, Ireland; CETMAR, Spain	Cofinanced with the support of the European Union ERDF Atlantic Area Programme	The Biotecmar project supports the development of a chain for the production of valuable ingredients using underexploited marine products
The Food Programme (MATPROGRAMMET), Norway. High-value products from low-grade by-products of animal and marine origin: process monitoring of industrial enzymatic protein hydrolysis **01.01.2013-31.12.2015**	Nils Kristian Afseth, FFL JA-finansiert prosjekt, The Research Council of Norway	The Research Council of Norway and the Norwegian Government	The R&D program Norwegian food from fjords and farms aims to strengthen industrial development through research and innovation in Norway's food industries
Utilisation and stabilisation of by-products from cod species FISHERY BY-PRODUCTS **(2000–2004)**	EU FP5 funding contract number QLK1-2000-01017 SINTEF Fisheries and Aquaculture, Norway; Icelandic Fisheries Laboratories, Iceland; Department of Food Science, Food Technology and Nutrition, UCC, Cork, Ireland; Novozymes, Denmark; Netherlands Institute for Fisheries Research, The Netherlands; Laboratoire de Biochimie et Molécules Marines, Nantes, France	EU FP5 funding	The main objective of this work was to increase the utilization of by-products from cod species. The project aimed to develop systems to sort, handle, and store by-products on board vessels, to develop preservation methods, and to extract biomolecules for use in pharmaceuticals and food

demand for fisheries products will result in increased fish production and, therefore, an increase in fish processing, which will result in greater quantities of fish discard/by-products or what should really be termed "coproducts" (Delgado et al. 2003). Marine by-products can be the starting materials for new and naturally sourced value-added ingredients that can be used as functional foods or cosmeceutical or pharmaceutical ingredients. This chapter will focus on recent advances in the recovery of valuable bioactive compounds, including proteins, peptides, lipids, and small molecules, from marine discard and by-products, and current and potential applications of these bioactive compounds.

4 Strategies for Fish Protein Recovery from By-product Material

Fish bycatch is a rich source of valuable protein, but, at present, the majority of fish bycatch is used for the manufacture of fish meal or as a fertilizer. The production of fish by-products consists essentially of the hygienic collection of mince and the separation of solids, oil, and water using steps including boiling, pressing, evaporation, and drying (Sanmartín et al. 2009). In order to avoid rapid spoilage, the water and oil content of this fish "mince" must be reduced to below 10 %. This ensures that autolytic degradation by endogenous enzymes and lipid oxidation does not occur. In order to successfully recover proteins from fish, it is necessary to choose the correct technique, as failure to do so may result in poor quality protein with little nutritive value and poor functional applications.

5 Fish Protein Concentrates (FPC)

Fish protein concentrate was one of the first products developed from fish protein and were first made in Sweden in 1936. FPC are produced using ensilage techniques and there are two types, known as A and B. Type A FPC is produced by solvent extraction of fish meat using an alkali or solvents such as ethyl alcohol. It is poorly soluble in water and has poor emulsifying properties. Type A FPC is odorless and colorless and contains less than 1 % fat. Type B FPC is produced by isoelectric precipitation and this FPC has a fish odor and contains higher levels of lipids. Mild processing techniques such as enzymatic recovery of protein from fish are the most widely used methods for the recovery of proteins for industrial use.

Fish proteins and fish meal have also been used historically as a good source of protein and minerals for the dairy, pig, and poultry industries. It is a naturally good source of the essential amino acids Lysine and Methionine. Trace elements of Iodine and Selenium are also present. The use of fish protein in animal feed products is set to continue and the demand will increase if the technical issues of purity, salt content, and undesirable nitrogen compound levels are resolved.

6 Chemical and Enzymatically Hydrolyzed Fish Protein

During the chemical and/or enzymatic hydrolysis of fish protein, peptides are recovered which have a number of different beneficial bioactivities. Chemical hydrolysis using acid or alkali result in poor products with bitter tastes and reduced nutritional qualities. Enzymatic hydrolysis employs commercially available enzymes such as papain, alcalase, ficin, trypsin, pancreatin, and neutrase. Proteolytic lactic acid bacteria (LAB) may also be used. Proteolytic enzymes are classified by their hydrolyzing mechanism into endopeptidases and exopeptidases. Endopeptidases hydrolyze the peptide bonds within the parent protein at random to produce large peptides. Exoproteases remove amino acids from either the N- or C-terminus by hydrolyzing the terminal peptide bonds. For a controlled enzymatic process, it is necessary to inactivate using heat or pH the endogenous proteases contained in fish muscle prior to the addition of enzymes for controlled hydrolysis. The use of added external enzymes allows for control of the hydrolysis process and can improve the functional, sensory, and physicochemical properties of the fish protein. As commercially available enzymes are expensive, it is valuable for processors to first carry out in silico analysis prior to the generation of a bioactive hydrolysate. Computer programs, including ExPASy PeptideCutter (http://web.expasy.org/peptide_cutter/), are useful in predicting the outcome of a protein hydrolysis with a chosen enzyme. However, this analysis method is only possible if the sequence of the protein that will be hydrolyzed is already known and available in protein databanks such as PubMed. The use of fish exogenous enzymes is widely applied in the fish sector. For example, trypsin isolated from skipjack tuna was used previously to degrade sardine proteins (Klomklao et al. 2006). More recently, press cakes obtained from three discarded species in the west Mediterranean, sardine (*Sardina pilchardus*), mackerel (*Scomber colias*), and horse mackerel (*Trachurus trachurus*), were hydrolyzed using two serine endoprotease enzymes, one of bacterial origin (subtilisin, EC 3.4.21.62) and another from an animal source (trypsin EC3.4.21.4). These hydrolysates were found to contain acceptable antioxidant activities, with DPPH inhibition values ranging from 35 % to 45 % (García-Moreno et al. 2013). Press cakes were obtained by heating the viscera and gonads of the three fish species at 40 ºC for 30 min and pressing in an electric press, where the mix was subjected to three presses under pressure at 150 bar. Press cake peptides were also found to have antihypertensive activities.

7 Autolysis

Autolysis using endogenous enzymes was used with some success previously. Kristinsson and Rasco (2000) used endogenous enzymes to produce fish sauces and silage. However, the primary limitation of the autolytic process for value-added ingredient recovery is the diminished functionality of the final product and the fact that a homogenous end product (hydrolysate) is difficult to obtain.

8 Processing of Stickwater

Three types of waste are generated during the processing of fish and the production of fish meal. These are: bail water, blood water, and stickwater. Stickwater represents approximately 60 % of the processed fish weight (García-Sifuentes et al. 2011) and is composed of solids, which should be removed before being discarded at sea (Fernández et al. 2003). Solids represent approximately 6–10 % of stickwater and consist of proteins, fats, and inorganic ions, and these could be used for food ingredients for humans (García-Sifuentes et al. 2009). One of the primary problems with stickwater recovery is its variable nature. Stickwater depends upon species, storage conditions, and the processing plant conditions (Guerrero et al. 1998; García-Sifuentes et al. 2011). Treatments such as stickwater evaporation consume a lot of energy and can result in fouled pipes.

Cross-flow membrane technologies for effluent protein recovery have been previously reported (Afonso et al. 2002). Membrane filtration using low molecular weight cutoff and ultrafiltration methods is an alternative to recover solids from effluent. García-Sifuentes et al. (2011) investigated the effect of a multistep stickwater treatment method that employed centrifugation, pH shift, and ultrafiltration steps. The method was successful to a degree, as the removal of most solids was attained and a clearer effluent resulted.

9 Collagen and Gelatine Extraction

Collagen is the major structural protein in the connective tissue of skin and bone, giving strength and support to tissues. The collagen molecule is a triple helix, with three α-chains that adopt a three-dimensional structure. It contains a high percentage of glycine, hydroxyproline, and proline, which make up the most common repeating triplet in the molecule, with glycine always occupying every third position. Gelatine is the hydrolyzed form of collagen and is a high-value functional protein. Collagen and gelatine have many uses in the food, cosmetic, and pharmaceutical industries, including their uses as gelling and stabilizing agents and their use as nutritional enhancement agents. They are most commonly sourced from porcine and bovine skin, with fish skin accounting for less than 1 % of the worldwide market. However, fish skin may potentially be a good alternative source of collagen and gelatine. Fish skin-sourced collagen and gelatine are considered suitable for communities with Kosher and Halal religious beliefs.

There is an increasing worldwide demand for collagen and gelatine, with the market currently estimated to be approximately 326,000 tons (Karim and Bhat 2009), and fish skin is an excellent source of both. The extraction of collagen from fish skin provides a perfect opportunity for marine processors to reduce waste from fish processing while simultaneously providing a product that is in demand globally.

The extraction of collagen ordinarily involves three steps: acid/alkaline pretreatment, extraction, and purification. Previous studies indicate that the largest amount of

total collagen content (90 %) can be extracted using 0.5 M acetic acid at 4 °C for 24 h using a ratio of skin to solvent of 1:40 (w/v), without any pretreatment process. In recent years, gelatine with good gelling properties was extracted from the skin and bones of fish species, including giant catfish (Jongjareonrak et al. 2010), bigeye snapper (Benjakul et al. 2009), cuttlefish (Aewsiri et al. 2008), greater lizardfish (Taheri et al. 2009), hoki (Mohtar et al. 2010), grouper (Rahman and Al-Mahrouqi 2009), striped catfish (Singh et al. 2011), bream (Sitthipong et al. 2011), and unicorn leatherjacket (Ahmad et al. 2010). Recently, gelatine extraction was successfully carried out from catfish bones (*Clarias gariepinus*) using 3.35 % HCl for 14.5 h pretreatment of bones, followed by hot water extraction at 67.23 % for 5.2 h (Sanaei et al. 2013).

In Europe, farmed salmon has become a significant fish resource. Over the last two decades, the production of farmed salmon has multiplied approximately 40 times, with the UK being the third largest farmed salmon consumer in the world, accounting for 12 % (100,000 t) of worldwide consumption (UN Food and Agricultural Organization, Fisheries Global Information System). In the past, salmon was sold mostly as a skin-on product, so salmon skin was not available in large quantities, but in more recent years, the market for skinless salmon products has grown rapidly (Arnesen and Gildberg 2007). Salmon skin, which comprises about 5 % of the whole fish, has been studied briefly with regard to collagen (Kolodziejska et al. 2008; Simpson et al. 1999) and gelatine (Yunoki et al. 2003; Arnesen and Gildberg 2007). These studies have indicated that salmon skin is an excellent high-yielding source of collagen. Studies on the bioactive peptide production potential of salmon skin involving enzymatic hydrolysis have been carried out recently (Rui-Zeng Gu et al. 2011), and although this area of research is still in its infancy, the results of the studies have indicated that salmon skin collagen peptides may be useful in functional foods and as antihypertensive agents.

9.1 Collagen and Gelatine Applications

Collagen and gelatine are of value not only for their well-known applications in food, pharmaceuticals, and cosmetics where it is a growing trend to replace synthetic agents with more natural ones, but in recent years, they have also become of interest for the production of bioactive peptides. Several studies have investigated the enzymatic hydrolysis of collagen for the production of bioactive peptides, and oral intake studies conducted on animal and human models have determined the absorption and metabolism of hydroxyproline containing peptides to be excellent (Gómez-Guillén et al. 2011).

The rheological properties of gelatine will determine its suitability for a particular application (Stainsby 1987). The basic properties that define gelatine are the composition parameters, transparency, solubility, color, odor, and taste, but the attributes that determine the commercial quality of gelatine are its gel strength and thermal stability (Stainsby 1987). Gel strength and thermal stability are largely dependent on the molecular properties of gelatine, for the most part with respect to the molecular weight distribution (which results mainly from processing

conditions) and the species-specific amino acid composition (Gómez-Guillén et al. 2002). The proline and hydroxyproline content of gelatine is very important with regard to the gel strength, with the triple helical structure stability reported to be proportional to the total pyrrolidine imino acid content (Ledward 1986). It has also been reported that the total Gly-Pro-Hyp sequence content is one of the main factors affecting thermal stability (Burjandze 2000).

Applications of gelatine include adhesives, nutraceutical agents, and in cosmeceuticals, as well as others as documented in the next sections.

9.2 Adhesive Applications

Traditionally, gelatine was used in the manufacture of glue (Karim and Bhat 2009). For adhesion to take place, a warm gelatine solution must be used and the gelatine must not have gelled before the surfaces to be glued are brought together. An example of this is in the pharmaceutical or confectionery tableting business and in liquorice allsorts, where it can be used to join the layers (Cole 2000).

9.3 Gelling Applications

The most common use of gelatine is for its thermally reversible gelling properties with water; for example, in the production of table jellies. In confectionery, gelatine is used as the gelling binder in gummy products such as wine gums (Marrs 1982). In the manufacture of these products, gelatine is combined with water, sugar, and glucose syrups. To these extracts, flavorings, colors, and texture modifiers may be added. Gelatine is widely used in confectionery because it gels, foams, or solidifies into a piece that dissolves slowly in the mouth, gently releasing flavors and creating a smooth taste sensation (Karim and Bhat 2009). However, sometimes, incompatibility between gelatine and glucose syrup can occur. In products with low water content, competition between gelatine and glucose polymers for water can result in the precipitation of the gelatine, or, at least, a marked loss in the gelling properties or hardness of the product. Gelatines with similar properties in water can have very different properties in confectionery formulations (Cole 2000).

9.4 Gelatine Use as an Anti-foam Agent

Gelatine is a very efficient foam stabilizer, and this property is exploited in the manufacture of marshmallows (Cole 2000). Foam stabilizing properties differ from gelatine to gelatine, so, for this use, the gelatine needs to be carefully selected. However, the foaming properties can be standardized by the use of sodium lauryl sulfate (Johnston-Banks 1990).

9.5 Protective Colloid/Crystal Modifying Properties of Collagen/Gelatine

If a gelled jelly is frozen, the product will suffer from syneresis (the extraction or expulsion of a liquid from a gel) and, on thawing, the clear jelly will disintegrate with much exuded water. However, if water containing 0.5 % gelatine is frozen, the water will freeze as millions of small discrete crystals, instead of forming a single solid block of ice. This effect is most desirable in "ice lollies" and is also used in ice cream manufacture to obtain a smooth product with small ice crystals and also to ensure that any lactose precipitates as fine crystals, avoiding the development of graininess with time (Marrs 1982).

9.6 Gelatine as a Nutritional Supplement

Gelatine has increased proportions of certain amino acids, such as lysine (Karim and Bhat 2009). Studies have shown that the consumption of 7–10 g/day of lysine can significantly improve nail growth rate and strength (Schwimmer and Mulinos 1957) and it also promotes hair growth (Silvestrini and Bruno 1988). Gelatine has also been shown to benefit arthritis sufferers in a large proportion of cases (Adam 1991), and when mixed with beef protein, it can increase the net protein value from 84 % to 99 % (Rama-Rao et al. 1964). Adding to these benefits the fact that gelatine is low calorie (3.5 kcal/g) and has excellent digestibility (Johnston-Banks 1990) makes it an ideal ingredient for food product supplementation.

9.7 Gelatine as a Texture Agent

Gelatine is used in dried soups to provide an appropriate mouth feel (viscosity) to the final product (Karim and Bhat 2009). In the manufacture of yoghurt, gelatine helps to avoid the floury texture of starches, while in ice creams, it controls the excess formation of ice crystals. In both yoghurt and ice cream, as with similar dairy desserts or iced confectionary, gelatine that melts at body temperature provides a smooth creamy sensation and allows the full release of flavors and aromas (Cole 2000).

9.8 Polyelectrolyte Properties of Gelatine

The clarification of apple juice with gelatine is a common industrial practice (Benitez and Lozano 2007). It works by sticking to the particles or by using charged ions to cause particles to stick to each other, in any case, making them heavy enough to sink to the bottom by the action of gravity. What is left is a transparent though not a clear juice (Benitez and Lozano 2007).

9.9 Use of Collagen as a Beauty and Cosmetic Product Ingredient

Collagen is widely used for soft tissue (e.g., skin), cartilage, and bone repair (Salgado et al. 2004). Its intrinsically high water retention capacity has been taken advantage of in creams for treating rough skin. Natural collagen triggers low immune responses, so it is a good substrate for cell adhesion, with good capacity to interact with host tissues (Salgado et al. 2004). Collagen is used in the manufacture of biocompatible glues (which consist of a mixture of collagen with citric acid as a cross-linking agent) that are particularly powerful at binding tissue–tissue interfaces and yield less toxicity than such semi-synthetic and synthetic formulations as gelatine–aldehyde and cyanoacrylate-based glues (Taguchi et al. 2006).

9.10 Bioactive Peptides from Marine By-products

Marine by-products are a known source of bioactive compounds that may have potential for use in the food, pharmaceutical, and cosmeceutical sectors. The health benefits of marine-derived peptides are well known, and several studies have isolated heart-beneficial peptides, such as angiotensin-I-converting enzyme inhibitors (Fitzgerald et al. 2011), antioxidant peptides (Ranathunga et al. 2006), anticoagulant peptides, and others, such as antiobesity, antidiabetic, and anticancer peptides (Ngo et al. 2011).

Discarded collagen containing materials from the fish processing industry have been found to be valuable sources of hydrolysates and peptides with bioactive properties (Cheng et al. 2009). Gastrointestinal digestion, food processing, or fermentation can liberate biologically active peptides from the parent protein sequence in which they were inactive (Gómez-Guillén et al. 2011). Once liberated, these peptides were shown to positively affect numerous physiological functions of the organism. Collagen and gelatine from marine sources have been studied as a source of biologically active peptides with promising health benefits for nutritional and pharmaceutical applications (Wang et al. 2010). Enzymatically hydrolyzed fish skin gelatine has shown greater antihypertensive and antioxidative ability in comparison to fish muscle protein peptides (Kim et al. 2001; Mendis et al. 2005). It is presumed that these greater antihypertensive and antioxidative properties are due to the unique amino acid composition of fish skin gelatine peptides, which contain high levels of Gly-Pro-Hyp sequences in their structure (Gómez-Guillén et al. 2011). These peptides were also shown to increase the rate of absorption of dietary calcium in animal models and, hence, improved calcium bioavailability.

Marine-derived bioactive peptides have also been shown to have anticoagulant activities (Jo and Jung 2008), antimicrobial activities, mineral-binding capacity, immunomodulatory activity, a lipid-lowering effect, and beneficial effects on skin, bone, and joint health (Gómez-Guillén et al. 2011), further increasing their potential as ingredients for health-promoting functional foods and nutraceuticals. In vivo studies have demonstrated that some peptides from marine sources act protectively

against ultraviolet radiation-induced damage on mice skin, and salmon skin collagen hydrolysates have been reported to affect lipid absorption and metabolism in rats (Saito et al. 2009).

Enzymatic hydrolysis is most commonly used to obtain gelatine- and collagen-derived hydrolysates and peptides, and it is the preferred method over solvent extraction and microbial fermentation due to the lack of residual organic solvents or toxic chemicals in the products.

A number of commercial proteases were used previously for the production of these hydrolysates and peptides, including trypsin, chymotrypsin, pepsin, alcalase, properase E, pronase, collagenase, bromelain, and papain (Kim et al. 2001; Lin and Li 2006; Mendis et al. 2005; Yang et al. 2008). The specific proteases used will affect the free amino acid composition of the peptides obtained, hence, their amino acid sequences, which influence the biological activity of the hydrolysates (Chen et al. 1995; Wu et al. 2003). Alcalase, which is a commercial protease from a microbial source, was used in numerous studies dealing with gelatine/collagen hydrolysis because of its broad specificity, as well as the high degree of hydrolysis that can be achieved in a relatively short time under moderate conditions (Benjakul and Morrissey 1997; Diniz and Martin 1998). During the hydrolysis of skin gelatine from Alaskan pollock, squid *Todarodes pacificus*, and giant squid, alcalase displayed extensive proteolytic activity, producing hydrolysates with low average molecular weights, which exhibited high antioxidant activities and angiotensin-converting enzyme (ACE) inhibitory activities (Giménez et al. 2009; Kim et al. 2001; Nam et al. 2008).

The potency of marine-derived peptides for ACE-I inhibition is expressed as an IC_{50} value, which is the ACE inhibitor concentration leading to 50 % inhibition of ACE-I activity. The IC_{50} values for several ACE-I inhibitory peptides derived from marine sources have been determined (Table 7.2).

The biological properties of protein hydrolysates are largely determined by their molecular weights (Jeon et al. 1999). An ultrafiltration membrane system was used previously to obtain peptide fractions with the desired molecular sizes and higher bioactivity levels, and also as a purification step (Lin and Li 2006; Mendis et al. 2005).

9.11 Antihypertensive and ACE-I Inhibition of Marine Peptides

Hypertension is a worldwide problem that affects 15–20 % of all adults. Its treatment is effective in reducing the risk of cardiovascular diseases, such as arteriosclerosis, stroke, and myocardial infarction.

ACE is a circulating vasoactive enzyme that plays a key role in the regulation of blood pressure. ACE is secreted by pulmonary and renal endothelial cells and participates in the body's renin–angiotensin system (RAS), which mediates arterial vasoconstriction and the extracellular volume of blood plasma, lymph, and interstitial fluid. The enzyme catalyzes both the production of angiotensin and inactivation

Table 7.2 ACE-I inhibitory values of gelatine-derived peptides

Source	Hydrolysis enzyme	Amino acid sequence	Activity	IC_{50} (μM)	Reference
Alaska pollock skin	Serial digestion (alcalase, pronase, collagenase)	Gly-Pro-Leu	Antihypertensive	2.6	Byun and Kim (2001)
		Gly-Pro-Met	ACE-I inhibitory	17.13	
Salmon skin	Alcalase, papain	Ala-Pro	ACE-I inhibitory	60	Rui-Zeng Gu et al. 2011
Chicken leg	Protease	Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro	Antihypertensive, ACE-I inhibitory	29	Saiga et al. (2008)
Sea bream scale	Protease	Val-Ile-Tyr	ACE-I inhibitory	7.5	Fahmi et al. (2004)
Sole skin	α-Chymotrypsin	Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu	Antioxidant		Giménez et al. (2009)
Bovine skin	Serial digestion (alcalase, pronase E, collagenase)	Gly-Pro-Val	Antihypertensive, ACE-I inhibitory	4.67	Kim et al. (2001)
		Gly-Pro-Leu		2.55	
Porcine skin	Protease	Gly-Phe-Hyp-Gly-Pro	Antihypertensive, ACE-I inhibitory	91	Ichimura et al. (2009)
		Gly-Pro		360	

of the vasodilator bradykinin. ACE inhibition is considered to be an effective therapeutic approach to treating hypertension.

ACE-I inhibitory peptides were originally discovered in snake venom and, since then, synthetic ACE-I inhibitors such as enalapril and captopril have been synthesized and are widely used to treat hypertension and heart failure in humans (Ondetti et al. 1977; Patchett et al. 1980). In the last 10 years however, researchers have looked for alternative more innovative natural sources of these heart-healthy peptides, due to the negative side effects, such as coughing, taste disturbances, skin rashes, and angioneurotic edema, which are associated with the synthetic ACE inhibitors (Atkinson and Robertson 1979). The major natural source of ACE inhibitory peptides identified to date is milk, but these peptides have also been isolated from many other animal and plant protein sources, such as blood proteins (Mito et al. 1996), maize (Miyoshi et al. 1991), chickpea (Yust et al. 2003), ovalbumin (Miguel et al. 2004), soy (Wu and Ding 2001), and muscle proteins from pig, cattle, fish, and chicken (Ahmad et al. 2010; Fujita and Yoshikawa 1999).

Collagen and gelatine have been shown to be good sources of ACE inhibitors (Table 7.2). Potent ACE inhibitory hydrolysates and peptides were obtained previously from collagenous materials, not only from land-based sources such as porcine skin collagen (Gómez-Guillén et al. 2011; Ichimura et al. 2009), bovine skin gelatine (Kim et al. 2001), and chicken legs, but also from marine sources such as fish skin (Nagai et al. 2006; Park et al. 2009), fish scales (Fahmi et al. 2004), fish cartilage (Nagai et al. 2006), squid tunics (Alemán et al. 2011), and sea cucumbers (Zhao et al. 2007).

ACE inhibitory peptides have certain common features (Gómez-Guillén et al. 2011). Most of them are relatively short sequences with low molecular mass, as the active site of the ACE cannot accommodate large peptide molecules (Gómez-Guillén et al. 2011). Binding to ACE is strongly influenced by the C-terminal tripeptide sequence, which may interact with subsites at the active site of the enzyme. ACE prefers substrates or inhibitors that contain hydrophobic amino acid residues (aromatic or branched side chains) at each of the three C-terminal positions (Cheung et al. 1980; Murray et al. 2007). The presence of arginine or lysine on the C-terminal position has also been reported to contribute substantially to the inhibitory activity (Cheung et al. 1980; Meisel 2003).

The ACE inhibitory activity described for collagen and gelatine hydrolysates and peptides may be related to the high concentration of hydrophobic amino acids, as well as to high proline levels. Proline seems to be one of the most effective amino acids for increasing ACE inhibitory activity and has been identified in many of the naturally occurring ACE inhibitors (Contreras et al. 2009; Gómez-Ruiz et al. 2004a, b; Pihlanto et al. 2008; Quirós et al. 2007).

In vivo, the effects of antihypertensive peptides are usually tested using spontaneously hypertensive rats, which constitute an accepted model for human essential hypertension (FitzGerald et al. 2004), and many of the ACE inhibitory peptides from collagenous sources have already been tested in vivo, with an antihypertensive effect being reported. Spontaneously hypertensive rats experienced a significant decrease in blood pressure following the oral administration of both a chicken leg

collagen hydrolysate and the isolated octapeptide Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro (Iwai et al. 2008; Saiga et al. 2008). Bovine and porcine collagen hydrolysates produced a considerable reduction in blood pressure in spontaneously hypertensive rats after oral administration (Faria et al. 2008). In another study, administering the lowest fraction of sea cucumber gelatine hydrolysate significantly reduced the blood pressure of renal hypertensive rats (Zhao et al. 2007). ACE inhibitory peptides Gly-Pro and Gly-Phe-Hyp-Gly-Pro, isolated from porcine skin collagen hydrolysates, also had antihypertensive effects in spontaneously hypertensive rats (Ichimura et al. 2009).

9.12 Antioxidant Activities

Synthetic antioxidants are viewed with suspicion by the public due to reports concerning potential toxicity and carcinogenic effects, so natural antioxidants are preferred (Yangthong et al. 2009). Fish protein hydrolysates and bioactive peptides were identified previously as having antioxidant properties. Indeed, such antioxidants were isolated from capelin, cod, mackerel, Alaskan pollock, and rockfish. Fish-derived antioxidants are equally as efficient as antioxidants sourced from other foods, such as polyphenols, vitamins E and C, and carotenoids, in the prevention of oxidative stress-related diseases (Kaur and Kapoor 2001). There are over 20 analytical methods for determining the antioxidant activity of hydrolysates or extracts (Thaipong et al. 2006). There are two main categories of antioxidant capacity assays; the hydrogen atom transfer (HAT) reaction-based assays and the electron transfer (ET) reaction-based assays.

References

Adam M (1991) Osteoarthritis therapy with gelatin preparations: results of a clinical study. Therapiewoche 38:2456–2461

Aewsiri T, Benjakul S, Visessanguan W, Tanaka M (2008) Chemical compositions and functional properties of gelatin from pre-cooked tuna fin. Int J Food Sci Technol 43(4):685–693

Afonso MD, Brites Alves AM, Mohsen M (2002) Crossflow microfiltration of marble processing wastewaters. Desalination 149:153–162

Ahmad M, Benjakul S, Nalinanon S (2010) Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (*Aluterus monoceros*). Food Hydrocolloid 24(6–7):588–594

Alemán A, Giménez B, Pérez-Santin E, Gómez-Guillén MC, Montero P (2011) Contribution of Leu and Hyp residues to antioxidant and ACE inhibitory activities of peptide sequences isolated from squid gelatine. Food Chem 125:334–341

Arnesen JA, Gildberg A (2007) Extraction and characterisation of gelatine from Atlantic salmon (*Salmo salar*) skin. Bioresour Technol 98:53–57

Atkinson AB, Robertson JIS (1979) Captopril in the treatment of clinical hypertension and cardiac failure. Lancet 2:836–839

Benitez EI, Lozano JE (2007) Effect of gelatin on apple juice turbidity. Latin Am Appl Res 37:261–266
Benjakul S, Morrissey MT (1997) Protein hydrolysates from Pacific Whiting Solid Waste. J Agric Food Chem 45:3423–3430
Benjakul S, Oungbho K, Visessanguan W, Thiansilakul Y, Roytrakul S (2009) Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem 116(2):445–451
Burjanadze TV (2000) New analysis of the phylogenetic change of collagen thermostability. Biopolymers 53:523–528
Chen H, Muramoto K, Yamauchi F (1995) Structural analysis of antioxidative peptides from soybean B-conglycinin. J Agric Food Chem 43(3):574–578
Cheng FY, Wan TC, Liu YT, Chen CM, Lin LC, Sakata R (2009) Determination of angiotensin-I converting enzyme inhibitory peptides in chicken bone protein hydrolysate with alcalase. Anim Sci J 80:91–97
Choi SS, Regenstein JM (2000) Physicochemical and sensory characteristics of fish gelatin. J Food Sci 65:194–199
Cole CGB (2000) Gelatin. In: Frederick JF (ed) Encyclopedia of food science and technology, 4 vols. Wiley, New York, pp 1183–1188
Delgado CL, Wada N, Rosegrant MW, Meijer S, Ahmed M (2003) Outlook for fish to 2020: meeting global demand. International Food Policy Research Institute, Food Policy Report, pp 1–36
Diniz FM, Martin HM (1998) Influence of process variables on the hydrolysis of shark muscle protein. Food Sci Technol Int 4:91–98
FAO (2009) Fish as feed inputs for aquaculture: practices, sustainability and implications. In: Hasan MR, Halwart M (eds) FAO Fisheries and Aquaculture Technical Paper, No. 518, Rome, p 40
Fernández-Dıaz MD, Montero P, Gómez-Guillén MC (2003) Effect of freezing fish skins on molecular and rheological properties of extracted gelatin. Food Hydrocolloid 17:281–286
FitzGerald RJ, Murray BA, Walsh DJ (2004) Hypotensive peptides from milk proteins. J Nutr 134(4):980S–988S
Fitzgerald C, Gallagher E, Tasdemir D, Hayes M (2011) Heart health peptides from Macroalgae and their potential use in functional foods. J Agric Food Chem 13(59):6829–6836
Fujita H, Yoshikawa M (1999) LKPNM: a prodrug-type ACE inhibitory peptide derived from fish protein. Immunopharmacology 44(1):123–127
García-Moreno PJ, Pérez-Gálvez R, Espejo-Carpio FJ, Muñío MM, Guadix A, Guadix EM (2013) Lipid characterization and properties of protein hydrolysates obtained from discarded Mediterranean fish species. J Sci Food Agri. doi:10.1002/jsfa.6266
García-Sifuentes CO, Pacheco-Aguilar R, Lugo-Sánchez M, García-Sánchez G, Ramirez-Suárez JC (2009) Properties of recovered solids from stick-water treated by centrifugation and pH shift. Food Chem 114:197–203
García-Sífuentes CO, Aguilar RP, Ramirez Suárez JC, Ruíz GC (2011) Stickwater multi-step treatment: effect on organic material removal. Biotecnia XIII 1:10–16
Giménez B, Alemán A, Montero P, Gómez-Guillén MC (2009) Antioxidant and functional properties of gelatine hydrolysates obtained from skin of sole and squid. Food Chem 114(3):976–983
Gómez-Guillén MC, Turnay J, Fernández-Díaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloid 16(1):25–34
Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocolloid 25(8):1813–1827
Guerrero FL, Omil F, Mendez R, Lema JM (1998) Protein recovery during the overall treatment of wastewaters from fish-meal factories. Bioresour Technol 63:221–229
Hayes M (2013) Biological activities of proteins and marine-derived peptides from byproducts and seaweeds. In: Kim S-K (ed) Marine proteins and peptides: biological activities and applications. Wiley, West Sussex, pp 139–167

Jeon YJ, Byun H-G, Kim S-K (1999) Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochem 35(5):471–478

Jo HY, Jung WK et al (2008) Purification and characterization of a novel anticoagulant peptide from marine echiuroid worm, *Urechis unicinctus*. Process Biochem 43(2):179–184

Johnston-Banks FA (1990) Gelatin. In: Harris P (ed) Food gels. Elsevier, London, pp 233–289

Jongjareonrak A, Rawdkuen S, Chaijan M, Benjakul S, Osako K, Tanaka M (2010) Chemical compositions and characterisation of skin gelatin from farmed giant catfish (*Pangasianodon gigas*). LWT Food Sci Technol 43:161–165

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloid 23:563–576

Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables – the millennium's health. Int J Food Sci Technol 36(7):703–725

Kim SE, Kim Y, Byun H, Nam K, Joo D, Shahidi F (2001) Isolation and characterisation of antioxidant peptides from gelatine hydrolysate of Alaska pollock skin. J Agric Food Chem 49(4):1984–1989

Klomklao S, Benjakul S, Visessanguan W, Kishimura H, Simpson BK (2006) Proteolytic degradation of sardine (*Sardinella gibbosa*) proteins by trypsin from skipjack tuna (*Katsuwonus pelamis*) spleen. Food Chem 98:14–22

Kołodziejska I, Skierka E, Sadowska M, Kołodziejski W, Niecikowska C (2008) Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem 107(2):700–706

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40:43–81

Kubota K, Shijimaya H, Kobayashi A (1986) Volatile components of roasted shrimp. Agric Biol Chem 50(11):2867–2873

Kubota K, Matsukage Y, Sekiwa Y, Kobayashi A (1996) Identification of the characteristic volatile flavor compounds formed by cooking squid (*Todarodes pacificus* Steenstrup). Food Sci Technol Int 2(3):163–166

Ledward DA (1986) Gelation of gelatin. In: Ledward DA, Mitchell JR (eds) Functional properties of food macromolecules. Elsevier, New York, pp 171–201

Lin L, Li B (2006) Radical scavenging properties of proteins hydrolysates from Jumbo flying squid skin gelatin. J Sci Food Agric 86(14):2290–2295

Marrs WM (1982) Gelatin/carbohydrate interactions and their effect on the structure and texture of confectionery gels. In: Phillips GO, Williams PA, Wedlok DJ (eds) Progress in food science and nutrition 6. Pergamon Press, Oxford/New York/Toronto/Sydney/Paris/Frankfurt, pp 259–268

Mendis E, Rajapakse N, Byun H, Kim S (2005) Investigation of jumbo squid (*Dosidicus eschrichitii Steenstrup*) skin gelatine. J Sci Food Agric 86(14):2290–2295

Mito K, Fujii M, Kumahara M, Matsumura N, Shimizu T, Sugano S, Karaki H (1996) Antihypertensive effect of angiotensin-I converting enzyme inhibitory peptides derived from haemoglobin. Eur J Pharmacol 304:93–98

Miyoshi S, Ishikawa H, Kanebo T, Fukui F, Tanaka H, Maruyama S (1991) Structure and activity of angiotensin-converting enzyme inhibitors of an α-zein hydrolysate. Agric Biol Chem 55:1313–1318

Mohtar NF, Perera C, Quek S-Y (2010) Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS-PAGE. Food Chem 122(1):307–313

Morrissey K, O'Donoghue C, Hynes S (2011) Quantifying the value of multi-sectoral marine commercial activity in Ireland. Mar Policy 35:721–727

Nalinanon S, Benjakul S, Kishimura H, Osako K (2011) Type I collagen from the skin of ornate threadfin bream (*Nemipterus hexodon*): characteristics and effect of pepsin hydrolysis. Food Chem 125:500–507

Nam KA, You SG, Kim SM (2008) Molecular and physical characteristics of squid (*Todarodes pacificus*) skin collagens and biological properties of their enzymatic hydrolysates. J Food Sci 73(4):C249–C255

Ngo DH, Wijesekara I, Vo TS, Ta QV, Kim SK (2011) Marine food-derived functional ingredients as potential antioxidants in the food industry: an overview. Food Res Int 44:523–529

Ondetti MA, Rubin B, Cushman DW (1977) Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. Science 196:441–444

Rahman MS, Al-Mahrouqi AI (2009) Instrumental texture profile analysis of gelatin gel extracted from grouper skin and commercial (bovine and porcine) gelatin gels. Int J Food Sci Nutr 60(s7):229–242

Rama-Rao PB, Norton HW, Johnson B (1964) The amino acid composition and nutritive value of proteins V. Amino acid requirements as a pattern for protein evaluation. J Nutr 82:88–93

Ranathunga S, Rajapakse N, Kim SN (2006) Purification and characterisation of antioxidant peptide derived from muscle of conger eel (*Conger myriaster*). Eur Food Res Technol 222:310–315

Gu R-Z, Li C-Y, Liu W-Y, Yi W-X, Cai M-Y (2011) Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (*Salmo salar* L.) skin. Food Res Int 44:1536–1540

Saito M, Kiyose C, Higuchi T, Uchida N, Suzuki H (2009) Effect of collagen hydrolysates from salmon and trout skins on the lipids profile in rats. J Agric Food Chem 57(21):10477–10482

Salgado AJ, Coutinho OP, Reis RL (2004) Bone tissue engineering: state of the art and future trends. Macromol Biosci 4:743–765

Sanaei AV, Mahmodami F, See SF, Yusop SM, Babji AS (2013) Optimisation of gelatine extraction and physico-chemical properties of catfish (*Clarias gariepinus*) one gelatine. Int Food Res J 20(1):423–430

Sanmartín E, Arboleya JC, Villamiel M, Moreno FJ (2009) Recent advances in the recovery and improvement of functional proteins from fish processing by-products: use of protein glycation as an alternative method. Compr Rev Food Sci Food Safety 8:332–344

Schwimmer M, Mulinos MG (1957) Antibiot Med Clin Ther IV(7):403–407

Silvestrini, Bruno (1988) United States Patent 4, 149, 684

Simpson BK, Awafo V, Ramaswamy H (1999) Biotechnological approaches for the production of value-added substances from by-products of seafood harvesting. Trends in food science and technology. Proceedings of the 4th international food convention (IFCON'98), Jwalamukhi Job Press, Bangalore, pp 151–157

Singh P, Benjakul S, Maqgood S, Kishimura H (2011) Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianadon hypophthalmus*). Food Chem. doi:10.1016/j.foodchem.2010.05.111

Stainsby G (1987) Gelatin gels, advances in meat research. In: Pearson AM, Dutson TR, Bailey AJ (eds) Collagen as a food. Van Nostrand Reinhold Company, New York, pp 209–222

Taguchi T, Saito H, Aoki H, Uchida Y, Sakane M, Kobayashi H, Tanaka J (2006) Biocompatible high-strength glue consisting of citric acid derivative and collagen. Mater Sci Eng C 26:9–13

Taheri A, Abedian Kenari AM, Gildberg A, Behnam S (2009) Extraction and physicochemical characterization of greater lizardfish (*Saurida tumbil*) skin and bone gelatin. J Food Sci 74(3):E160–E165

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevellos L, Byrne-Hawkins D (2006) Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity for guava fruit extracts. J Food Compos Anal 19(6–7):669–675

Uhlmann SS, van Helmond ATM, Stefánsdóttir EK, Sigurðardóttir S, Haralabous J, Bellido JM, Carbonell A, Catchpole T, Damalas D, Fauconnet L, Feekings J, Garcia T, Madsen N, Malloid S, Margeirsson S, Margeirsson S, Palialexis A, Readdy L, Valeiras J, Vassilopoulou V, Rochet M-J (2013) Discarded fish in European waters: general patterns and contrasts. ICES J Mar Sci. doi:10.1093/icesjms/fst030

Wang J, Wang Y, Tang Q, Wang Y, Chnag Y, Zhao Q, Xue C (2010) Antioxidant activities of low molecular weight gelatine hydrolysate isolated from the sea cucumber *Stichopus japonicus*. J Ocean Univ China 9(1):94–98

Weissenberger J (2013) Discarding fish under the common fisheries policy, library briefing. Library of the European Parliament, 130436REV1, 13 May 2013
White E, Minto C, Nolan CP, King E, Mullins E, Clarke M (2011) First estimates of age, growth, and maturity of boarfish (*Capros aper*): a species newly exploited in the Northeast Atlantic. ICES J Mar Sci 68:61–66
Wu J, Ding X (2001) Hypotensive and physiological effect of angiotensin converting enzyme inhibitory peptides derived from soy protein on spontaneously hypertensive rats. J Agric Food Chem 49(1):501–506
Wu H-C, Chen H-M, Shiau C-Y (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of Mackerel (*Scomber austriasicus*). Food Res Int 36(9–10):949–957
Yang J, Ho H, Chu Y, Chow C (2008) Characteristic and antioxidant activity of retorted gelatine hydrolysate from Cobia (*Rachycentron canadum*) skin. Food Chem 110(1):128–136
Yust M, Pedroche J, Giron-Calle J (2003) Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase. Food Chem 81(3):363–369
Yunoki S, Suzuki T, Takai M (2003) Stabilization of low denaturation temperature collagen from fish by physical cross-linking methods. J Biosci Bioeng 96:575–577

Chapter 8
Recovery of Gelatin with Improved Functionality from Seafood Processing Waste

Venkateshwarlu Gudipati and Nagalakshmi Kannuchamy

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1 Introduction

The utilization of waste from the fish processing industry for value-added products has attracted substantial attention, since it addresses environmental pollution issues apart from providing added economic returns to the industry. Being a unique hydrocolloid with exceptional characteristics, fish gelatin has found a wide range of applications in the food industry. It has been extensively employed as a gelling, thickening, foaming, fining, emulsifying, and microencapsulating agent. Recently, an increasing number of new applications have been found for gelatin in products such as colloid stabilizers, hydrogels, and biodegradable packaging materials, as per the consumer demand to replace synthetic agents with more natural ones. Gelatin is also considered a highly digestible dietary food ideal for certain types of diets, particularly as nutritional supplements. Consequently, gelatin is classified as food in its own right and it is, therefore, not subjected to the food additives legislation in Europe (European Parliament and Council 2006).

Several comprehensive reviews have been published on fish gelatin illustrating its biomedical and nutraceutical applications (Kim and Mendis 2006), potential of fish processing by-products for the production of gelatin (Wasswa et al. 2007), possible gelatin alternatives for the food industry (Karim and Bhat 2008), fish gelatin as an alternative to mammalian gelatin (Karim and Bhat 2009), valorization of fish gelatin from marine by-products (Ferraro et al. 2010), and functional and bioactive properties of gelatin from alternative sources (Gómez-Guillén et al. 2011).

Though fish gelatin has been highlighted as a better alternative to mammalian gelatins from ethical and religious point of views, the production of fish gelatin is still in its infancy, contributing only about 1 % of the annual world gelatin production (Arnesen and Gildberg 2006). However, in the last few years, several attempts have been made to identify potential sources for the production of fish gelatin and develop appropriate and optimal extraction methods for the recovery of gelatin with desirable functional properties to suit various applications. In this chapter, efforts are made to discuss the intricacies of extraction conditions and their influence on extracted gelatin with respect to its functionality, so that the information generated can be directed at the effective utilization of fish gelatin.

Gelatin is extensively used in the food industry, especially in confectionery, low-fat spreads, dairy products, bakery products, meat products, beverages, desserts, and ice cream. The suitability of a gelatin for a particular application depends largely on the functional properties, such as gel strength, viscosity, and melting point (Stainsby

1987). These functional properties depend on the chemical and structural features of gelatin, particularly the molecular weight distribution and the amino acid composition (Muyonga et al. 2004). In turn, these chemical composition and structural features of gelatin depend on the extraction process.

2 Sources of Gelatin

The principal sources of gelatin are mainly animal waste. The most abundant sources of gelatin are pig skin (46 %), bovine hide (29.4 %), and pork and cattle bones (23.1 %). Fish gelatin accounted for less than 1.5 % of the total gelatin production in 2007, but this percentage was double that of the market data for 2002, indicating that gelatin production from alternative non-mammalian species had grown in importance. Apart from the well-known sociocultural and environmental aspects, the rising interest in utilizing by-products from the seafood industry for economical use is one of the reasons why exploring different species and optimizing the extraction of fish gelatin has attracted the attention of researchers in the last decade (Gómez-Guillén et al. 2002; Karim and Bhat 2009). However, because of the raised health concerns with bovine spongiform encephalopathy (BSE) and the religious feelings that some of the population does not consume products from cattle and pig sources, there was a search for alternative sources of gelatin. This resulted in the extraction of gelatin from poultry and fish waste. The results from the continuing research effort revealed that gelatin from marine sources could be a better alternative to mammalian gelatin.

Seafood processing discards, by-catch, and underutilized fish species are the promising sources for the extraction of fish gelatin. Fish processing discards generally include skin, bones, and scales. Several research groups attempted the extraction of gelatin from various fish and fish processing wastes. Gelatin has been extracted from the skin and bones of various warmwater fishes (e.g., tuna, catfish, tilapia, Nile perch, shark, and megrim), coldwater fishes (e.g., cod, hake, Alaska pollock, and salmon), and cephalopods.

3 Gelatin from Seafood Processing Waste

The utilization of seafood processing waste for the commercial production of gelatin is a good waste management practice, along with earning some economic benefit. Fish processing leads to the generation of a large biomass of fish waste (e.g., skin, bones, and scales), which is generally discarded (~7.3 million tons/year) (Kelleher 2005). It has been reported that the solid waste from surimi processing constitutes up to 50–70 % of the original raw material, depending on the method of meat extraction from the carcass (Morrissey et al. 2005). The extraction of gelatin from fish waste by different methods has been done and attempts are made to improve the functional properties of the extracted fish gelatin. At present, methods

are standardized, which could yield fish gelatin that exhibits desirable functional properties comparable to mammalian gelatin.

The solid waste from surimi processing, which may range from 50 % to 70 % of the original raw material (Morrissey et al. 2005), could also be the initial material for obtaining gelatin or collagen from underutilized fish resources. The offal from further processing of semi-processed fish products, such as skin from salted and marinated herring or cold-smoked salmon, has been studied as a source of gelatin (Kołodziejska et al. 2008). Smoking did not significantly alter the susceptibility of collagen to thermal denaturation, as the resulting gelatin was degraded considerably less and had higher gel strength than gelatins from the skin of marinated and salted herrings.

Scales, small rigid plates that grow out of fish skin to provide protection, are biocomposites of highly ordered type I collagen protein and hydroxyapatite (Ikoma et al. 2003). These scales constitute important fish industry residue and may account for around 5 % of the material contained in fish collagenous waste (Wang and Regenstein 2009). The degradation of discarded fish scales takes a longer time owing to their high organic content coupled with their hard and rigid nature, causing serious environmental pollution issues. Thus, the preparation of gelatin from these by-products has assumed importance, as it increases the economic returns for the fishing industry and decreases the load of environmental pollution. Recently, the exploration of fish scales as a prospective source of gelatin has been undertaken by Wangtueai and Noomhorm (2009) on lizardfish scales and Zhang et al. (2011) on grass carp scales.

Squid, one of the largest and most abundant cephalopods, in which only the mantle is used for human consumption and the rest of the animal is mostly discarded without any effort directed to their further recovery. Among the squid wastes and by-products, around 30 % are rich in collagen, which is the main fibrous component of the connective tissue and the most abundant protein in any organism (Brinckmann 2005). Gelatin extraction from cuttlefish was also attempted by Hoque et al. (2010).

4 Gelatin Production

Gelatin is prepared by the thermal denaturation of collagen by using either acid or alkali. The conversion of collagen to gelatin takes place due to the cleavage of a number of intra- and intermolecular covalent cross-links present in collagen. In addition, some amide bonds in the elementary chains of collagen molecules undergo hydrolysis (Bailey and Light 1989). The extraction process can influence the length of the polypeptide chains and the functional properties of the gelatin. This depends on the processing parameters (extraction temperature, time, and pH), the pretreatment, and the properties and preservation method of the starting raw material.

Gelatin production from any source generally involves two main steps:

- Pretreatment of raw material
- Extraction of gelatin

4.1 Pretreatment of Raw Material

Insoluble native collagen needs to be pretreated before it is converted into a suitable form for extraction, which is normally done by hot water. A chemical pretreatment will break non-covalent bonds so as to disorganize the protein structure, thus producing adequate swelling and collagen solubilization (Stainsby 1987). Subsequent heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Djabourov et al. 1992; Gómez-Guillén et al. 2002). The degree of collagen conversion into gelatin is related to the severity of both the pretreatment and the warm-water extraction process, as a function of pH, temperature, and extraction time (Johnston-Banks 1990).

The degree of collagen cross-linking is a key factor in order to decide the pretreatment process required for gelatin manufacture, and it is highly dependent on a number of factors, such as collagen type, tissue, animal species, etc. In the gelatin extraction from mammalian waste, the raw materials are pretreated with either dilute acid or alkali. These two production systems generate two types of gelatin. Type A gelatin is produced by the acid process and type B gelatin is produced by the alkali processing. The type of pretreatment clearly affects the characteristics of the extracted gelatin and typical differences between these types are the isoelectric point, viscosity, and textural and nanostructural properties. Acidic treatment has been found to be the most suitable for the less covalently cross-linked collagens, such as pig skin, and alkaline treatment has been found to be the most suitable for the complex collagens, such as bovine hides. However, in case of seafood waste, the collagen is different from that of mammals, and several studies showed that a combination of alkaline and acid pretreatments before extraction was helpful for the improvement of yield and functional properties of the extracted gelatin (Grossman and Bergman 1992; Gudmundsson and Hafsteinsson 1997; Zhou and Regenstein 2005). Further, the combination of an alkaline pretreatment followed by an acid pretreatment has been shown to remove the non-collagenous proteins and also to provide proper pH conditions for extraction, during which some cross-linkages could be further destroyed to yield gelatin in high amounts with good gel properties (Zhou and Regenstein 2005).

4.2 Extraction of Fish Gelatin

The increasing concern regarding fish gelatin production has necessitated the adoption of a variety of alkali, acid, and extraction parameters by researchers all over the world for different fish species, as well as different sources, such as skin, scales, and bones. Though the concept of gelatin production is the same, the procedures have slight variations among them. In general, the extraction of gelatin from fish skin typically involves a mild chemical pretreatment of the raw material and mild temperature conditions during the extraction process (Fig. 8.1). For bones and scales, degreasing

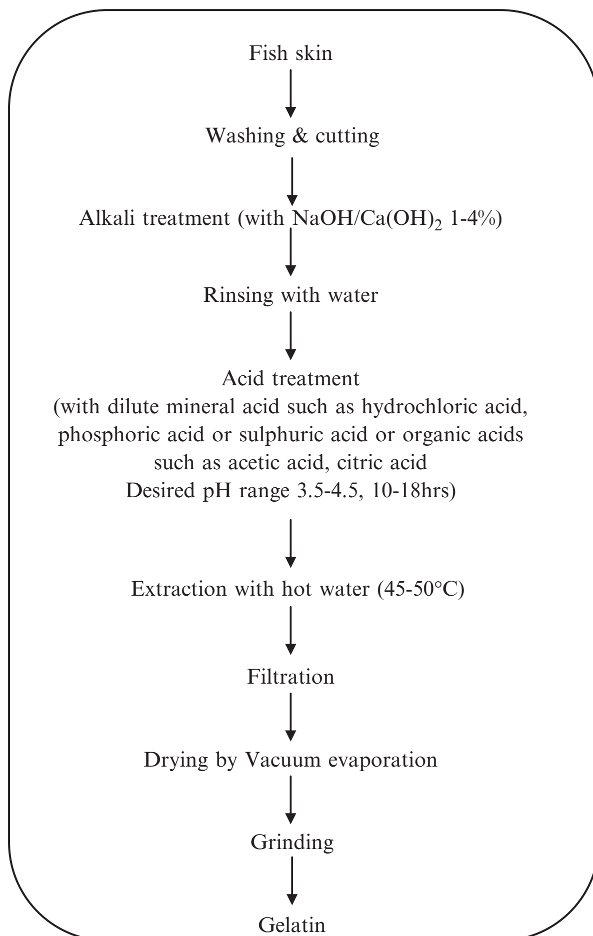

Fig. 8.1 Flow chart for gelatin extraction from fish skin

followed by demineralization or decalcifying treatment is generally employed prior to the acid treatment (Fig. 8.2). In this process, the raw material, bones and scales, are immersed in chemical compounds such as EDTA, acid, or alkali. Muyonga et al. (2004) used 3 % HCl for 9–12 days for Nile perch bones and Arafah et al. (2008) used 4–6 % HCl for 24–48 h for treating snakehead fish bones, whereas, Wangtueai and Noomhorm (2009) used low alkaline concentration (0.1–0.9 %) at 30 °C for 1–5 h for treating lizardfish scales and Duan et al. (2010) treated carp scales with 0.1 M NaOH. Demineralization of fish bones has been attempted by different chemicals such as butyl alcohol, hexane, etc. (Duan et al. 2010; Wang and Regenstein 2009).

Though fish waste has been considered a potential source for the production of gelatin, mammalian gelatin is usually preferred because of its better functional properties (Choi and Regenstein 2000; Cho et al. 2005), particularly high gelling and melting temperatures. Hence, several researchers have investigated various approaches to extract fish gelatin with improved functionality, several of which are illustrated next.

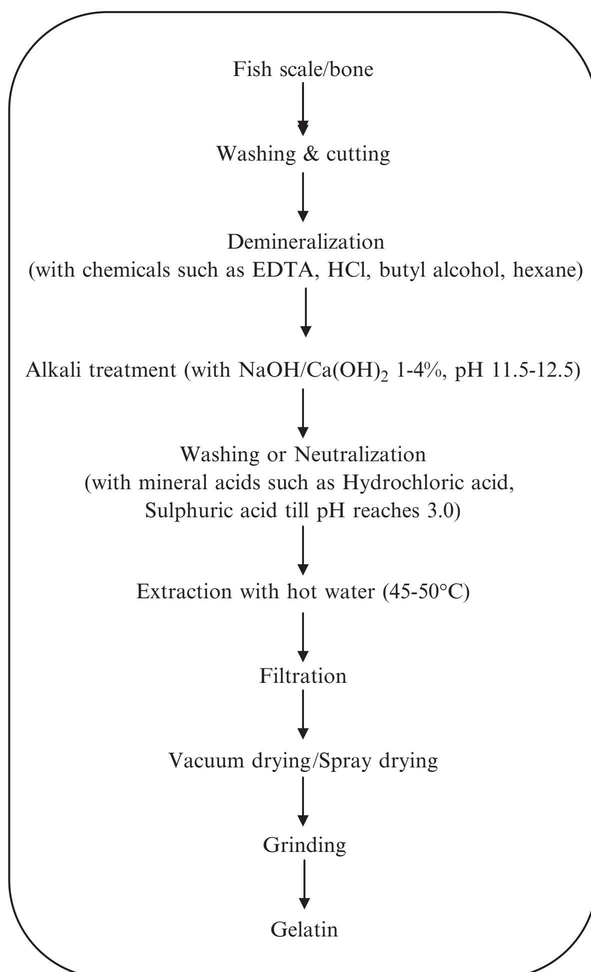

Fig. 8.2 Flow chart for gelatin extraction from fish scales and bones

4.3 Method of Grossman and Bergman (1992)

Grossman and Bergman (1992) followed the same procedure for both fish skin and bones. In this method, 100 g of fish skin or bones are cleaned and washed with tap water to remove superfluous materials. The fish skin and bones were soaked in 0.25 % (w/v) sodium hydroxide for 40 min. After washing out the sodium hydroxide, two successive acid incubations were performed, each for 40 min, first in a sulfuric acid (0.25 %, v/v) and then in a citric acid solution (1.1 %, w/v). The acid solutions were drained and then samples were washed with cold water till the pH was neutral. The final extraction of gelatin was performed in distilled water at 45 °C for 18 h. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No.1), followed by vacuum oven drying and made into powder by pestle and mortar, and packed in an air-tight container.

4.4 Method of Gudmundsson and Hafsteinsson (1997)

The method followed by Grossman and Bergman (1992) for the extraction of fish gelatin was modified by Gudmundsson and Hafsteinsson (1997) with respect to the concentrations of NaOH, sulfuric acid, and citric acid. They used this procedure for extracting gelatin from cod skin (*Gadus morhua*). Thawed skin was thoroughly cleaned and rinsed with excess water to remove superfluous material and treated by soaking with 0.2 % (w/v) sodium hydroxide solution for 40 min. Then, it was soaked with 0.2 % (w/v) sulfuric acid for 40 min. This was followed by soaking with 1.0 % (w/v) citric acid. After each soaking treatment, the skin was washed under running tap water until a pH of about 7 was reached. Each soaking and washing treatment was repeated three times, with a total time of 2 h for each treatment. The ratio of skin to washing liquid used was 1 kg (wet weight of skin) to 7 L of acid or alkali solution for each treatment. The skin was then subjected to a final wash with distilled water to remove any residual matter. The final extraction was carried out in distilled water at a controlled temperature of around 45 °C for 12 h. The ratio used was 1 kg (weight of wet skin) to 3 L of distilled water. The clear extract obtained was filtered, followed by vacuum oven drying and made into powder.

4.5 Method of Gómez-Guillén and Montero (2001)

Among the several extraction methods described by different authors, the most widely adopted method for the extraction of fish gelatin is the method developed by Gómez-Guillén and Montero (2001). In this method, the skin and bones of fish are washed in tap water and 0.8 M NaOH for 35 min at 5 °C, rinsed with plenty of tap water and treated with 0.05 M acetic acid for 90 min, and extraction is done with distilled water for 12 h at 45 °C. The clear extract obtained is filtered, followed by vacuum drying. The process involves a mild acid pretreatment for collagen swelling, followed by extraction in water at moderate temperatures. The extracted gelatin has been reported to exhibit high gelling capacity.

4.6 Method of Giménez et al. (2005)

Giménez et al. (2005) used this method for extracting gelatin from the hoki (*Macruronus novaezelandiae*) fish. The method is similar to that of Grossman and Bergman (1992), until the acid wash stage. In this method, 0.05 M lactic acid was used for 18 h at 45 °C instead of citric acid. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No.1), vacuum dried, powdered by pestle and mortar, and then packed in an air-tight container.

4.7 Method of Zhou and Regenstein (2005)

Zhou and Regenstein (2005) extracted gelatin from the Alaska pollock (*Theragra chalcogramma*). In this method, the skin and bones were maintained at a temperature below 10 °C with six volumes (w/v) of 0.2 M $Ca(OH)_2$ for 1 h. This was followed by soaking in six volumes of 0.1 M citric acid at the same temperature for 3 h. After each soaking treatment at below 10 °C, the skin and bone waste was washed with excess cold water until a pH of about 7 was reached. The skin and bones were subjected to a final wash with distilled water to remove any residual matter. Finally, extraction was done with three volumes of distilled water at 50 °C for 3 h. The extract was then filtered with cheese cloth and dried in an oven at 90 °C. After drying in a tray, the sheet of gelatin formed was reconstituted in distilled water, stabilized at 90 °C for 1 h. The reconstituted gelatin was centrifuged at 3,500 rpm for 15 min, filtered and vacuum dried for 12 h at 70 °C, ground to a powder form, and packed in an air-tight container.

4.8 Method of Liu et al. (2008)

The extraction of gelatin from the channel catfish (*Ictalurus punctaus*) was done by Liu et al. (2008). Fish skin was treated with ten volumes (w/v) of 0.1 % calcium hydroxide solution for 72 h. The treated skin and bones were washed with distilled water and 1 M H_2SO_4 was used to neutralize calcium hydroxide in the skin and bones. Then, the skin was washed again to remove deposits of calcium sulfate. Gelatin was subsequently extracted from skin in distilled water at temperatures around 45 °C for 6 h. The extracted solution was filtered and concentrated with a rotary evaporator. The remainders of the concentrated solution were dried until the moisture content was less than 10 % and dry gelatin was then ground and packed in an air-tight container.

4.9 Method of Kołodziejska et al. (2008)

This method was followed for the extraction of gelatin from Baltic cod (*Gadus morhua*), salmon (*Salmo solar*), and herring (*Clupea harengus*) by the authors. Fish skin was stirred with 0.45 M NaCl for 3 min at 4 °C and washed with tap water, and extraction continued in distilled water for 60 min at 45 °C. The clear extract obtained was filtered in a Buchner funnel with Whatman filter paper (No.1), vacuum dried and made into a powder by pestle and mortar, and packed in an air-tight container.

4.10 Method of Rahman et al. (2008)

The extraction of gelatin from the hoki (*Macruronus novaezelandiae*) fish was done by this method. In this method, the skin was washed in tap water and 0.5 M NaCl. It was then soaked in 0.1 M NaOH for 40 min at 20 °C, rinsed with tap water, and extracted in 0.1 M acetic acid for 18 h at 50 °C. The clear extract obtained was filtered, vacuum dried, powdered, and packed in an air-tight container.

4.11 Method of Benjakul et al. (2009)

This method was used for extracting gelatin from the skin of big eye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*). Initially, the skin was soaked in 0.025 M NaOH solution. Then, the mixture was stirred for 2 h at room temperature, 25–28 °C. The alkaline solution was changed every hour to remove non-collagenous proteins and pigments. Alkaline-treated skin was then washed with tap water until neutral or faintly basic pH of the wash water was obtained. The skin was then soaked in 0.2 M acetic acid with a skin–bone solution ratio of 1:10 (w/v) for 2 h with gentle stirring. The acid solution was changed every 40 min to allow swelling of the collagenous material in the fish skin–bone matrix. Acid-treated skin was washed thoroughly, as previously described. After swelling, the swollen fish skin was soaked in ten volumes (w/v) of distilled water (45 °C) for 12 h with occasional stirring. The mixture was then filtered using two layers of cheese cloth. The clear extract obtained was filtered, vacuum dried, powdered, and packed in an air-tight container.

4.12 Enzyme-Assisted Extraction of Fish Gelatin

The enzymatic pretreatment/hydrolysis for the production of fish gelatin (Fig. 8.3) generally comprises the use of enzymes with a combination of alkali and acid treatments. Different researchers have used different protease enzymes for the production of fish gelatin and the results revealed the advantage of using enzymes for gelatin extraction from fish in terms of yield, gel strength, etc. Several enzymes, such as proteases, which catalyze hydrolytic cleavage of peptide bonds in proteins and polypeptides, have been used in the manufacture of gelatin. These include pepsin, trypsin, chymotrypsin, collagenases, and pronase. Liu (1979) determined the optimum conditions for the extraction of gelatin from shark skin treated with proteolytic enzymes (papain and pancreatin). It is important to remove traces of enzyme before extraction or else extended hydrolysis leads to peptide fragments, resulting in poor functional properties. By controlling the different process parameters like pH, time, and enzyme/substrate ratio, gelatin with the desired functional properties can be obtained.

Ofori (1999) attempted to extract gelatin from shark and salmon skin using citric acid along with pepsin at concentrations of 0.01, 0.055, 0.1, and 0.12 % and reported

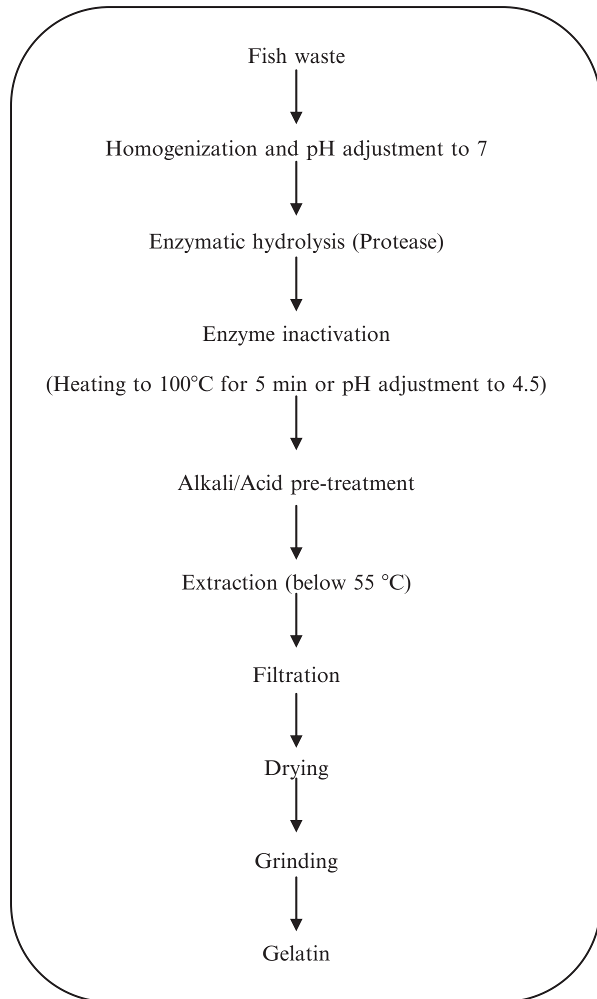

Fig. 8.3 Flow chart for enzyme-assisted extraction of gelatin

maximum gelatin yields of up to 20 % and 8 % from shark and salmon skins, respectively. Zhang et al. (2011) used protease A 2G to pretreat grass carp fish (*Ctenopharyngodon idella*) scales and response surface methodology (RSM) was adopted for the optimization. The optimal conditions obtained were temperature 30.73 °C, protease A 2G enzyme 0.22 % (w/w), and hydrolyze time 5.52 h, and the resulting gel strength was 276 ± 12 g. In the subsequent year, Jiang (2013) screened different enzymes and, among the selected types of protease (trypsin, neutral protease, papain, and alkaline protease), alkaline protease was found to be the most effective enzyme for gelatin extraction by the hydrolysis of fish scale collagen. By using RSM, the optimum hydrolysis conditions obtained were as follows: reaction time 6 h, temperature 50 °C, pH 9, and enzyme amount 3 % (w/w), and the gelatin yield reached 48.1 %.

Recently, Barve (2012) filed a patent with the World Intellectual Property Organization (WIPO) claiming that the use of protease enzyme for 4 h followed by alkali treatment, acid treatment, and extraction has provided a new and technically and/or economically significant method of producing gelatin from fish parts, including skin, bones, and scales, with better yield. By using specific enzymes for the purpose, the peptide bonds at specific locations can be broken, which will yield the final product with desirable functional properties. This is a major advantage for the food industry for a wide variety of products.

5 Functionality of Fish Gelatin

Functionality has been defined as "any property of a food or food ingredient, except its nutritional ones, that affects its utilization". In order to exhibit its functionality, the protein must interact with other components of the food system. These interactions may often require that the protein be free to either move throughout the system or to alter its structure in such a way to allow interactions with other components.

Gelatin exhibits the following functional properties:

- It is capable of forming and stabilizing hydrogen bonds to form a stable three-dimensional gel
- It exhibits an emulsifying property because of its amphiphilic nature
- It is a good film-forming material, with the potential to produce strong, flexible, edible, and biodegradable films

5.1 Gelation Behavior of Fish Gelatin

The most common use of gelatin in food products is in water gel desserts, due to its unique melt-in-the-mouth property. Certain other hydrocolloids also have thermoreversible characteristics, but they generally melt at higher temperatures. Gelatin desserts made from various gelatins may provide variety in texture and gel melting behavior, offering new product development opportunities. The desserts made from fish skin gelatin were found to be more similar to desserts made from high Bloom value mammalian gelatin. Furthermore, the lower melting temperature in gel desserts made from fish gelatin has been reported to accelerate flavor release (Zhou and Regenstein 2007).

5.2 Emulsifying Capacity of Fish Gelatin

The amphoteric nature and the presence of hydrophobic zones on the peptide chain enable gelatin to show strong emulsifying and emulsion-stabilizing properties

(Cole 2000). Consequently, it has been proven that gelatin has the ability to form a strong adsorbed layer at the interface to prevent droplet coalescence. Because of this property, it is widely used in the manufacture of toffees and water-in-oil emulsions, such as low-fat margarines, salad dressings, and whipped creams. A low-fat spread was prepared using fish gelatin and pectin by Cheng et al. (2008).

5.3 Film Formation Ability of Fish Gelatin

The film-forming ability of protein can be influenced by amino acid composition, distribution and polarity, ionic cross-links between amino and carboxyl groups, hydrogen bonding, and intra- and intermolecular disulfide bonds (Gennadios and Weller 1991). The interconnection of protein molecules during the drying process leads to the formation of film matrix and the extension or unfolding of protein molecule may favor the interaction among the molecules (Hoque et al. 2010). Hence, the selection of raw material and the extraction method influence the film-forming ability of the extracted gelatin.

6 Recovery of Fish Gelatin with Improved Functionality

It is a well-known fact that the method of extraction affects the physicochemical properties of gelatin. The degree of conversion of collagen into gelatin is related to the severity of both the pretreatment and the extraction processes, which depends on the temperature, time, and pH. Covalent cross-links between alpha chains, hydrogen bonds that stabilize the triple helix, and even peptide bonds in the primary structure are ruptured during these treatments (Hinterwaldner 1977; Ledward 1986; Veis 1964).

A wide range of potential functionality of fish gelatin is due to its specific structure consisting of amino acid composition and the distribution of the molecular weights of its polypeptide chains. Fish gelatin is different from mammalian gelatins in their properties, such as melting and gelling temperatures and gel strength. The difference is due to the different amino acid profiles, especially in respect of the contents of proline and hydroxyproline. Hydroxyproline is an imino acid derived from proline. Both of them are responsible for the stability of collagen structure. They form hydrogen bonds that stabilize the triple-helix structure by sharp twisting of the collagen structure. The content of these amino acids distinguishes the different types of gelatin sources. The lower the proline and hydroxyproline contents, the lower the melting and gelling temperatures of a gelatin (Haug et al. 2004). Different types of gelatins have different physicochemical characteristics that affect the thermal and rheological properties, including melting and gelling temperatures and Bloom strength.

The molecular weight distribution of gelatin mainly depends on the production process, which usually gives single α-chains, two α-chains covalently cross-linked to give β-chains, and three covalently cross-linked α-chains, named γ-chains

(Haug et al. 2004). The average molecular weight of one α-chain has been reported to be between 80 and 125 kDa and, for the β-form, from 160 to 250 kDa. The γ form has a molecular weight of 240–375 kDa. Large amounts of β- and γ -chains have been shown to negatively affect some of the functional properties of fish gelatins, such as lowering viscosity, lowering melting and setting points, and resulting in a longer setting time (Muyonga et al. 2004).

6.1 Effect of Resource (Cold Water/Warm Water) and Season on the Functionality of Fish Gelatin

A literature survey shows that gelatin has been extracted from several warmwater fishes, coldwater fishes, and other marine sources, such as squids (Gudipati 2013). The data generated on gel strength and melting point clearly demonstrated the variation of these important functional properties in fish gelatin extracted from different sources. The skin of warmwater fishes such as tilapia, yellowfin tuna, sole, and megrim exhibited very high gel strength. On the other hand, coldwater fishes, for example, cod and Alaska pollock, showed low gelling and melting points.

The films prepared from coldwater fish gelatin have been found to exhibit significantly lower water vapor permeability (0.93 gmm/m^{2}hkPa) compared to the films prepared from warmwater fish and mammalian gelatin (1.31 and 1.88 gmm/m^{2}hkPa, respectively). This was related to increased hydrophobicity due to reduced amounts of proline and hydroxyproline in coldwater fish gelatins. As expected, the gel strength and gel setting temperatures were lower for coldwater fish gelatin than either warmwater fish gelatins or mammalian gelatins. The study demonstrated significant differences in the physical, chemical, and rheological properties between mammalian and fish gelatins, which result in the permeability properties of the corresponding films. Lower water vapor permeability of fish gelatin films can be useful particularly for applications related to reducing water loss from encapsulated drugs and refrigerated or frozen food systems (Avena-Bustillos et al. 2006).

Duan et al. (2010) have studied the effect of season on the functional properties of fish gelatin by determining the physiochemical (molecular weight distribution and amino acid composition) and rheological (viscosity property, gel strength, and melting point) properties of gelatins from the skin of carp (*Cyprinus carpio*) caught in both winter and summer seasons. The gelatins from winter and summer fish skin have been extracted at 60, 70, and 80 °C. SDS-PAGE patterns for gelatins extracted under the same conditions showed that the degradation of gelatins from winter fish skin was more severe than that during the summer. The gelatins from summer fish presented higher melting points and gel strengths, as well as better viscosity properties than the winter equivalents ($P<0.05$). A huge difference in the temperature is usually observed between the winter and summer seasons in several areas, and, based on these results, it can be planned to extract gelatin from fish caught during the summer, which results in higher melting points and gel strengths.

6.2 Effect of Sample Preservation on the Functionality of Fish Gelatin

The use of by-products from fish for gelatin production, as an alternative to mammalian gelatin, creates several questions, such as the diversity of aquatic species and also the higher susceptibility of this collagen to deteriorate when compared to mammals. Further, after degutting and filleting of fish, the skin is kept with the rest of the by-products and subjected to rapid enzymatic and microbial damage, leading to a wide variation with respect to the quality of these gelatins. This problem urges the need for appropriate preservation of these by-products and studies to determine the quality changes in the gelatin when it is extracted from the skin preserved either by freezing or by drying.

In a study carried out by Montero et al. (1999), frozen (−20 °C) collagenous material from fish skin was shown to have high functional properties. In another study, Fernández-Díaz et al. (2003) investigated the effect of freezing temperature on the extracted gelatin. Flounder skin was frozen at −12 or −20 °C, and the resulting gelatin preparations were compared with a gelatin extracted from fresh skin. The viscoelastic properties of gelatin preparations were studied using cooling and heating ramps straight after preparation. The gelatin from frozen skin at −12 °C had lower gel strength values when compared to the fresh skin, but showed the highest melting point value. SDS-PAGE revealed that gelatin from fresh skin showed some high molecular weight aggregates and clear bands corresponding to α-, β-, and γ-components. Gelatin from frozen skin showed fewer α- and β-chains but more bands corresponding to lower molecular weight fragments; γ-components are less evident in both cases, especially in the case of skin frozen at −12 °C.

Freezing of fish skins affected molecular composition of the resulting gelatins, leading to a decrease of the amount of extracted β and γ-components, as well as higher molecular weight polymers. This effect was more severe with increased frozen temperature (−12 °C instead of −20 °C), with a concomitant prevalence of low molecular weight peptides, which could be easily extracted. The practical absence of high molecular weight polymers and γ-component in gelatins from frozen skins prevented a correct annealing of protein chains during maturation period, leading to decreased gel strength and decreased ulterior renaturation ability. The considerable higher amount of small peptides in gelatin from skins frozen at −12 °C led to broader thermal transitions curves, and revealed a scarce effect of the maturation time on the gel network development, in terms of elastic modulus (G'). This effect, however, was not found in gel strength, when compared to gelatin from skins frozen at −20 °C (Fernández-Díaz et al. 2003).

An alternative preservation method could be the drying of fish skin. Drying would allow a reduction in weight of the fish skin and storage at room temperature, which, in turn, may decrease transport, distribution, and storage costs when compared to freezing preservation. Drying has been evaluated as a preservation method for the storage of Dover sole (*Solea vulgaris*) skin. Fish skin was air dried by using ethanol, ethanol–glycerol mixture, and marine salt, and stored at room temperature

for 160 days. Gelatin extraction was carried out periodically during the storage time, and the quality of the resulting gelatin preparations was evaluated according to the molecular weight distribution, viscoelastic properties, gelling and melting temperatures, and gel strength. All the drying methods showed a similar effect on the gelatin quality characteristics evaluated. Although drying resulted in a slight decrease in viscoelastic properties as well as gelling and melting points, great protein stability was found throughout the storage period (Giménez et al. 2005). Drying scarcely affected the gel strength and other functional properties, demonstrating the suitability of this method for the storage of raw material for longer periods. Furthermore, the drying of fish skin involves important advantages compared to freezing, since dried fish skin is able to be preserved at room temperature and its weight is largely decreased, which leads to a considerable reduction in transport and distribution costs.

6.3 Effect of Extraction Method on the Functionality of Fish Gelatin

The effects of 18 different extraction conditions on yield, weight average molecular weight, dynamic storage modulus, gelling and melting temperatures, and helix recovery were studied by Eysturskard et al. (2009). Gelatins extracted from saithe (*Pollachius virens*) skin resulted in an average yield of 8.9 ± 0.8 %. High weight average molecular weight gelatins extracted at room temperature have been observed to exhibit higher dynamic storage modulus, higher gelling and melting temperatures, and more helix formation compared to highly hydrolyzed gelatins extracted under harsher conditions. The storage modulus was increased five times compared to commercial coldwater fish gelatin. The highest gelling and melting temperatures observed for 5 % (w/v) fish gelatin were 8 °C and 16 °C, respectively. Their study suggests that the dynamic storage modulus, gelling and melting temperatures, and helix content are related and increase with increasing weight average molecular weight up to about 250 kg/mol. The dynamic storage modulus correlates with the helix concentration according to the previously published correlation between the dynamic storage modulus and helix concentration, which has been defined as the master curve for gelatin (Eysturskard et al. 2009).

The effect of the extraction temperature has been investigated by studying the physicochemical properties, functional properties, and antioxidative activities of gelatin from the skin of brownbanded bamboo shark (BBS; *Chiloscyllium punctatum*) and blacktip shark (BTS; *Carcharhinus limbatus*) (Kittiphattanabawon et al. 2010). The α-amino acid group content and surface hydrophobicity of both gelatins from both species increased as the extraction temperature increased ($P < 0.05$). Both of the gelatins extracted at 60 °C exhibited the highest emulsion activity index (EAI), emulsion stability index (ESI), and foam expansion (FE). The lowest foam stability (FS) was obtained when gelatin was extracted at 75 °C ($P < 0.05$). The antioxidative activities of both gelatins increased with the coincidental increase in

α-amino group content as the extraction temperature increased ($P<0.05$). The BTS gelatin generally exhibited higher antioxidative activities compared with the BBS gelatin ($P<0.05$). Gelatin extracted at 60 °C showed the highest interfacial properties, while those extracted at higher temperature (75 °C) had enhanced antioxidative activities. The study, therefore, clearly revealed the influence of extraction temperature in regulating the functional properties.

6.4 *Effect of High Pressure on the Functionality of Fish Gelatin*

Generally, the extraction of gelatin is a time-consuming process requiring several hours to days, particularly in the pretreatment stage. Several attempts have been made to reduce the time requirement, and one of the successful methods is the application of high pressure (Gómez-Guillén et al. 2005). The high pressure, at 250 and 400 MPa for 10 or 20 min, was applied at either of two stages: during pretreatment in acid at 10 °C to facilitate destabilization of acid-labile cross-links or during extraction in water at 45 °C to accelerate collagen hydrolysis. The resulting gelatins were evaluated in terms of yield, molecular weight distribution, and viscoelastic properties. The pressure level and time of treatment were found to induce noticeable changes in the molecular weight distribution and, consequently, affected the viscoelastic properties. The use of high pressure to extract gelatin from fish skin has been recognized as a useful alternative to the conventional procedure. Its utility lies basically in shortening the longest phase of the treatment, and making it possible to produce a gelatin of high gelling quality within a few minutes (Gómez-Guillén et al. 2005).

6.5 *Effect of Bleaching on the Functionality of Fish Gelatin*

The extraction of gelatin from cuttlefish skin has been recognized as an economically viable activity. However, the presence of pigments in the skin poses a color problem, undermining its potential as a raw material the gelatin production. For removal of the color, bleaching of the skin prior to gelatin extraction has been attempted. Hydrogen peroxide is a potent oxidant that is widely used as a bleaching agent in seafood processing (Thanonkaew et al. 2008). The functional properties of gelatin from the dorsal and ventral skin of cuttlefish with and without bleaching by H_2O_2 at different concentrations [2 and 5 % (w/v)] for 24 and 48 h were studied. Gelatin from skin bleached with 5 % H_2O_2 for 48 h showed the highest yield (49.65 % and 72.88 % for dorsal and ventral skin, respectively). It has been observed that the bleaching not only improves the color of gelatin gel by increasing the L* value and decreasing the a* value, but it also enhances the Bloom strength and the emulsifying and foaming properties of the resulting gelatin. The gelatin from bleached skin contained protein with a molecular weight of 97 kDa and had increased

carbonyl content. A Fourier transform infrared spectroscopic study showed higher intermolecular interactions and denaturation of gelatin from bleached skin compared to that of the control. These results clearly indicate that hydrogen peroxide, an oxidizing agent, induces the oxidation of gelatin, resulting in the formation of gelatin cross-links, giving improved functional properties (Aewsiri et al. 2009).

7 Optimization of Extraction Variables for Improved Functional Properties

The functional properties of the extracted gelatin depend on the type of raw material, such as fish skin, scale, bone, fish species, and its composition, extraction method used, etc. Also, the extraction conditions maintained during the hydrolysis process, such as extraction temperature, time, pretreatment time, concentration of acid or alkali, enzyme/substrate ratio, pH, etc., play a vital role in deciding the quality of the gelatin.

When more than two factors influence the process, with different combinations of factors, optimization is vital in order to discover the best alternative or combination among all the possible combinations of factors or variables in the process. It reduces the overall cost, time, and effort, as statistically derived experimental designs are framed to develop the models. Also, it helps to achieve better and more desirable outputs as the outcome of the process or experiment. Zhou and Regenstein (2005) and Gudmundsson and Hafsteinsson (1997) have studied the effect of the pretreatment conditions on the resulting yield and observed yields ranging from 7 % to 16 % for pollock skins and from 11 to 14 % for cod skins, respectively. This indicates that the optimal gelatin with respect to both quantity and quality may be found by optimizing both the pretreatment and the extraction conditions.

7.1 Response Surface Methodology

RSM has been an effective tool in the optimization and monitoring of food processes (Wangtueai and Noomhorm 2009). It is a collection of mathematical and statistical modeling techniques that relate product treatment to the outputs and establish a regression equation to describe interrelations between input parameters and product properties (Cho et al. 2004). Various authors have attempted the extraction of fish gelatin from different species of fish by using RSM, as listed in Table 8.1. In general, the independent variables involved in the gelatin extraction process are extraction temperature, time, pretreatment time, concentration of acid or alkali, enzyme/substrate ratio, and pH, and the dependent variables include gelatin yield, gel strength, melting point, and other rheological and functional properties. The results of the different studies clearly show that improvements in the gelatin yield and gel strength could be obtained by optimization using RSM.

Table 8.1 Optimization of fish gelatin extraction by response surface methodology

Species	Optimal conditions of design variables					Maximum predicted value of response variables				Reference
	Alkali/acid conc. (%)	Treatment time (h)	Extraction temperature (°C)	Extraction time (h)	Pretreatment temperature (°C)	Yield (%)	Gel strength (g)	Viscosity (cP)	Melting point (°C)	
Lizardfish (*Saurida* spp.) scales	0.51 NaOH	3.1	78.5	3.02	–	10.7	240	5.61	–	Wangtueai and Noomhorm (2009)
Grass carp (*Ctenopharyngodon idella*) skin	1.19 HCl	24	52.61	5.12	–	19.83	267	–	26.8	Kasankala et al. (2007)
Catfish (*Clarias gariepinus*)	3.35 HCl	14.5	67.23	5.2	–	60.54	230.25	4.64	–	Sanaei et al. (2013)
Yellowfin tuna (*Thunnus albacares*)	1.89 NaOH	48.2	58.15	4.72	–	89.7	429.1	–	–	Cho et al. (2005)
Pollock (*Theragra chalcogramma*) skin	0.19 NaOH 0.09 HCl	–	50.0	–	2	18	460	6.20	–	Zhou and Regenstein (2004)
Nile tilapia (*Oreochromis niloticus*) skin	3.2 NaOH 0.7 HCl	2.3 84	–	–	–	20.4	260	–	22.4	Zeng et al. (2010)
Rainbow trout (*Onchorhynchus mykiss*) skin	0.25 NaOH 0.121 AcOH	3	–	–	–	9.36	459	3.2	20.4	Tabarestani et al. (2010)
Blackspotted croaker (*Protonibea diacanthus*)	0.23 NaOH	0.45	55.3	17.3	–	17.21	422	–	23.5	Jakhar et al. (2013)

Sanaei et al. (2013) used a central composite design, comprising 30 treatments including 24 factorial points, six replicates of the central point, and eight axial points, for extracting catfish bone gelatin. The four independent variables were the concentration of HCl, treatment time, extraction time, and extraction temperature. The catfish bone gelatin yield was improved from 17.52 to 60.54 %, and the gel strength was improved to 230.25 g. Similarly, the central composite design was adopted in the optimization of gelatin extraction from the dorsal skin of yellowfin tuna by Cho et al. (2005) and they could achieve a gel strength of 426 g, which was found to be higher than the mammalian gelatin (bovine 216 g, porcine 295 g) used in their study.

Recently, Tabarestani et al. (2010) used a central composite rotatable design for the extraction of rainbow trout skin gelatin. In this study, depending on the pretreatment, gelatin recoveries were in the range of 19.8–99.5 % and the optimum pretreatment conditions were found to be 0.148 N concentration of NaOH, 0.181 N concentration of acetic acid, and 2.71 h pretreatment time, which resulted in a gelatin recovery of 84.3 %. Kharyeki et al. (2011) extracted whitecheek shark (*Carcharhinus dussumieri*) skin gelatin using RSM by following a central composite design and the optimum extraction conditions were discovered to be 0.92 N NaOH concentration, 0.01 N HCl concentration, and 5.45 h extraction time, by which 93.062 (%) crude protein content and 10.208 (mPa.s) viscosity of the extracted gelatin was achieved.

Wangtueai and Noomhorm (2009) used RSM as a tool to optimize the extraction parameters for the scale gelatin of lizard fish and their results showed that the optimum conditions for the highest values of the three responses were as follows: concentration of NaOH 0.51 %, treatment time 3.10 h, extraction temperature 78.5 °C, and extraction time 3.02 h. The experimental values were 10.6 % extraction yield, 252 g gel strength, and 7.50 cP viscosity. Kasankala et al. (2007) extracted gelatin from grass carp skin adopting RSM for the optimization and they reported a 19.83 % yield and 267 g gel strength by the optimum conditions derived from the experiment: HCl concentration 1.19 %, pretreatment time 24 h, extraction temperature 52.61 °C, and extraction time 5.12 h.

The optimization of gelatin extraction from hoki *(Macruronus novaezelandiae*) skin was studied by Mohtar et al. (2010) and, with the obtained optimum conditions of 0.75 M NaCl for 9 min of pretreatment time and hot water extraction at 49.3 °C for 60 min, an experimental yield of 17.6 % and a gel strength of 197 g was reported. Similarly, different authors worked on the extraction of fish gelatin by optimizing the process parameters and observed significant improvements in the response variables, such as gelatin yield, gel strength, melting point, etc. Also, the regression models developed have been validated against different species of fish, and the results revealed the suitability or versatility of the models.

Fish gelatin has low gel strength when compared with mammalian gelatin, which limits its application in the food and pharmaceutical industry. By using these experimental design tools, the gel strength could be improved considerably, which enables fish gelatin to be on a par with mammalian gelatin. Thus, it helps make fish gelatin a better alternative to mammalian gelatin, which is the need of hour right now.

8 Improvement of Functional Properties of Extracted Gelatin

The functional properties of gelatins can be enhanced chemically and enzymatically. As discussed in the previous sections, the extraction methods influence the structural and functional properties of gelatin. Furthermore, the blending of fish gelatin with other biopolymers, such as κ-carrageenan, chitosan, and pectin (Chen et al. 2003; Haug et al. 2004; Uresti et al. 2003) and the addition of plasticizers, such as glycerol, sorbitol, sucrose, polyethylene glycol (Tanaka et al. 2001), and solutes (Fernández-Díaz et al. 2001; Haug et al. 2004) are a few other approaches available to improve the functional properties of fish gelatin. The addition of chemical cross-linking agents, such as glutaraldehyde, formaldehyde, and glyoxal (Bigi et al. 2001; De Carvalho and Grosso 2004), or enzymes, such as microbial transglutaminase (Yi et al. 2006), have also been shown to improve the properties of fish gelatin.

The influence of different salts on the melting temperature of mammalian gelatins has been known for a long time (Harrington and Von Hippel 1961). Following this, the effect of sugars on the gel strength of gelatin gels has also been observed (Naftalian and Symons 1974). In last decade, several reports have been published on the improvement of gelatins properties by enzymatic methods. Transglutaminase is an enzyme that catalyses an acyl transfer reaction that occurs between γ-carboxamide groups of glutamine residues as "acyl donor" and 3-amine groups of lysine residues as "acyl acceptor". The reaction results in the formation of 3-(γ-glutaminyl) lysine intra- and intermolecular cross-linking bonds in the proteins (De Jong and Koppelman 2002). Cross-linked bonding between γ-glutamine and lysine residues due to the presence of transglutaminase has been reported to increase the melting and gelling temperatures and gel strength, which could lead to the formation of products with enhanced rheological and functional properties (Kołodziejska et al. 2004; Fernández-Díaz et al. 2001).

The gel strength and melting point of fish gelatin could also be increased by following RSM with the incorporation of co-enhancers such as magnesium sulfate, sucrose, and transglutaminase (Koli et al. 2011, 2012). The addition of co-enhancers at different combinations as per the Box–Behnken design resulted in gel strength and melting point in the ranges 150.5–240.5 g and 19.5–22.5 °C, respectively. Based on these results, the mathematical models were developed by following RSM to determine the optimal concentrations and predicted maximum gel strength and melting point. By the addition of co-enhancers at the optimal concentrations in verification experiments, the gel strength and melting point could be improved from 170 to 240.89 g and 20.3 to 22.7 °C, respectively. The developed models help to produce desirable properties of fish gelatin suitable for a wide range of applications in the food industry. Further, there is tremendous scope for enhancing the functional properties of fish gelatin extracted particularly from coldwater fishes, which are known to possess low gel strength and melting point.

The effect of ultraviolet (UV) irradiation on the viscoelastic properties of fish gelatin was investigated by exposing the dry gelatin granules to UV light for 30 and 60 min (Bhat and Karim 2009). Irradiated samples exhibited significant

improvement in the gel strength, marked reduction in viscosity, with significant changes in the melting enthalpy, indicating the prospects of employing UV radiation as a potential method for enhancing the functional properties of fish gelatin.

9 Conclusions

In addition to well-known sociocultural advantages and environmental benefits, the increased interest in utilizing by-products from the seafood industry for economical use is another important reason for exploring different species and optimizing the extraction of fish gelatin. Further, compared to the invariable composition of land-based mammalian gelatin, fish gelatin can be extracted from the skin, bones, and scales of cold- or warmwater fish having great variation in composition, particularly with respect to amino acid profile and, accordingly, in their functional and visco-elastic properties, to find diverse applications in the food, pharmaceutical, and other industries. The extraction and evaluation of the functional properties of the extracted biopolymer is a continuous process. The development of improved recovery methods enhancing the desirable functional properties of extracted gelatin would certainly utilize the vast amount of seafood processing waste to produce fish gelatin with desirable quality attributes.

References

Aewsiri T, Benjakul S, Visessanguan W, Eun JB, Wierenga PA, Gruppen H (2009) Antioxidative activity and emulsifying properties of cuttlefish skin gelatin modified by oxidised phenolic compounds. Food Chem 117:160–168

Arafah E, Herpandi, Handayani T (2008) Characterization of gelatin from snakehead fish bone. In: Proceedings of the Seminar Nasional Tahunan V Hasil Penelitian Perikanan dan Kelautan, Semnaskan UGM, 26 July, pp 15–15

Arnesen JA, Gildberg A (2006) Extraction of muscle proteins and gelatine from cod head. Process Biochem 41:697–700

Avena-Bustillos RJ, Olsen CW, Olson DA, Chiou B, Yee E, Bechtel PJ, McHugh TH (2006) Water vapor permeability of mammalian and fish gelatin films. J Food Sci 71:E202–E207

Bailey AJ, Light ND (1989) Connective tissue in meat and meat products. Elsevier Applied Science, London/New York, pp 238–242

Barve S (2012) Method of producing gelatin from fish. WIPO Patent Application WO/2012/160575

Benjakul S, Oungbho K, Visessanguan W, Thiansilakul Y, Roytrakul S (2009) Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem 116(2):445–451

Bhat R, Karim AA (2009) Ultraviolet irradiation improves gel strength of fish gelatin. Food Chem 113:1160–1164

Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N (2001) Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. Biomaterials 22:763–768

Brinckmann J (2005) Collagens at a glance. Top Curr Chem 247:1–6

Chen T, Embree HD, Brown EM, Taylor MM, Payne GF (2003) Enzyme-catalyzed gel formation of gelatin and chitosan: potential for in situ applications. Biomaterials 24:2831–2841

Cheng LH, Lim BL, Chow KH, Chong SM, Chang YC (2008) Using fish gelatin and pectin to make a low-fat spread. Food Hydrocoll 22:1637–1640
Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB, Kim SB (2004) Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocoll 18:573–579
Cho SM, Gu YS, Kim SB (2005) Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocoll 19:221–229
Choi SS, Regenstein JM (2000) Physicochemical and sensory characteristics of fish gelatin. J Food Sci 65:194–199
Cole CGB (2000) Gelatin, encyclopedia of food science and technology, vol 4, 2nd edn. Wiley, New York, pp 1183–1188
De Carvalho RA, Grosso CRF (2004) Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocoll 18:717–726
De Jong GAH, Koppelman SJ (2002) Transglutaminase catalyzed reactions: impact on food applications. J Food Sci 67:2798–2806
Djabourov M, Lechaire JP, Gaill F (1992) Structure and rheology of gelatin and collagen gels. Biorheology 30:191–205
Duan R, Zhang J, Du X, Yao X, Konno K (2010) Properties of collagen from skin, scale and bone of carp (*Cyprinus carpio*). Food Chem 112:702–706
European Parliament and Council (2006) European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1995L0002:20060815:EN:PDF. Accessed 23 Feb 2013
Eysturskarð J, Haug IJ, Ulset AS, Draget KI (2009) Mechanical properties of mammalian and fish gelatins based on their weight average molecular weight and molecular weight distribution. Food Hydrocoll 23:2315–2321
Fernández-Díaz MD, Montero P, Gómez-Guillén MC (2001) Gel properties of collagens from skins of cod *(Gadus morhua*) and hake (*Merluccius merluccius*) and their modification by the coenhancers magnesium sulphate, glycerol and transglutaminase. Food Chem 74:161–167
Fernández-Díaz MD, Gomez-Guillen MC, Montero P (2003) Effect of freezing fish skin on molecular and rheological properties of extracted gelatine. Food Hydrocoll 17:281–286
Ferraro V, Cruz IB, Jorge RF, Malcata FX, Pintado ME, Castro PML (2010) Valorisation of natural extracts from marine source focused on marine by-products: a review. Food Res Int 43(9):2221–2233
Gennadios A, Weller CL (1991) Edible films and coatings from soymilk and soy protein. Cereal Foods World 36:1004–1009
Giménez B, Gómez-Guillén MC, Montero P (2005) The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted. Food Hydrocoll 19:951–957
Gómez-Guillén MC, Montero P (2001) Extraction of gelatin from megrim (*Lepidorhombus boscii)* skins with several organic acids. J Food Sci 66(2):213–216
Gómez-Guillén MC, Turnay J, Fernández-Díaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll 16:25–34
Gómez-Guillén MC, Giménez B, Montero P (2005) Extraction of gelatin from fish skins by high pressure treatment. Food Hydrocoll 19:923–928
Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocoll 25(8):1813–1827
Grossman S, Bergman M (1992) Process for the production of gelatin from fish skins. US Patent 5,093,474
Gudipati V (2013) Fish gelatin: a versatile ingredient for the food and pharmaceutical industries. In: Kim S-K (ed) Marine proteins and peptides: biological activities and applications, 1st edn. Wiley, Chichester, pp 271–296

Gudmundsson M, Hafsteinsson H (1997) Gelatin from cod skins as affected by chemical treatment. J Food Sci 62:37–39

Harrington WF, Von Hippel PH (1961) The structure of collagen and gelatin. In: Anfisen CB, Edsall JT, Richards FM (eds) Advances in protein chemistry. Academic, New York, pp 1–138

Haug IJ, Draget IK, Smidsrød O (2004) Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocoll 18:203–213

Hinterwaldner R (1977) Raw materials & technology of gelatin manufacture. In: Ward AG, Courts A (eds) The science and technology of gelatin. Academic, London, pp 295–361

Hoque MS, Benjakul S, Prodpran T (2010) Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J Food Eng 96:66–73

Ikoma T, Kobayashi H, Tanaka J, Walsh D, Mann S (2003) Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticas*. Int J Biol Macromol 32:199–204

Jakhar JK, Basu S, Sasidharan S, Chouksey MK, Gudipati V (2013) Optimization of process parameters for gelatin extraction from the skin of Blackspotted croaker using response surface methodology. J Food Sci Technol. doi:10.1007/s13197-012-0883-9

Jiang L (2013) Preparation of fish-scale gelatins by mild hydrolysis and their characterization. J Polym Environ 21(2):564–567. doi:10.1007/s10924-012-0546-y

Johnston-Banks FA (1990) Gelatin. In: Harris P (ed) Food gels. Elsevier Applied Science, London, pp 233–289

Karim AA, Bhat R (2008) Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends Food Sci Technol 19:644–656

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23:563–576

Kasankala LM, Xue Y, Weilong Y, Hong SD, He Q (2007) Optimization of gelatine extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresour Technol 98(17):3338–3343

Kelleher K (2005) Discards in the world's marine fisheries. An update, FAO fisheries technical paper no 470. FAO, Rome, p 131

Kharyeki ME, Rezaei M, Motamedzadegan A (2011) The effect of processing conditions on physico-chemical properties of whitecheek shark (*Carcharhinus dussumieri*) skin gelatin. Int Aquat Res 3:63–69

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39:383–393

Kittiphattanabawon P, Benjakul S, Visessanguan W, Shahidi F (2010) Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. Food Hydrocoll 24:164–171

Koli JM, Basu S, Nayak BB, Nagalakshmi K, Gudipati V (2011) Improvement of gel strength and melting point of fish gelatin by addition of coenhancers using response surface methodology. J Food Sci 76:E503–E509

Koli JM, Basu S, Nayak BB, Patange SB, Pagarkar A, Gudipati V (2012) Functional characteristics of gelatin extracted from skin and bone of Tiger-toothed croaker (*Otolithes ruber*) and Pink perch (*Nemipterus japonicus*). Food Bioprod Process 90(3):555–562

Kołodziejska I, Kaczorowski K, Piotrowska B, Sadowska M (2004) Modification of the properties of gelatin from skins of Baltic cod (*Gadus morhua*) with transglutaminase. Food Chem 86:203–209

Kołodziejska I, Skierka E, Sadowska M, Kołodziejski W, Niecikowska C (2008) Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem 107:700–706

Ledward DA (1986) Gelation of gelatin. In: Mitchell JR, Ledward DA (eds) Functional properties of food macromolecules. Elsevier, London, pp 171–201

Liu CL (1979) Studies on the extraction of gelatin fiom shark skin and skate skio. J Chin Agric Chem Soc 17(12):35–39

Liu HY, Li D, Guo SD (2008) Rheological properties of channel catfish *(Ictalurus punctaus)* gelatine from fish skins preserved by different methods. LWT Food Sci Technol 41:1425–1430

Mohtar NF, Perera CO, Quek S-Y (2010) Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS–PAGE. Food Chem 122:307–313

Montero P, Gómez-Guillén MC, Borderías AJ (1999) Functional characterization of muscle and skin collagenous material from hake (*Merluccius merluccius L.*). Food Chem 65:55–59

Morrissey MT, Lin J, Ismond A (2005) Waste management and by-product utilization. In: Park JW (ed) Surimi and surimi seafood, 2nd edn. CRC Press/Taylor and Francis Group, Boca Raton/Hoboken, pp 279–323

Muyonga JH, Cole CGB, Duodu KG (2004) Extraction and physico-chemical characterisation of Nile Perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocoll 18:581–592

Naftalin RJ, Symons MCR (1974) The mechanism of sugar-dependent stabilisation of gelatin gels. Biochimica Biophysica Acta 352:173–176

Ofori RA (1999) Preparation of gelatin from fish skin by an enzyme aided process. Thesis submitted to Department of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, Montreal, Canada, 146 pp

Rahman MS, Al-Saidi GS, Guizani N (2008) Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin. Food Chem 108:472–481

Sanaei AV, Mahmoodani F, See SF, Yusop SM, Babji AS (2013) Optimization of gelatin extraction and physico-chemical properties of catfish *(Clarias gariepinus*) bone gelatin. Int Food Res J 20(1):423–430

Stainsby G (1987) Gelatin gels. In: Pearson AM, Dutson TR, Bailey AJ (eds) Advances in meat research, vol 4, Collagen as food. Van Nostrand Reinhold, New York, pp 209–222

Tabarestani HS, Maghsoudlou Y, Motamedzadegan A, Mahoonak ARS (2010) Optimization of physico-chemical properties of gelatin extracted from fish skin of rainbow trout (*Onchorhynchus mykiss*). Bioresour Technol 101(15):6207–6214

Tanaka M, Iwata K, Sanguandeekul R, Handa A, Ishizaki S (2001) Influence of plasticizers on the properties of edible films prepared from fish water-soluble proteins. Fish Sci 67:346–351

Thanonkaew A, Benjakul S, Visessanguan W, Decker EA (2008) The effect of antioxidants on the quality changes of cuttlefish (*Sepia pharaonis*) muscle during frozen storage. LWT Food Sci Technol 41(1):161–169

Uresti RM, Ramírez J, López-Arias N, Vázquez M (2003) Negative effect of combining microbial transglutaminase with low methoxyl pectins on the mechanical properties and colour attributes of fish gels. Food Chem 80:551–556

Veis A (1964) The macromolecular chemistry of gelatin. Academic Press, New York

Wang Y, Regenstein JM (2009) Effect of EDTA, HCl, and citric acid on Ca salt removal from Asian (silver) carp scales prior to gelatin extraction. J Food Sci 74(6):C426–C431

Wangtueai S, Noomhorm A (2009) Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT Food Sci Technol 42:825–834

Wasswa J, Tang J, Gu X (2007) Utilization of fish processing by-products in the gelatin industry. Food Rev Int 23:159–174

Yi JB, Kim YT, Bae HJ, Whiteside WS, Park HJ (2006) Influence of transglutaminase-induced cross-linking on properties of fish gelatin films. J Food Sci 71:E376–E383

Zeng S, Yan X, Cao W, Hong P, Zhang C, Li L (2010) Optimisation of extraction conditions and characteristics of skin gelatin from Nile tilapia (*Oreochromis niloticus*). Int J Food Sci Technol 45:1807–1813

Zhang F, Xu S, Wang Z (2011) Pre-treatment optimization and properties of gelatin from freshwater fish scales. Food Bioprod Process 89(3):185–193

Zhou P, Regenstein JM (2004) Optimization of extraction conditions for pollock skin gelatin. J Food Sci 69:C393–C398

Zhou P, Regenstein JM (2005) Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. J Food Sci 70(6):C392–C396

Zhou P, Regenstein JM (2007) Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. J Food Sci 72:C192–C201

Chapter 9
Utilization of Fish Waste for the Making of Fish Sauce

Siswa Setyahadi

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1 Introduction

The agricultural production sector of developing countries has traditionally been given considerable attention by planners and policymakers, with relatively little focus on the development of agro-industry. Over the years, widespread failure of the survival of medium- to large-scale food-processing enterprises in developing countries has led to growing recognition of the need to foster the development of small-scale food industry. The role of the small-scale food processing as a subsector that can contribute significantly to the development of the rural economy is increasingly being realized (Dietz 1999).

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Fishery is one of the agricultural sectors and can be regarded as a great and valuable source of proteins for both animal and human nutrition. Fish meal, fish sauce, surimi, and fish silage are traditional protein-based products. The FAO State of World Fisheries and Aquaculture report 2008 states that the aquaculture sector comprised about 3.06 million tons (or 56.0 %) of the world fishmeal production and 0.78 million tons (or 87.0 %) of the total fish oil production in 2006. Thus, the total amount of fishmeal and fish oil used in aquafeeds is estimated to have grown more than threefold between 1992 and 2006, from 0.96 million tons to 3.06 million tons and from 0.23 million tons to 0.78 million tons, respectively. Aquafeed manufacturers are increasing their use of fishmeal and fish oil at the expense of all other sectors (e.g., human consumption, industrial, and pharmaceutical).

Globally, the demand for and use of fishmeal has increased rapidly, especially in some of the emerging aquaculture countries in Asia. China is the single largest user of fishmeal. In 2004, it used 1.6 million tons, with 1.2 million tons being imported and the remainder coming from domestic production. Of this total amount, about 75 % was used for aquaculture production. The Asia-Pacific aquaculture sector uses about 2.4 million tons of fishmeal (equivalent to about 10.3 million tons of raw material) as a feed source. (GAFTA 2012). Fishmeal production in IFFO countries increased by 40 % during 2011 as catches in South America returned to normal levels. Northern European production fell as a higher percentage of catches was taken up by direct human consumption. Fresh fish prices in the local market have increased recently due to low supplies, particularly of fresh fish imported from Indonesia. The recent declining catches, escalating operating costs, and growing demand in the country of origin have reduced fish imports from neighboring Sumatra, Indonesia. Malaysia imported around 71 million tons of fresh fish last year, mainly from Thailand and Indonesia, a decline by almost 50 % compared with the amount imported in 2010 (IFFO 2011).

Over 6 million tons of fishmeal is produced worldwide each year from about 25–30 million tons of industrial fish. The demand is increasing with the growth in aquaculture and the price has been rising (Klickhardt 2006). Fish sauce is produced in a quantity of about 0.4 million tons each year (Dissaraphong et al. 2006). Fish silage is almost entirely used for feed. Norway is the major fish silage producer, producing about 0.14 million tons per year, mainly from aquaculture by-products (Rustad 2003).

There are promising opportunities in the upgrading of marine by-products and underutilized fish by using mild processing techniques to convert them into protein and peptide ingredients both to improve yield in traditional seafood and to be used as nutraceuticals or in functional foods.

2 Increase the Value-Added Fish Production

About 76 % of the global fish supply was used for human consumption in 2002 (FAO 2004). About 40 million tons were used for manufacturing products for direct human consumption. But up to 50–70 % of the fish may end up as co-products, as

the yield in filleting operations ranges from 30 to 50 % (Arason 2003; Kristinsson et al. 2006; Mackie 1982). About 6 million tons of trimmings and by-products from food fish processing are processed into fish meal (Fish meal Information Network 2007) and the rest is used in fish silage or as a fertilizer or discarded.

Great additional economic, nutritional, and environmental values can obtained by increasing the yield of raw material in fish filleting operations and in utilizing fish waste for producing by-product as fish sauce.

The meat, poultry, and fish industries in the USA and other parts of the world comprise up to 12 % of brine to modify both fresh retail and further processed products. This is done to improve quality, firmness, and juiciness, and to meet increasing consumers' demand for convenience food items while at the same time increasing yield. The brine is made up of water, salt, phosphates, and sometimes also other functional or flavor ingredients, like sodium lactate, polysaccharide gums, hydrolyzed whey, and soy proteins and modified starches (Xiong 2005).

Technological innovations in fish product development by utilizing fish waste need to be done in order to provide added value to small industries. A prospect which is very promising is the manufacture of fish sauce with high protein content, so that it can be used for the food industry.

3 Type of Fermented Fish

Fermented fish is a fish curing process done in traditional or modern fashions. In the traditional method, processing is done with salt and is left for some time, while in the modern approach, biopreservation, lactic acid bacteria is added to the fish in order for it to be fermented. This produces active antimicrobials such as lactic and acetic acid, hydrogen peroxide, and peptide bacteriocins. It can also produce the antimicrobial nisin, a particularly effective preservative.

In Indonesia, there are many different types of fermented fish, such as: (1) dried fish (jambal fermented fish, peda, ikan tungkai); (2) moist fish (bekasam, naniura, and picungan); (3) lumped pounded fish/shrimp (terasi); (4) semi liquid (kecap ikan, bekasang, and cincaluk).

3.1 Dried Fish

Peda is processed using mackerel (*Rastrelliger neglectus*) as the raw material. The basic method of peda processing is a salting process with two steps. The first salting step takes several weeks to develop its characteristic flavor and texture, and this is followed by a maturation phase. Salting is carried out using a 1:3 salt to fish ratio. Fish and salt are arranged in layers alternately in a salting tank. By the end of the process, the fish are soaked in saturated brine pickle, with coarse salt remaining at the bottom and the top covering the fish. This salting process normally takes 3 days. Maturation is performed by mixing the fish and salt thoroughly. The amount of salt

used is a one-third of the fish weight. During maturation, the fish are kept in bamboo baskets, with the process taking around 1–2 weeks (Rahayu 1992). Better products can be obtained by using eviscerated fish (Hanafiah 1987) and without salt addition in the second fermentation (Hanafiah 1987; Irianto 1990; Irianto and Brooks 1994). Microbiological studies on peda conducted by Hanafiah (1987) revealed that Gram-positive cocci predominated and some were identified as lactic acid bacteria. The isolation of halotolerant bacteria from peda by Suwandi (1988) concluded that bacterial growth in peda was characterized as Gram-positive cocci, which were non-motile, aerobes or facultative aerobes, catalase-positive, non-indole producers, and oxidase-negative. They can utilize citrate as the only carbon source, ferment glucose, and show proteolytic activity. Some of them are able to reduce nitrate. These bacteria can be classified as mesophiles and require a pH of 6–8. They show variations in salt tolerance and can be divided on the basis of salt tolerance as weak, moderate, and halotolerant bacteria. Idawati (1996) showed that lactic acid bacteria isolated from peda were homofermentative and heterofermentative *Lactobacillus* sp., *Leuconostoc* sp., and *Streptococcus* sp.

Jambal roti is processed from marine catfish (*Arius thalassinus*). In the processing, after being beheaded and eviscerated, the fish were washed and soaked in fresh water for 24 h and drained. The fish are arranged in a basin. Each layer of fish is sprinkled with salt and the bellies are also filled with salt. The total amount of salt used for salting is 30–35 % of the fish weight and the salting process takes around 24 h. After salting, the fish are freed from excessive salt and washed. Clean fish are soaked in fresh water for 15–20 min and subsequently split into a butterfly form. The split fish is sun dried for 3–5 days.

Ikan tukai is a traditional fermented fish product which is mostly processed from barracuda (*Sphyranea* sp.). The traditional processing method of ikan tukai is unique. Barracuda is washed and soaked in 20 % brine for around 2 h. After draining, the fish are dried for a day. Dried fish are then wrapped with taro leaves. The wrapped fish are kept underground for 2 days to allow fermentation and then sun dried until dry.

3.2 Moist Fish

Bekasam is a fermented fish product processed from freshwater fish by mixing with chilli and sugar. In bekasam processing, in spite of the salt addition, carbohydrate sources are also incorporated to stimulate the growth of lactic acid bacteria by decomposing into simpler compounds. Sources of carbohydrate used are cooked rice, roasted rice, and sticky rice.

A similar product is called naniura. In naniura processing, fish are firstly soaked in lemon juice or 25 % acetic acid solution for 3 h. After that, ground boiled rice is added and the fish are then packed and allowed to ferment. Another product similar to bekasam is picungan. This product is processed using marine fish. Picungan seeds are used as a carbohydrate source. The seeds are cut into small pieces before mixing with fish and salt.

3.3 Lumped Pounded Fish

Terasi is consumed in small quantities as a flavor. The product is not only for local consumption, but is also exported, mainly to the Netherlands and Suriname, in powder form. Terasi is usually made from planktonic shrimp "rebon" *(Atyas* sp. or *Mycis* sp.) (Budhyatni et al. 1982) or mixing waste shrimp shell and fish. Terasi can be processed in two ways: (1) with salt and (2) with mixing salt and ingredients.

In the processing of terasi with salt, firstly, rebon is washed, drained, and dried, until half dried. During drying, impurities such as small fish, mussel shells, and coral are removed. After that, semi dried rebon is sifted to separate sand and other undesirable materials. The rebon is then left overnight at ambient temperature and pounded the next day. During the first pounding, salt is added (around half of the total salt required during processing). The total amount of salt used in terasi processing is 2–5 % of the dry weight of rebon, which should be added as solution. Pounded rebon is sun dried and subsequently kept in a container at ambient temperature for 2–3 days. The stored rebon is then pounded for a second time, while the remaining salt is added. After that, the pounded rebon is sun dried and kept at ambient temperature for 2–3 days until soft. It is then ground by many passes through a meat grinder until it is fine. Fine rebon is formed in cubes or cylinders of 1 kg in weight and subsequently fermented for a week or more at ambient temperature (Yunizal 1998).

The other processing method of terasi with mixing salt and ingredients is similar to the processing method of terasi with salt. The difference is in the second pounding, where the salt solution is mixed with 2.5 % (w/w) coconut sugar and 2 % (w/w) tamarind prior to be added to fresh rebon. The additional ingredients accelerate the fermentation process (Yunizal 1998).

Microorganisms in terasi powder during ambient storage are those of *Lactobacillus* sp., *Staphylococcus* sp., *Bacillus* sp., and *Proteus* sp., but *Salmonella* sp., *Clostridium* sp., *Vibrio* sp., and *E. coli* are not found (Budhyatni et al. 1982).

3.4 Semi Liquid

Bekasang is a traditional product processed from the viscera of skipjack (*Katsuwonus pelamis*) and waste from cakalang fufu (smoked skipjack). The processing method of bekasang is as follows. Skipjack viscera obtained from processed cakalang fufu is washed and mixed with salt at a ratio of 2.5:l. The mixture of viscera and salt is kept for a week to allow fermentation. After fermentation ceases, the fermented viscera are boiled for 2 h and filtered using a gauze.

Cincaluk is the other traditional fermented fresh shrimp mixed with boiled rice and salt in a pan. For 1 kg of shrimp, the rice added is around 200–300 g, while the salt quantity is approximately 300 g. The pan is then sealed with the lid to avoid air entry and kept for 4 days until liquid release. After that, the mixture is put into bottles and eventually sealed tightly. Another method to process cincaluk is by mixing fresh shrimp with a ratio of 20:1:1 for tapioca flour, salt, and sugar. In its

processing, shrimp are descaled and then washed. Tapioca flour is dissolved in water, gelatinized, and allowed to cool. Shrimp are then mixed thoroughly with salt, sugar, and gelatinized tapioca flour. The mixture is placed into washed bottles and sealed firmly and fermented at ambient temperature for 1–2 weeks.

Fish sauce is processed traditionally from oil sardine (*Sardinella lemuru*) by fermentation using high salt concentration.

4 Fish Sauce Processing

Although fermented fish sauce itself may not be directly used for a physiological functional food because of its high concentration of sodium chloride, the sauce may be useful as a source of biologically active substances, traditional food supplements in the diet, and are widely used around the world as condiments, as flavoring, material, and sometimes as a substitute for soy bean sauce (Watanabe et al. 2004). It was concluded that summer capelin may be successfully utilized for the production of fish sauce without added enzymes. Capelin harvested during the summer season was more suitable as a raw material than capelin harvested during the winter season, due to its higher proteolytic activity. Reduced salt content in processing may help increase the fermentation rate as well as improve nutritional properties by reducing the sodium content (Hjalmarsson et al. 2007). Many fermented fish products are prepared in different parts of the world and the method of processing depends upon various factors; availability of raw materials, consumers' preferences, and the climatic conditions of the region. In addition, fish sauce is a product that can be made cheaply from various fish raw materials, which are not normally used for food. Also, it is a clear brown liquid hydrolysate from salted fish (Amano 1962; Beddows 1985), and is a liquid seasoning commonly used in most parts of Southeast Asia. Fish sauce is called by different names, such as patis in the Philippines, shottsuru in Japan, budu in Malaysia, nam pla in Thailand, nước mắm in Vietnam, kecap ikan in Indonesia, yuilu in China, and ngapi in Burma, and it contains a mixture of amino acids and other protein degradation products. (Fukami et al. 2002).

Different countries have different recipes for making fish sauce. Nam pla (Thai fish sauce), which is the most dominant in the world market, is mainly produced from anchovies (*Stolephorus* sp.), mackerel (*Rastrelliger* sp.), and herring (*Clupea* sp.) (Wilaipan 1990).

4.1 Traditional Process

There are two major ingredients in fish sauce production, fish and salt. Traditionally, nam pla is produced by mixing one part salt with two or three parts fish and fermenting under static atmospheric conditions in an underground concrete tank at ambient temperature (30–40 °C) for up to 18 months. The process of salting the traditional fish processing will result in the loss of fish protein by 5 %, depending on the levels

of salt and the salting time. It is recommended that the added salt does not exceed 40 parts by weight of the fish. The supernatant from the fermentation tank is filtered and ripened under the sun for 2–4 weeks (Wilaipan 1990).

The other process can use marine and freshwater fish as a raw material for fish sauce production. Fish and sea salt are mixed at a weight ratio of approximately 2:1 for marine fish and 3:1 for freshwater fish. Traditionally, whole fish is dumped on a concrete floor, and the excess liquid is allowed to drain off. Fish are thoroughly mixed with salt. The mixture is then placed into a tank. Another layer of salt is spread on top. After 1 week, the fish may float and rise to the top of the fermentation tank. It is necessary to keep the fish immersed at all times, otherwise they will become rancid. While the salting progresses, hydrolysis of fish tissues by fish gut enzymes provides the necessary nutrients for bacterial fermentation to begin. The time needed for the full flavor of fish sauce to develop varies from 8 to 18 months at 30–35 °C (Owens and Mendoza 1985; Lopetcharat et al. 2001; Fukami et al. 2004). The finished product is a clear dark-brown color, with pH 5.5–5.8 and 20.5–27.4 % salt, and has a distinct aroma and flavor. The flavor and aroma determine its quality and these characteristics develop progressively as the fermentation process advances (Lopetcharat et al. 2001).

The ratio between salt and fish is very different, depending on the country, ranging from 1:6 to 1:2 (w/w). In Indonesia, the procedure of fish sauce processing is as follows. Whole fish were washed with 2 % saline solution and then chopped. The minced fish is inserted into a barrel and mixed with 5 % salt. Whole fish were washed with 2 % saline solution and then chopped up and put into the tank and mixed with 5 % salt. 60 % (v/v) of acetic acid was added to the mixture of salt and fish to pH 3–4. The addition of vinegar aims to reach medium acidic conditions (pH 3–4). Highly acidic conditions will increase the activity of the enzyme protease, which would break down proteins in the fish tissue.

The time required to make fish sauce depends on several factors, such as the type of acid. From various studies, the time required ranged from 4 to 10 weeks. The addition of protease enzymes from the outside can reduce the time required. According to Gilberg et al. (1984), the addition of the enzyme pepsin in the manufacture of fish sauce can reduce the processing time to get good quality fish sauce to just 3–6 h.

Microorganisms generally increase during the early fermentation stage and then decrease gradually as the fermentation time is extended (Saisithi et al. 1966; Ijong and Ohta 1995). Beddows et al. (1980) reported that budu (Malaysian fish sauce) produced in the presence of rifampicin does not have the unique aroma of budu. Microorganisms such as *Bacillus* and *Staphylococcus*, which were isolated from nam pla, kecap ikan or bakasang (Indonesian fish sauce), and patis (Philippine fish sauce), produced a significant amount of volatile acids (Saisithi et al. 1966; Ijong and Ohta 1995).

Fermentation takes place in tanks at temperatures slightly above room temperature for 1–5 days. After fermentation completed, filtering for solids separation was conducted. During the process of fermentation, competition among microorganisms occurs, with bacteria and microorganisms that survive participating in the process of fermentation. High amounts of salt causes a long fermentation time and the resulting soy products have a very salty taste. To overcome these problems various

improvements have been made that is utilize proteolytic enzymes. The enzyme can work optimally with reducing concentration of salt and optimal fermentation conditions (Yunizal et al. 1982).

The liquid fish sauce can be added with sodium carbonate for a pH of 6–7 and be boiled and seasoned as needed. The product can also be add with a thickening agent such as carboxymethyl cellulose (CMC) before being packaged into bottles and becoming ready for the market.

4.2 Enzymatic Process

Making traditional fish sauce requires a relatively long time. Microorganisms producing protease enzyme adaptation takes a long time to be able to live in high salinity environments and other abnormal conditions.

During the fermentation process, the fish tissue undergoes hydrolysis by enzymes produced by microorganisms. The role of these enzymes is bond breaker polypeptides, to become simpler bonds. Microorganisms that develop during the fermentation of fish are not fully known. Nevertheless, estimated species of lactic acid bacteria such as *Laucosotic mesenterides*, *Pediococccus cerevisiae*, and *Lactobacillus plantarum* are growing. Some types of yeast are also thought to have evolved in the fermentation.

The addition of proteolytic enzymes before fermentation can shorten the fermentation time of fish sauce processing. In this case, it is no longer necessary to time the adaptation of microorganisms to produce enzymes that can hydrolyze proteins.

The high price of pure proteolytic enzymes becomes a constraint to produce fish sauce quickly, easily, and inexpensively. However, by utilizing the sap of papaya and pineapple extracts, the role of pure proteolytic enzymes can be replaced.

Papaya sap contained in the proteolytic enzyme is called papain. Papain has a high activity for hydrolyzing proteins. In the food industry, papain is used for retaining the freshness of beer, softening meat, and removing proteins in food, while in pineapples, especially young pineapples, bromelain is another proteolytic enzyme. Its ability to hydrolyze proteins is not dissimilar to papain.

However, fish sauce produced enzymatically has scent and color that are very different to the fish sauce made traditionally, although it is not very different in terms of nutritional content.

5 Quality of Fish Sauce

It is high in protein (as much as 10 %), and this protein is a complete one containing all the essential amino acids that the body requires for growth and regeneration. Top-quality fish sauce also contains a rich supply of B vitamins, particularly B 12,

Table 9.1 Nutrient content based on the quality of fish sauce (g/100 g)

Component	Quality I	Quality II
Salt	28.4	28.4
Protein	0.92	1.90
Amino nitrogen	5.75	5.58
Nitrogen organic	0.62	1.13

Table 9.2 Differences in the nutritional values of fish sauce on the different types of fish used

	Proximate, (%)				
Kind of fish used	Protein	Lipid	Carbohydrate	Water	Salt
Petek (*Leiognathus* sp.)	5.57	0.84	0.46	88.07	5.04
Kuniran (*Upeneus sulphureus*)	5.40	0.77	0.24	87.54	6.04

pantothenic acid, riboflavin, and niacin. Other beneficial nutrients include calcium, phosphorous, iodine, and iron (Table 9.1).

According to Cole et al. (1985), good fish sauce is considered to have a protein content of 2.3 % and a pH below 6, but standard fish sauce in every country is different.

Whether fresh water or salt water, fish for making fish sauce are usually small ones that otherwise would have little value for consumption. Among the most common marine fish used are anchovies and a few related species of schooling fish, from 2 to 5 in. in length, found in plentiful supply in the rich gulf waters. Larger varieties of fish, such as mackerel and sardines, also make good fish sauce, but because they are relatively more expensive due to their value as a food fish, they are seldom used in the commercial production of fish sauce. For fish sauce to develop a pleasant, fragrant aroma and taste, the fish must be very fresh. The different types of fish used showed differences in nutritional value (Table 9.2).

The protein content of fish sauce from Petek (*Leiognathus* sp.) is higher than the protein content of fish sauce from kuniran (*Upeneus sulphureus*), which is caused by the different salt contents. Salt is known to inhibit the activity of enzymes in the process of breakthrough of the tissue into soluble protein. Some researchers reported that, the tissues of fish, especially the internal organs, contained a variety of enzymes. Some researchers reported that protease enzymes in the body tissues of fish are most active in acidic conditions (pH 3–4), while others are most active in base conditions (pH 7–8). Meanwhile, Cole et al. (1985) reported that endopeptidase enzymes and lipase are active at salt levels up to 15 %, and will decrease in activity if the salinity is increased. The results of the amino acid analysis of fish sauce protein shows there are 19 different amino acids, with the highest content being glutamic acid, amounting to 20.57 % of the total protein (Table 9.3). The high content of glutamic acid in the protein composition showed that fish sauce can use as a flavor enhancer.

During the process of making fish sauce, other flavor ingredients can be added. Thus, the created fish sauce can be adjusted to suit various tastes and it will increase the value of this product.

Table 9.3 Amino acids composition in fish sauce

No	Amino acids	% of total protein
1	Glycine	3.80
2	Alanine	1.10
3	Valine	6.24
4	Leucine	11.09
5	Isoleucine	5.33
6	Proline	9.78
7	Phenylalanine	5.51
8	Tyrosine	4.83
9	Tryptophan	0.60
10	Serine	4.58
11	Threonine	3.02
12	Methionine	2.75
13	Arginine	2.70
14	Histidine	6.42
15	Lysin	5.22
16	Aspartic acid	4.70
17	Glutamine	0.94
18	Glutamic acid	20.57
19	Asparaginic acid	0.55

Isnawan et al. (2001)

References

Amano K (1962) The influence of fermentation on the nutritive value of fish with special reference to fermented fish products of South-East Asia. In: Heen E, Kreuzer R (eds) Fish nutrition. Fishing News Books, London, 180 pp
Arason S (2003) Utilization of fish by-products in Iceland. In: Bechtel PJ (ed) Advances in seafood by-products, 2002 conference proceedings. University of Alaska, Alaska, pp 43–62
Beddows CG (1985) Fermented fish and fish products. In: Wood BJB (ed) Microbiology of fermented foods, vol 2. Elsevier Applied Science, New York, pp 1–39
Beddows CG, Ardeshir AG, Daud WJB (1980) Development and origin of the volatile fatty acids in Budu. J Sci Food Agric 31:86–92
Budhyatni S, Murtini JT, Peranginangin R (1982) The microflora of terasi powder. Laporan Penelitian Teknologi Perikanan 16:25–33 (in Indonesian)
Cole RC, Clucas IJ, Bostock TW, Rogers JF, Stephens AD (1985) Fish handling, preservation and processing in the tropics: part 2. Tropical Development and Research Institute, London
Dietz MH (1999) The potential of small-scale food processing for rural economies. Courier 174:89–92
Dissaraphong S, Benjakul S, Visessanguan W, Kishimura H (2006) The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation. Bioresour Technol 97(16):2032–2040
FAO (2004) The state of world fisheries and aquaculture. FAO, Rome
Fishmeal Information Network (2007) Fishmeal facts and figures. Fishmeal Information Network Scott 18, April at http://www.gafta.com/fin/finfacts3.html
Fukami K, Funatsu Y, Kawasaki K, Watabe S (2004) Improvement of fish-sauce odor by treatment with bacteria isolated from the fish-sauce mush (moromi) made from frigate mackerel. J Food Sci 69(2):fms45–fms49

Fukami K, Ishiyama S, Yaguramaki H, Masuzaw T, Nabeta Y, Endo K, Shimoda M (2002) Identification of distinctive volatile compounds in fish sauce. J Agric Food Chem 50: 5412–5416
GAFTA (2012) http://www.gafta.com/fin/pdfs/FACTSANDFIGURES.pdf. Accessed 23 Aug 2012
Gildberg A, Espejo-Hermes J, Magno-Orejana F (1984) Acceleration of autolysis during fish sauce fermentation by adding acid and reducing the salt content. J Sci Food Agric 35:1365–1369
Hanafiah TAR (1987) Factors affecting quality of pedah siam. Master thesis, University of Washington, Seattle
Hjalmarsson GH, Park JW, Kristbergsson K (2007) Seasonal effects on the physicochemical characteristics of fish sauce made from capelin (*Mallotus villosus*). Food Chem 103:495–504
Idawati (1996) Isolation and selection of lactic acid bacteria having antibacterial activities from *peda* and kecap ikan. Sarjana thesis, Faculty of Agricultural Technology-Bogor Agricultural University, Bogor (in Indonesian)
IFFO (2011) http://www.infofish.org/News.aspx?Type=Market. Accessed 23 Aug 2012
Ijong FG, Ohta Y (1995) Amino acid compositions of bakasang, a traditional fermented fish sauce from Indonesia. LWT-Food Sci Technol 28:236–237
Irianto HE (1990) Studies on the processing of "pedah", a traditional Indonesian fermented fish product. Diploma thesis, Massey University, New Zealand
Irianto HE, Brooks JD (1994) Investigation on the optimum conditions in pedah processing. Jurnal Penelitian Pasca Panen Perikanan 8(1):18–29
Isnawan H, Siswa Setyahadi, Suwendo H (2001) Teknologi Pembuatan Kecap Ikan secara Enzimatik Terkendali untuk Industri Skala Menengah dan Rumah Tangga. Jurnal Sains dan Teknologi Indonesia 3(3):8–13
Klinkhardt M (2006) Demand for fish meal and fish oil rising. Eurofish Magazine, 6
Kristinsson HG, Theodore AE, Ingadottir B (2006) Chemical processing methods for protein recovery from marine by-products and underutilized species. In: Shahidi F (ed) Maximising the value of marine by-products. Woodhead Publishing, Cambridge, pp 144–168
Lopetcharat K, Choi YJ, Park JW, Daeschel MA (2001) Fish sauce products and manufacturing: a review. Food Rev Int 17(1):65–88
Mackie IM (1982) Fish protein hydrolysates. Proc Biochem 17(1):26–32
Owens JD, Mendoza LS (1985) Enzymically hydrolysed and bacterially fermented fishery products. Int J Food Sci Technol 20:273–293
Rahayu S (1992) Processing of peda. In: Suparno, Nasran S, Setiabudi E (eds) Compilation of research results of fishery post-harvest. Pusat Penelitian dan Pengembangan Perikanan, Jakarta, pp 133–134 (in Indonesian)
Rustad T (2003) Utilisation of marine by-products. Electron J Environ Agric Food Chem 2:458–463
Saisithi P, Kasemsarn RO, Liston J, Dollar AM (1966) Microbiology and chemistry of fermented fish. J Food Sci 31(1):105–110
Suwandi I (1988) Studies on the physiological characteristics of halotolerant bacteria isolated from peda. Sarjana thesis, Bogor Agricultural University, Bogor. (in Indonesian)
Watanabe F, Michihata T, Takenaka S, Kittaka-Katsura H, Enomoto T, Miyamoto E, Adachi S (2004) Purification and characterization of corrinoid compounds from Japanese fish sauce. J Liq Chromatogr Relat Technol 27(13):2113–2119
Wilaipan P (1990) Halophilic bacteria producing lipase in fish sauce. MSc thesis, Chulalongkorn University, Bangkok
Xiong YLL (2005) Role of myofibrillar proteins in water-binding in brine-enhanced meats. Food Res Int 38(3):281–287
Yunizal (1998) Processing of shrimp terasi. Warta Penelitian dan Pengembangan Pertanian XX(1):4–6 (in Indonesian)
Yunizal NS, Muljanah I, dan Peranginangin R (1982) Studies on preparation of lactic acid bacteria starter. Laporan Penelitian Teknologi Perikanan 18:31–36 (in Indonesian)

Chapter 10
Enzymes from Fish Processing Waste Materials and Their Commercial Applications

Se-Kwon Kim and Pradeep Dewapriya

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1 Introduction

Enzymes are biocatalysts responsible for thousands of chemical reactions in cellular systems. Due to their high reactivity and specificity, they have become an important tool for biological as well as industrial reactions. Despite the credit of the pioneering work on enzymes going to the French chemist Anselme Payen for discovery of diastase, the German physiologist Wilhelm Kühne (1837–1900) first used the term

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"enzyme" to describe biocatalysts (Gutfreund 1976). Even though the exact function of the enzymes was clear at the time, enzymes have been used since ancient times to produce food products, such as cheese, sourdough, beer, wine, vinegar, and for the manufacturing of commodities such as leather, indigo, and linen. With the advances of knowledge on enzymes and their superior catalytic power, today, enzymes have become an integral part of many industrial chemical reactions. Many plant- and animal-derived enzymes are currently used in the industrial production of various commodities. However, fermentative production of industrially important enzymes using selected microorganisms have made it possible to produce a large amount of relatively pure enzymes more easily than extraction from plant or animal sources and, hence, this has become a popular method for commercial enzyme production (Kirk et al. 2002). Further, recombinant DNA technology and recent developments of protein chemistry enabled to develop tailor-made enzymes that have high catalytic power and adaptive physical properties (Olempska-Beer et al. 2006).

A wide range of applications of enzymes in bioindustry has considerably increased the global market for enzymes, and the production of industrial enzymes has become a cash cow for many companies. However, in general, the use of enzymes in industrial processes makes it a costly addition due to its high cost, and maintaining the enzyme-specific conditions during the process adds considerable cost to the production process. Therefore, enzymes with high activity at very low concentrations and under mild conditions of pH and temperature are of great interest. Further, the increasing sophistication of commodities, including food products, demands a broad variety of enzymes having properties compatible with processing conditions as a catalyst in production processes. Thus, there is an increasing demand for novel as well as highly reactive and specific enzymes. A wide variety of genetic material in the marine environment has made marine resources promising for the discovery of novel enzymes with properties that could not be found previously (Lee et al. 2010). Further, the prevailing extreme conditions in the marine environment have also contributed to the evolution of novel enzymes in marine resources. In this chapter, we aim to discuss the potentials of marine resources for the extraction of enzymes for industrial processes, paying specific attention to fish processing waste as a source of enzymes.

2 Marine Resources as a Source of Enzymes

Marine microorganisms, representing over 90 % of the marine biomass, are the most fascinating living creatures in the marine environment. It is a well-known fact that marine microorganisms play an active part in the mineralization of complex organic matter that contributes to the secondary production of the marine environment. To accomplish these sequenced and complex chemical processes, marine microorganisms have been reinforced with a vast array of enzymes. Thus, marine microorganisms are a promising source of marine enzymes (Zhang and Kim 2010). Especially, extremophiles and symbiotic microorganisms are capable of producing

novel enzymes with their habitat-related characteristics, such as salt tolerance, hyperthermostability, barophilicity, and cold adaptivity (Trincone 2011). In addition, marine microalgae have also been explored as a source of marine enzymes.

Apart from microorganisms, many other marine organisms such as fishes, prawns, crabs, snakes, plants, and algae produce unique marine enzymes that have biotechnological potentials. For instance, Zonase X™ is a well-proven exfoliating and skin rejuvenation product developed by Aqua Biotechnology using an enzyme derived from the hatching fluid of salmon. Originally, the enzyme degrades the egg shell surrounding the fish larvae during early embryogenesis. The experimental results showed that this enzyme is capable of gently removing the outer dead layer of human skin without destroying the skin itself (Sarkar et al. 2010). This clearly shows the potential of marine-derived enzymes and their applications. It has been recognized that fish processing leftovers and underutilized marine fish species are potential sources of different enzymes, such as proteases, lipases, cellulase, and collagenase (Table 10.1). Head, skin, viscera, bones, blood, and stickwater, which account for as much as 70–80 % of the total catch, could be utilized for the extraction of enzymes. Particularly, fish processing leftovers are a rich source of proteases, which have high commercial value, and, thus, low-cost fishery leftover has gained much attention in the extraction of industrial proteases (Klomklao 2008). Further, approximately 30 % of the total landing of fish is considered as underutilized, bycatch, or unconventional. Even though many conventional approaches have been developed to optimize utilization, low income generated through such approaches demand novel directions to produce value-added products. Therefore, the use of these types of fish for commercially important enzyme production brings new insight to the world fishing industry. Moreover, it has been found that marine fish and invertebrates are common sources of some specific enzymes. Polyphenolases from lobster and shrimp, urease from fish liver, thymidylate kinase from sea urchin, and trimethylamine oxide demethylase from gadoid fish such as hake and flounder have been recovered as major enzymes (Shahidi and Janak Kamil 2001). Most of the enzymes that have been identified from marine fish and leftovers also present in terrestrial sources, but characteristic differences in the molecular weight, amino acid composition, optimum pH, optimum temperature, stability, and catalytic power of the enzymes have brought a unique value to those of marine origin. In this chapter, we mainly focus on different types of marine fish and fishery leftover-derived enzymes, their unique features, and industrial applications.

3 Enzymes from Fish and Processing By-products

3.1 Digestive Enzymes

As mentioned earlier, proteases are the most common enzymes present in marine animals. Table 10.1 is a summary of digestive enzymes identified from marine fish and fish leftover. Proteases of marine animals are polyfunctional enzymes which

Table 10.1 Examples of enzymes isolated and purified from fish

Enzyme	Fish species	Origin	Molecular weight	Optimum pH and temperature	Reference
Pepsin	Albacore tuna	Stomach	32.5 kDa	pH 2, 50 °C	(Nalinanon et al. 2010)
	Smooth hound	Stomach	35 kDa	pH 2, 40 °C	(Bougatef et al. 2008)
	Sea bream	Stomach	30 kDa	pH 3–3.5, 40–50 °C	(Zhou et al. 2007)
	European eel	Stomach	30 kDa	pH 2, 35–40 °C	(Wu et al. 2009)
Trypsin	Pirarucu	Viscera	28 kDa	pH 6,5–11, 55 °C	(Freitas-Júnior et al. 2012)
	Mackerel	Viscera	24 kDa	pH 8, 60 °C	(Chun et al. 2011)
	Skipjack tuna	Spleen	24 kDa	pH 8.5, 60 °C	(Klomklao et al. 2007)
	Yellowfin tuna	Spleen	24 kDa	pH 8.5, 55–65 °C	(Klomklao et al. 2006b)
	Tongol tuna	Spleen	24 kDa	pH 8.5, 65 °C	(Klomklao et al. 2006a)
Lipase	Chinook salmon	Pyloric caeca	79.6–54.9 kDa	pH 8–8.5, 35 °C	(Kurtovic et al. 2010)
	Hoki	Pyloric caeca	44.6 kDa	pH 8–8.5, 35 °C	(Kurtovic et al. 2010)
	Grey mullet	Dorsal part	46.5, 41.2 kDa	pH 8–8.5, 33–35 °C	(Islam et al. 2008)
	Carp	Liver	74 kDa	pH 8, 37 °C	(Görgün and Akpınar 2012)
Collagenase	Mackerel	Internal organs	14.8 kDa	pH 7.5, 55 °C	(Park et al. 2002)
	Filefish	Internal organs	27 kDa	pH 7.0–8.0, 55 °C	(Kim et al. 2002)
	Rayfish	Viscera	NA	pH 6, 40 °C	(Murado et al. 2009)
	Mixture of fish	Processing waste	60 kDa	pH 7.5, 35 °C	(Daboor et al. 2012)
Cathepsin L	Blue scad	Skeletal muscle	30 kDa	pH 5.5, 55 °C	(Zhong et al. 2012)
Gelatinolytic proteinases	Carp	Dark muscle	64–75 kDa	NA	(Wu et al. 2008)
Chitinase	Nile tilapia	Stomach, intestine, and serum	75 kDa	pH 7.0, NA	(Molinari et al. 2007)

NA: data not available

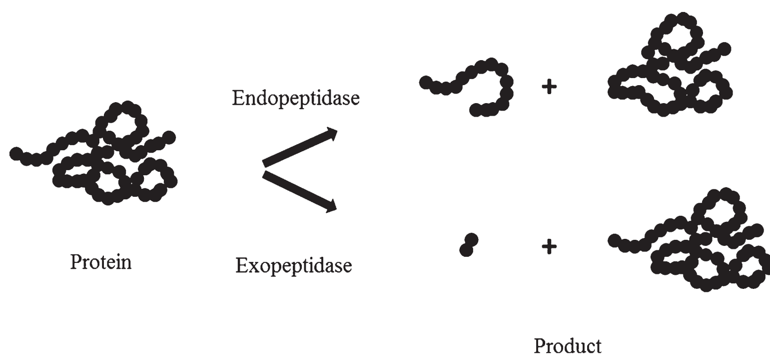

Fig. 10.1 Action of endopeptidases and exopeptidases in protein breakdown

catalyze the hydrolytic degradation of proteins. This type of protein is commonly found in the digestive system of marine animals. Interestingly, the distribution and catalytic activity of the same enzyme varies depending on the organ and the species. Mainly, pepsin, gastricsin, trypsin, chymotrypsin, collagenase, elastase, carboxy-peptidase, and carboxyl esterase are common digestive enzymes of marine animals. All digestive enzymes of marine animals are classified based on the same criteria applied to other protein classifications (Haard and Simpson 2000). Based on the proteolytic nature of the digestive enzymes, proteases belong to two main classes: exopeptidases and endopeptidases or proteinases. Endopeptidases are responsible for the cleavage of polypeptide chains at peptide bonds of non-terminal amino acids, whereas exopeptidases hydrolyze one amino acid from the N-terminus (amino peptidases) or the C-terminus (carboxypeptidases) (Fig. 10.1) (Klomklao 2008). In addition, based on the nature of the catalytic site, proteases are classified into four main groups: acid or aspartate proteinases, serine proteinases, thiol or cysteine proteinases, and metalloproteinases.

3.1.1 Pepsin

Pepsin, belonging to the aspartic endopeptidase group, is one of three principal protein-degrading enzymes found in the fish stomach. The catalytic activity of pepsin greatly depends on the pH where pepsin is active and is most stable at acidic conditions. Based on the optimal pH, fish-derived pepsin is categorized into two groups, pepsin I and pepsin II. Several pepsin and pepsin-like enzymes have been isolated from fish, including cold- and warmwater fish. The temperature of the natural habitat of some of the fish was below 4 °C. Some of them were found at around 600–1,500 m depth. Hence, pepsin extracted from such fish species (such as *Pectoral rattail*) shows great catalytic power under high-pressure processing conditions at low temperature than enzymes extracted from warm-blooded animals (Klomklao et al. 2007). Despite pepsin being composed of a single polypeptide chain of 321 amino acids with a molecular weight of 35 kDa, pepsins derived from marine animals show molecular

weights ranging from 27 to 42 kDa. Pepsin is used for a variety of applications in the food manufacturing and pharmaceutical industries, making use of the advantage of the strong biochemical digestive action of pepsin. Commercially, pepsin is extracted from the glandular layer of hog stomachs. With the high demand for pepsin and high cost associated with hog pepsin, considerable attention has been directed in order to find substitutes. Many reports have showed that marine fish viscera-derived pepsin is a competitive option in the substitution of hog pepsin.

3.1.2 Trypsin and Chymotrypsin

Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) are members of the serine protease family of enzymes, which are commonly found in juice from the pancreas. Trypsin catalyzes the hydrolysis of peptide bonds, breaking down proteins into smaller peptides, which could be further degraded by other proteases. Chymotrypsin has the ability to break down both proteins and polypeptides, but its catalytic power is relatively low (Klomklao 2008). Trypsin and trypsin-like proteolytic enzymes have been purified and characterized from the viscera of several fish species. Even though trypsin from marine animal fish species resembles mammalian trypsin with respect to their physical characteristics, some reports have shown that marine trypsin is more stable under harsh conditions (high temperature and pH) and in the presence of surfactants or oxidizing agents. Generally, the optimum pH of marine-derived trypsin varies from 6 to 11.5, while their optimum temperature for hydrolysis ranges from 35 °C to 65 °C (Freitas-Júnior et al. 2012). Interestingly, Atlantic cod-derived trypsin III is better adapted to lower temperatures than any other known trypsin.

Trypsin and chymotrypsin have various commercial uses, especially as a dietary supplement in the food industry and in substitutive therapies for patients with pancreatic diseases. In addition, trypsin is commonly used in biomedical experiments as a cell detacher and has been successfully used in the detergent enzyme industry, as they are capable of removing protein stains like blood, eggs, dairy products, juices, juice, etc. It has been shown that these enzymes can maintain around 80 % of their catalytic activity even after 1 h of incubation with chemical detergent (Espósito et al. 2009). With this significant industrial importance, pure trypsin and chymotrypsin have gained high commercial demand. Cod-derived trypsin is already used in several industries like food and cosmeceuticals. Further, trypsin derived from Pacific and Atlantic cod has successfully been used as a catalyst in enzymatic peptide synthesis (Gudmundsdóttir and Pálsdóttir 2005). The pyloric caeca, intestine, and spleen of fish are popular sources for the extraction of marine trypsin. However, lipids in the tissues have created several difficulties in extracting, preparing, and purifying trypsin from fish leftover. A recent research has shown that supercritical carbon dioxide could be used as an effective defatting agent in the extraction of trypsin from fishery by-products and it is an effective alternative for common defatting agents such as chemical solvents (Chun et al. 2011). Even though less attention has been paid to marine-derived chymotrypsin, it has been revealed that fishery waste material is a promising source for the extraction of chymotrypsin. Further, many reports suggest that fishery leftovers are a competitive alternative for expensive fresh cattle or swine

pancreas-derived chymotrypsin. Zhou et al. 2011 have comprehensively reviewed the extraction, purification, and characterization of fish chymotrypsin and its commercial applications related to its unique physical properties.

3.1.3 Chymosins

Chymosin or rennin (EC 3.4.23.4) is generally produced by ruminant animals in the lining of the abomasum. It is responsible for the breaking down of peptide bonds between phenylalanine and methionine in casein. Therefore, this property of chymosin is used to coagulate milk. As the active enzyme found in rennet that is extensively used in the milk industry, chymosin has great commercial value. Generally, rennet is commercially extracted from slaughtered young claves or in recombinant *Escherichia coli* and *Aspergillus* spp. (Chitpinityol and Crabbe 1998). However, considerable attention has been focused on finding a suitable alternative for calf rennet with its high associated cost. Many reports have shown that some marine animals produce chymosin and chymosin-like enzymes. Chymosin has been extracted from carp and harp seal stomach (Shahidi and Janak Kamil 2001). Further, some recent research has pointed out that proteolytic and peptidase extracted from some crustaceans could be used for the production of cheese, and cheddar-type cheeses manufactured using the enzymes extracted from the crustacean *Munida* show improved physical and sensory qualities compared to cheese produced from traditional chymosin (Rossano et al. 2005, 2011).

3.1.4 Lipases

Lipase is an enzyme that catalyzes the hydrolysis of ester bonds in various substrates such as triglycerides (TGs), phospholipids, cholesteryl esters, and vitamin esters. Lipases are present in most living organisms and play a critical role in the digestion of lipids. Traditionally, lipases have been used for the preparation of milk products. Owing to the hydrolysis of milk fat, lipases contribute to flavor enhancement in cheese, cream, and many other milk products. Recently, lipases have been involved in many industrial processes, such as baking and laundry detergents manufacturing (Reetz 2002). The ability of lipase to remove lipid stains more efficiently than conventional cleaning chemicals has reduced the environment chemical load. Further, lipases have been successfully used as biocatalysts in various synthesis processes. Different kinds of surfactants/emulsifiers could be economically produced using lipases, particularly microbial lipases. The industrial values of lipases have been discussed and reviewed in many reports (Hasan et al. 2006).

Commercially, lipases are extracted from the pancreas and serous glands of ruminants, especially from young calves and pigs. With the many advantages of microbial lipases, the production of industrial lipases using numerous species of bacteria, yeasts, and molds has received increased attention (Hasan et al. 2006). In addition to that, oilseeds, oily fruits, and cereal grains are used for the extraction of plant lipases. During the past decade, aquatic animals also emerged as a prospective

source of lipases. In an investigation of different tissues which could be used as a source of lipases, Nayak et al. 2003 found that fish intestine and liver could be a potential source. It has been noticed that the quantity and catalytic activity of lipases vary with origin and fish species. Active fishes such as mackerel and scup lipase show relatively high activity, and fishes whose diets contain high amounts of phospholipids and wax esters show high amounts of lipase in extraction. Several recent studies have shown that sardine (Smichi et al. 2010), carp (Görgün and Akpınar 2012), gray mullet (Aryee et al. 2007), and salmon and hoki (Kurtovic et al. 2010) (Table 10.1) are good sources for the extraction of fish lipases. All of the isolated enzymes have shown unique physicochemical characteristics. Lipases extracted from salmon and hoki were stable in several water-immiscible solvents. Further, Rivera-Pérez et al. 2011 reported that some crustaceans could also be used to extract digestive lipases. Owing to the unique characteristics of fish-derived lipases, Kurtovic et al. 2011 revealed that fish lipases show similar activity to commercially available lipases in terms of fatty acid release and flavor and odor development in dairy cream. Further, the study demonstrated the potential for flavor enhancement in dairy products using fish lipases. Another interesting application of lipases is the production of ω-3 polyunsaturated fatty acids (PUFAs) concentrate using marine oils (Kahveci et al. 2010). Due to the high substrate specificity of lipases, commercially available lipases are not suitable for the production of ω-3 concentrates using long-chain PUFAs. However, it has been found that the fish lipase-immobilized system works well in concentrating PUFAs with long-chain PUFAs. In addition to the fish digestive lipases, fish tissue localized lipases have also been isolated. Lipases in red muscle, adipose tissue, and liver have been identified as potential tissues for the extraction of tissue lipases (Nayak et al. 2003; Kurtovic et al. 2009).

3.2 Collagenase from Fish

Collagen is a group of naturally occurring proteins that is commonly found in the connective tissues of animals. Usually, collagen is found in a triple-helix structure that often consists of glycine, proline, and hydroxyproline in a sequenced manner. Inter- and intramolecular cross-links formed by hydrogen bonds stabilize the unique structure of collagen. Among the different collagens, types I and III are the most abundant. Interestingly, only very few enzymes are capable of breaking down collagens. Few kinds of collagenolytic enzymes could be found in animals. Elastase is the well-studied collagenolytic enzyme that belongs to the serine protease enzyme. It is responsible for the breakdown of the connective tissue protein elastin. Cathepsin K is the most potent mammalian collagenase and this enzyme breaks down collagen type I in an acidic medium. All the other collagenolytic enzymes are classified under the group of metalloproteinases. These types of collagenase can act in neutral pH conditions and characteristically differ from the serine protease family of collagenases due to differences in their catalytic sites. Generally, metalloproteinases hydrolyze types I, II, and III tropocollagens (Daboor et al. 2010).

Collagenases have been identified from both vertebrates and microbial sources. Bacterial collagenases differ from vertebrate collagenases in their substrate specificity and catalytic power. It has been found that microbial collagenase can break down almost all collage types. Traditionally, collagenases are prepared from *Clostridium histolyticum* culture supernatants, but several other animal sources have also been identified as sources of extractable collagenases. Collagenase has considerable commercial value as a proteolytic enzyme. Different kinds of collagen have been used in the tannery and fur industries. In the processing of squid, collagenases are used as skinning agents. Collagenase is often used as a stabilizer and clarifier in the food industry. Further, collagen is used as medicine and as a chemical agent in biomedical research (Daboor et al. 2012).

The isolation and characterization of collagenases from fish sources has been an interesting topic, and various types of collagenases have been identified from marine animals. Marine as well as freshwater fish species such as carp, catfish, Atlantic cod, and rayfish have been studied for extractable collagenases (Shahidi and Janak Kamil 2001). Recently, interest has re-emerged with the high commercial demand for proteolytic enzymes and increasing attention for the effective utilization of fishery leftovers. Murado et al. 2009 revealed that overcooled acetone precipitation is a simple and sustainable method to partially purify collagenase from rayfish viscera. A decade ago, the same technique was applied by Park and his research team to isolate collagenases from mackerel, *Scomber japonicus*. Kim et al. 2002 showed that ammonium precipitation followed by chromatographic purification could also be used for the isolation of collagenase from fish waste. Recently, collagenases belonging to the group metalloproteinases have been isolated from a mixture of fish waste (haddock, herring, groundfish, and flounder) using a similar procedure (Daboor et al. 2012). Not only digestive collagenases but also tissue collagenases have been isolated from marine fish waste. Collagenase, particularly cathepsin B, present in fish tissue is mainly responsible for the quality deterioration of fish. It hints that fish muscle waste, which has high vulnerability to degrading quickly, could be a promising source for the extraction of collagenases (Sovik and Rustad 2006). Cathepsin L, which has optimal pH and temperature at 5.5 °C and 55 °C, respectively, has been purified from the skeletal muscle of blue scad (*Decapterus maruadsi*) (Zhong et al. 2012). Moreover, gelatinolytic proteinases, which have potent ability to hydrolyze native type I collagen even at 4 °C, have been isolated from common carp (Wu et al. 2008). All these findings suggest that fish processing waste, internal organs, digestive tissues, and cutoffs are potential sources for the extraction of commercially valuable collagenases.

3.3 Chitinase

Chitinases are hydrolytic enzymes that have the ability to break down glycosidic bonds in chitin polymer. However, some lysozymes also possess chitinolytic activity. True chitinases are categorized into chitinase and chitobiase based on the end

product of the enzymes. These types of enzymes are generally found in organisms that either need to reshape their own chitin or digest chitinous substances ingested as food. Especially, animals that ingest worms and arthropods as their main food use chitinases for the digestion of their food. Many microbial species are also capable of producing chitinases and, hence, they act as pathogenic organisms of arthropods. Several kinds of chitinases have been isolated and characterized from the digestive systems or related organs of marine fish (Gutowska et al. 2004; Ikeda et al. 2009). However, the origin of the fish chitinases has become questionable with some research findings. Some findings have suggested that gastrointestinal chitinases of marine fish are from gut microflora. Many recent reports have shown that marine fish species are capable of producing chitinases, and the production of chitinases varies with depth distribution, origin, and the fish species. Chitinase extracted from the stomach of deeper living fish has shown greater activity than intestinal chitinases. Fish species that consume more crustaceans as food produce high amounts of chitinase. In cobia, a highly prized food fish, crustaceans account for 78 % of the stomach contents and over 34 % of carbohydrates are utilized for nutritional requirements. Fines and Holt 2010 have found that this fish species produces high amounts of both chitinase and chitobiase. Further, their findings suggested that fish use both chitinase and chitobiase to digest and absorb nutrients from chitin food. Using chitinase and chitobiase, chitin food is degraded to soluble and absorbable N-acetyl-glucosamine (Molinari et al. 2007). With the interesting findings on the biological activities of chitin and chitosan oligomers, chitinolytic enzymes have gained great attention, and fish chitinase could be used for the production of a range of chitin-derived health products. Further, due to the inconsistent physicochemical properties of chitin products produced with acid or alkyl hydrolysis, cheap chitinolytic enzymes with improved catalytic power are continuously in demand. In this regard, marine fishery waste is a potential source for extracting chitinases with high catalytic power.

4 Applications of Fish Enzymes

Enzymes have become an indispensable component of food, pharmaceutical, and cosmeceutical production processes with their great catalytic power. In addition, some enzymes have healing properties or can be used as therapeutic agents in disease conditions in which the endogenous enzyme activity is reduced. Therefore, enzymes have great commercial value. Table 10.2 summarizes some of the potential applications of fish waste-derived enzymes. Among the different applications of enzymes, fish-derived enzymes could be used as a processing aid for many seafood products. With the high availability of fish waste in their own processing plants, these enzymes could be effectively used at a lower cost compared to other applications. Enzymatic pealing is a popular application of enzymes in the food as well as cosmetic industries. Some enzymes are capable of dissolving only the outer layer of muscles, without damaging the original muscle tissue, taking advantage of the biochemical differences between the skin and muscle cells. This type of enzyme is

Table 10.2 Potential application of fish-derived enzymes

Enzyme	Area of application	Application
Pepsin	Pharmaceutical, cosmeceuticals	Collagen extraction, therapeutic agents, fish hydrolysate, and bioactive peptide production
	Dairy	Cheese production
	Fishery	Fish silage, caviar production, descaling of fish
	Leather	To remove hair and residual tissue
Trypsin	Food, fishery	Production of seafood flavorant, extraction of carotenoprotein, fish sauce
	Dairy	Cheese production
	Paint	Production of pearl essence
	Pharmaceutical	Therapeutic agents, wound healing, veterinary medicine
Chymotrypsin	Food processing	Meat tenderizing, improving food nutritional value, protein hydrolysate production
	Fishery	Bone protein removal
	Dairy	Cheese production
	Leather	Dehairing, bating, and soaking
	Detergent	Decontaminating agent, cleaning agent
Collagenase	Fishery	Squid peeling and softening, surimi processing, fish ripening, production of protein hydrolysates
	Food processing	Stabilizer and clarifier
Lipase	Detergent	As a cleaning agent
	Food	Flavor enhancer of cream
Chitinase	Pharmaceutical, nutraceutical	Production of chitin and chitosan oligomers, glucosamine production

applied in the formulation of skin-peeling agents in the cosmetics industry. Moreover, peeling enzymes are commonly used in the removal of undesirable parts from many economically valuable marine fish species, such as tuna, skate, filefish, squid, and many other shellfish. In addition to that, the descaling of fish skin and purification of fish roe are other potential applications for these enzymes (Kandasamy et al. 2012). Enzymes derived from fish and shellfish have been applied for several production processes of the dairy industry. Particularly, fish digestive enzymes could be used as a cheap and alternative source for cheese production. In addition to this, many industrial applications of fish-derived enzymes have been reported (Shahidi and Janak Kamil 2001). The ability of the fish enzymes to work at low temperature and high pressure are the main characteristic features that have brought about their great potential to be used as a competitive alternative for traditional enzymes.

5 Concluding Remarks

In addition to the above-mentioned enzymes that have been isolated and identified from fish processing waste, many more novel enzymes with significant catalytic power have been reported. Due to the high availability of fish processing waste and

the high industrial value for enzymes, the utilization of fish processing waste would be an ideal option to overcome the drawbacks associated with traditional fish processing by-products. Moreover, this could be a cheap way to produce industrially important enzymes. But to make this dream come true, there are many hurdles that need to be overcome. Even though different kinds of fish enzymes have been isolated from processing waste, their physicochemical properties such as pH, thermal stability, enzyme turnover, substrate specificity, inhibition of activity, etc. must be finalized. Further, the optimization of extraction and purification processes of the enzymes is vital in order to run this business as a profitable one. On the other hand, extensive research should be conducted to optimize the use of these enzymes in different applications to make the use of fish processing waste-derived enzymes an economically viable approach, because conventional processing parameters cannot be applied to the habitat-related characteristics of fish enzymes.

References

Aryee ANA, Simpson BK, Villalonga R (2007) Lipase fraction from the viscera of grey mullet (*Mugil cephalus*): isolation, partial purification and some biochemical characteristics. Enzyme Microb Technol 40(3):394–402

Bougatef A, Balti R, Zaied SB, Souissi N, Nasri M (2008) Pepsinogen and pepsin from the stomach of smooth hound (*Mustelus mustelus*): purification, characterization and amino acid terminal sequences. Food Chem 107(2):777–784

Chitpinityol S, Crabbe MJC (1998) Chymosin and aspartic proteinases. Food Chem 61(4): 395–418

Chun B-S, Kishimura H, Nalinanon S, Klomklao S, Benjakul S (2011) Mackerel trypsin purified from defatted viscera by supercritical carbon dioxide. J Amino Acids 2011:1–7

Daboor SM, Budge SM, Ghaly AE, Brooks SL, Dave D (2010) Extraction and purification of collagenase enzymes: A critical review. Am J Biochem Biotechno 6:239–263

Daboor SM, Budge SM, Ghaly AE, Brooks MS, Dave D (2012) Isolation and activation of collagenase from fish processing waste. Adv Biosci Biotechnol 3(3):191–203

Espósito TS, Amaral IP, Buarque DS, Oliveira GB, Carvalho LB Jr, Bezerra RS (2009) Fish processing waste as a source of alkaline proteases for laundry detergent. Food Chem 112(1):125–130

Fines BC, Holt GJ (2010) Chitinase and apparent digestibility of chitin in the digestive tract of juvenile cobia, *Rachycentron canadum*. Aquaculture 303(1–4):34–39

Freitas-Júnior ACV, Costa HMS, Icimoto MY, Hirata IY, Marcondes M, Carvalho LB Jr, Oliveira V, Bezerra RS (2012) Giant Amazonian fish pirarucu (*Arapaima gigas*): its viscera as a source of thermostable trypsin. Food Chem 133(4):1596–1602

Görgün S, Akpınar MA (2012) Purification and characterization of lipase from the liver of carp, *Cyprinus carpio* L. (1758), living in Lake Tödürge (Sivas, Türkiye). Turk J Fish Aquat Sci 12:207–215

Gudmundsdóttir Á, Pálsdóttir HM (2005) Atlantic cod trypsins: from basic research to practical applications. Mar Biotechnol 7(2):77–88

Gutfreund H (1976) Wilhelm Friedrich Kühne; an appreciation. FEBS Lett 62(Supplement):E1–E2

Gutowska MA, Drazen JC, Robison BH (2004) Digestive chitinolytic activity in marine fishes of Monterey Bay, California. Comp Biochem Physiol A 139(3):351–358

Haard NF, Simpson BK (2000) Seafood enzymes: utilization and influence on postharvest seafood quality, vol 97. CRC Press, Boca Raton

Hasan F, Shah AA, Hameed A (2006) Industrial applications of microbial lipases. Enzyme Microb Technol 39(2):235–251

Ikeda M, Miyauchi K, Mochizuki A, Matsumiya M (2009) Purification and characterization of chitinase from the stomach of silver croaker *Pennahia argentatus*. Protein Express Purif 65(2):214–222

Islam MA, Absar N, Bhuiyan AS (2008) Isolation, purification and characterization of lipase from grey mullet (*Liza parsia* Hamilton, 1822). Asian J Biochem 3:243–255

Kahveci D, Falkeborg M, Gregersen S, Xu X (2010) Upgrading of farmed salmon oil through lipase-catalyzed hydrolysis. Open Biotechnol J 4:47–55

Kandasamy N, Velmurugan P, Sundarvel A, Jonnalagadda Raghava R, Bangaru C, Palanisamy T (2012) Eco-benign enzymatic dehairing of goatskins utilizing a protease from a *Pseudomonas fluorescens* species isolated from fish visceral waste. J Clean Prod 25:27–33

Kim S-K, Park P-J, Kim J-B, Shahidi F (2002) Purification and characterization of a collagenolytic protease from the filefish, *Novoden modestrus*. J Biochem Mol Biol 35(2):165–171

Kirk O, Borchert TV, Fuglsang CC (2002) Industrial enzyme applications. Curr Opin Biotechnol 13(4):345–351

Klomklao S (2008) Digestive proteinases from marine organisms and their applications. Songklanakarin J Sci Technol 30:37–46

Klomklao S, Benjakul S, Visessanguan W, Kishimura H, Simpson BK (2006a) Purification and characterization of trypsin from the spleen of tongol tuna (*Thunnus tonggol*). J Agr Food Chem 54(15):5617–5622

Klomklao S, Benjakul S, Visessanguan W, Kishimura H, Simpson BK, Saeki H (2006b) Trypsins from yellowfin tuna (*Thunnus albacores*) spleen: purification and characterization. Comp Biochem Physiol B 144(1):47–56

Klomklao S, Kishimura H, Yabe M, Benjakul S (2007) Purification and characterization of two pepsins from the stomach of pectoral rattail (*Coryphaenoides pectoralis*). Comp Biochem Physiol B 147(4):682–689

Kurtovic I, Marshall SN, Zhao X, Simpson BK (2009) Lipases from mammals and fishes. Rev Fish Sci 17(1):18–40

Kurtovic I, Marshall SN, Zhao X, Simpson BK (2010) Purification and properties of digestive lipases from Chinook salmon (*Oncorhynchus tshawytscha*) and New Zealand hoki (*Macruronus novaezelandiae*). Fish Physiol Biochem 36(4):1041–1060

Kurtovic I, Marshall SN, Miller MR, Zhao X (2011) Flavour development in dairy cream using fish digestive lipases from Chinook salmon (*Oncorhynchus tshawytscha*) and New Zealand hoki (*Macruronus novaezealandiae*). Food Chem 127(4):1562–1568

Lee HS, Kwon KK, Kang SG, Cha S-S, Kim S-J, Lee J-H (2010) Approaches for novel enzyme discovery from marine environments. Curr Opin Biotechnol 21(3):353–357

Molinari LM, Pedroso RB, Scoaris DO, Ueda-Nakamura T, Nakamura CV, Dias Filho BP (2007) Identification and partial characterisation of a chitinase from Nile tilapia, *Oreochromis niloticus*. Comp Biochem Physiol B 146(1):81–87

Murado MA, González MP, Vázquez JA (2009) Recovery of proteolytic and collagenolytic activities from viscera by-products of rayfish (*Raja clavata*). Mar Drugs 7(4):803–815

Nalinanon S, Benjakul S, Kishimura H (2010) Biochemical properties of pepsinogen and pepsin from the stomach of albacore tuna (*Thunnus alalunga*). Food Chem 121(1):49–55

Nayak J, Viswanathan Nair PG, Ammu K, Mathew S (2003) Lipase activity in different tissues of four species of fish: rohu (*Labeo rohita* Hamilton), oil sardine (*Sardinella longiceps* Linnaeus), mullet (*Liza subviridis* Valenciennes) and Indian mackerel (*Rastrelliger kanagurta* Cuvier). J Sci Food Agr 83(11):1139–1142

Olempska-Beer ZS, Merker RI, Ditto MD, DiNovi MJ (2006) Food-processing enzymes from recombinant microorganisms—a review. Regul Toxicol Pharmacol 45(2):144–158

Park P-J, Lee S-H, Byun H-G, Kim S-H, Kim S-K (2002) Purification and characterization of a collagenase from the mackerel, *Scomber japonicus*. J Biochem Mol Biol 35(6):576–582

Reetz MT (2002) Lipases as practical biocatalysts. Curr Opin Chem Biol 6(2):145–150

Rivera-Pérez C, García-Carreño FL, Saborowski R (2011) Purification and biochemical characterization of digestive lipase in whiteleg shrimp. Mar Biotechnol 13(2):284–295

Rossano R, Piraino P, D'Ambrosio A, O'Connell OF, Ungaro N, McSweeney PLH, Riccio P (2005) Proteolysis in miniature cheddar-type cheeses manufactured using extracts from the crustacean *Munida* as coagulant. J Biotechnol 120(2):220–227

Rossano R, Larocca M, Lamaina A, Viggiani S, Riccio P (2011) The hepatopancreas enzymes of the crustaceans *Munida* and their potential application in cheese biotechnology. LWT Food Sci Technol 44(1):173–180

Sarkar S, Pramanik A, Mitra A, Mukherjee J (2010) Bioprocessing data for the production of marine enzymes. Mar Drugs 8(4):1323–1372

Shahidi F, Janak Kamil YVA (2001) Enzymes from fish and aquatic invertebrates and their application in the food industry. Trends Food Sci Technol 12(12):435–464

Smichi N, Fendri A, Chaâbouni R, Rebah FB, Gargouri Y, Miled N (2010) Purification and biochemical characterization of an acid-stable lipase from the pyloric caeca of sardine (*Sardinella aurita*). Appl Biochem Biotechnol 162(5):1483–1496

Sovik SL, Rustad T (2006) Effect of season and fishing ground on the activity of cathepsin B and collagenase in by-products from cod species. LWT Food Sci Technol 39(1):43–53

Trincone A (2011) Marine biocatalysts: enzymatic features and applications. Mar Drugs 9(4):478–499

Wu J-L, Lu B-J, Du M-H, Liu G-M, Hara K-J, Su W-J, Cao M-J (2008) Purification and characterization of gelatinase-like proteinases from the dark muscle of common carp (*Cyprinus carpio*). J Agr Food Chem 56(6):2216–2222

Wu T, Sun L-C, Du C-H, Cai Q-F, Zhang Q-B, Su W-J, Cao M-J (2009) Identification of pepsinogens and pepsins from the stomach of European eel (*Anguilla anguilla*). Food Chem 115(1):137–142

Zhang C, Kim S-K (2010) Research and application of marine microbial enzymes: status and prospects. Mar Drugs 8(6):1920–1934

Zhong C, Cai Q-F, Liu G-M, Sun L-C, Hara K, Su W-J, Cao M-J (2012) Purification and characterisation of cathepsin L from the skeletal muscle of blue scad (*Decapterus maruadsi*) and comparison of its role with myofibril-bound serine proteinase in the degradation of myofibrillar proteins. Food Chem 133(4):1560–1568

Zhou Q, Fu X-P, Zhang L-J, Su W-J, Cao M-J (2007) Purification and characterization of sea bream (*Sparus latus* Houttuyn) pepsinogens and pepsins. Food Chem 103(3):795–801

Zhou L, Budge SM, Ghaly AE, Brooks MS, Dave D (2011) Extraction, purification and characterization of fish chymotrypsin: a review. Am J Biochem Biotechnol 7:104–123

Chapter 11
Functional Proteins and Peptides from Fish Skin

S.W.A. Himaya and Se-Kwon Kim

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1 Introduction

One-third of fish harvest is discarded annually as processing by-products. Fish is processed into fillets, canned, and surimi, where it results in an immense quantity of by-products, which include trimmings, belly flaps, heads, frames, fins, skin, and viscera. However, the nutrient contents in these by-products are not lower compared to the consumed fish parts. Moreover, discarding these by-products would largely contribute to environmental pollution. Therefore, the need to develop efficient by-product recovery systems has both economic and environmental benefits (Kim and Mendis 2006; Wilson et al. 2011). The amount of fish by-products discarded as

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waste is enormous enough to generate raw materials for the development of other large-scale processing units. The utilization of by-products is both environmentally and commercially favorable practice. Among fish processing by-products, fish skin accounts for around 30 % (Jamilah et al. 2011). Fish skin is largely processed to produce proteins and fish oil.

As a main macronutrient in foods, protein provides essential amino acids to fulfill basic nutritional requirements. For a long time, proteins have been rated and categorized based on their composition and biological value. Nevertheless, certain proteins exert physiological significance influencing human well-being, mainly in a beneficial way, beyond their nutritional values. Further, specific degradation of basic proteins results in biologically active protein hydrolysates and peptides, which possess a broad spectrum of health-promoting abilities. Since seafood is considered a worldwide prime source of proteins with high biological value, fish processing waste is an ideal and cheap source for the extraction tremendously valuable ingredients, especially fish skin generated from commercial fish filleting, which is nutritionally valuable and easily digestible, with a well-balanced amino acid composition (Harnedy and FitzGerald 2012). Collagen and gelatin are the main proteins extracted from fish skin and, apart from their uses as gelling agents, functional properties of fish skin-derived proteins have been discovered. This chapter focuses on the functional materials isolated from fish skin and their effect on human well-being.

2 Fish Collagen and Gelatin

Collagen, the most abundant protein of animal origin, has earned much interest as a biomaterial in medical applications due to its biodegradability and weak antigenicity. Type I, out of the 28 types of collagen, is the most naturally abundant collagen in animals and is found in skin, tendons, vascular ligature, organs, and bone. Collagen is composed of three similarly sized triple-helix polypeptide chains, which consist of around 1,000 amino acids residues. Gelatin is a structurally different form of the same macromolecules that make up collagen and, particularly, a hydrolyzed form of collagen. Common sources of collagen and gelatin include bovine hide, pig skin, or chicken waste; they have, however, recently faced several constraints related to biological contaminants and religious issues (Aberoumand 2010). This is the main reason why an alternative source for collagen and gelatins for commercial purposes is sought. Raw materials from fish waste have received considerable attention in recent years as an alternative for collagen and gelatin extraction, due to its unique features. Fish skin and bones mainly used for collagen extraction and three main extraction methods, neutral salt solubilization, acid solubilization, and enzyme solubilization, are used based on the characteristics of the waste and end products. During the extraction of collagen, triple-helix structure, which contributes its unique properties, has to be secured. Generally, extracted collagen is solubilized with hot water treatment by breaking down the hydrogen and covalent bonds of the triple-helix, resulting in helix-to-coil transition and conversion into soluble gelatin (Gómez-Guillén et al. 2011).

Table 11.1 Biomedical potentials of fish collagen and gelatin

Fish source	Waste material	By-product	Biochemical potentials	Reference
Rohu and *Catla catla*	Scale	Type I collagen	High thermal stability	Pati et al. (2010)
Nile tilapia	Skin	Collagen drink	Attenuate UVA damage	Kato et al. (2011)
Nile tilapia	Scale	Peptide	Free radical scavenging	Ngo et al. (2010)
Alaska pollock	Skin	Peptide	ACE inhibition	Byun and Kim (2001)
Pacific cod	Skin	Peptide	Antioxidant and ACE inhibition	Ngo et al. (2011)
Unicorn leatherjacket	Skin	Gelatin film	Antimicrobial	Ahmad et al. (2012)
Chum salmon	Skin	Peptide	Memory enhancement	Pei et al. (2010)

2.1 Biomedical Applications of Collagen and Gelatin

Several unique applications of collagen and gelatin derived from fish by-products have been reported, due to the enriched properties of fish collagen (Table 11.1). The high hydroxyproline content of collagen plays a role in reducing pain in osteoarthritis patients supplemented with collagen/gelatin hydrolysate (Moskowitz 2000). Moreover, collagen also shows great advantages as a carrier molecule of drugs, proteins, and genes through long-term maintenance of the concentration and controlled release at target sites. Further, collagen serves as a main scaffold for biotechnological applications. Detailed studies revealed that collagen type I, with selective removal of its telopeptides, exhibit characteristic features of bioscaffolds for bone regeneration. The experimental data confirm that collagen and gelatin derived from fish by-products have inherent properties of collagen and can be used as an alternative to mammalian products (Senaratne et al. 2006). Collagen is mainly extracted from the skin of cod, haddock, and salmon. Moreover, tilapia fish scales are also being used as a source of low molecular weight collagen (around 1,000 KDa) (Lee et al. 2011). In addition, the enzymatic hydrolysis of fish collagen and gelatin produces small peptides, which have potent biological activities. The repeated Gly-Pro-Ala sequence of gelatin peptides has reinforced the peptide with high antioxidant and antihypertensive properties. Numerous studies have been conducted to reveal the biological activity of peptides derived from fish collagen and gelatin.

3 Fish Protein Hydrolysates

After removal of the valuable portion, the remaining fish skin and muscles are solubilized by means of several chemical and physical methods to obtain hydrolyzed fish proteins. Although fish protein hydrolysate (FPH) has classically been used for agricultural purposes, advanced technological developments have made it possible to apply these FPHs as functional ingredients in food and pharmaceuticals. Likewise, the hydrolysate is also a rich source of biologically active small peptides that have

been proved for various therapeutic potentials. The hydrolysis of protein is achieved through acids, alkalis, or enzymes mediated breakdown of parent proteins in the waste into smaller protein fractions, peptides, and free amino acids. Acid hydrolysis makes the product unpalatable due to tryptophan destruction and the formation of sodium chloride after the neutralization. Alkaline hydrolysis produces several toxic compounds which are undesirable for human consumption. Among protein hydrolyzing methods, enzymatic hydrolysis offers several advantages over others (Kristinsson and Rasco 2000).

Proteolytic enzymes come from several sources, such as plants (papain, ficin, bromelain), animals (trypsin, pancreatic enzymes), or microbials (pronase, alcalase), and are employed for hydrolysis, depending on the type of processing waste and the desired functionality of the end product. In the conventional enzymatic method, commercial enzymes are directly applied under predefined conditions such as pH, temperature, incubation time, and enzyme/substrate ratio (Bhaskar et al. 2008). The fermentative approach for producing fish protein hydrolysates is not a novel concept. Proteases producing microbial strains are used as starter culture and incubated in preferred conditions to grow microbes on fish processing waste. Physicochemical as well as functional properties of enzymatic hydrolysate vary with the degree of hydrolysis, which determines the size of protein fractions. Overhydrolysis may impair some functional properties of food proteins or cause the development of off-flavors in hydrolysates (Balti et al. 2010). Further, improvements in the functional properties and therapeutic value of protein hydrolysates have been obtained through a selective molecular weight cutoff. An ultrafiltration membrane system equipped with a molecular cutoff has been identified as an effective method to purify protein hydrolysates based on the molecular weight of protein factions (Jeon et al. 1999). Serial enzymatic digestions in a system consisting of a multistep recycling membrane reactor combined with an ultrafiltration membrane system have been developed to produce protein hydrolysates with the desired molecular weights while preserving expensive photolytic enzymes (Byun and Kim 2001).

3.1 Biological Properties of Fish Protein Hydrolysates

The main goal of the preparation of FPHs, i.e., improving the functional properties of fish proteins, is accompanied with advanced health-promoting abilities due to the improvement in functionality achieved through the high amount of polar groups and the solubility of hydrolysate and bioavailability. Thus, dozens of studies have been carried out to prove various kinds of biomedical applications of FPHs (Table 11.2). Particularly, FPHs possess potent antioxidant activity, which attenuate oxidative damages that take place in the body where the endogenous antioxidant defense mechanism is not good enough. As an economically viable product, FPHs seem to be good candidates to combat the production of superoxide anion (O^{2-}) and hydroxyl ($OH^{1-)}$) radicals, which are considered as causative agents for the initiation of chronic diseases such as heart disease, stroke, arteriosclerosis, diabetes, and cancer (Perera and Bardeesy 2011). The highlighted advantage of the product is in

Table 11.2 Biomedical potentials of fish protein hydrolysates

Fish source	Method of hydrolysis	Bioactivity	Reference
Blue whiting	pH shift extraction	Antiproliferative	Picot et al. (2006)
Pacific hake	Autolysis	Antioxidant	Samaranayaka and Chan (2008)
Round scad	Flavourzyme hydrolysis	Antioxidant	Thiansilakul et al. (2007)
Brownstripe red snapper	Pyloric caeca proteases	Antioxidant	Khantaphant et al. (2011)
Ornate threadfin bream	Pepsin hydrolysis	Antioxidant	Nalinanon et al. (2011)
Cod	Enzyme hydrolysis	ACE inhibitory	Jeon et al. (1999)
Yellowfin sole	Enzyme hydrolysis	Anticoagulant	Rajapakse et al. (2005)
Blue whiting	Enzyme hydrolysis	Satiating effect	Cudennec et al. (2012)

providing protection against oxidative damage while providing additional nutritional value. Additionally, protein hydrolysates generated from fish processing waste reduce the risk of cardiovascular diseases (CVD) by triggering several key associated processes, including blood clot and platelet formation, angiotensin 1-converting enzyme activity, and cholesterol metabolism. Moreover, a recent study (Cudennec et al. 2012) has demonstrated that FPHs derived from blue whiting suppress appetite via the enhancement of cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) secretion in in vitro STC-1 cells as well as in vivo experiments. The biological effects of CCK and GLP-1 stimulation lead to promising effects on body weight reduction, which has gained much attention in developed countries. All these findings clearly indicate that FPHs have potential for use in disease risk reduction. Thus, the identified potential sources are currently used in the production of biologically active FPHs in the form of nutraceutical and functional food.

4 Bioactive Peptide Isolation from Fish Skin

In an attempt to demonstrate the biological consequence of FPHs and gelatin hydrolysates, it was found that small peptides present in the hydrolysates mediate biochemical pathways to exert defined health properties of the hydrolysates. Detailed studies revealed that small protein fragments show this potent activity. Most of the bioactive peptides are 2–20 amino acids long. The amino acid composition of the sequence plays a critical role in its bioactivity. At present, there is an increasing interest in the utilization of food-derived biologically active peptides as nutraceuticals or nutritional supplements. Bioactive peptides exert physiological hormone-like effects on humans beyond their nutritional value (Himaya et al. 2012a, b; Erdmann et al. 2008). Generally, the bioactive peptides remain latent within the parent protein molecule and are released by hydrolysis.

It is popularly known that the protein hydrolysis method and types of proteinase employed are crucial factors affecting the biological activity of the peptide. The ultrafiltration membrane system has been identified as a useful tool to purify active peptides based on molecular weight. Sequential enzymatic digestions with several different enzymes are employed to achieve the desired functionality of

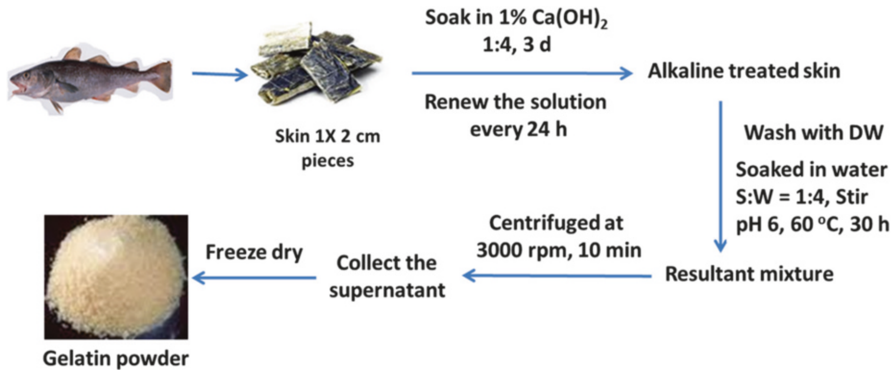

Fig. 11.1 Process of extracting gelatin from fish skin for hydrolysis on the laboratory scale. The resulting gelatin could be hydrolyzed with enzyme, chemical, or physical means using optimal conditions depending on the method of hydrolysis

peptides (Kim et al. 2007). The biological activities of peptides derived from fish skin range from simple antioxidant activity to the prevention and cure of serious chronic diseases.

Several studies have reported that peptides derived from fish skin showed antihypertensive activity inhibiting the activity of angiotensin I-converting enzyme (ACE), which plays a vital role in the regulation of blood pressure. ACE participates in the body's renin–angiotensin system by converting inactive angiotensin I into an active vasopressor angiotensin II. This conversion increases blood pressure, triggering the function of the blood vessel dilator bradykinin (Huang et al. 2005). After the discovery of the critical role of ACE in blood pressure regulation, several commercial ACE inhibitors, such as captopril, enalapril, alacepril, and lisinopril, were synthesized and employed to treat hypertension and heart failures. Among the natural ACE inhibitors that have been isolated from various food and natural sources, ACE inhibitors derived from fish processing by-products is of great interest due to the preferred sequence of peptides for the potent inhibition of ACE (Lee et al. 2010). In addition, several peptides which cause a significant impact on the causative agents of chronic disease have been isolated.

Gelatin isolated from fish skin has been used as a common source of biologically active peptides. The scheme of gelatin extraction and hydrolysis for peptide production is shown in Fig. 11.1. Most of the peptides derive from fish skin gelatin act as effective antioxidant and antihypertensive agents in both in vitro and in vivo models (Table 11.3). The biological activities of these peptides are attributed to their unique amino acid composition.

The factor determining the activity of a peptide is the type of amino acids present in its sequence. It has been found that the binding of inhibitory peptide to the ACE is strongly supported by the presence of hydrophobic amino acids at the carboxy terminal and branched-chain aliphatic amino acids at the amino terminal (Cushman and Cheung 1971; Qian et al. 2007). Furthermore, it has been suggested that proline, lysine, or arginine are the preferred amino acids at the C-terminal for increased ACE

Table 11.3 Biologically active peptides isolated from fish skin gelatin hydrolysates

Source	Peptide sequence	Activity	Reference
Alaska pollock skin gelatin	Gly-Pro-Leu	ACE inhibitory activity	Byun and Kim (2001)
Alaska pollock skin gelatin	Gly-Pro-Hyp	Antioxidant activity	Kim et al. (2001)
Jumbo squid skin gelatin	Phe-Asp-Ser-Gly-Pro-Ala-Gly-Val-Leu	Antioxidant activity	Mendis et al. (2005)
	Asn-Gly-Pro-Leu-Gln-Ala-Gly-Gln-Pro-Gly-Glu-Arg		
Hoki skin gelatin	His-Gly-Pro-Leu-Gly-Pro-Leu	Antioxidant activity	Mendis et al. (2005)
Japanese flounder skin gelatin	Gly-Gly-Phe-Asp-Met-Gly	Antioxidant activity	Himaya et al. (2012b)
Pacific cod skin gelatin	Thr-Cys-Ser-Pro	Antioxidant and ACE inhibitory activity	Ngo et al. (2011)
	Thr-Gly-Gly-Gly-Asn-Val		
Pacific cod skin gelatin	Leu-Leu-Met-Leu-Asp-Asn-Asp-Leu-Pro-Pro	Antioxidant and ACE inhibitory activity	Himaya et al. (2012a)

inhibitory potency of the peptide (Meisel 1997). For a peptide to have potent antioxidant activity, the molecular weight should be around 500–1,500 Da. Peptides with molecular weights in this range are more potent antioxidants compared to peptides with molecular weights above 1,500 Da and below 500 Da (Li et al. 2008). Increased hydrophobicity of the peptide enhances its antioxidant activity, as it allows the peptide to reach hydrophobic targets, such as cell membranes (Hsu 2010). Furthermore, the positioning of Leu at the N-terminal of the sequence is thought to be important for its antioxidant activity (Ranathunga et al. 2006).

5 Concluding Remarks

Commercial fish processing generates ample amounts of waste materials annually, which demands an effective disposal method to minimize environmental and economic concerns. Thus, the utilization of fisheries waste materials for the development of by-products is an effective method for the sustainable use of aquatic resources while minimizing environmental issues. Although various kinds of fish processing by-products have been developed, their commercial value is comparatively low and, thus, discourage the use of waste materials. The identification of biologically active materials and their potential application in growing fields such as biomedical, nutraceutical, and functional food has brought new insight to fish processing by-products. Thus, comprehensive studies on identified potential ingredients from fish skin, especially collagen and gelatin, to develop commercially viable materials would facilitate a successful use of fish processing by-products in the biomedical field.

References

Aberoumand A (2010) Isolation and characteristics of collagen from fish waste material. World J Fish Mar Sci 2:471–474

Ahmad M, Benjakul S, Prodpran T, Agustini TW (2012) Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocol 28:189–199

Balti R, Bougatef A, Ali NE-H, Zekri D, Barkia A, Nasri M (2010) Influence of degree of hydrolysis on functional properties and angiotensin I-converting enzyme-inhibitory activity of protein hydrolysates from cuttlefish (*Sepia officinalis*) by-products. J Sci Food Agr 90:2006–2014

Bhaskar N, Benila T, Radha C, Lalitha RG (2008) Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (*Catla catla*) for preparing protein hydrolysate using a commercial protease. Bioresour Technol 99:335–343

Byun H-G, Kim S-K (2001) Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (*Theragra chalcogramma*) skin. Process Biochem 36:1155–1162

Cudennec B, Fouchereau-Peron MF, Ferry F, Duclos E, Ravallec R (2012) In vitro and in vivo evidence for a satiating effect of fish protein hydrolysate obtained from blue whiting (*Micromesistius poutassou*) muscle. J Funct Foods 4:271–277

Cushman DW, Cheung HS (1971) Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 20:1637–1648

Erdmann K, Cheung BWY, Schröder H (2008) The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutr Biochem 19:643–654

Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocol 25:1813–1827

Harnedy PA, FitzGerald RJ (2012) Bioactive peptides from marine processing waste and shellfish: a review. J Funct Food 4:6–24

Himaya SWA, Ngo DH, Ryu B, Kim SK (2012a) An active peptide purified from gastrointestinal enzyme hydrolysate of Pacific cod skin gelatin attenuates angiotensin-1 converting enzyme (ACE) activity and cellular oxidative stress. Food Chem 132:1872–1882

Himaya SWA, Ryu B, Ngo DH, Kim SK (2012b) Peptide isolated from Japanese flounder skin gelatin protects against cellular oxidative damage. J Agr Food Chem 60:9112–9119

Hsu KC (2010) Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product. Food Chem 122:42–48

Huang R, Mendis E, Kim S-K (2005) Improvement of ACE inhibitory activity of chitooligosaccharides (COS) by carboxyl modification. Bioorg Med Chem 13:3649–3655

Jamilah B, Tan KW, Umi Hartina MR, Azizah A (2011) Gelatins from three cultured freshwater fish skins obtained by liming process. Food Hydrocol 25:1256–1260

Jeon Y-J, Byun H-G, Kim S-K (1999) Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochem 35:471–478

Kato S, Matsui H, Saitoh Y, Miwa N (2011) Fish collagen-containing drink is subcutaneously absorbed and attenuates the UVA-induced tissue-integrity destruction and DNA damages in 3D-human skin tissue model. J Funct Food 3:50–55

Khantaphant S, Benjakul S, Ghomi MR (2011) The effects of pretreatments on antioxidative activities of protein hydrolysate from the muscle of brownstripe red snapper (*Lutjanus vitta*). LWT Food Sci Technol 44:1139–1148

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39:383–393

Kim S-K, Kim Y-T, Byun H-G, Nam K-S, Joo D-S, Shahidi F (2001) Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. J Agric Food Chem 49:1984–1989

Kim S-Y, Je J-Y, Kim S-K (2007) Purification and characterization of antioxidant peptide from hoki (*Johnius belengerii*) frame protein by gastrointestinal digestion. J Nutr Biochem 18:31–38

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40:43–81

Lee S-H, Qian Z-J, Kim S-K (2010) A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. Food Chem 118:96–102

Lee CM, Barrow CJ, Kim S-K, Miyashita K, Shahidi F (2011) Global trends in marine nutraceuticals. Food Technol 65:22–31

Li XX, Han LJ, Chen LJ (2008) In vitro antioxidant activity of protein hydrolysates prepared from corn gluten meal. J Sci Food Agr 88:1660–1666

Meisel H (1997) Biochemical properties of regulatory peptides derived from milk proteins. Biopolymers 43:119–128

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

Moskowitz RW (2000) Role of collagen hydrolysate in bone and joint disease. Semin Arthritis Rheum 30:87–99

Nalinanon S, Benjakul S, Kishimura H, Shahidi F (2011) Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. Food Chem 124:1354–1362

Ngo D-H, Qian Z-J, Ryu BM, Park JW, Kim S-K (2010) In vitro antioxidant activity of a peptide isolated from Nile tilapia (*Oreochromis niloticus*) scale gelatin in free radical-mediated oxidative systems. J Funct Food 2:107–117

Ngo D-H, Ryu BM, Vo T-S, Himaya SWA, Wijesekara I, Kim S-K (2011) Free radical scavenging and angiotensin-I converting enzyme inhibitory peptides from Pacific cod (*Gadus macrocephalus*) skin gelatin. Int J Biol Macromol 49:1110–1116

Pati F, Adhikari B, Dhara S (2010) Isolation and characterization of fish scale collagen of higher thermal stability. Bioresour Technol 101:3737–3742

Pei X, Yang R, Zhang Z, Gao L, Wang J, Xu Y, Zhao M, Han X, Liu Z, Li Y (2010) Marine collagen peptide isolated from Chum Salmon (*Oncorhynchus keta*) skin facilitates learning and memory in aged C57BL/6J mice. Food Chem 118:333–340

Perera MR, Bardeesy N (2011) Cancer: when antioxidants are bad. Nature 475:43–44

Picot L, Bordenave S, Didelot S, Fruitier-Arnaudin I, Sannier F, Thorkelsson G, Bergé JP, Guérard F, Chabeaud A, Piot JM (2006) Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochem 41:1217–1222

Qian Z-J, Jung W-K, Lee S-H, Byun H-G, Kim S-K (2007) Antihypertensive effect of an angiotensin I-converting enzyme inhibitory peptide from bullfrog (*Rana catesbeiana* Shaw) muscle protein in spontaneously hypertensive rats. Process Biochem 42:1443–1448

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

Ranathunga S, Rajapakse N, Kim S-K (2006) Purification and characterization of antioxidative peptide derived from muscle of conger eel (*Conger myriaster*). Eur Food Res Technol 222:310–315

Samaranayaka AGP, Li-Chan ECY (2008) Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (*Merluccius productus*). Food Chem 107:768–776

Senaratne LS, Park P-J, Kim S-K (2006) Isolation and characterization of collagen from brown backed toadfish (*Lagocephalus gloveri*) skin. Bioresour Technol 97:191–197

Thiansilakul Y, Benjakul S, Shahidi F (2007) Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem 103:1385–1394

Wilson J, Hayes M, Carney B (2011) Angiotensin-I-converting enzyme and prolyl endopeptidase inhibitory peptides from natural sources with a focus on marine processing by-products. Food Chem 129:235–244

Chapter 12
Seafood Processing By-products: Collagen and Gelatin

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1 Introduction

Collagen is defined as a fibrous protein of connective tissue and bones that yield gelatin on boiling, derived from the Greek word *kolla* for glue (Grant 2007). However, there is no agreed definition for a collagen. Gelse (2003) described the name “collagen” as a generic term, which can form a typical triple helix of three polypeptide chains. Also, all members of the collagen family can form those supramolecular structures in the extracellular matrix, despite their considerably varying size, function, and tissue distribution. The main formation of collagen molecules is constituted by three polypeptide chains (α-chains) and at least one domain composed of repeating Gly-X-Y sequences in each chain (Myllyharju 2004). Gelatin is defined as a product obtained by the partial acid/alkaline hydrolysis (type A and type B gelatin, respectively) of collagen derived from skin, bone, and cartilage (Johnston-Banks 1990; Zarai et al. 2012).

Collagen from animal sources is the most abundant protein, accounting for approximately 30 % of the total animal protein, which is generally found in skin, bone, and other connective tissues (Dong et al. 2008; Liu et al. 2007; Ogawa et al. 2004). Gelatin, derived from a cheap and resourceful meat and fish by-product collagen, is widely used as a food additive to improve the texture, water-holding capacity, and stability of several food products (Jongjareonrak et al. 2005).

Gelatin from plant sources does not exist, and there is no chemical action between gelatin and other materials considered as vegetable gelatin, like seaweed extracts (GMIA 2012; Karim and Bhat 2008, 2009). There are more than 19 types of collagen, named types I to XIX. All connective tissue, including bone and skin, contains type I – a heteropolymer of “two 1 chains and one 2 chain”. Among them, types I, II, III, and V are the fibrous collagens (Aberoumand 2010, 2012; Palpandi et al. 2010). In contrast, Brinckmann (2005) and Veit (2005) reported that 28 types of

collagen consisting of at least 46 different polypeptide chains have been identified in vertebrates. Furthermore, Myllyharju (2004), Pataridis et al. (2008), Schreiber and Gareis (2007), and Schumacher (2006) have reported at least 27 collagen types with 42 distinct polypeptide chains, at least 20 additional proteins with collagen-like domains, and about 20 isoenzymes of various collagen-modifying enzymes.

A significant amount of collagen and gelatin is used in the food, pharmaceutical, and photographic industries, as well as for various technical uses (Jung et al. 2006; Pati et al. 2010). Especially in the food industry, gelatin is utilized as emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials, and microencapsulating agents, coinciding with the growing trend to replace synthetic agents with more natural ones (Gómez-Guillén et al. 2011).

The development of new industrial applications stimulates the increasing consumption of collagen and gelatin (Woo et al. 2008). Collagen and gelatin from livestock organs such as bovine and porcine skin and bone have been widely used. However, the outbreak of mad cow disease or bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) (Wangtueai and Noomhorm 2009), foot-and-mouth disease (FMD) (Jongjareonrak et al. 2005), and avian influenza (Nagai 2010) resulted in panic among the consumers regarding collagen and its related products obtained from land animals (Jongjareonrak et al. 2005; Nagai 2010).

In addition, in many countries, the collagen derived from bovine and porcine skin or bone is forbidden to be used as a component of some foods due to esthetic and religious concerns (Herpandi et al. 2011; Kołodziejska et al. 2004). Sikhs and Hindus cannot take collagens obtained from bovine sources, while Muslims and Jews cannot have porcine collagen (Xuan et al. 2007). Moreover, collagen peptide from bovine and porcine and other land animals may cause a food allergen (Shon et al. 2011).

An alternative to the mammalian gelatin, which is not likely associated with infections such as BSE, TSE, FMD, influenza A virus, and accepted as a food additive by those religious groups or an allergenic source, is the aquatic animal (Nagai 2010), which is abundant around the world. Collagen and gelatin, especially when extracted from the by-products of aquatic animals, such as skin, bone, or scale, can help reduce harmful environmental effects (Haug et al. 2004a, b; Shon et al. 2011). Therefore, the utilization of alternative sources has attracted increasing attention, especially by-products of aquatic animals, to produce collagen and gelatin (Gómez-Guillén et al. 2011; Herpandi et al. 2011; Karim and Bhat 2009; Xuan et al. 2007).

2 Gelatin Production from Fish

The most common raw materials for collagen and gelatin extraction are skins or hides, bones, tendons, and cartilage (Gómez-Guillén et al. 2011; Herpandi and Adzitey 2011). The first raw material used for the manufacturing of gelatin in the

1930s was pig skin, and it continues to be the most important material for large-scale industrial production (Karim and Bhat 2009). Fish raw materials have received considerable attention recently, but their price is higher than mammalian gelatins, due to their limited production (Gómez-Guillén et al. 2011; Gómez-Guillén et al. 2009). The intrinsic characteristics of fish species gelatin raised the interest of the scientific community in optimizing the extracting conditions, characterizing the yields, and ensuring physicochemical and functional properties of the resulting gelatins obtained from the skin, scale, fins, offal, and solid waste from surimi processing species (Aberoumand 2010; Aewsiri et al. 2009; Ahmad et al. 2010; Gómez-Guillén et al. 2009; Karim and Bhat 2008, 2009).

A large amount of skin was used to extract gelatins, including bigeye snapper (*Priacanthus macracanthus*) (Benjakul et al. 2009), bigeye snapper (*Priacanthus tayenus*) (Sukkwai et al. 2011), bigeye snapper (*Priacanthus hamrur*) (Binsi et al. 2009), cuttlefish (*Sepia pharaonis*) (Aewsiri et al. 2009), greater lizardfish (*Saurida tumbil*) (Taheri et al. 2009), hoki (*Macruronus novaezelandiae*) (Mohtar et al. 2011), blacktip shark (*Carcharhinus limbatus*), and giant catfish (*Pangasianodon gigas*) (Jongjareonrak et al. 2010). Others species that have been studied as a source of gelatin include coldwater fish like, cod, Atlantic salmon, haddock, Alaska pollock, or hake, tropical or subtropical species, for example black or red tilapia, Nile perch, yellowfin tuna, channel catfish, sin croaker, shortfin scad, grass carp, and skate, and flat species like megrim and Cephalopods species like giant squid (Aberoumand 2012; Aewsiri, et al. 2009; Benjakul et al. 2009; Binsi et al. 2009; Jongjareonrak et al. 2010; Mohtar et al. 2011; Taheri et al. 2009; Zúñiga and Aguilera 2009; Zhou et al. 2006).

Scales from different species are used to extract collagen or gelatin, including sea bream and red tilapia (Ikoma et al. 2003), black drum and sheep's head (Ogawa et al. 2004), sardine (Nomura et al. 1996), Asian (Silver) carp (Wang and Regenstein 2009), and lizard fish (Wangtueai and Noomhorm 2009). Precooked fins from canned tuna processing (Aewsiri et al. 2008), solid waste from surimi processing (Jongjareonrak et al. 2005; Morrissey et al. 2005), and offal from further processing, such as the skin from salted and marinated herring or cold-smoked salmon and bones (Kołodziejska et al. 2008), have also been studied as a source of gelatin.

True comparisons are difficult since methodologies may differ considerably from one work to another. Coldwater fish gelatins generally have low gelling (~4–12 °C) and melting (<17 °C) temperatures, in contrast to warmwater species with higher values of gelling (~18–19 °C) and melting (~24–29 °C) temperatures (Aberoumand 2012). Similarly, comparable gel strength and shear viscosity values for coldwater species are relatively lower than 100 g and 2 MPa, respectively, whereas those from warmwater species normally have higher gel strength and viscosity values of up to 487.61 g for tilapia and 5.24 MPa for catfish, respectively (Gómez-Guillén et al. 2009; See et al. 2010).

The skin of striped catfish (*Pangasianodon hypophthalmus*) contains acid-soluble collagen and pepsin-soluble collagen. Based on the wet weight of skin, the yields of acid-soluble collagen and pepsin-soluble collagen were 5.1 % and 7.7 %, respectively, and 12.8 % in total. Both collagens had the highest solubility in acidic conditions. They comprise two different α-chains (α1 and α2) are characterized as

type I, which contain imino acids of 206 and 211 imino acid residues/1,000 residues, respectively. There were slight differences in composition and structure between acid-soluble collagen and pepsin-soluble collagen, which led to slightly different properties (Singh et al. 2011).

In another study, when acid-soluble collagen and pepsin-soluble collagen were extracted from Nile tilapia skin by the two methods of Noitup and Ogawa, the biochemical properties and thermal stability were compared. The amino acid profiles of two collagens obtained from both methods were similar. Except for the lower yield obtained from the Ogawa method, there were no differences in the molecular weight, denaturation temperature, and amino acid composition between acid-soluble collagen and pepsin-soluble collagen acquired from both methods (Potaros et al. 2009).

When gelatin was extracted from dried cobia skin (*Rachycentron canadum*), the yield was higher than that of frozen skin. There were significant differences in the contents of protein, ash, and fat. This gelatin was snowy white in color, with bright appearance and gave higher gel strength, while that of frozen skin was darker. However, there was no significant difference in the gelling temperature and amino acid composition between both gelatins. The results showed that drying could be a better method for preserving cobia skin compared to freezing, prior to gelatin extraction (Amiza and Siti 2011). The same results were obtained from fresh and sun-dried seawater fish skin (Pranoto et al. 2011).

Unlike skin, scales are rich in calcium phosphate compounds such as hydroxyapatite and calcium carbonate. Therefore, the pretreatment removal of Ca from fish scales is critical in order to obtain a better gel strength of the gelatin (Wang and Regenstein 2009). Decalcification of more than 90 % resulted from pretreatment with 0.2 mol/L EDTA, with a gelatin yield of 22 % and gel strength of 152 g, which are considerably higher than those obtained with 0.20 mol/L HCl or 1.2 g/L citric acid (Wang and Regenstein 2009).

Precooked tuna caudal fin, an important waste product from canned tuna processing in Thailand, has been proposed as a promising source for gelatin extraction, but its yield was low (1.99 %) (Aewsiri et al. 2008). Tuna fin gelatin (TFG) contains a high protein content of 89.54 %, with a hydroxyproline content of 14.12 mg/g. However, TFG presented an ash content as high as 40 %; consequently, an exhaustive demineralization step was needed. Furthermore, during the steam heating in precooking, the collagen might have undergone denaturation somewhat, in terms of damage to the yield and the properties of the extracted gelatin, which exhibited inferior gelling, emulsifying, foaming, and film-forming properties in comparison to those of commercial pig skin gelatin.

Solid fish waste constitutes 50–70 % of the original raw material in surimi processing (Jongjareonrak et al. 2005; Morrissey et al. 2005). This waste consisted of skin and bones with high collagen content. They might be the initial material for obtaining gelatin or collagen from underutilized fish resources and help to eliminate harmful environmental impacts (Gómez-Guillén et al. 2009). Moreover, refining discharge from the Pacific whiting surimi process represented around 4–8 % of whole fish, consisting of muscle (95 %), skin (2.1 %), bone (2.9 %), and trace amounts of scale fragments (Kim and Park 2005). Crude collagen from refiner discharge, extracted in the form of acid-soluble collagen or recovered in a simpler way

as partially purified collagen, was found to present higher functional properties (emulsifying activity, cooking stability, water and oil absorption capacity) compared with the other by-products (skins and frames) generated in the same manufacturing process (Kim and Park 2004, 2005).

The acid-soluble collagen from Alaska pollock surimi refining discharge has a thermal denaturation temperature slightly higher than that for Alaska pollock skin (Park et al. 2007). Three solid by-products (skin, frame, and refiner discharge) from Pacific whiting surimi manufacturing are a good resource for collagen extraction due to their total protein concentrations and other biochemical properties. The denaturation temperature of acid-soluble collagen was 23.3 °C, 21.7 °C, and 20.6 °C for refiner discharge, skin, and frame, respectively. Acid-soluble collagen was considered as the best on the basis of functional properties and showed potential to be an ingredient in food processed manufacturing (Kim and Park 2004).

Moreover, fish collagen has gained considerable attention for their potential as an ingredient in processed functional food manufacturing and cosmetic, biomedical, and pharmaceutical applications. The extraction and functional characterization of acid- and/or pepsin-soluble collagen has been reported for different fish species, such as Pacific whiting (Kim and Park 2004), plaice (*Pleuronectes platessa*) (Montero et al. 1995), squid (*Illex coindetii*) (Ruiz-Capillas et al. 2002), Alaska pollock (Park et al. 2007), deep-sea redfish (Lin et al. 2008), threadfin bream (Nalinanon et al. 2008), walleye pollock (Yan et al. 2008), brownstripe red snapper (Jongjareonrak et al. 2005), or unicorn leatherjacket (*Aluterus monoceros*) (Ahmad et al. 2010).

3 Collagens Structure and Composition

Collagens exist in the form of the collagen fibril or collagen fiber in vivo. The basic institutional units of collagen are protocollagens, whose relative molecular weight is 2.85×10^5. Generally, collagen consists of three helical polypeptide chains, while every collagen chain is a left-handed helical. Collagen is characterized as repeating Gly-X-Y sequences (Canty 2005; Myllyharju 2004). Every three amino acid has glycine residue. Frequently, proline is found in the X position and hydroxyproline in the Y position. The amino acid composed of collagen is considerably different from the typical globular protein. The major components of collagen molecules are glycine, proline, and hydroxyproline. They cannot form α-helix and β-folding, but can shape the characteristic triple-helical structure that is coiled by three chains called α-chains (Kadler et al. 2007), which is a right-handed super helical cable, making the final structure a rope-like rod.

3.1 Nomenclature and Classification

Generally, collagen molecules are substantially enormous groups. So far, 28 different types of collagen have been found (Friess 1998; Pace et al. 2003). Among them,

collagen I is the largest, which comprises more than 90 % of the content of the total collagen protein. Collagen is classified according to the formation of collagen fibril with periodic lateral cuts and can be divided into two kinds, archetypal fibrillar collagen protein and no archetypal fibrillar collagen protein, respectively. The former contains types I, II, III, IV, V, and XI (Ricard-Blum 2010; Djabourov et al. 1992). Types I, II, III, IV, and V collagens are easily separated and their production is relatively high. The others are contained in the no archetypal fibrillar collagen protein. However, the latter are further divided into six kinds. They are FACT collagens, mesh collagen protein, moniliform fibril collagen, anchor fibril collagen, and transmembrane collagens, in proper the order. No fibrillar collagen proteins have non-triple-helical domains at their N- and C-termini. These domains are called 'non-collagenous' (NC) domains, but also triple-helical domains, which are regarded as collagens domains (Kadler et al. 2007). Collagen proteins are also sorted into two kinds on the base of dissolving capacity. The first kind is soluble collagen protein, while another kind is dissoluble collagen protein. Nevertheless, the former is classified into acidic soluble collagen protein, basic soluble collagen protein, and neutral soluble collagen protein. In addition, gene recombination collagen protein is included.

3.2 Vertebrate Collagens Are Classified by Function and Domain Homology

3.2.1 Fibril-Forming Collagens

According to complex structure diversity, the collagen protein family, which has about 21 members, is divided into collagen fiber, basement membrane collagen, microfiber collagen, anchor collagen, six-side mesh collagen, non-fiber collagen, transmembrane collagen, basement membrane collagen, and others. Most of the collagen family comprises fibril-forming collagens. Fibril-forming collagens contain the widely distributed types I, III, and V, types II and XI, especially in cartilage and eye, and the newly discovered types XXIV and XXVII (Boot-Handford 2003; Koch 2003). All of the 12 fibril-forming α-chains share a long continuous collagenous domain flanked by N- and C-terminal non-collagenous propeptides. The α-chains assemble into at least 12 type-specific protomers, characterized as homo- and heterotrimers. The terminal propeptides are further cleaved by specific proteases, which support oligomerization and fibril formation (Khoshnoodi et al. 2006).

Fibril-forming collagens are synthesized as procollagens containing N- and C-propeptides at each end of the triple-helical domain. Cleavage of the C-propeptides is required for fibrillogenesis. The C-propeptides are cleaved by procollagen C-proteinases, which are identical to the BMP-1/tolloid proteinases (Greenspan 2005). In the case of collagen V, the pro-α1 (V) chain is cleaved by furin to release the C-propeptide. The N-propeptides are cleaved by procollagen N-proteinases, which are identical to the ADAMTS 2, ADAMTS 3, and ADAMTS 14 proteinases (Colige et al. 2005). The pro-α1 (V) chain of collagen V is the exception, which is

cleaved by BMP-1 (Greenspan 2005). Cleavage of the propeptides exposes telopeptide sequences that are short, non-triple-helical extensions of the polypeptide chains. The telopeptides contain binding sites for fibrillogenesis (Prockop and Fertala 1998). The fibrillar collagens are stabilized by non-reducible covalent cross-links that involve residues in the triple helix and in telopeptides (Eyre et al. 1984). The cross-links are indispensable for the normal mechanical properties of collagen-containing tissues (Kadler et al. 2007).

3.2.2 Fibril-Associated Collagens with Interrupted Triple Helices (FACITs)

The FACIT collagens include types IX, XII, XIV, XVI, XIX, XX, XXI, and XXII. Collagen IX is composed of three α-chains, which is characterized by short collagenous domains interrupted by several NC domains (Ricard-Blum 2010). The protomer of collagen IX is a heterotrimer, while all others are homotrimers, accounting for at least nine distinct protomers. Unlike the fibril-forming collagens, the FACITs have significantly shorter C-NC domains: 75 residues for collagen XII and fewer than 30 residues for collagen IX, whereas those of fibrillar collagen include about 260 residues.

3.2.3 Network-Forming Collagens

Collagen IV is the typical network-forming collagen. It forms a connected network in basement membranes, where it has an important molecular filtration function. Collagen IV is comprised of a third chain (α3) together with the two classical chains (α1 and α2). The α3 (IV) chain exists within the same triple-helical molecule together with the α1(IV) and α2 (IV) chains and/or within a separate triple-helical molecule, exclusive of α1 (IV) and α2 (IV) chains, but connected through the NC1 domains to the classical triple-helical molecule comprised of α1 (IV) and α2 (IV) chains (Than et al. 2002). Collagen VIII is one of the two α-chains. The gene product is a short-chain collagen and a major component of the basement membrane of the corneal endothelium. The type VIII collagen fibril can be either a homo- or a heterotrimer. Alternatively spliced transcript variants encoding the same isoform have been observed. Type VIII collagen may be unique among the collagens for its tissue distribution and biosynthetic properties. It was first detected and designated endothelial cell collagen in biosynthetic studies of bovine aortic endothelial cells and rabbit corneal endothelial cells, but not all endothelial cells synthesize type VIII collagen, as the protein is restricted to the vascular endothelium (Stephan et al. 2004).

3.2.4 Transmembrane Collagens

Transmembrane collagens are often used as matrix components and cell surface receptors, which have long interrupted triple-helical extracellular domains and a short cytosolic N-terminal domain (Franzke et al. 2005). They contain types XIII,

XVII, XXIII, and XXV and other collagen-related proteins, such as the macrophage scavenger receptor and the MARCO receptor (Elomaa et al. 1998).

All transmembrane collagens are trimers of identical polypeptides, called α-chains, whose protomer is a homotrimer. The longest α-chains commonly have 1,497 amino acids, for example, collagen XVII, while the shortest only has 135 amino acids, like isoform of ectodysplasin A. The α-chains include a single transmembrane stretch, an N-terminal intracellular domain, and a large extracellular C-terminus. The extracellular linker subdomain has an α-helical coiled coil, which can prompt the trimerization and the folding of the triple-helical domain. Some researchers have suggested that the N-NC domains of collagens XIII and XVII are essential for triple helix formation (Snellman et al. 2000). Furthermore, nucleation of the triple helix usually appears in the N-terminal region and proceeds in an N- to C-terminal direction (Snellman et al. 2000). Collagen XIII has been confirmed to have a short sequence of 21 residues at the juxtamembrane linker subdomain and a putative identification site for trimerization (Khoshnoodi et al. 2006).

3.2.5 Endostatin-Producing Collagens

Collagen XV is a basement membrane collagen of a subfamily of multiplexins with multiple triple-helix domains and interruptions. It can form a great variety of oligomeric assemblies and bridge adjacent collagen fibrils near the basement membranes (Amenta et al. 2007). Besides, collagen XVIII has also been recently discovered in some basement membranes (Marneros and Olsen 2005). The cleavage of part of the NC1 domains of collagens XV and XVIII can release endostatins, which will reduce tumor growth in animals, inhibit the migration of endothelial cells, and control neuronal guidance (Marneros and Olsen 2005).

3.2.6 Anchoring Fibrils

Anchoring fibrils have been discovered in the subbasal lamina fundamental epithelia of some outside tissues (Chen et al. 2002). They are indispensable for functional integrity and are also essential to the dermoepidermal junction. They could fortify the attachment of the epidermis to the dermis from their appearance. This hypothesis could be enhanced by the observational results that anchoring fibrils were diminished, abnormal, and absent from individuals with malnourished epidermolysis bullosa. Consequently, the molecular constituents of anchoring fibrils and their interactions with other dermal components and basement membrane might result in gene defects, at least some forms of epidermolysis bullosa.

Recently, collagen VII has been regarded as the major protein constituent of the anchoring fibrils, which are confined to the basement zone under the stratified squamous epithelia (Woodley et al. 2007). In general, collagen VII has three identical α-chains, including a collagenous domain of 1,530 residues, which has 19 interruptions flanked by C-NC and N-NC domains (Christiano et al. 1994).

3.2.7 Beaded-Filament-Forming Collagen

The prototypal beaded-filament-forming collagen is collagen VI, which can be found in most tissues, where it can form structural links with cells. The primary fibrils of collagen VI assemble within the cell to antiparallel, overlapping dimers, which then cross link into tetramers. These tetramers can aggregate into long molecular chains known as microfibrils with a beaded repeat of 105 nm (Kadler et al. 2007). Collagen VI has three different α-chains (α1, α2, α3) with short triple-helical domains, while the protomer is a α-chains heterotrimer. The α1- and α2-chains are very similar in size and include one N-NC domain and a C-NC domain. However, the α3-chain is nearly as twice as long as the other two chains for large C- and N- terminal globular domains. For example, the N-NC domain has ten subdomains and five C-NC subdomains (Lamande et al. 2006). Those extended domains will undergo comprehensive posttranslational processing and alternative mosaicing, both inside and outside the cell. Some related reports have interpreted that the α3-chain involves sequences necessary for the formation of protomers and the association of chains (Khoshnoodi et al. 2006; Lamande et al. 2006).

4 Physical and Chemical Properties of Collagen and Gelatin

Collagen is a hydrophilic colloid of an aqueous form, and is also an important insoluble fiber protein in an extracellular form. Normally, collagen is a brittle solid, which is almost tasteless, odorless, and vitreous, and its specific gravity is about 1.3–1.4 (GMIA 2012; Saranjit et al. 2002). Gelatin is a kind of modified product derived by acid treatment, alkali treatment, high-temperature treatment, or enzymolysis of collagen, contains 18 different amino acid radicals like collagen, but has no biological activity and could dissolve in aqueous solutions of polyhydric alcohols such as glycerol and propylene glycol (Midorikawa et al. 2012). Many factors, such as pH, temperature, ash content, and method of manufacture and concentration, can influence the behavior of gelatin solutions (Gómez-Guillén et al. 2002; Songchotikunpan et al. 2008). Gelatin can be used not only as a natural nutrient food thickener with high protein and no fat and cholesterol, but also as aspic, additives, and candy in the food field and plywood, gauze, and sandstone in industry. Beyond that, it can be used in medicine, for example, hard and soft capsule, surgical dressing, and hemostatic sponge.

4.1 Amphoteric Properties

Collagen can produce chemical reactions with acid, alkali, and salt for different amino acid characteristic, as it is a typical protein which acts as both an acid and a base (Jongjareonrak et al. 2005; Saranjit et al. 2002). Gelatin contains relatively few ionizable groups that can be titrated, while the imidazolium of histidine, the

carboxyl groups of aspartic and glutamic acids, and the guanidinium of arginine produce several ionizable groups (GMIA 2012). In addition, there are terminal amino and carboxyl groups (Saranjit et al. 2002).

Gelatin with acidic solutions is positively charged and migrates in an electric field as a cation (type A gelatin), while gelatin with alkaline solutions is negatively charged and migrates as an anion (type B gelatin). Type A gelatin has a broad isoelectric range (pH 3.8–6.0; isoelectric point 6–8), while type B has a narrower isoelectric range (pH 5.0–7.4; isoelectric point 4.7–5.3) (GMIA 2012; Johnston-Banks 1990; Saranjit et al. 2002; Zarai et al. 2012). There is no non-colloidal ion in gelatin solution other than the known isoionic gelatin, such as H+and OH-, whose pH is known as the isoionic point (pI). The ion exchange resins may possibly use these solutions (GMIA 2012).

4.2 Chemical Derivatives

Chemical treatments can bring about significant changes in the physical and chemical properties of materials, such as gelatin and collagen, showing different properties. These changes could cause structural modifications and/or chemical reactions (Ren et al. 2009). For improving the biocompatibility and mechanical properties of gelatin, chemical modifications usually include acylation, esterification, cross-linking reagent, and deamination of the amino group (Ren et al. 2009; Zarai et al. 2012; Zhu et al. 2012).

4.3 Gel Strength

Gel usually refers to mixture, whose particle size is between 1 and 10 Å. Gelatin has an important property regarding the formation of thermoreversible gels in water (Brenner et al. 2009; Tosh and Marangoni 2004; Chiou et al. 2006). When a polymer solution is cooled, thermoreversible gels form. When gels are reheated, they melt (Tosh and Marangoni 2004). This phenomenon is due to the low-energy interactions that stabilize the gel network (Brenner et al. 2009).

When an aqueous solution of gelatin concentration greater than approximately 0.5 % is cooled to approximately 35–40 °C, first, its viscosity increases and it then forms a gel later. So, the gelatin concentration can decide the rigidity or strength of the gel, i.e., the intrinsic strength of the gelatin (Brenner et al. 2009).

4.4 Viscosity

Gelatin is a polyampholyte, based on the structural protein collagen by hydrolysis (Zarai et al. 2012). Like other polymers, gelatin increases the solution viscosity.

Although gelatin solutions are gel at low temperatures, they are Newtonian at higher temperatures (typically > 40 °C) (Gómez-Guillén et al. 2009). In general, gelatin is adsorbed at charged or hydrophobic surfaces in water, imparting steric effects and charge stability to colloidal particles, in addition to increasing the effective volume occupied. Due to these properties, the viscosity of a suspension of hydrophobic or charged colloidal particles in aqueous gelatin can have a greater viscosity than the same suspension with a shortage of gelatin (Hone and Howe 2002). The suspension rheological property depends on several factors, including concentration, size of the colloidal particles, and molecular weight of the gelatin used (Hone and Howe 2002).

4.5 Protective Colloidal Action

Gelatin is a typical hydrophilic colloid which can stabilize a variety of hydrophobic materials. The efficiency of gelatin as a protective colloid is proved by its gold number, which is the lowest of any of the other colloids. This property is especially important in the photographic industries (GMIA 2012).

4.6 Coacervation

Complex coacervation is a liquid–liquid phase separation phenomenon that occurs when mixing oppositely charged polyelectrolyte solutions. Proteins and polysaccharides are commonly used for complex coacervation for entrapping targeted materials in the concentrated polymer phase (Kizilay et al. 2011; Klemmer et al. 2012). In particular, gelatin is widely used in combination with polyanions, such as gum acacia (Mayya et al. 2003), sodium dodecyl sulfate (Li et al. 2009), and chitosan (Kim et al. 2006) to produce high-potential microencapsulation systems.

4.7 Color

The color of gelatin relies on the properties of the crude materials extracted and whether the gelatin is further extracted. Gelatins are usually faintly yellow in color (Saranjit et al. 2002). In general, the color does not affect the characteristics of the gelatin or reduces its advantages (GMIA 2012).

4.8 Ash

The content of ash in gelatin varies depending on the kinds of crude materials used and the production techniques. Unlike skin, the high concentration of calcium

phosphate compounds, such as hydroxyapatite and calcium carbonate in scales, made the pretreatment removal of calcium from fish scales necessary, which is extremely important in order to achieve the desired final yield, purity, and gel strength of the gelatin (Wang and Regenstein 2009).

5 Functional Properties of Collagen and Gelatin

The gel properties, such as construction, gumminess, and texture, are closely related with each other, while they are determined mainly by the structure, molecular size, and temperature of the organization. Collagen and gelatin are different forms of the same macromolecule; however, they are often confused by non-specialists. Actually the gelation mechanism of gelatin, along with the formed gel network structure, differ significantly from that of collagen.

5.1 Gelling and Water-Binding Properties

During the process of in vitro self-assembly, which leads to collagen gelatin, the size of the fibrils, their chemical interactions, along with the resulting microstructure, are regulated by three main experimental conditions: pH, ionic strength, and temperature (Achilli and Mantovani 2010). For the period of collagen gelatin, the process of collagen molecule accumulation and fibril creation takes place at the same time. They are prompted by changes in the ionic strength, pH, and also temperature. Throughout the collagen gelatin procedure, there is an interval stage where the primary combinations (dimers and trimers of collagen molecules) are nucleated. Then, microfibrillar aggregation starts with the adjacent aggregation of subunits until equilibrium has been reached. The self-assembly procedure of type I collagen from vertebrates occurs when the gelatin temperature is raised from 20 °C to 28 °C (Widmer et al. 2012; Yang et al. 2010). In contrast, the rudimentary mechanism of gelatin is related to the converse coil-to-helix transition activated by cooling solutions below 30 °C. During this process, the helices are created, like the collagen triple helix. Because of this, no equilibrium is reached. The gelation process for both collagen and gelatin is thermoreversible, although in opposite directions: collagen gels melt by lowering the temperature, while gelatin gels melt by raising the temperature (Gómez-Guillén et al. 2011).

Because gelatin can melt quickly in the mouth, it is widely used in water gel sweets. Though other hydrocolloids have this property, they commonly melt at higher temperatures. Sweets made from various gelatins can offer diversities in texture and gel melting behavior, resulting in developing all kinds of food. By increasing gelatin concentrations or by using gelatin mixtures (of coldwater and warmwater fish), desserts made from fish skin gelatin were found to be more similar to desserts made from high-Bloom pork skin gelatin (Karim and Bhat 2009). What's more, the lower melting temperature in gel sweets made from fish gelatins can speed up flavor release.

Gelatin can offer plenty of flexibility in terms of textures and forms for product development, in which they introduce a gas phase into gelatin-gel-based products, such as fruit jellies. In addition, the distributed air makes portions less calorie-rich, by reducing the energy consumed per unit volume. The combination of gas (air, nitrogen, or helium) in the form of bubbles can weaken the construction of gelatin gels to obtain some unique mechanical properties and an opaque white appearance (Zúñiga and Aguilera 2009). Among the three gases tested, air-filled gels offered the lowest densities, the largest bubble diameters, and the weakest structures in comparison with the other gas-filled gels.

Gelatin or collagen cables in solution can link to form matrices capable of swelling in the presence of aqueous solutions, creating what are commonly known as gelatin hydrogels. Hydrogels have the ability to swell to an equilibrium volume while preserving their shape because they are hydrophilic and insoluble in water. The chemical cross-linkers used may be either polyfunctional macromolecules or relatively small bifunctional molecules, for example, glutaraldehyde (Deiber et al. 2009). Presently, because natural polyampholyte hydrogels have the ability to absorb large quantities of water, it is widely used in applications such as gelatin casings. Such a property is becoming more important in the fields of medicine, pharmacy, agriculture, and biodegradable food packaging. Gelatin is very popular in forming hydrogel packaging because it is comparatively cheap and eco-friendly. The properties of gelatin–pectin hydrogels have been shown to depend strongly on the pH in the reaction mixture and on the charge balance (determined by the gelatin–pectin ratio), which will influence the degree of electrostatic associations and ionic interactions in the gelling system (Farris et al. 2009).

The properties of gelatin hybrid hydrogels, such as super-swelling produced by mixing with synthetic polymers, have been exhaustively revised, particularly with reference to their swelling capacity, degradation rate, and controlled release of drugs (Zohuriaan-Mehr et al. 2009). Transglutaminase (TGase) can be used to prompt enzymatic cross-linking. TGase can catalyze an acyl transfer reaction between the ε-amine group of lysine residues of peptide chains and the γ-carboxamide group of glutamine residues (Karim and Bhat 2009). Actually its concentration, incubation time, and degree of enzyme heat inactivation may control the thermoreversibility of the gelatin gel. Increasing the concentration of TGase could increase the elasticity and raise the melting temperature and cohesiveness of megrim skin gelatin gels, but because of excessively rapid gel network formation, there was a lowering of the gel strength and hardness (Gómez-Guillén et al. 2001). The addition of TGase has different effects on different gelatins. For gelatin extracted from Baltic cod skin, the addition of TGase effectively produced stable gels at room temperature, as well as after heating in boiling water for a period of less than 30 min (Kołodziejska et al. 2004).

Though *k*-carrageenan and/or gellan (Haug et al. 2004a, b), or hydroxypropyl methylcellulose (Chen et al. 2009) are different polysaccharides, they have the same functions, i.e., increase fish gelatin gel strength, setting time, and thermostability, with the aim of enhancing their cold and thermal gelation properties. In this, they can lead to a non-thermoreversible character. Similarly, gelatin or hydrolyzed collagen materials could be hardened by reaction with chemically modified polysaccharides, such as dialdehyde starch (Langmaier et al. 2008).

Fish gelatin as an Alaska pollock surimi processing additive was used to improve water retention in heat-set gels. Expressible moisture was improved when 7.5–15 g/kg of fish gelatin was contained in Alaska pollock surimi gels. However, when adding 15 g/kg, the effect was destructive. It indicated that a high concentration of fish gelatin is unfavorable to the textual properties of the gel, more so when using the highest quality surimi. (Hernández-Ledesma et al. 2007). By adding gelatin (0.5 %) from bigeye snapper skin, the gel-forming ability of threadfin bream (*Nemipterus japonicus*) mince was clearly improved (Binsi et al. 2009). In contrast, gelatin additions of 5 % and 10 % reduced the elastic modulus (G') values significantly, possibly owing to the fact that water molecules were unavailable for protein gelatin because of their entrapment by high gelatin concentrations. Accordingly, gelatin has little or no interaction between filler particles and gel matrix, and was considered to be an inactive binder. However, when chicken myosin and pork skin gelatin mixtures were heated from 25 °C to 80 °C, the G' values were markedly higher than those of pure myosin. It indicated that an interaction, possibly electrostatic nature, between myosin and gelatin had caused higher gel elasticity. Therefore, there is an advantage in using this gelatin in restructured (chicken) meat products (Yang et al. 2007).

5.2 Surface Properties

The surface properties of collagen and gelatin are on the base of the charged groups in the protein side chains and on some parts of the collagen sequence concluding either hydrophilic or hydrophobic amino acids. Both hydrophobic and hydrophilic parts tend to migrate towards surfaces, such that weakening the surface tension of aqueous systems and forming the required identically charged film around the components of the gel formation can additionally strengthen the dispersed phase (Schreiber and Gareis 2007).

In a relatively high isoelectric point (pI = 7.0), type A gelatins used as a treatment of animal raw material with dilute acid are more suitable for creating oil-in-water emulsions with a positive charge over a wider range of pH values compared with conventional protein emulsifiers, such as soy, casein, or whey proteins (Dickinson and Lopez 2001). In the aspect of gelling properties, the emulsion capacity of gelatin from fish species is generally lower than that from mammalians. Besides the distribution of charge, gel firmness is a significant criterion in selecting a suitable gelatin type. At the same temperature and concentration, the gel-like protective sheath around the oil droplets is increased as the gel firmness is strengthened (Schreiber and Gareis 2007). For both types of gelatin, the emulsion capacity is strengthened by increasing the protein concentration from 2 to 5 %, with high protein concentrations facilitating more protein adsorption at the surface. It is reported that the value of emulsion capacity becomes higher with increasing gelatin concentrations (0.1 % and 0.2 %), because a higher degree of polypeptide is unstretched during the shearing involved in the emulsifying stage (Binsi et al. 2009).

Apart from the protein concentration, the molecular weight could be a key factor influencing the ability of gelatin to form and stabilize oil-in-water emulsions.

For example, the emulsion of low molecular weight fish gelatin (~55 kDa) had more large droplets and greater oil destabilization compared with high molecular weight fish gelatin (~120 kDa) (Sun et al. 2009).

Being an insoluble protein, it is of little significance to presume that collagen as is emulsifier, as isolating or partially purifying collagen needs to be in a soluble form in order to display its surface-active properties. Acid-soluble collagen is the best in terms of the immediate emulsifying activity, while pepsin-soluble collagen is the best regarding emulsion stability. The acid-soluble collagen from Pacific whiting surimi refiner discharge was found to be higher compared with both acid-soluble collagen from skin and the commercial emulsifier Tween-80 (Kim and Park 2004).

Soluble collagen and gelatin exhibit suitable foaming properties, even without gelling, since they can weaken the surface tension at the liquid–air interface by enhancing the viscosity of the aqueous phase (Schreiber and Gareis 2007). The characteristics of the raw material have a great influence on the foaming properties. For adsorption at the air–water interface, molecules should contain hydrophobic regions which become more exposed upon protein unfolding, thus facilitating foam formation and stabilization (Townsend and Nakai 1983). It is found that the foam capacity of gelatin from farmed giant catfish was higher than that from calf skin, because the former contained a higher content of hydrophobic amino acid residues. It had almost four times greater viscosity than that of calf skin (Jongjareonrak et al. 2010).

5.3 Film-Forming Properties

Gelatins are used to develop biodegradable films as an outer covering instead of synthetic plastic materials for the characteristics of not only environmental protection but also protecting foodstuffs against drying, light, and oxygen (Gómez-Guillén et al. 2009; Zaman et al. 2012). In addition, gelatin is one of the first materials used as a carrier of bioactive components. Compared to films from other resources, most fish gelatins are observed to have great film-forming properties, such as being transparent, nearly colorless, water soluble, and highly extensible. However, gelatin films cannot resist liquid and, hence, physical damage. The mechanism of forming strong mechanical and waterproof gelatin films is a challenge faced by the food industry.

Casting and extrusion are the two dominant edible films-processing methods (Hernandez-Izquierdo and Krochta 2008). The casting method involves dissolving the biopolymer and blending it with plasticizers and/or additives to obtain a film-forming solution, followed by casting onto plates and drying. The extrusion method would be the better choice when dealing with the thermoplastic behavior of proteins at low moisture levels. Films can be produced by extrusion followed by heat pressing at temperatures that are ordinarily higher than 80 °C (Gómez-Guillén et al. 2009).

The amino acid composition and molecular weight distribution could be the main factors affecting the physical properties of fish gelatin and then fish gelatin films (Gómez-Guillén et al. 2002). Specifically, the amino acid composition results mainly from the species of resources, while the molecular weight distribution results mainly from the films-processing conditions.

Obviously, the various amino acid compositions from various fish species may result in the differences between the properties of films. Low molecular weight fragments correspond to weak and deformable films, due to the protein heat degradation during the water extraction step or during the evaporation step (Muyonga et al. 2004; Carvalho et al. 2008). Besides, films made using a gelatin of lower molecular weight were found to be more plasticized, as a result of the higher plasticizer biopolymer molar ratio (Carvalho et al. 2008).

A large amount of scientific literature has been published indicating that films can be enhanced to obtain better mechanical and water resistance properties by blending other biopolymers, which includes lipids (Bertan et al. 2005; Pérez-Mateos et al. 2009), biopolymers like soy protein isolates (Cao et al. 2009; Denavi et al. 2009), polysaccharides like gellan (Lee et al. 2004), chitosan (Arvanitoyannis et al. 1998), pectins (Farris et al. 2009), konjac glucomannan (Li et al. 2006), and plasticizers (Cao et al. 2009), synthetic polymers like poly (vinyl) alcohol (Carvalho et al. 2009) and polyethylene (Haroun and Migonney 2010), and cross-linking agents like glutaraldehyde (Bigi et al. 2001) and TGase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Kołodziejska and Piotrowska 2007).

Adding natural antioxidants and antimicrobial substances to fish gelatin films can make a great contribution to extend the functional properties of an active packaging biomaterial. Apart from this, plant extracts as natural sources of polyphenolic compounds have captured people's attention more and more, and films blended with plant extracts seem "natural" and are welcomed. Studies have been published about the performance advantages of rosemary (*Rosmarinus officinalis*) and oregano (*Origanum vulgare*) (Gómez-Estaca et al. 2009a, b), aqueous extracts from the leaves of murta (*Ugni molinae*) (Gómez-Guillén et al. 2007), and water/alcohol extract from borage (*Borago officinalis*) seeds (Gómez-Estaca et al. 2009a, b).

5.4 Microencapsulation

Microencapsulation is produced by firstly refining liquid, solid, or gas, and taking the droplets (particles) as the core, making the composition film-forming material (wall material) deposited on the core, then coating it to form a thin film, which packages the heart droplets (particles) (Yan et al. 2008). It is interesting that gelatin coacervates can combine with anionic polymers in the form of microcapsules because of their ability to destroy functional components in a carrier and provide protection against oxidation or degradation in the process of storage. Furthermore, encapsulation can control the release of functional components from the food product or the bioactive packaging when ingested. Coacervates are made when a mixed dilute solution of gelatin and an anionic polyelectrolyte are brought to a certain pH, under the condition that polyelectrolytes have opposite net charges (Schreiber and Gareis 2007). Under these conditions, the microencapsulation formed.

The complex systems and natural cross-linkers have been used as a means of reinforcing gelatin microcapsules (Gómez-Guillén et al. 2011). Under oxidizing

conditions to form covalent cross-links, plant-derived polyphenols and flavonoids (contained in instant coffee and grape juice) have been shown to react with gelatin–pectin-based microparticular coacervates, which result in presenting a structure with greater mechanical strength and thermal stability, and less capacity to expand and absorb water. Furthermore, a complex coacervation microcapsule system has been applied in frozen baked foods, as they can encapsulate baking flavor oil in a gelatin gum Arabic system (Yeo et al. 2005). Besides, the incorporation of probiotic bacteria in functional food products has become an increasingly overwhelming trend due to their ability to exert beneficial effects on the intestinal microflora (Gobbetti et al. 2010).

In addition, on the incorporation of bioactive compounds, encapsulation technology could contribute to the development of active packaging materials. Apart from protecting the active potential of the natural extract and invoking its release into the covered food product, encapsulation may be used for the purposes of taste and odor masking, because vegetal extracts often have very strong flavors (Gómez-Guillén et al. 2011). As a first step in the development of new material with applications in the biomedical field, a gelatin/poly (L-lactide) (PLLA) composite has been suggested for producing homogeneous and well-shaped nanofibers by electrospinning, using gelatin extracted from channel catfish skin (An et al. 2010).

6 Applications of Fish Collagen and Gelatin

Fish gelatin has been widely used in the industry; it has been produced by acid extraction since 1960. Gelatin has been extracted from the skin and bones of various coldwater and warmwater fish (Karim and Bhat 2009).

Coldwater fish gelatin can be used in frozen or refrigerated products that are consumed quickly following removal from the fridge or defrosting (Karim and Bhat 2008, 2009) because the gelatin produced from the skin of fish living in cold waters does not gel at room temperature – its gelling temperature is below 8–10 °C (Gómez-Guillén et al. 2009). Gelatins with low melting points could also be used in dry products (Gómez-Guillén et al. 2011). In recent years, small volumes of fish gelatin have been used to make soft-gel capsules. The use of fish gelatin soft capsules is the most common in nutrition supplements (Karim and Bhat 2009).

The low gelling temperature of gelatin from coldwater fish can be used for the light-sensitive coatings, which are significant to the electronics trade (Karim and Bhat 2009). As a protein, gelatin is low in calories, but melts in the mouth to give brilliant sensory properties resembling fat, so it is ideal for use in low-fat products. Owing to a good pricing structure, a large volume of fish gelatin is considered by manufacturers.

Warmwater fish gelatin has a Bloom value of 200–250 g. For example, tuna is a good source, but the skin has a lot of fat, while gelatin must be fat-free. Tuna or tilapia gelatins have a melting point of 25–27 °C. Consequently, these gelatins should be stored at low room temperatures. These gelatins more closely resemble bovine or pig

gelatin, which melts at 32–35 °C. A sensory study on gelatin gel desserts recommended that fish gelatin with lower gel melting temperatures had a better release of aroma and offered a stronger flavor (Choi and Regenstein 2000). By increasing the concentration of gelatin, desserts made from fish gelatins would be more comparable to desserts made from high-Bloom pork skin gelatin (Zhou and Regenstein 2007).

Enzymatically cross-linked fish gels did not melt during heating in a boiling water bath (Kołodziejska et al. 2004). Fish gelatin could be used as a gelling component of sterilized products if the gel structure is not destroyed at higher temperature. Combinations of fish gelatin with other common hydrocolloids can extend the application of fish gelatin as a food ingredient. For instance, fish gelatin and pectin have been used to make a low-fat spread (Chen et al. 2009). An increase in bulk density, firmness, compressibility, adhesiveness, elasticity, and meltability is due to a decrease in the fish gelatin to pectin ratio. Fish gelatin has also been used in pharmaceutical products. It can be used for hard capsules (Park et al. 2007). Owing to the low gelling temperature property of fish, TGase can be used for cross-linking, which circumvented the problems of gelatin. Fish gelatin (Bloom value higher than 100) can also be used as an ingredient in drug tablets.

Gelatins can be used to establish harmlessness and latent functionality in food packaging applications, such as making edible films (Núñez-Flores et al. 2013). As gelatins have special texture and melt-in-the-mouth perception, it is one of the most versatile gelling agents. In addition to the food industry, gelatin can also be applied to the pharmaceutical and photographic industries (Haug et al. 2004a, b).

7 Bioactive Properties of Fish Gelatin Hydrolysates

Bioactive peptides can be used as food-derived components (genuine or generated) because of their nutritional value, which exert a physiological effect on the body (Ryan et al. 2011). Bioactive peptides from meat sources could be produced by using all kinds of proteases of bacterial, animal, and plant origin (Jang et al. 2008; Jang and Lee 2005; Liu et al. 2008). Bioactive peptides in the parent protein are inactive or latent, but they can display an active form after proteolytic digestion (Korhonen 2009). Digestive enzymes (Hernández-Ledesma et al. 2007), bacterial proteases (You et al. 2009; Zhu et al. 2006), and following microbial fermentation (Hayes et al. 2006; Tsai et al. 2008) are put into use in the proteolytic digestion of parent proteins, including hydrolysis. Dietary gelatin from a multitude of animals, especially fish gelatin, are a source of biologically active peptides, which are inactive in the parent protein sequence but can be liberated during gastrointestinal digestion, food processing, or fermentation (Erdmann et al. 2008; Gómez-Guillén et al. 2011; Nagpal et al. 2011; De Noni et al. 2009). Once they are released, bioactive peptides are considered to promote numerous physiological functions, such as opioids, mineral binding, immunomodulatory, as well as antimicrobial, antioxidative, antithrombotic, hypocholesterolemic, and antihypertensive activity (Guan et al. 2007; Hajirostamloo 2010; Sharma et al. 2011; Kittiphattanabawon et al. 2012).

7.1 Production of Bioactive Peptides

Due to promising health benefits for nutritional or pharmaceutical applications, collagen and gelatin have been considered as an important source of biologically active peptides (Gómez-Guillén et al. 2011). Aquatic animals, including freshwater and marine fish and mollusks as fish processing by-products, including heads, skin viscera, blood, offal, and tails, as well as seafood shells, coupled with their ready availability, are used to produce the mammalian hydrolysates and peptides with bioactive properties, which is accepted as a food additive by religious groups and for allergenic reasons (Chen et al. 2009; Haug et al. 2004a, b; Shon et al. 2011). The isolation of peptides from other aquatic animals with important biological activities includes collagen and gelatin obtained from sources like freshwater rotifers (*Brachionus calyciflorus*), jellyfish, bullfrog, or sea cucumber, and chum salmon (*Oncorhynchus keta*) (Sai et al. 2012; Qian et al. 2008; Lee 2010; Liang et al. 2010; Zhuang et al. 2009).

Gelatin and collagen-derived hydrolysates and peptides are generally acquired by means of enzymatic proteolysis. A number of commercial proteases have been used for the production of these hydrolysates and peptides, including trypsin, chymotrypsin, pepsin, alcalase, flavourzyme, properase E, pronase, collagenase, bromelain, and papain (Kim and Mendis 2006; Yang et al. 2008a; Haug et al. 2004a, b; Kechaou et al. 2009; Jongjareonrak et al. 2005; Pihlanto 2006). Besides commercial proteases, enzymatic extracts from fish viscera have been used to obtain bioactive hydrolysates from the skin and bones of different fish species (Herpandi and Adzitey 2011; Yang et al. 2008b; Gómez-Guillén et al. 2011; Nalinanon et al. 2011). The biological activity of the hydrolysates is affected by size, amount, free amino acid composition, peptides, and their amino acid sequences of protease (Cuerrier 2005; Santos et al. 2009). Fish protein is hydrolyzed efficiently according to proteases such as alcalase and flavourzyme (Safari et al. 2012; Dumay et al. 2006; Kristinsson and Rasco 2000; Normah et al. 2005), which have broad specificity and a high degree of hydrolysis is achieved in a relatively short time under optimum conditions (40 °C, 60 min, pH 8–9) (Cigić and Zelenik-Blatnik 2004; Kamara et al. 2011). These enzymes manifested extensively proteolytic activity during the hydrolysis of gelatin from tuna skin, muscle of brownstripe red snapper, Korean rockfish, cod (*Gadus morhua*) backbones, and pyloric caeca extract from bigeye snapper for producing hydrolysates with high antioxidant activity and inhibitory capacity which exhibited low average molecular weight (Kim et al. 2011; Khantaphant and Benjakul 2008; Khantaphant et al. 2011; Nalinanon et al. 2011; Phanturat et al. 2010).

The biological properties of protein hydrolysates are seriously affected by their average molecular weight (Gómez-Guillén et al. 2011; Šližytė et al. 2009). Peptide fractions from protein hydrolysates may be different in their effectiveness for a given biological activity. An ultrafiltration membrane system that could obtain peptide fractions with a desired molecular size and a higher bioactivity is a useful and industrially advantageous method (Ajibola et al. 2011; Picot et al. 2010).

A majority of the studies regarding collagen and gelatin-derived peptides is related to their mineral binding, opioid, antimicrobial, antioxidative, inhibitory, immunomodulatory, antithrombotic, hypocholesterolemic, and antihypertensive

activity (Kim and Mendis 2006; Gómez-Guillén et al. 2011; Guan et al. 2007; Hajirostamloo 2010; Sharma et al. 2011). The unique Gly-Pro-Hyp sequences have been repeated in their structure for these peptides, and this unique amino acid composition played an important role in the antioxidative and antihypertensive properties of these peptides (Kim and Mendis 2006).

With the hydrolysis of the protein substrate, various bioactivities are assayed from the hydrolysates. After the bioactivity of the crude protein hydrolysates was detected, ultrafiltration is used to separate the hydrolysates by peptide size (Iroyukifujita et al. 2000; Kim et al. 2001; Li et al. 2004). Individual peptides are obtained by means of purifying the hydrolysate fraction with the highest bioactivity using different techniques, most notably, reverse-phase high-performance liquid chromatography (RP-HPLC) or gel permeation chromatography (Hernández-Ledesma et al. 2007; Pihlanto et al. 2008; Qian et al. 2007). The combined techniques of mass spectrometry and protein sequencing are used to identify individual peptide fractions. Finally, a combining version of the peptide is synthesized, while its bioactivity is verified by repeating the assay (Saiga et al. 2006; Tsai et al. 2008; Rutherfurd and Gill 2000).

7.2 Antihypertensive Peptides

Antihypertensive peptides are peptide molecules which may lower blood pressure when ingested via the inhibition of vasoactive enzymes such as the angiotensin-converting enzyme (ACE). ACE can transfer angiotensin I to angiotensin II, which can constrict blood vessels and inactivate bradykinin, which is a vasodilator (Anthony et al. 2010). Therefore, ACE plays an important role in the regulation of blood pressure by means of the rennin–angiotensin system, while the inhibition of this enzyme is considered to be a useful therapeutic approach in the treatment of hypertension (Li et al. 2004; Liu et al. 2012; Wijesekara and Kim 2010).

Synthetic ACE inhibitors such as captopril have emerged at the right moment. Moreover, captopril and other synthetic ACE inhibitor drugs often cause various side-effects, such as coughing, skin rashes, and taste disturbances (Pihlanto 2006; Vermeirssen et al. 2002). Hypertension, a disease affecting one-third of the Western worlds' population, is often called a "silent killer" because people with hypertension are often asymptomatic for years (Gao et al. 2010). Thus, it is recognized as a risk factor for developing cardiovascular disease, which has motivated the ongoing exploitation of food-derived antihypertensive peptides, and those peptides are usually considered as antihypertensive components in nutraceuticals and functional foods (López-Fandiño et al. 2006; Vermeirssen et al. 2002).

Collagen and gelatin from marine sources such as fish skin (Byun and Kim 2001; Kim et al. 2011), scales (Fahmi et al. 2004), squid tunics (Alemán et al. 2011), and sea cucumbers (Zhao et al. 2007) have been shown to be good sources of antihypertensive peptides by enzymatic digestion. However, collagen and gelatin from marine sources have been studied less extensively compared to those from other sources. The peptide sequences identified from collagenous materials are shown in Table 12.1.

Table 12.1 Antihypertensive peptides derived from marine collagen and gelatin fish by-products

Source	Characteristics	References
Lampern (*Lampetra fluviatilis*)		
Brain, gill, gonad, gut, heart, liver, skeletal muscle, skin, kidney, and plasma		(Cobb et al. 2004)
Alaska pollock (*Theragra chalcogramma*)		
Skin, bronchial heart, gill, kidney, duct, liver, whole brain, and gut	Gly-Pro-Leu	(Byun and Kim 2001) (Cobb et al. 2004)
Sea bream		
Scale	Gly-Tyr, Val-Tyr, Gly-Phe, and Val-Ile-Tyr	(Fahmi et al. 2004)
Sardinelle (*Sardinella aurita*)		
Heads and viscera	Phe, Arg, Gly, Leu, Meth, Hist, Try	(Bougatef et al. 2008)
Grass carp fish (*Ctenopharyngodon idella*)		
Scale		(Zhang et al. 2009)
Tilapia		
Fillet		(Raghavan and Kristinsson 2009)
Tuna		
Frame proteins	Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro	(Lee 2010)
Shrimp (*Pandalopsis dispar*)		
Shells, tails, and heads	Tyr, Phe, Leu, Ile, Val and Lys	(Cheung and Li-Chan 2010)
Squid (*Dosidicus eschrichitii Steenstrup*)		
Skin		(Lin et al. 2012)
Atlantic salmon (*Salmo salar*) and wild caught cod (*Gadus morhua*)		
Muscle, skin, frame, brain, eye, heart, gill, kidney, stomach and/or intestine, pyloric caeca, bile, liver, spleen, sperm, eggs, and blood		(Gu et al. 2011) (Dragnes et al. 2009)
Cuttlefish (*Sepia officinalis*)	Tyr-Ala-Pro, Val-Ile-Ile-Phe and Met-Ala-Trp	
Muscle		(Balti et al. 2010)
Freshwater clam (*Corbicula fluminea, Müller*)		
Muscle	Val-Lys-Pro and Val-Lys-Lys	(Tsai et al. 2006)
Bullfrog (*Rana catesbeiana Shaw*)	Gly-Ala-Ala-Glu-Leu-Pro-Cys-Ser-Ala-Asp-Trp-Trp	
Muscle		(Qian et al. 2007)

7.3 Antioxidant

Uncontrolled production of free radicals with increased production of reactive oxygen species (ROS) that attack macromolecules such as membrane lipids, proteins, and DNA may lead to many health disorders, such as cancer, diabetes mellitus, and neurodegenerative and inflammatory diseases, with severe tissue injuries (Erdmann et al. 2008). Additionally, the oxidation of lipids or rancidity can result in the formation of undesirable secondary lipid peroxidation products that can eventually cause the deterioration of some foods (Kim and Wijesekara 2010).

Antioxidants can protect the human body against molecules known as ROS, which have an adverse affect on membrane lipids, protein, and DNA. This, in turn, can be a causative factor in many diseases, such as cardiovascular disease, diabetes, cancer, and Alzheimer's disease. The deterioration of food quality and a reduction in the shelf life of a food product are caused by lipid oxidation, while people have various diseases, such as cancers, diabetes, and cardiovascular disease, after consuming foods containing lipid oxidation (Giménez et al. 2009; Waris and Ahsan 2006).

Many synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG), have been extensively used to minimize the hazards brought about by lipid oxidation in foods. However, synthetic antioxidants in vivo can lead to potential health risks. Therefore, the exploitation of natural antioxidants in foodstuffs has aroused researchers' interests (Moure et al. 2001; Pihlanto 2006; Vercruysse et al. 2009). In the past several years, antioxidant peptides extracted from livestock and poultry have been the focus of much research. However, due to the outbreak of animal diseases (Wangtueai and Noomhorm 2009; Jongjareonrak et al. 2005) and esthetic and religious objections (Shon et al. 2011), an alternative to mammalian gelatin in the form of aquatic animals has been considered and further processing to yield collagen can help reduce harmful environmental effects (Haug et al. 2004a, b; Shon et al. 2011).

Recently, a number of studies have demonstrated that peptides derived from different marine collagen and gelatin hydrolysates act as potential antioxidants and have been isolated from marine organisms such as jumbo squid (Mendis et al. 2005; Rajapakse et al. 2005), oyster (Qian et al. 2008), blue mussel (Rajapakse et al. 2005), hoki (Je et al. 2005; Kim and Mendis 2006; Mendis et al. 2005), tuna (Je et al. 2007), cod (Šližytė et al. 2009), mackerel (Wu et al. 2003), Alaska pollock (Je et al. 2005; Jia et al. 2010), yellowstripe trevally (Klompong et al. 2009), mackerel muscle protein (Wu et al. 2003), conger eel (Ranathunga et al. 2006), squid skin gelatin (Mendis et al. 2005; Rajapakse et al. 2005), fish skin gelatin (Mendis et al. 2005), tuna backbone (Je et al. 2007), tuna cooking juice (Hsu et al. 2009), cobia (*Rachycentron canadum*) (Yang et al. 2008b), microalgae (Sheih et al. 2009), and sole (Giménez et al. 2009), as well as from several squid species, such as giant squid (*Dosidicus gigas*) (Rajapakse et al. 2005), jumbo flying squid (*Dosidicus eschrichitii Steenstrup*) (Lin and Li 2006), or squid (*Todarodes pacificus*) (Giménez et al. 2009).

The beneficial effects of marine collagen and gelatin peptides are well known in scavenging free radicals and ROS or in preventing oxidative damage by interrupting the radical chain reaction of lipid peroxidation (Mendis et al. 2005; Qian et al. 2008;

Rajapakse et al. 2005). Bioactive peptides isolated from collagen and gelatin from jumbo squid exhibited the inhibitory activity in lipid peroxidation, and its activity has been determined using a linoleic acid model system, which was much higher than a-tocopherol and close to that of BHT (Mendis et al. 2005). The hydrophobic amino acids in the peptide mostly play a vital role in these antioxidant activities (Mendis et al. 2005). In addition, the bioactive antioxidant collagen and gelatin peptides Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu, isolated from oyster (*Crassostrea gigas*), showed a stronger inhibitory activity against polyunsaturated fatty acid peroxidation than a-tocopherol (Qian et al. 2008).

Radicals formed during peroxidation, metal-chelating ability, or oxygen-containing compounds can be eliminated by antioxidant peptides. Furthermore, peptides derived from marine fish collagen and gelatin peptides have greater antioxidant properties than a-tocopherol in different oxidative systems (Jung et al. 2005; Rajapakse et al. 2005). Peptides derived from marine gelatin can exhibit higher antioxidant activities compared to other antioxidant peptides sequences, due to the higher emulsifying ability, which is attributed to the abundance of hydrophobic amino acids (Mendis et al. 2005).

The antioxidant collagen and gelatin peptides have been considered as lipid peroxidation inhibitors, free radical scavengers, and chelators of transition metal ions. However, there are no published studies reporting the exact mechanism underlying the antioxidant activity of collagen and gelatin peptides (Gómez-Guillén et al. 2011). Gelatin peptides can show a higher inhibitory ratio of lipid peroxidation compared to other antioxidative peptides sourced from many other proteins (Gómez-Guillén et al. 2011). The challenge for the development of antioxidant peptides is to create novel functional foods and medicines without the undesired side effects (Ngo et al. 2012).

8 Conclusions and Future Outlook

Because of its unique physiological function and physical and chemical characteristics, collagens are widely used in value-added products such as medicine, food, and cosmetics. The consumption of fish collagen and gelatin has increased with the development of new industrial applications. Extracting collagens or collagen peptides from aquatic products is gradually becoming a hot worldwide research topic. Because of several animal diseases, as well as esthetic and religious objections, developing collagen from new raw materials has increasingly aroused the interest of researchers and manufacturers alike. With the development of fishery, researchers and manufacturers have paid more attention to aquatic comprehensive utilization. Collagen is rich in fish processing by-products such as skin, bone, scales, and fins. Therefore, the waste from fish processing is an excellent raw material for preparing collagen.

In the pharmaceutical industry, due to its good biological characteristics and low immunogenicity, collagen can be used in the medical areas of cells, tissue, trauma, or even organ repair.

In the field of cosmetics, collagen has a very safe record for nutritional repair, moisturizing, compatibility, and other features. For example, collagen can increase its nutritional moisturizing effect by adding in cosmetic emulsion, it can be used as a raw material for the production of cosmetic fillers, and the condensation of oleoyl chloride production of the anionic surfactant can be modified for shampoos.

In the food industry, collagen is mainly used in the healthy production of food packaging, meat additives, nutritional food, and so on. At present, fish gelatin has been widely used in canned drinks, dairy products, meat products, fruit wine thickeners, emulsifiers, stabilizers, and clarifying agents.

However, the undesirable physicochemical properties of fish gelatin, including the temperature in the formation of fish gelatin, higher economic cost, and lower yield compared to mammal gelatin, severely limit its large-scale production.

The development of lower cost and higher quality fish gelatin with minimal or even no contaminants (chemical or microbial) is at the top of the agenda.

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References

Aberoumand A (2010) Isolation and characteristics of collagen from fish waste material. World J Fish Mar Sci 2(5):471–474

Aberoumand A (2012) Comparative study between different methods of collagen extraction from fish and its properties. World Appl Sci J 16(3):316–319

Achilli M, Mantovani D (2010) Tailoring mechanical properties of collagen-based scaffolds for vascular tissue engineering: the effects of pH, temperature and ionic strength on gelation. Polymer 2(4):664–680

Aewsiri T, Benjakul S, Visessanguan W, Tanaka M (2008) Chemical compositions and functional properties of gelatin from pre-cooked tuna fin. Int J Food Sci Technol 43(4):685–693

Aewsiri T, Benjakul S, Visessanguan W (2009) Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. Food Chem 115(1):243–249

Ahmad M, Benjakul S, Nalinanon S (2010) Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (*Aluterus monoceros*). Food Hydrocoll 24(6–7):588–594

Ajibola CF, Fashakin JB, Fagbemi TN, Aluko RE (2011) Effect of peptide size on antioxidant properties of African Yam bean seed (*Sphenostylis stenocarpa*) protein hydrolysate fractions. Int J Mol Sci 12(10):6685–6702

Alemán A, Giménez B, Montero PA, Gómez-Guillén MC (2011) Antioxidant activity of several marine skin gelatins. LWT Food Sci Technol 44(2):407–413

Amiza MA, Siti Aishah D (2011) Effect of drying and freezing of Cobia *(Rachycentron canadum)* skin on its gelatin properties. Int Food Res J 18:159–166

An K, Liu H, Guo S, Kumar DNT, Wang Q (2010) Preparation of fish gelatin and fish gelatin/poly(L-lactide) nanofibers by electrospinning. Int J Biol Macromol 47(3):380–388

Anthony CS, Corradi HR, Schwager SLU, Redelinghuys P, Georgiadis D, Dive V, Ravi Acharya K, Sturrock ED (2010) The N domain of human angiotensin-I-converting enzyme: the role of N-glycosylation and the crystal structure in complex with an N domain-specific phosphinic inhibitor, RXP407. J Biol Chem 285(46):35685–35693

Arvanitoyannis IS, Nakayama A, Aiba S (1998) Chitosan and gelatin based edible films: state diagrams, mechanical and permeation properties. Carbohydr Polym 37:371–382

Balti R, Nedjar-Arroume N, Bougatef A, Guillochon D, Nasri M (2010) Three novel angiotensin I-converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) using digestive proteases. Food Res Int 43(4):1136–1143

Benjakul S, Oungbho K, Visessanguan W, Thiansilakul Y, Roytrakul S (2009) Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chem 116(2):445–451

Bertan LC, Tanada-Palmu PS, Siani AC, Grosso CRF (2005) Effect of fatty acids and 'Brazilian elemi' on composite films based on gelatin. Food Hydrocoll 19:73–82

Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N (2001) Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. Biomaterials 22:763–768

Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK (2009) Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel-forming ability of fish mince. Food Hydrocoll 23(1):132–145

Boot-Handford RP, Tuckwell DS, Plumb DA, Rock CR, Poulsom R (2003) A novel and highly conserved collagen (pro(alpha)1(XXVII)) with a unique expression pattern and unusual molecular characteristics establishes a new clade within the vertebrate fibrillar collagen family. J Biol Chem 278(33):31067–31077

Bougatef A, Nedjar-Arroume N, Ravallec-Plé R, Leroy Y, Guillochon D, Barkia A, Nasri M (2008) Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (*Sardinella aurita*) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases. Food Chem 111(2):350–356

Brenner T, Nicolai T, Johannsson R (2009) Rheology of thermo-reversible fish protein isolate gels. Food Res Int 42(8):915–924

Brinckmann J (2005) Collagens at a glance. Top Curr Chem 247:1–6

Byun H-G, Kim S-K (2001) Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (*Theragra chalcogramma*) skin. Process Biochem 36:1155–1162

Canty EG, Kadler KE (2005) Procollagen trafficking, processing and fibrillogenesis. J Cell Sci 118(7):1341–1353

Cao N, Yang X, Fu Y (2009) Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. Food Hydrocoll 23:729–735

Carvalho RA, Maria TMC, Moraes ICF, Bergo PVA, Kamimura ES, Habitante AMQB, Sobral PJA (2009) Study of some physical properties of biodegradable films based on blends of gelatin and poly (vinyl alcohol) using a response-surface methodology. Mater Sci Eng C 29:485–491

Chen M, Costa FK, Lindvay CR, Han YP, Woodley DT (2002) The recombinant expression of full-length type VII collagen and characterization of molecular mechanisms underlying dystrophic epidermolysis bullosa. J Biol Chem 277(3):2118–2124

Chen H-H, Lin C-H, Kang H-Y (2009) Maturation effects in fish gelatin and HPMC composite gels. Food Hydrocoll 23(7):1756–1761

Cheung IWY, Li-Chan ECY (2010) Angiotensin-I-converting enzyme inhibitory activity and bitterness of enzymatically-produced hydrolysates of shrimp (*Pandalopsis dispar*) processing byproducts investigated by Taguchi design. Food Chem 122(4):1003–1012

Chiou B-S, Avena-Bustillos R, Shey J, Yee E, Bechtel PJ, Imam SH, Glenn GM, Orts WJ (2006) Rheological and mechanical properties of cross-linked fish gelatins. Polymer 47(18):6379–6386

Choi S-S, Regenstein JM (2000) Physicochemical and sensory characteristics of fish gelatin. J Food Sci 65:194–199

Christiano AM, Greenspan DS, Lee S, Uitto J (1994) Glycine substitutions in the triple-helical region of type VII collagen result in a spectrum of dystrophic epidermolysis bullosa phenotypes and patterns of inheritance. J Biol Chem 269:20256–20262

Cigić BA, Zelenik-Blatnik M (2004) Preparation and characterization of chicken egg white hydrolysate. Acta Chim Slov 51:177–188

Cobb CS, Frankling SC, Thorndyke MC, Jensen FB, Rankin JC, Brown JA (2004) Angiotensin I-converting enzyme-like activity in tissues from the Atlantic hagfish (*Myxine glutinosa*) and detection of immunoreactive plasma angiotensins. Comp Biochem Physiol Part B Biochem Mol Biol 138(4):357–364

Colige A, Ruggiero F, Vandenberghe I, Dubail J, Kesteloot F, Van Beeumen J, Beschin A, Brys L, Lapière CM, Nusgens B (2005) Domains and maturation processes that regulate the activity of ADAMTS-2, a metalloproteinase cleaving the aminopropeptide of fibrillar procollagens types I–III and V. J Biol Chem 280(41):34397–34408

Cuerrier D, Moldoveanu T, Davies PL (2005) Determination of peptide substrate specificity for mu-calpain by a peptide library-based approach: the importance of primed side interactions. J Biol Chem 280(49):40632–40641

De Noni I, FitzGerald RJ, Korhonen HJT et al (2009) Review of the potential health impact of β-casomorphins and related peptides. EFSA Sci Rep 231:1–107

Deiber JA, Ottone ML, Piaggio MV, Peirotti MB (2009) Characterization of cross-linked polyampholytic gelatin hydrogels through the rubber elasticity and thermodynamic swelling theories. Polymer 50(25):6065–6075

Denavi GA, Pérez-Mateos P, Añón MC, Montero P, Mauri AN, Gómez-Guillén MC (2009) Structural and functional properties of soy protein isolate and cod gelatin blend films. Food Hydrocoll 23:2094–2101

Dickinson E, Lopez G (2001) Comparison of the emulsifying properties of fish gelatin and commercial milk proteins. J Food Sci 66:118–123

Djabourov M, Lechaire JP, Gaill F (1992) Structure and rheology of gelatin and collagen gels. Biorheology 30(3–4):191–205

Dong S, Zeng M, Wang D, Liu Z, Zhao Y, Yang H (2008) Antioxidant and biochemical properties of protein hydrolysates prepared from Silver carp (*Hypophthalmichthys molitrix*). Food Chem 107(4):1485–1493

dos Santos SDA, Martins VG, Salas-Mellado M, Prentice C (2009) Evaluation of functional properties in protein hydrolysates from bluewing searobin (*Prionotus punctatus*) obtained with different microbial enzymes. Food Bioprocess Technol 4(8):1399–1406

Dragnes BT, Stormo SK, Larsen R, Ernstsen HH, Elvevoll EO (2009) Utilisation of fish industry residuals: screening the taurine concentration and angiotensin converting enzyme inhibition potential in cod and salmon. J Food Compos Anal 22(7–8):714–717

Dumay J, Donnay-Moreno C, Barnathan G, Jaouen P, Bergé JP (2006) Improvement of lipid and phospholipid recoveries from sardine (*Sardina pilchardus*) viscera using industrial proteases. Process Biochem 41(11):2327–2332

Elomaa O, Sankala M, Pikkarainen T, Bergmann U, Tuuttila A, Raatikainen-Ahokas A, Sariola H, Tryggvason K (1998) Structure of the human macrophage MARCO receptor and characterization of its bacteria-binding region. J Biol Chem 273:4530–4538

Erdmann K, Cheung BWY, Schröder H (2008) The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutr Biochem 19(10):643–654

Eyre DR, Paz MA, Gallop PM (1984) Cross-linking in collagen and elastin. Annu Rev Biochem 53:717–748

Fahmi A, Morimura S, Guo HC, Shigematsu T, Kida K, Uemura Y (2004) Production of angiotensin I converting enzyme inhibitory peptides from sea bream scales. Process Biochem 39(10):1195–1200

Farris S, Schaich KM, Liu L, Piergiovanni L, Yam KL (2009) Development of polyion-complex hydrogels as an alternative approach for the production of bio-based polymers for food packaging applications: a review. Trends Food Sci Technol 20(8):316–332

Franzke CW, Bruckner P, Bruckner-Tuderman L (2005) Collagenous transmembrane proteins: recent insights into biology and pathology. J Biol Chem 280(6):4005–4008

Friess W (1998) Collagen–biomaterial for drug delivery. Eur J Pharm Biopharm 45(2):113–136

Gao D, Chang T, Li H, Cao Y (2010) Angiotensin I-converting enzyme inhibitor derived from cottonseed protein hydrolysate. Afr J Biotechnol 9:8977–8982

Gelse K, Pöschl E, Aigner T (2003) Collagens–structure, function, and biosynthesis. Adv Drug Deliv Rev 55(12):1531–1546

Giménez B, Alemán A, Montero P, Gómez-Guillén MC (2009) Antioxidant and functional properties of gelatin hydrolysates obtained from skin of sole and squid. Food Chem 114(3):976–983

GMIA (2012) Gelatin handbook. Gelatin Manufacturers Institute of America

Gómez-Estaca J, Bravo L, Gómez-Guillén MC, Alemán A, Montero P (2009a) Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chem 112:18–25

Gómez-Estaca J, Giménez B, Montero P, Gómez-Guillén MC (2009b) Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. J Food Eng 92:78–85

Gómez-Guillén MC, Sarabia AI, Solas MT, Montero P (2001) Effect of microbial transglutaminase on the functional properties of megrim (*Lepidorhombus boscii*) skin gelatin. J Sci Food Agric 81:665–673

Gómez-Guillén MC, Turnay J, Fernández-Dıaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll 16:25–34

Gómez-Guillén MC, Ihl M, Bifani V, Silva A, Montero P (2007) Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae Turcz*). Food Hydrocoll 21:1133–1143

Gómez-Guillén MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez B, Montero P (2009) Fish gelatin: a renewable material for developing active biodegradable films. Trends Food Sci Technol 20:3–16

Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocoll 25:1813–1827

Gobbetti M, Cagno RD, De Angelis M (2010) Functional microorganisms for functional food quality. Crit Rev Food Sci Nutr 50(8):716–727

Grant ME (2007) From collagen chemistry towards cell therapy – a personal journey. Int J Exp Pathol 88(4):203–214

Greenspan DS (2005) Biosynthetic processing of collagen molecules. Top Curr Chem 47:149–183

Gu R-Z, Li C-Y, Liu W-Y, Yi W-X, Cai M-Y (2011) Angiotensin I-converting enzyme inhibitory activity of low-molecular-weight peptides from Atlantic salmon (*Salmo salar* L.) skin. Food Res Int 44(5):1536–1540

Hajirostamloo B (2010) Bioactive component in milk and dairy product. World Acad Sci Eng Technol 72:162–166

Haroun AA, Migonney V (2010) Synthesis and in vitro evaluation of gelatin/hydroxyapatite graft copolymers to form bionanocomposites. Int J Biol Macromol 46:310–316

Haug IJ, Draget KI, Smidsrød O (2004a) Physical behaviour of fish gelatin-κ-carrageenan mixtures. Carbohydr Polym 56(1):11–19

Haug IJ, Draget KI, Smidsrød O (2004b) Physical and rheological properties of fish gelatin compared to mammalian gelatin. Food Hydrocoll 18:203–213

Hayes M, Ross RP, Fitzgerald GF, Hill C, Stanton C (2006) Casein-derived antimicrobial peptides generated by *Lactobacillus acidophilus* DPC6026. Appl Environ Microbiol 72(3):2260–2264

Hernández-Izquierdo VM, Krochta JM (2008) Thermoplastic processing of proteins for film formation—a review. J Food Sci 73:R30–R39

Hernández-Ledesma B, Quirós A, Amigo L, Recio I (2007) Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. Int Dairy J 17(1):42–49

Herpandi NH, Adzitey F (2011) Fish bone and scale as a potential source of halal gelatin. J Fish Aquat Sci 6(4):379–389

Herpandi NH, Rosma A, Wan Nadiah WA (2011) The tuna fishing industry: a new outlook on fish protein hydrolysates. Compr Rev Food Sci Food Saf 10(4):195–207

Hone JHE, Howe AM (2002) Viscosity of colloidal suspensions in aqueous gelatin. J Colloid Interface Sci 251:193–199

Hsu K-C, Lu G-H, Jao C-L (2009) Antioxidative properties of peptides prepared from tuna cooking juice hydrolysates with orientase (*Bacillus subtilis*). Food Res Int 42(5–6):647–652

Ikoma T, Kobayashi H, Tanaka J, Walsh D, Mann S (2003) Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticas*. Int J Biol Macromol 32(3–5):199–204

Iroyukifujita H, Eiichiyokoyama K, Yoshikawa M (2000) Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. J Food Sci 65:564–569

Jang A, Lee M (2005) Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. Meat Sci 69:653–661

Jang A, Jo C, Kang KS, Lee M (2008) Antimicrobial and human cancer cell cytotoxic effect of synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. Food Chem 107:327–336

Je J-Y, Park P-J, Kim S-K (2005) Antioxidant activity of a peptide isolated from Alaska pollack (*Theragra chalcogramma*) frame protein hydrolysate. Food Res Int 38(1):45–50

Je J-Y, Qian Z-J, Byun H-G, Kim S-K (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42(5):840–846

Jia J, Zhou Y, Lu J, Chen A, Li Y, Zheng G (2010) Enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. J Sci Food Agric 90(4):635–640

Johnston-Banks FA (1990) Gelatin. In: Harris P (ed) Food gels. Elsevier Applied Science, London, pp 233–289

Jongjareonrak A, Benjakul S, Visessanguan W, Tanaka M (2005) Isolation and characterization of collagen from bigeye snapper (*Priacanthus macracanthus*) skin. J Sci Food Agric 85(7):1203–1210

Jongjareonrak A, Rawdkuen S, Chaijan M, Benjakul S, Osako K, Tanaka M (2010) Chemical compositions and characterisation of skin gelatin from farmed giant catfish (*Pangasianodon gigas*). LWT Food Sci Technol 43(1):161–165

Jung W-K, Park P-J, Byun H-G, Moon S-H, Kim S-K (2005) Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. Food Chem 91(2):333–340

Jung W-K, Karawita R, Heo S-J, Lee B-J, Kim S-K, Jeon Y-J (2006) Recovery of a novel Ca-binding peptide from Alaska Pollack (*Theragra chalcogramma*) backbone by pepsinolytic hydrolysis. Process Biochem 41(9):2097–2100

Kadler KE, Baldock C, Bella J, Boot-Handford RP (2007) Collagens at a glance. J Cell Sci 120(12):1955–1958

Kamara MT, Amadou I, Kexue Z, Kelfala Foh MB, Huiming Z (2011) The influence of debittering and desalting on defatted foxtail millet (*Setaria italica* L.) protein hydrolysate. Am J Biochem Mol Biol 1(1):39–53

Karim AA, Bhat R (2008) Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends Food Sci Technol 19(12):644–656

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll 23(3):563–576

Kechaou ES, Dumay J, Donnay-Moreno C, Jaouen P, Gouygou J-P, Bergé J-P, Amar RB (2009) Enzymatic hydrolysis of cuttlefish (*Sepia officinalis*) and sardine (*Sardina pilchardus*) viscera using commercial proteases: effects on lipid distribution and amino acid composition. J Biosci Bioeng 107(2):158–164

Khantaphant S, Benjakul S (2008) Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with antioxidative activity. Comp Biochem Physiol Part B Biochem Mol Biol 151(4):410–419

Khantaphant S, Benjakul S, Ghomi MR (2011) The effects of pretreatments on antioxidative activities of protein hydrolysate from the muscle of brownstripe red snapper (*Lutjanus vitta*). LWT Food Sci Technol 44(4):1139–1148

Khoshnoodi J, Cartailler JP, Alvares K, Veis A, Hudson BG (2006) Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem 281(50):38117–38121

Kim SK, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39(4):383–393

Kim J-S, Park JW (2004) Characterization of acid-soluble collagen from pacific whiting surimi processing byproducts. J Food Sci 69(8):C637–C642

Kim JS, Park JW (2005) Partially purified collagen from refiner discharge of Pacific whiting surimi processing. J Food Sci 70(8):c511–c516

Kim S-K, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2(1):1–9

Kim S-K, Byun H-G, Park P-J, Shahidi F (2001) Angiotensin I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. J Agric Food Chem 49:2992–2997

Kim J-C, Lee HY, Kim MH, Lee H-J, Kang H-Y, Kim SM (2006) Preparation and characterization of chitosan/gelatin microcapsules containing triclosan. Colloids Surf B Biointerfaces 52:52–56

Kim H-J, Park K-H, Shin J-H, Lee J-S, Heu M-S, Lee D-H, Kim J-S (2011) Fractionation and characterization of fractions with high antioxidative activity from the gelatin hydrolysates of Korean rockfish *Sebastes schlegelii* skin. Fish Aquat Sci 14(3):168–173

Kittiphattanabawon P, Benjakul S, Visessanguan W, Shahidi F (2012) Gelatin hydrolysate from blacktip shark skin prepared using papaya latex enzyme: antioxidant activity and its potential in model systems. Food Chem 135(3):1118–1126

Kizilay E, Kayitmazer AB, Dubin, PL (2011) Complexation and coacervation of polyelectrolytes with oppositely charged colloids. Adv Colloid Interfac 167(1–2):24–37

Klemmer KJ, Waldner L, Stone A, Low NH, Nickerson MT (2012) Complex coacervation of pea protein isolate and alginate polysaccharides. Food Chem 130(3):710–715

Klompong V, Benjakul S, Yachai M, Visessanguan W, Shahidi F, Hayes KD (2009) Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe trevally (*Selaroides leptolepis*). J Food Sci 74:C126–C133

Koch M (2003) Collagen XXIV, a vertebrate fibrillar collagen with structural features of invertebrate collagens: selective expression in developing cornea and bone. J Biol Chem 278(44):43236–43244

Kołodziejska I, Piotrowska B (2007) The water vapour permeability, mechanical properties and solubility of fish gelatin–chitosan films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and plasticized with glycerol. Food Chem 103:295–300

Kołodziejska I, Kaczorowski K, Piotrowska B, Sadowska M (2004) Modification of the properties of gelatin from skins of Baltic cod (*Gadus morhua*) with transglutaminase. Food Chem 86(2):203–209

Kołodziejska I, Skierka E, Sadowska M, Kołodziejski W, Niecikowska C (2008) Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem 107(2):700–706

Korhonen H (2009) Milk-derived bioactive peptides: from science to applications. J Funct Foods 1(2):177–187

Kristinsson HG, Rasco BA (2000) Kinetics of the hydrolysis of Atlantic salmon (*Salmo salar*) muscle proteins by alkaline proteases and a visceral serine protease mixture. Process Biochem 36:131–139

Lamandé SR, Mörgelin M, Adams NE, Selan C, Allen JM (2006) The C5 domain of the collagen VI α3 (VI) chain is critical for extracellular microfibril formation and is present in the extracellular matrix of cultured cells. J Biol Chem 281(24):16607–16614

Langmaier F, Mokrejs P, Kolomaznik K, Mládek M (2008) Biodegradable packing materials from hydrolysates of collagen waste proteins. Waste Manag 28(3):549–556

Lee KY, Shim J, Lee HG (2004) Mechanical properties of gellan and gelatin composite films. Carbohydr Polym 56:251–254

Lee JK, Yun JH, Jeon J-K, Kim S-K, Byun H-G (2010) Effect of antioxidant peptide isolated from *Brachionus calyciflorus*. J Korean Soc Appl Biol Chem 53(2):192–197

Li G-H, Le G-W, Shi Y-H, Shrestha S (2004) Angiotensin I–converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. Nutr Res 24(7):469–486

Li B, Kennedy JF, Jiang QG, Xie BJ (2006) Quick dissolvable, edible and heatsealable blend films based on konjac glucomannan – gelatin. Food Res Int 39:544–549

Li G-H, Qu M-R, Wan J-Z, You J-M (2007) Antihypertensive effect of rice protein hydrolysate with in vitro angiotensin I-converting enzyme inhibitory activity in spontaneously hypertensive rats. Asia Pac J Clin Nutr 16(Suppl 1):275–280

Li W, Wu G, Chen H, Wang M (2009) Preparation and characterization of gelatin/SDS/NaCMC microcapsules with compact wall structure by complex coacervation. Colloids Surf A Physicochem Eng Asp 333:133–137

Liang J, Pei XR, Wang N, Zhang ZF, Wang JB, Li Y (2010) Marine collagen peptides prepared from chum salmon (*Oncorhynchus keta*) skin extend the life span and inhibit spontaneous tumor incidence in Sprague–Dawley rats. J Med Food 13(4):757–770

Lin L, Li B-F (2006) Radical scavenging properties of protein hydrolysates from Jumbo flying squid (*Dosidicus eschrichitii* Steenstrup) skin gelatin. J Sci Food Agric 86(14):2290–2295

Lin W, An X, Yang F, Xin Z, Zhao L, Hu Q (2008) Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*). Food Chem 108(2):616–623

Lin L, Lv S, Li B (2012) Angiotensin-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. Food Chem 131(1):225–230

Liu HY, Li D, Guo SD (2007) Studies on collagen from the skin of channel catfish (*Ictalurus punctaus*). Food Chem 101(2):621–625

Liu Z, Dong S, Xu J, Zeng M, Song H, Zhao Y (2008) Production of cysteine-rich antimicrobial peptide by digestion of oyster (*Crassostrea gigas*) with alcalase and bromelin. Food Control 19:231–235

Liu L, Lu B, Liu L, Wu X, Zhang Y (2012) Studies on bioactive peptide from Chinese soft-shelled turtle (*Pelodiscus sinensis*) with functionalities of ACE inhibition and antioxidation. Afr J Biotechnol 11(25):6723–6729

López-Fandiño R, Otte J, Van Camp J (2006) Physiological, chemical and technological aspects of milk-protein-derived peptides with antihypertensive and ACE-inhibitory activity. Int Dairy J 16(11):1277–1293

Marneros AG, Olsen BR (2005) Physiological role of collagen XVIII and endostatin. FASEB J 19(7):716–728

Mayya KS, Bhattacharyya A, Argillier J-F (2003) Micro-encapsulation by complex coacervation: influence of surfactant. Polym Int 52:644–647

Mendis E, Rajapakse N, Byun H-G, Kim S-K (2005) Investigation of jumbo squid (*Dosidicus gigas*) skin gelatin peptides for their in vitro antioxidant effects. Life Sci 77(17):2166–2178

Midorikawa T, Lawal OS, Sasaki Y, Fukae R (2012) Structure and physical properties of gelatin fibers prepared by gel-spinning in ethylene glycol. J Appl Polym Sci 125(S2):332–338

Mohtar NF, Perera CO, Quek SY (2011) Utilisation of gelatine from NZ hoki (*Macruronus novaezelandiae)* fish skins. Int Food Res J 18(3):1062–1066

Montero P, Álvarez C, Martí MA, Borderías AJ (1995) Plaice skin collagen extraction and functional properties. J Food Sci 60:1–3

Morrissey MT, Lin J, Ismond A (2005) Waste management and by-product utilization. In: Park JW (ed) Surimi and surimi seafood, 2nd edn. CRC Press, Boca Raton, pp 279–323

Moure AS, Cruz JM, Franco D, Manuel Domínguez J, Sineiro J, Domínguez H, José Núñez M, Carlos Parajó J (2001) Natural antioxidants from residual sources. Food Chem 72:145–171

Muyonga JH, Cole CGB, Duodu KG (2004) Characterisation of acid soluble collagen from skins of young and adult nile perch (*Lates niloticus*). Food Chem 85:81–89

Myers JC, Amenta PS, Dion AS, Sciancalepore JP, Nagaswami C, Weisel JW, Yurchenco PD (2007) The molecular structure of human tissue type XV presents a unique conformation among the collagens. Biochem J 404(3):535–544

Myllyharju J, Kivirikko KI (2004) Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet 20(1):33–43

Nagai T, Suzuki N, Tanoue Y, Kai N, Nagashima T (2010) Characterization of acid-soluble collagen from skins of surf smelt (*Hypomesus pretiosus japonicus* Brevoort). Food Nutr Sci 1:59–66

Nagpal R, Behare P, Rana R, Kumar A, Kumar M, Arora S, Morotta F, Jain S, Yadav H (2011) Bioactive peptides derived from milk proteins and their health beneficial potentials: an update. Food Funct 2(1):18–27

Nalinanon S, Benjakul S, Visessanguan W, Kishimura H (2008) Tuna pepsin: characteristics and its use for collagen extraction from the skin of threadfin bream (*Nemipterus* spp.). J Food Sci 73(5):C413–C419

Nalinanon S, Benjakul S, Kishimura H, Shahidi F (2011) Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. Food Chem 124(4):1354–1362

Ngo DH, Vo TS, Ngo DN, Wijesekara I, Kim SK (2012) Biological activities and potential health benefits of bioactive peptides derived from marine organisms. Int J Biol Macromol 51: 378–383

Nomura Y, Sakai H, Ishii Y, Shirai K (1996) Preparation and some properties of type I collagen from fish scales. Biosci Biotechnol Biochem 60:2092–2094

Normah I, Jamilah B, Saari N, Che Man Yaakob B (2005) Optimization of hydrolysis conditions for the production of threadfin bream (*Nemipterus japonicus*) hydrolysate by alcalase. J Muscle Foods 16:87–102

Núñez-Flores R, Giménez B, Fernández-Martín F, López-Caballero ME, Montero MP, Gómez-Guillén MC (2013) Physical and functional characterization of active fish gelatin films incorporated with lignin. Food Hydrocoll 30:163–172

Ogawa M, Portier RJ, Moody MW, Bell J, Schexnayder MA, Losso JN (2004) Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). Food Chem 88(4):495–501

Pace JM, Corrado M, Missero C, Byers PH (2003) Identification, characterization and expression analysis of a new fibrillar collagen gene, *COL27A1*. Matrix Biol 22:3–14

Palpandi C, Ramasamy P, Rajinikanth T, Vairamani S, Shanmugam A (2010) Extraction of collagen from mangrove archeaogastropod *Nerita* (*Dostia*) *crepidularia* Lamarck, 1822. Am Eur J Sci Res 5(1):23–30

Pan S-K, Yao D-R, Zhou M-Q, Wu S-J (2012) Hydroxyl radical scavenging activity of peptide from sea cucumber using enzyme complex isolated from the digestive tract of sea cucumber. Afr J Biotechnol 11(5):1214–1219

Park C-H, Park J-W, Kang K-T, Lee J-H, Kim J-S (2007) Characterization of acid-soluble collagen from Alaska pollock surimi processing by-products (refiner discharge). Food Sci Biotechnol 16:549–556

Pataridis S, Eckhardt A, Mikulíková K, Sedláková P, Mikšík I (2008) Identification of collagen types in tissues using HPLC-MS/MS. J Sep Sci 31(20):3483–3488

Pati F, Adhikari B, Dhara S (2010) Isolation and characterization of fish scale collagen of higher thermal stability. Bioresour Technol 101(10):3737–3742

Pérez-Mateos M, Montero P, Gómez-Guillén MC (2009) Formulation and stability of biodegradable films made from cod gelatin and sunflower oil blends. Food Hydrocoll 23:53–61

Phanturat P, Benjakul S, Visessanguan W, Roytrakul S (2010) Use of pyloric caeca extract from bigeye snapper (*Priacanthus macracanthus*) for the production of gelatin hydrolysate with antioxidative activity. LWT Food Sci Technol 43(1):86–97

Picot L, Ravallec R, Fouchereau-Péron M, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, Guérard F, Chabeaud A, LeGal Y, Martinez Alvarez O, Bergé J-P, Piot J-M, Batista I, Pires C, Thorkelsson G, Delannoy C, Jakobsen G, Johansson I, Bourseau P (2010) Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties. J Sci Food Agric 90(11):1819–1826

Pihlanto A (2006) Antioxidative peptides derived from milk proteins. Int Dairy J 16(11): 1306–1314

Pihlanto A, Akkanen S, Korhonen HJ (2008) ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chem 109(1):104–112

Potaros T, Raksakulthai N, Runglerdkreangkrai J, Worawattanamateekul W (2009) Characteristics of collagen from nile tilapia (*Oreochromis niloticus*) skin isolated by two different methods. Kasetsart J (Nat Sci) 43:584–593

Pranoto Y, Marseno DW, Rahmawati H (2011) Characteristics of gelatins extracted from fresh and sun-dried seawater fish skins in Indonesia. Int Food Res J 18(4):1335–1341

Prockop DJ, Fertala A (1998) Inhibition of the self-assembly of collagen I into fibrils with synthetic peptides. Demonstration that assembly is driven by specific binding sites on the monomers. J Biol Chem 273:15598–15604

Qian Z-J, Jung W-K, Lee S-H, Byun H-G, Kim S-K (2007) Antihypertensive effect of an angiotensin I-converting enzyme inhibitory peptide from bullfrog (*Rana catesbeiana* Shaw) muscle protein in spontaneously hypertensive rats. Process Biochem 42(10):1443–1448

Qian Z-J, Jung W-K, Kim S-K (2008) Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, *Rana catesbeiana* Shaw. Bioresour Technol 99(6):1690–1698

Raghavan S, Kristinsson HG (2009) ACE-inhibitory activity of tilapia protein hydrolysates. Food Chem 117(4):582–588

Rajapakse N, Mendis E, Byun H-G, Kim S-K (2005) Purification and in vitro antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. J Nutr Biochem 16(9):562–569

Ranathunga S, Rajapakse N, Kim S-K (2006) Purification and characterization of antioxidative peptide derived from muscle of conger eel (*Conger myriaster*). Eur Food Res Technol 222:310–315

Ren L, Wang X, Taotao Q, Zhou G, Li F (2009) Synthesis, characterization and application of novel aminated gelatin hydrolysate. J Chem Soc Park 31(3):447–452

Ricard-Blum S (2010) The collagen family. Cold Spring Harb Perspect Biol 3(1):a004978

Ruiz-Capillas C, Moral A, Morales J, Montero P (2002) The effect of frozen storage on the functional properties of the muscle of volador (*Illex coindetii*). Food Chem 78(2):149–156

Rutherfurd KJ, Gill HS (2000) Peptides affecting coagulation. Br J Nutr 84(Suppl 1):99–102

Ryan JT, Ross RP, Bolton D, Fitzgerald GF, Stanton C (2011) Bioactive peptides from muscle sources: meat and fish. Nutrients 3:765–791

Safari R, Motamedzadegan A, Ovissipour M, Regenstein JM, Gildberg A, Rasco B (2012) Use of hydrolysates from yellowfin tuna (*Thunnus albacares*) heads as a complex nitrogen source for lactic acid bacteria. Food Bioprocess Technol 5(1):73–79

Saiga A, Okumura T, Makihara T, Katsuda SI, Morimatsu F, Nishimura T (2006) Action mechanism of an angiotensin I-converting enzyme inhibitory peptide derived from chicken breast muscle. J Agric Food Chem 54:942–945

Schreiber R, Gareis H (2007) Gelatine handbook: theory and industrial practice. Wiley-VCH, Weinheim

Schumacher MA (2006) The crystal structure of a collagen-like polypeptide with 3(S)-hydroxyproline residues in the xaa position forms a standard 7/2 collagen triple helix. J Biol Chem 281(37):27566–27574

See SF, Hong PK, Ng KL, Wan Aida WM, Babji AS (2010) Physicochemical properties of gelatins extracted from skins of different freshwater fish species. Int Food Res J 17:809–816

Sharma S, Singh R, Rana S (2011) Bioactive peptides: a review. Int J Bioauto 15(4):223–250

Sheih IC, Wu TK, Fang TJ (2009) Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems. Bioresour Technol 100:3419–3425

Shon J, Eo JH, Hwang SJ, Eun JB (2011) Effect of processing conditions on functional properties of collagen powder from skate (*Raja kenojei*) skins. Food Sci Biotechnol 20(1):99–106

Singh P, Benjakul S, Maqsood S, Kishimura H (2011) Isolation and characterisation of collagen extracted from the skin of striped catfish (*Pangasianodon hypophthalmus*). Food Chem 124(1):97–105

Šližytė R, Mozuraitytė R, Martínez-Alvarez O, Falch E, Fouchereau-Peron M, Rustad T (2009) Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones. Process Biochem 44(6):668–677

Snellman A, Tu H, Väisänen T, Kvist A-P, Huhtala P, Pihlajaniemi T (2000) A short sequence in the N-terminal region is required for the trimerization of type XIII collagen and is conserved in other collagenous transmembrane protein. EMBO J 19:5051–5059

Songchotikunpan P, Tattiyakul J, Supaphol P (2008) Extraction and electrospinning of gelatin from fish skin. Iint J Biol Macromol 42(3):247–255

Stephan S, Sherratt MJ, Hodson N, Shuttleworth CA, Kielty CM (2004) Expression and supramolecular assembly of recombinant α1 (VIII) and α2 (VIII) collagen homotrimers. J Biol Chem 279(20):21469–21477

Sukkwai S, Kijroongrojana K, Benjakul S (2011) Extraction of gelatin from bigeye snapper (*Priacanthus tayenus*) skin for gelatin hydrolysate production. Int Food Res J 18(3): 1129–1134

Sun H, Liu D, Li S, Qin Z (2009) Transepithelial transport characteristics of the antihypertensive peptide, Lys-Val-Leu-Pro-Val-Pro, in human intestinal caco-2 cell monolayers. Biosci Biotechnol Biochem 73(2):293–298

Taheri A, Abedian Kenari AM, Gildberg A, Behnam S (2009) Extraction and physicochemical characterization of greater lizardfish (*Saurida tumbil*) skin and bone gelatin. J Food Sci 74(3):E160–E165

Than ME, Henrich S, Huber R, Ries A, Mann K, Kühn K, Timpl R, Bourenkov GP, Bartunik HD, Bode W (2002) The 1.9-A crystal structure of the noncollagenous (NC1) domain of human placenta collagen IV shows stabilization via a novel type of covalent Met-Lys cross-link. Proc Natl Acad Sci U S A 99(10):6607–6612

Tosh SM, Marangoni AG (2004) Determination of the maximum gelation temperature in gelatin gels. Appl Phys Lett 84(21):4242

Townsend A-A, Nakai S (1983) Relationships between hydrophobicity and foaming characteristics of food proteins. J Food Sci 48:588–594

Tsai JS, Lin TC, Chen JL, Pan BS (2006) The inhibitory effects of freshwater clam (*Corbicula fluminea*, Muller) muscle protein hydrolysates on angiotensin I converting enzyme. Process Biochem 41(11):2276–2281

Tsai JS, Chen TJ, Pan BS, Gong SD, Chung MY (2008) Antihypertensive effect of bioactive peptides produced by protease-facilitated lactic acid fermentation of milk. Food Chem 106(2):552–558

Veit G, Kobbe B, Keene DR, Paulsson M, Koch M, Wagener R (2005) Collagen XXVIII, a novel von Willebrand factor a domain-containing protein with many imperfections in the collagenous domain. J Biol Chem 281(6):3494–3504

Vercruysse L, Smagghe G, Beckers T, Van Camp J (2009) Antioxidative and ACE inhibitory activities in enzymatic hydrolysates of the cotton leafworm, *Spodoptera littoralis*. Food Chem 114(1):38–43

Vermeirssen V, Van Camp J, Verstraete W (2002) Optimisation and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. J Biochem Biophys Methods 51:75–87

Wang Y, Regenstein JM (2009) Effect of EDTA, HCl, and citric acid on Ca salt removal from Asian (silver) carp scales prior to gelatin extraction. J Food Sci 74(6):C426–C431

Wangtueai S, Noomhorm A (2009) Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT Food Sci Technol 42(4):825–834
Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinogen 5(1):14
Widmer C, Gebauer JM, Brunstein E, Rosenbaum S, Zaucke F, Drögemüller C, Leeb T, Baumann U (2012) Molecular basis for the action of the collagen-specific chaperone Hsp47/SERPINH1 and its structure-specific client recognition. Proc Natl Acad Sci 109(33):13243–13247
Wijesekara I, Kim S-K (2010) Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: prospects in the pharmaceutical industry. Mar Drugs 8(4):1080–1093
Woo J-W, Yu S-J, Cho S-M, Lee Y-B, Kim S-B (2008) Extraction optimization and properties of collagen from yellowfin tuna (*Thunnus albacares*) dorsal skin. Food Hydrocoll 22(5):879–887
Woodley DT, Remington J, Chen M (2007) Autoimmunity to type VII collagen: epidermolysis bullosa acquisita. Clin Rev Allergy Immunol 33:78–84
Wu H-C, Chen H-M, Shiau C-Y (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res Int 36(9–10):949–957
Xuan Ri S, Hideyuki K, Koretaro T (2007) Characterization of molecular species of collagen in scallop mantle. Food Chem 102(4):1187–1191
Yan M, Li B, Zhao X, Ren G, Zhuang Y, Hou H, Zhang X, Chen L, Fan Y (2008) Characterization of acid-soluble collagen from the skin of walleye pollock (*Theragra chalcogramma*). Food Chem 107(4):1581–1586
Yang YL, Zhou GH, Xu XL, Wang Y (2007) Rheological properties of myosin–gelatin mixtures. J Food Sci 72(5):C270–C275
Yang J-I, Ho H-Y, Chu Y-J, Chow C-J (2008a) Characteristic and antioxidant activity of retorted gelatin hydrolysates from cobia (*Rachycentron canadum*) skin. Food Chem 110(1):128–136
Yang H, Wang Y, Zhou P, Regenstein JM (2008b) Effects of alkaline and acid pretreatment on the physical properties and nanostructures of the gelatin from channel catfish skins. Food Hydrocoll 22(8):1541–1550
Yang YL, Motte S, Kaufman LJ (2010) Pore size variable type I collagen gels and their interaction with glioma cells. Biomaterials 31(21):5678–5688
Yeo Y, Bellas E, Firestone W, Langer R, Kohane DS (2005) Complex coacervates for thermally sensitive controlled release of flavor compounds. J Agric Food Chem 53:7518–7525
You L, Zhao M, Cui C, Zhao H, Yang B (2009) Effect of degree of hydrolysis on the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates. Innov Food Sci Emer Technol 10(2):235–240
Zaman HU, Khan MA, Khan RA (2012) Studies on the thermo-mechanical properties of gelatin based films using 2-hydroxyethyl methacrylate by gamma radiation. Open J Comp Mater 2(1):15–21
Zarai Z, Balti R, Mejdoub H, Gargouri Y, Sayari A (2012) Process for extracting gelatin from marine snail (*Hexaplex trunculus*): chemical composition and functional properties. Process Biochem 47(12):1779–1784
Zhang F, Wang Z, Xu S (2009) Macroporous resin purification of grass carp fish (*Ctenopharyngodon idella*) scale peptides with in vitro angiotensin-I converting enzyme (ACE) inhibitory ability. Food Chem 117(3):387–392
Zhao Y, Li B, Liu Z, Dong S, Zhao X, Zeng M (2007) Antihypertensive effect and purification of an ACE inhibitory peptide from sea cucumber gelatin hydrolysate. Process Biochem 42(12):1586–1591
Zhou P, Regenstein M (2007) Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. J Food Sci 72(4):C196–C201
Zhou P, Mulvaney SJ, Regenstein JM (2006) Properties of Alaska pollock skin gelatin: a comparison with tilapia and pork skin gelatins. J Food Sci 71(6):C313–C321
Zhu D, Jin L, Wang Y, Ren H (2012) Swelling behavior of gelatin-based hydrogel cross-linked with microbial transglutaminase. J Aqeic 63(2):11–20

Zhu K, Zhou H, Qian H (2006) Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (WGPH) prepared with alcalase. Process Biochem 41(6):1296–1302

Zhuang Y, Sun L, Zhao X, Wang J, Hou H, Li B (2009) Antioxidant and melanogenesis-inhibitory activities of collagen peptide from jellyfish (*Rhopilema esculentum*). J Sci Food Agric 89(10):1722–1727

Zohuriaan-Mehr MJ, Pourjavadi A, Salimi H, Kurdtabar M (2009) Protein- and homo poly(amino acid)-based hydrogels with super-swelling properties. Polym Adv Technol 20(8):655–671

Zúñiga RN, Aguilera JM (2009) Structure–fracture relationships in gas-filled gelatin gels. Food Hydrocoll 23(5):1351–1357

Chapter 13
Bycatch Utilization in Asia

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Acronyms

ASEAN	Association of Southeast Asian Nations
DANIDA	Danish Development Assistance Program
FAO	Food and Agriculture Organization
MFRD	Marine Fisheries Research Department
MT	Metric tons
SE	Southeast
SEAFDEC	Southeast Asian Fisheries Development Center

1 Introduction

Bycatch is one of the greatest and most pervasive threats to marine life and a major problem in fisheries management. Fisheries activities, whether on a large industrial scale or a small artisanal scale, create vast amounts of bycatch. The amount of bycatch has been increased tremendously after the development of bigger and low-cost fishing gear, faster boats, and thousands of kilometers of nets and lines during last ~50 years. This modern fishing gear efficiently catch fish as they cover an extensive area; however, many other creatures are also caught in addition to the target species, hence, they can be considered as highly unselective and detrimental for a considerable part of marine resources. Poor management practices and non-implementation of fisheries policies, like legal bans on fishing, has also been a cause of bycatch landings and discards. Bycatch includes dolphins, marine turtles, seabirds, corals, starfish, sharks, juvenile fish, and small fish with little or no commercial value. Billions of unwanted animals are caught all over the world by fishing boats, which are discarded back into the ocean, dead or alive. Due to bycatch, valuable natural resources are being wasted, resulting in dramatic declines in the populations of many marine species (Gulf of Mexico Fishery Management Council 2006). The bycatch of endangered and threatened species is of particular concern for biologists and conservationists. In some fisheries, bycatch kills wildlife at such a scale that it may affect the structure and function of marine systems at all levels; that is, population, community, and ecosystem (Alverson et al. 1994). Bycatch may be defined as incidental catch which is not targeted but is still retained and marketed, along with the discarded and released catch (Clucas 1997). The term "bycatch" has a different meaning and definition to different scientists and fishery managers. Alverson et al. (1994) stated that a range of bycatch definitions has been put forward by different groups, as given below:

1. The capture of non-target species (and discarded juveniles of target species) in fishing gear (WWF, World Wildlife Fund).
2. Discarded catch of any living marine resource plus retained incidental catch and unobserved mortality due to direct encounter with fishing gear (NOAA, National Marine Fisheries Service; McCaughran 1992).

3. Fish or other fauna (e.g., birds or marine mammals) that are caught during fishing, but which are not sold or kept for personal use. In commercial fishing, these include both fish discarded for economic reasons (economic discards) and because regulations require it (regulatory discards). Fish released alive under catch-and-release fishery management programs are not normally considered as bycatch (Review of Fisheries in OECD Countries: Glossary, February 1998).
4. Bycatch generally refers to unintended catch that is taken of non-target species and also the target species that are under a desirable size (International Sea Food Sustainability Foundation).
5. The TOTAL CATCH is that quantity taken by the fishing gear which reaches the deck of the fishing vessel. DISCARD is the portion thrown away at sea (for one reason or another). The remainder is the LANDED CATCH or RETAINED CATCH (i.e., which is brought ashore) that can further be subdivided into TARGET CATCH and INCIDENTAL CATCH, bearing in mind the volume, value, incidence of species caught, and nature of the fishing operations. The same species can move from one category to another depending on size, market demand, season, or other criteria; at the same time, other species may be undesirable or of limited value (FAO 1996).

These precise definitions of bycatch are not accepted by all scientists and the term has caused confusion generally due to two different opinions; anything that is caught and discarded at sea (including fish that were targeted by the fishing trip, but are discarded because they are unmarketable due to being damaged or being too small); or anything that is caught and taken back to port, but that was not the target of the fishing trip (these are known as "non-target" species); or a combination of both. According to Davies et al. (2009), the term needs to be redefined as a consistent definition that establishes what should be considered as bycatch. He put forward a new definition as, "bycatch is the catch that is either unused or unmanaged". Applying this definition to the global marine fisheries data, bycatch represents 40.4 % of global marine catches, exposing systemic gaps in fisheries policy and management (Davies et al. 2009). There are at least four different ways in which the word "bycatch" is used in fisheries (FAO Technical guidelines for Responsible Fisheries 2009).

- Catch which is retained and sold but which is not the target species for the fishery
- Species/sizes/sexes of fish which fishermen discard (Karp et al. 2011)
- Non-target fish, whether retained and sold or discarded (OECD 1997)
- Unwanted invertebrate species, such as echinoderms and non-commercial crustaceans, and various vulnerable species groups, including seabirds, sea turtles, marine mammals, and elasmobranchs (sharks and their relatives).

The discrepancies in the definitions of bycatch has been felt globally and it has also been depicted in the Technical Guidelines for Responsible Fisheries (FAO 2009) that "it is not possible to develop a standard international definition of bycatch because of the very diverse nature of the world's fisheries, historical differences in how bycatch has been defined nationally, ambiguities associated with bycatch

related terminologies and choices of individual fishers on how different portions of their catch will be used". Scientists paid attention to the issue of bycatch and discards only during the last 40–50 years, and the matter has been taken as the under-utilization of some stocks or the overexploitation of others (Bricklemyer et al. 1989/1990; Saila 1983). The bycatch and discard issues have surfaced during the early 1990s as high-priority fisheries management issues in developing and developed countries and an international law was designed to address the discard problems (Burke et al. 1993; Perra 1992). A plethora of studies has later been directed towards discards and associated economic (Queirolo et al. 1995) and biological losses (Chan and Liew 1986; Harris and Poiner 1990; Hall 1996; Hall et al. 2000), and considerable efforts have been made to examine management strategies and technological solutions to reduce incidental harvests of discards of overfished and threatened species and allow small, juvenile fish to survive to become a part of the fish harvest. The latest international guidelines on bycatch management and reduction of discards were developed and adopted by FAO technical consultation. These are intended to assist states and regional fisheries management organizations in the management of bycatch and reduction of discards in conformity with the FAO Code of Conduct for Responsible Fisheries (2009). On the other hand, Australian Government legislation for the organization for economic cooperation and development on policy options for fisheries bycatch, where the bycatch has immediate or potential commercial value, is unavoidable in the course of fishing and does not include marine wildlife species subject to separate nature conservation legislation, or utilization does not undermine broader management arrangements, it should be fully utilized (Truelove 1997; FAO Code of Conduct for Responsible Fisheries 2009). A substantial reduction in discards have been observed and documented in recent years, globally. The associated factors are the reduction in unwanted catch and increased utilization of bycatch. The improvement in processing technologies and expanding market opportunities for low-value species has increased the utilization of bycatch for human and animal food. A "no-discards" approach to fisheries management is a key feature and the need for a balance between bycatch reduction and bycatch utilization have considerably reduced the losses to fisheries and, in turn, to the fishermen communities.

Certain measures have been suggested to reduce the volume of bycatch by adopting technical changes to fishing operations and through management measures to make their capture less likely, such as species/time/area restrictions. Proven solutions do exist, such as modifying fishing gear and mesh size regulation to prevent the catch of undersize fish (Armstrong et al. 1990; Dawson 1991a, b, c; Caddy 1989; DeAlteris et al. 1990) so that either fewer non-target species are caught or non-target species can escape. In many cases, these modifications are simple and inexpensive, with the best innovations usually coming from the fishers themselves. The recent reduction in bycatch is largely a result of the use of more selective fishing gear, introduction of bycatch and discard regulations, and improved enforcement of regulatory measures. The National Oceanic and Atmospheric Administration Fisheries Service (NOAA) formulated the Bycatch Reduction Engineering Program (BREP). The mission of the BREP is to develop technological solutions and

Table 13.1 Estimates of total and percentage bycatch in Asian countries

	Country	Total catch (t)	Bycatch (t)	Year	Bycatch rate (%)
1.	Bangladesh	1,261,600	1,207,000	2000–2003	95.7[a]
2.	China	7,300,000	5,000,000	1999	68.5
3.	India	4,000,000	2,250,000	2000	56.3
4.	Indonesia	7,031,000	4,392,000	2000–2003	62.6
5.	Japan	6,900,000	900,000	1997	13.0
6.	Malaysia	1,283,000	718,480	2004	56.0
7.	Myanmar	1,790,000	870,000	2000–2003	48.6
8.	Pakistan	320,000	192,000	2003	60.0
9.	Philippines	1,300,000	406,000	2000–2003	31.2
10.	Sri Lanka	140,000	72,000	2000–2003	51.4
11.	Thailand	2,600,000	1,664,000	2000–2004	64.0
12.	Vietnam	1643,000	930,000	1999–2004	80[a]

Source: Data derived from Davies et al. (2009)
[a]The estimates have been made as per the definition of all undersize catch and, in case of Vietnam and Bangladesh, the entire trawl fleet is geared to catch very small shrimp and immature fish in ways that fail to conform to the regulation minimum net mesh size

investigate changes in fishing practices designed to minimize bycatch of fish (including sponges and deep-sea and shallow tropical corals) and protected species (including marine mammals, seabirds, and sea turtles), as well as minimize bycatch injury and mortality (including post-release injury and mortality).

Alverson et al. (1994) estimated an amount of 27 million tons of global discards annually. The share of Asian countries represented 47 % of the global discards. A revised estimation of global discards of 6.8 million tons was published by (Kelleher 2005). There is still no reliable discard estimation globally. The reason for this is the chaotic definitions of the terms used, such as “bycatch” and “discards” (Matsuoka 2008). The bycatch/discards further increased tremendously for some countries (Table 13.1) as per the redefinition of bycatch by Davies et al. (2009), who estimated 40 % discards, that is, 38,505,242 t out of the total global marine landings of 95,231,270 t.

2 Status of Bycatch in Asia

Asia dominates the fishery industry in quantities of both capture and aquaculture. The number of people employed and the number of vessels in operation is the highest. Over 85 % of all fishers and fish farmers in 2008 were Asian, as were six of the top ten producer countries in capture fishing. These are China, Indonesia, Japan, India, Philippines, and Myanmar (FAO 2010). Other important fishing nations of Asia are Malaysia, Thailand, Bangladesh, and Vietnam. In Asian countries, marine fisheries production is based almost exclusively on trawl net and a huge amount of bycatch is not unusual. Trawling, purse seining, and gillnetting are fishing methods

considered as destructive and indiscriminate, renowned for the generally high percentage of bycatch they produce. The most recent estimates of bycatch have been made by Davies et al. (2009), according to which the bycatch comprises 31–90 % of the total landings (Table 13.1), depending on the fishing area, fishing gear, and fishing season. This has also been accepted at regional forums that SE Asia had the greatest achievements in the utilization of bycatch and, hence, reduced the losses due to discards (James 1998; Clucas and Teutscher 1999). It is noteworthy that, in most of the developing Asian countries, there is full catch utilization and little discarding of fish, for example, Myanmar, Philippines, Thailand, etc. The reasons for the low discards in the SE Asian region include the fact that trash fish is still used for direct human consumption, such as in Indonesia, Philippines, Thailand, and Myanmar. Many studies have been conducted by international and regional fishery organizations, such as FAO/DANIDA, SEFDEC-MFRD, ASEAN, and Asia-Pacific Fishery Commission, for the utilization of bycatch to provide technical assistance to fisher folk (Min 1998; James 1998; Clucas and Teutscher 1999; Pangsorn et al. 2007). The MFD–SEAFDEC conducted a project from 2002 to 2005 on maximizing the utilization of fish catch from both marine and freshwater environments under the ASEAN–SEAFDEC Special Five-Year Program (MFRD 2005, 2006). In addition, great contributions have been made by the organizations and societies on a national level, particularly in India, where several seminars, workshops, and symposia have been organized and research findings have been disseminated in the form of proceedings and books (Ghadi et al. 1976; Govindan 1985; Basu et al. 1987; Gopakumar 1989, 1991, 1992, 1997; Venugopal and Shahidi 1995; Sen 2005) and a large number of individual publications are referred in this document.

3 Bycatch Composition and Utilization

In Asian countries, bycatch is characterized by its rich biodiversity and a large number of species of fish, shrimp, crab, squid, cuttlefish, and paste shrimp have been identified. The number of fish species ranged from 30 to more than a hundred. The large number of fish species represented in the bycatch belongs to a small number of families; some of the families are common to the bycatch in many parts of the world. The most common families that occur in the bycatch of shrimp trawlers throughout the topical world are Ariidae (catfish), Carangidae (jack), Clupeidae (herring, shad, sardines, menhaden), Gerreidae (mojarras), Sciaenidae (croakers), Synodontidae (lizardfish), Nemipteridae (threadfin), Bothidae (lefteye flounder), Dasyatidae (ray), and Trichiuridae (ribbonfish). Most of these species mature at sizes under 20 cm and weighing less than 100 g (Alverson et al. 1994). Table 13.2 presents the species composition of bycatch in Asian countries.

Previously, in shrimp trawl fisheries of the tropics, anything other than shrimp was considered as bycatch and was usually discarded. However, later socioeconomic factors forced the fishers to make use of the bycatch as direct food, value-added products, feeds, and fertilizers, hence reducing the discards in order to obtain

Table 13.2 Bycatch species composition in Asian countries

	Country	Species composition in the bycatch
1.	Bangladesh	Indian salmon, pomfret, catfish, jewfish, shark, ray, Bombay duck, and other marine fish (source: Clucas and Teutscher (eds) 1999)
2.	South China Sea	Cephalopods, crab, spiral shell, fishes of Stromateidae, Periopthalmidae, Nemipteridae, and Cynoglossidae, *Collichthys lucidus*, *Coilia grayii*, *Odontamblyopus rubicundus*, *Harpodon* spp., *Anchoviella heteroloba*, *Argyrosomus* spp. (Lin 1999)
3.	India	Carangid, anchovy, ribbonfish, Clupeidae, Bombay duck, croaker, Gobiidae, *Squilla*, shrimp, crab, soles, drift fish, puffer fish, lizardfish, *Acetes*, *Solenocera*
4.	Indonesia	Out of 72 species from bycatch, the predominant ones are: *Leiognathus decorus*, *Sardinella lemuru*, *Trichiurus lepturus*, *Caranx bucculentus*, *Nemipterus isacanthus*, *Psettodes erumei*, *Upeneus sulphureus*, *Austronibea oedogenys*, and *Johnius coitor* (Chasana 1999)
5.	Japan	
6.	Malaysia	*Rastrelliger brachysoma*, *Apogon* spp., *Secutor insidiator*, *Kowala macrolepis*, *Stolephorus andhraensis*, *Pellona* spp., *Pennahia macrocephalus*, *Trichiurus haumela*, *Triacanthus brevirostris*, *Siganus oramin*, *Thrissocles* spp., *Leiognathus* spp., *Selar kalla*, *Secutor ruconius* (Clucas 1997)
7.	Myanmar	Lizardfish, terapon, hammerhead shark, stingrays, sawfish, guitarfish, trevally, scad, shad, sardine, sole, anchovies, spadefish, flutefish, mojarras, Bombay duck, ponyfish, moonfish, threadfin, angelfish, whiting, croakers (Aye and Pyo 1998)
8.	Pakistan	*Sardinella longiceps*, *S. sindensis*, *S. gibbosa*, *S. tumbil*, *Liza subviridis*, *Sphyraena barracuda*, *Rastrelliger kanagurta*, *Otolithus ruber*, *Sillago cheme*, *Scomeroides*, *Charonutoll*, *Nematolosa nasus*, *Harpodon nehereus*, *Lepturacanthus savala*, *Upeneus vittatus*, *Hilsa kelee*, *Terapon jarbua*, *Euthynnus affinis*, *Lactarius lactarius*, *Rhinobatos granulates*, *Arius thalassinus*, *Thryssa malabarica*, *Billegoi scheme*, *Caranx dussumieria*, *Mugil cephalus*, *Himantura uarnak*, *Nemipterus japonicas*, *Squilla*, shrimp, crab, squid, octopus (Source: Shakir and Bano 1999)
9.	Philippines	Shrimp, crab, *Squilla* spp., *Apogon* spp., *Dactyloptena orientalis*, eel, shellfish, sea cucumber, octopus [out of about 80 species listed, only those species making up more than 1 % of the total catch (shrimp trawl bycatch)] *Platycephalus* spp., *Leiognathus* spp., *Saurida* spp., *Bothidae*, *Priacanthus hamrur*, *Nemipterus* spp., Mellidae, Gerridae, Lutjanidae, Carangidae (otter trawl bycatch) (Source: Legaspy 1999)

(continued)

Table 13.2 (continued)

	Country	Species composition in the bycatch
10.	Sri Lanka	
11.	Thailand	*Therapon* spp., *Mene maculata*, *Priacanthus*, *Diodon* spp., *Aluterus* spp., *Alepes* spp., *Leiognathus* spp., *Gazza minuta*, *Muraenesox* spp., *Congresox* spp., *Ablennes* spp., *Tylosurus* spp., *Xiphocheilus* spp., *Saurida*, *Thysanophrys* spp., *Nemipterus* spp., *Alectis* spp., *Upeneus*, *Psettodes* spp. *Gymnocranius elongates*, *Parupenaeus* spp., *Parapercis xanthozona*, *Siganus* spp., *Aurelia* spp., *Paraplagusia* spp., *Apogonichthys* spp., *Stolephorus* spp., *Lagocephalus* spp., *Sardinella* spp., *Epinephelus* spp., *Cociella cordelia*, *Plotosus lineatus*, *Trichiurus lepturus*, *Lutjanus* spp., *Chiloscyllium punctatum*, *Dasyatis* spp., *Scomberomorus* spp., *Otolithes* spp., *Eleutheronema* spp., *Sphyraena* spp., *Ogcocephalus* spp., *Rastrilliger* spp., *Scorpaena* spp., *Dactyloptena* spp., *Lutjanus lineolatus*, *Uranoscopus* spp., *Chirocentrus dorab*, *Lutjanus fulviflamma*, *Selar boops*, *Pterocaesio* spp., *Gerres* spp., *Anodontostoma chacunda*, *Oratosquilla* spp., *Thenus* spp., shrimp juveniles, *Loligo* spp., *Sepia* spp., *Portunus pelagicus*, *Thalamita* spp. (Source: Kungsuwan 1999)
12.	Vietnam	*Cantherines modestus*, *Trichiurus lepturus*, *Paramonacanthus* spp., *Leiognathus* spp., *Decapterus kurroides*, *D. maruadsi, Selaroides leptolepis*, *Saurida undosquamis*, *Decapterus macrosoma*, *Saurida*, *Tumbil*, *Trachinocephalus myops*, *Upeneus sulphureus*, *U. bensasi*, *Priacanthus macracanthus*, *Dasyatis* spp., *Evynnis cardinalis*, *Pristotis jerdoni*, *Pristipomoides microdon*, *Acropoma japonica*, *Ariomma indica*, *Lutjanus sanguineus*, *Apogon* spp., *Loligo* spp. (Source: MOFI/DANIDA 1998)

additional income (Kelleher 2005). These former discards are no longer considered as bycatch; rather, it is treated as multispecies fisheries by the fishers. According to Murawski (1992), "yesterday's bycatch may be tomorrow's target catch". However, from a management viewpoint, such slogans cannot single-handedly change the definition of bycatch and are not in accordance with the principles of sustainable fisheries management.

The Code of Conduct for Responsible Fisheries (FAO 1995) suggests that states should improve the use of bycatch as long as doing so is consistent with responsible fisheries management, and this is considered as an option in many deliberations concerning policy on fisheries management and mitigation of the discards problem. The utilization is indispensible to an extent which is consistent with responsible fisheries management practices.

In Asia, the utilization of bycatch varies from country to country, due to social, cultural, and religious differences, though there are a few common products, such as frozen fillet and dried and salted dried fish. Salted dried fish is the most commonly and widely used method in Asian countries due to the convenience of the processing methods, which do not need sophisticated machinery and storage facilities; hence, it is practiced as a small-scale household business.

Generally, a major part of the bycatch is utilized for the production of animal and aquaculture feeds in East Asia (China), value-added/novel products in SE Asia, and as fresh fish, traditional products, and animal feeds in South Asia. In SE Asia, the product development is based on the availability of bycatch species. Small pelagic fish like sardines, scads, and croakers are often salted, dried, and marketed widely as a low-cost source of protein. Minced fish meat with low gel strength is also used in a variety of indigenous products, usually as a binder in deep-fried fish cakes, rolls, etc. It has been well recognized that SE Asia has achieved the greatest success in utilizing the bycatch and discard, and the previous bycatch species have attained the status of target species as a result of market-driven demand (Ordonez 1985; Suwanrangsi 1986; James 1998). Table 13.3 gives an account of the products made and fish species utilized from bycatch in Asian countries. For Sri Lanka, although located in South Asia, the information on bycatch composition and utilization is not available in any of the regional or FAO documents and the subject of bycatch encompass turtle and mammal bycatch (MRAG 2012). The case is similar in Japan, for which scant information was found with regard to bycatch and its utilization.

Technological advances are almost non-existent and the techniques adopted for bycatch utilization are based mainly on simple, traditional, and cost-effective methods in the majority of Asian countries, except for Japan, where technological advances have been made for the development of value-added fishery products using high-value fish and sophisticated equipment. The quantity of bycatch is quite low, as mentioned above.

The technologies developed and tested to use low-value fish in products include the efficient recovery of fish meat by mechanical means (Flick et al. 1990). There are many types of deboners utilized to obtain meat (Agarwal et al. 1986; Ghadi et al. 1976; Suwanrangsi 1987; Grantham 1981; Gopakumar 1987; Burt et al. 1992). The maximum recovery of fish meat has significance for the development of a large variety of minced fish and surimi-based products. The low cost and simplest form of solar dryers have been designed for bycatch drying for rural and coastal communities (Cheapok and Pornnareay 1998; Sengar et al. 2009). The processing of smoked fish has been improved in Indonesia through the technical support under an FAO project; an improved smoking kiln is designed that has the potential to improve the production process, resulting in better quality products (Infofish International, 6/2010).

Surimi is composed of stabilized myofibrillar proteins that have been blended with a cryoprotectant for long-term frozen storage. Surimi is referred to as a fish-based food product intended to mimic the texture and color of the meat of lobster, crab, and other shellfish. In Japan, surimi industrial technology developed in the early 1960s promoted the growth of the modern surimi industry. The main raw material used was Alaska pollock (or walleye pollock), which is not considered as bycatch; hence, the surimi production of Japan is not included here. Fresh surimi products were in use in SE Asia for centuries, but the invention of a process for frozen surimi in 1960 provided the impetus for expansion of the industry.

Table 13.3 Products developed from bycatch fish species in Asian countries

	Country	Product	Fish	Reference
1	Bangladesh	Battered and breaded products, fish burgers breaded and unbreaded, precooked frozen burgers, fish balls, fish fingers, fish cakes	Sardine meat Surimi from unknown species	Etoh (1986), Yu and Siah (1996, 1997)
2	China	Fish cakes, fish balls, surimi and its products, condiments, salted dried and smoked fish, and fish snacks, fresh fish for human consumption, as well as fish culture, fish meal	Mixed species	
		Shark fin, dried sharkskin, extruded sharkskin, shark leather, shark fillets, shark meatballs, dried shark meat floss, shark cartilage powder, shark cartilage chondroitin, shark liver oil, vitamin A and D capsules and dogfish alkene	Sharks: *Hypoprion macloti*, *Carcharias latistomus*, *Carcharias pleurotaenia*, *Carcharhinus menisorrah*, and *Carcharhinus sorrah*	Vannuccini (1999)
3	India	Block-frozen fish mince	Low-value fish	Gopakumar (1998)
		Fish fingers	*Sciaenid* spp. *Nemipterus* spp.	Reddy et al. (1990), James (1998)
		Fish cutlets	Threadfin bream, lizardfish, Jewfish	Joseph et al. (1984)
		Fish cakes, semi-dried fish cakes	(*Otolithus ruber*)	Rao (1981), Durairaj and Pichiah (1985), Sankar et al. (1992), Venkatanarasimha and Chidambaram (1987), Basu et al. (1987), Yu (1993)
		Quick-salted fish cakes, intermediate moisture fish cakes	Sea bream, ribbonfish	
		Salted and dried minces	Threadfin bream, Indian oil sardine	Sudhakaran and Sudhakara (1985)
		Fish manure, Fish meal	Mixed fish, low quality	
		Surimi	*Saurida tumbil* *Johnius dussumieri* *Sphyraena* spp., *Trichiurus lepturus*, *Scomberoides lysan*, *Megalaspis cordyla*, and *Upeneus vittatus* *Nemipterus bleekeri*	Gopakumar et al. (1992), Muraleedharan and Gopakumar (1997), Jasmine et al. (1995)

4	Indonesia	Fish balls	Shark meat	Nasran et al. (1986)
		Fish satay	Mullidae	
		Fish surimi	*Nemipterus* spp.	
			Saurida spp.	
			Priacanthus spp.	
			Gerres kapas	
5	Japan			
6	Malaysia	Surimi	*Nemipterus japonicus*	Pangsorn et al. (2007)
			Priacanthus tayenus	
			Upeneus, *Pennahiaor*, *Johnius* spp., *Saurida tumbil*, *Sphyraena*	
			Trichiurus	
		Fertilizer, animal feed, satay	*Upeneus sulphureus*	Chasanah (1999)
		Satay	*Upeneus sulphureus*	Min (1998)
		Dehydrated laminates	*Harpodon nehereus*	Doke et al. (1996)
		Surimi-based products, fish balls, fish cakes, kamaboko, fish sausages, battered and breaded products, such as burgers, nuggets, fish fingers, imitation crab meat, fish filament, fish noodles, fish roll, chikuwa	*Upeneus sulphureus*, *Pentapalion longimahus*, *Eutheropa therapa*, *Decapterus russelli*, *Leiognathus equulus*	Yu (1993)
		Salted dried	Croakers	Chee (1996)
		Barbecue and snack food	Mullids	Do
		Fish balls	Synodontids, bullseyes	Do
7	Myanmar	Fish meal, dried, salted, minced, fish paste, fish sauce, fish crackers, fish balls from surimi	Trevally, scad, shad, sardines, sole, anchovies, spadefish, flutefish, mojarras, Bombay duck, hump-head, whitefish, ponyfish, moonfish, goatfish, threadfin bream, flatfish, barbel eel, big eye, croaker	Aye and Pyo (1998)

(continued)

Table 13.3 (continued)

	Country	Product	Fish	Reference
		Surimi	*Nemipterus* spp.	Pangsorn et al. (2007)
			Saurida spp.	
			Priacanthus spp.	
			Pennahia spp.	
			Johnius spp.	
			Upeneus spp.	
			Sphyraena spp.	
			Ilisha megaloptera	
8	Pakistan	Fish meal, fish protein hydrolyzate, fish syrups	Mixed species, processing waste from fishery industry	Shakir and Bano (1999)
9	Philippines	Fresh fish market	Mixed species, crispy crablets	Legaspy (1999)
		Salted dried	*Leiognathus* spp.	Legaspy (1999)
			Saurida spp.	
			Pricanthus spp.	
			Nemipterus spp.	
			Mullidae, *Decapterus* spp.	
			Cynoglossus spp.	
			Soleid spp.	
			Sillago spp.	
			Tetraodon spp.	
		Sausages	*Sardinella longiceps*	Ravishankar et al. (1993a, b)
		Fish balls, fish noodles	Mixed species, low-value fish	Marfori et al. 1991
		Fish sauce and fish paste, fish meal	Low-value fish	Adams et al. (1985)
				Legaspy (1999)
10	Sri Lanka	Salted dried fish	Mixed species	
11	Thailand	Fish noodles	*Saurida* spp.	Phithakpol et al. (1986)
		Fish satay	*Saurida* spp.	Yean (1998)

		Fish paste	*Leiognathus* spp., *Caranx* spp.	Saisithi (1987)
			Sardinella spp.	Choorit et al. (1991)
		Fish biscuits	*Decapterus russelli*	Yu and Kaur (1992)
		Fish balls	Nemipteridae, Synodontidae, Platycephalidae, Leiognathidae, Mullidae	Pruthiarenum et al. (1985), Pruthiarenum (1986)
		Salted dried	Ray, scad, barebreast jack, and sole	Kungsuwan,(1999)
		Fish surimi	*Nemipterus* spp.	
			Saurida spp.	
			Priacanthus spp.	
			Pennahia spp.	
			Johnius spp.	
			Upeneus spp.	
			Muraenesox spp.	
			Leiognathus spp.	
			Sardinella spp.	
			Gerres spp.	
			Scolopsis spp.	
		Karasaki ika	Surimi based	Min 1998
		Fish crackers	Sardines	Do
		Fish meal	Trash fish, processing waste from the fishery industry, and sardines	Do
12	Vietnam	Fish sauce, fish sauce concentrate	*Stolephorus* spp.	Thuc (1999)
			Common sprat, Atlantic round herring, gilt sardine	
		Fish cakes, fish balls (minced fish products)	Lizardfish, goatfish, ponyfish, snakefish	Do

(continued)

Table 13.3 (continued)

Country	Product	Fish	Reference
	Dried fish, chilled fish, fish meal, fish surimi-based products, fish meal	Mixed fish	Do
	Fish seasoned products	Yellowspotted trevally, furlong spined tripod fish, giant catfish	Do
	Surimi	*Nemipterus* spp.	
		Saurida spp.	
		Priacanthus spp.	
		Pennahia spp.	
		Johnius spp.	
		Upeneus spp.	
		Muraenesox spp.	
		Sphyraena spp.	

Japan was the first country to produce surimi on a commercial scale. Later, the Republic of Korea and Thailand entered the field of surimi production and showed considerable growth potential, which further stimulated the development of surimi production in Malaysia, Myanmar, Vietnam, Indonesia, and India. Fish species with white meat, such as threadfin bream (*Nemipterus* spp.), bigeye (*Priacanthus* spp.), goatfish (*Upenneus*), jewfish (*Pennahia* or *Johnius* spp.), barracuda (*Sphyraena*), ribbonfish (*Trichiurus*), and lizardfish (*Saurida* spp.), have been considered as the most suitable for processing export-quality surimi in Thailand, Malaysia, Vietnam, Myanmar, and Indonesia, whereas barracuda seem to be one of the main raw materials only in Malaysia. The SEAFDEC conducted a detailed survey on the "Surimi Industry in SE Asia", under the ASEAN–SEAFDEC program SEAFDEC/TD (Pangsorn et al. 2007). In surimi production, the quality depends largely on the freshness of fish.

3.1 Bangladesh

Bangladesh produced the highest amount of bycatch, comprising 90 % of the total landings (Davies et al. 2009), whereas Kungsuwan (1999) estimated it to be 43 %, categorized as trash fish, 26 % and discard 10 %, shrimp 5 %, and commercial finfish 12 %. The fisheries are categorized as artisanal and the use of estuarine set bag nets, drift nets, and trawl nets is the major factor contributing to bycatch production. Bycatch is composed of threadfin bream, sea catfish, illisha shad, Bombay duck, croaker, and goatfish; the trash fish include russelli scad, longfin silver biddy, ponyfish, puffer fish; and the discarded species include cuttlefish, squids, crabs, rays, and sharks. It has been concluded that 83 % was the bycatch, 5 % was shrimp, and 12 % was fish of commercial value (Kungsuwan 1999).

In Bangladesh, the most popular products are breaded fish burgers, frozen precooked burgers, and fish fingers, followed by fish cakes and fish balls made of trash fish from shrimp bycatch (Etoh 1986; Ihm et al. 1992). Some of the low-value fish is not generally used for human consumption. The use of three fish species, that is, sea catfish (*Tachysurus thalassinus*), silver belly (*Leiognathus bindus*), and jewfish (*Otolithoides microdon*), in the manufacture of surimi was investigated by measuring their gel-forming ability. It was concluded that *T. thalassinus* and *O. microdon* are suitable for use in surimi production (Nowsad et al. 1998).

3.2 China

The capture fisheries are diverse, using many methods, a large variety of gear, and produce multispecies catches. Reliable estimates of bycatch are not available; the estimate given for 1999 by Davies et al. (2009) is 68 % (Table 13.1). The species composition of the bycatch from the South China Sea area (Table 13.2) has been

reported by Lin (1999). Bycatch has been utilized generally in aquaculture and particularly for fish culture in China. The small trash fish species have been used in floating cages and at the nursery stage in shrimp culture and reduced to fish meal for advanced aquaculture operations. Some of the bycatch is processed for novel value-added products and traditional products like fish balls, fish cakes, surimi and its products, condiments, salted dried and smoked fish, and fish snacks. High-quality species are sorted for use in human consumption as fresh fish.

Sharks are caught as bycatch by set gillnets and drift nets. The species caught are *Hypoprion macloti*, *Carcharias latistomus*, *Carcharias pleurotaenia*, *Carcharhinus menisorrah*, and *Carcharhinus sorrah*. In areas of shark abundance, they account for 30 % of the total catch, whereas in other areas, the proportion is very small. Trawlers also capture them as a bycatch. The species mainly caught are *C. sorrah*, *C. menisorrah*, *Scoliodon* spp., Sphyrnidae, *Chiloscyllium* spp., and, occasionally, big *Rhincodon typus* and *Cetorhinus maximus*. It is estimated that shark caught as a bycatch of trawling amounts to 70–80 % of the total shark landings (Vannuccini 1999).

3.3 India

The total marine catch was 2.81 million tons during 2004 (FAO 2004). In the traditional gillnet fishery, the amount of bycatch is almost negligible. The considerable amount of bycatch is produced by longlines, shore seines, tidal nets, shrimp seed collection by small mesh nets, and shrimp trawl nets. The bycatch is 38 % as estimated from data given by Gopakumar (1998) and 56 % by Davies et al. (2009) of the total landings. Bycatch is well handled and processed by local communities. The utilization of bycatch has been improved to a great extent in India during the 1990s due to the economic liberalization process, which resulted in new markets for underutilized fish and the importance so gained forced fishers to carefully handle their bycatch. The economic improvement of urban communities also played a key role for the market growth of novel value-added products.

In India, several conventional products like burgers, patties, sausages, fingers, pastes, fritters, loaves, wafers, cutlets, fish cakes, and pickled products have been developed from minced fish for human consumption. Details of these product developments have been given by many scientists (Setty 1987; Regier and Raizin 1988; Gopakumar 1987; Joseph et al. 1984; Rao 1981; Shenoy et al. 1983; Durairaj and Pichiah 1985; Venkatanarasimha and Chidambaram 1987; Salagrama 1999; and Reddy et al. 1990). In addition, Basu et al. (1987) developed intermediate moisture fish cakes using various humectants and Sankar et al. (1992) developed semi-dried fish cakes with 7 % and 10 % salt levels and a shelf life of 18 and 21 days, respectively. The processes for instant soup powder from smoked, dried, and powdered sardines and restructured sardine meat by the addition of alginates were developed by Oh et al. (1988) and Nakayama et al. (1988). The stabilization of minced fish has been performed for storage at ambient temperature by adding tapioca starch, soy protein, and salt, followed by dehydration at 71–82˚C (Venugopal and Shahidi 1995).

3.4 Indonesia

In Indonesia, 72 species have been identified from bycatch; several predominant species are listed in Table 13.2. The ratio of bycatch to the total catch is 62 % (Davies et al. 2009). It has been reported that fish smaller than 10 cm were discarded soon after catching, which resulted in a miscalculation of bycatch. The discards were estimated to be 88 % of the bycatch (Kungsuwan 1999), although it is reported that shrimp nets were equipped with bycatch excluding devices (BEDs) in the Arafura Sea and adjacent regions. No data are available on the effectiveness of bycatch after mounting the BEDs. A shrimp to bycatch ratio of 1:13 was obtained from a 16-day fishing trip with 128 hauls.

In Indonesia, fish salting was the only method for bycatch utilization till 1999. Research has been conducted to explore the gelling capabilities of different fish species for surimi production (Chasana 1999). Data on the commercial production of surimi has been available since 2003. The combined surimi production from eight processing units was ~8,000 metric tons (MT), utilizing ~24,000 MT of fish as raw material in 2005 (Pangsorn et al. 2007).

3.5 Japan

The information available from Japan on bycatch utilization is meager. Queirolo et al. (1995) reported Pacific halibut and herring from Japan. Another study by Matsuoka (1997) mentioned several fishes as discards (trigger fish, filefish, dolphinfish, trevally, jacks; sharks: blue, Walbeem's sharpnose, oceanic whitetip; sculpins, snailfish, stingrays, walleye pollock, thornyhead, rockfish, and pacific cod; juveniles of commercial species such as grunt, striped beakperch, barracuda, red bullseye, anchovy, leatherjacket, sardines, conger eel, marbled sole, fine-spotted flounder, dogfish, cardinalfish, ponyfish, gobies, dragonet, brown sole, longsnout flounder, pointed flounder) with target species (such as skipjack tuna, walleye pollock, deepwater shrimps, mantis shrimp, tanner crab) in some fisheries (longline, surround net, trawl net, set net, bottom gillnets, boat seines, etc.), who tried to assess the quantities of the various species discarded. They concluded that the major discards problems are associated with the small trawl and boat seine fisheries in the coastal waters of Japan. There are also major discards of fish associated with the distant water longline tuna fleet. The discard reported by Matsuoka (1997) was 0.9 million tons from a total marine landing of 6.0 million tons; the discard rate is 14.2 %. Davies et al. (2009) reported an almost similar discard level of 13 % (Table 13.1), which is based on the report of Kelleher (2005).

3.6 Malaysia

From Malaysia, about 19 species have been documented from bycatch by Clucas (1997). Davies et al. (2009) reported 56 % bycatch (Table 13.1), whereas 66 % discards were documented by Clucas (1997).

There are several specific value-added products made from previously discarded species of trawl bycatch, such as salted dried fish, fish satay, barbecue snack food, and fish balls (Chee 1996). The fish species utilized in these products are given in Table 13.3. It has been reported that *Rastrelliger* spp., *Apogon* spp., and *Secutor* spp. were the predominant species during spring tide fishing and during neap tides fishing, *Stolephorus andhraensis* made up over 40 % of the trash fish; all of these fishes are utilized in fish meal production. The criteria for the marketing of fish as human food or conversion to fish meal are based mainly on the size of fish, with small specimens being sent for fish meal production and the larger ones for human consumption, either fresh or dried.

In Malaysia, the overall production of surimi products in 2005 was around 100,000 MT for the six main surimi processing plants. The quantity of raw materials supplied to the surimi processing plants was 618,100 MT. Surimi products are used in local surimi-based products and are also exported to many countries; the main importers are USA, Chile, Japan, and Singapore (Pangsorn et al. 2007). A study undertaken on freeze-dried surimi powder in Malaysia showed the superior functional properties of powder made from threadfin bream over the purple-spotted bigeye and lizardfish-based surimi (Huda et al. 2001). Malaysian surimi is categorized from medium to low grade, though a large number and variety of surimi-based products has been developed. Some of the common products are fish balls, fish cakes, kamaboko, fish sausage battered and breaded products, e.g., nuggets, burgers, fish fingers, patented shrimp, imitation crab meat, fish filament fish noodles, fish roll, and chikuwa (Pangsorn et al. 2007).

3.7 Myanmar

The survey conducted by (Aye and Pyo 1998) revealed a fish catch to shrimp ratio of 70:30 %. Of the 70 % fish catch, 60 % was considered as bycatch, 10 % trash, and 5 % discard, whereas 25 % comprised the high-quality fish. The bycatch comprises over 100 species of fish, squid, cuttlefish, spiny lobster, and elasmobranchs. The term “bycatch” was unknown until 1979–1980, when the FAO provided a consultancy on shrimp handling and processing techniques through the ODA (DFID). As per the criteria and estimate of Davies et al. (2009), bycatch proportion is 48.6 % of the total catch. The most abundant species are lizardfish, *Terapon*, hammerhead shark, stingray, sawfish, guitarfish, trevally, scad, shad, sardine, sole, anchovies,

spadefish, flutefish, mojarras, Bombay duck, ponyfish, moonfish, threadfin, angelfish, whiting, croakers (Table 13.2). Almost all of the catch is being utilized as fresh or processed into fish meal, animal feed, and traditional products like salted, minced, and pickled fish meat.

In Myanmar, the first surimi plant was established in Yangon in 1994–1995 to produce surimi from bycatch. Fishes such as lizardfish, small barracuda, blackspot threadfin, goatfish, small sea bass, threadfin bream, and big-head pennah croaker are used for surimi processing, with a 50 % predominance of the threadfin bream over all the other fish species. In 2007, there were five surimi processing plants, which processed 11,454 MT of fish to produce surimi. Myanmar exported a total of 4,230 MT during 2004–2005: 80 % of the total surimi production was exported to Japan, 18 % to China, and the remaining 2 % to Australia, Singapore, Taiwan, and Malaysia (Pangsorn et al. 2007).

3.8 Pakistan

The Karachi fish harbor handles 90 % of Pakistan's marine catch. As per observations made by Pitcher (2005), a three-fold increase in bycatch of non-target species in the shrimp trawl fishery has been found, which has raised the level of bycatch to more than 60 % of the landings. Marine fishery landings produce 192,000 MT of bycatch in 2003 (Davies et al. 2009), estimated from a total marine catch of 320,000 MT (Table 13.1). The species diversity of fish and shellfish is high (Table 13.2). The bycatch is predominantly utilized for the production of fish meal. About 90 % of the bycatch is utilized by the fish meal processing industry; on average, 178,000 MT (1987–2003) of fish is reduced to fish meal by 30 manufacturing units (Shakir and Bano 1999). In addition to the bycatch, fish processing discards are also included in the processing of fish meal. The produced fish meal is usually used as poultry feed. Pakistan has a capacity of producing 52,000 MT of fish meal annually. Besides China, Pakistan exports fish meal to Sri Lanka, Egypt, Malaysia, Indonesia, Russia, and Vietnam. It exported 42,500 MT in 2008/2009, while exports declined to 35,200 MT in 2009/2010, due to the lack of fish catch and less production (Khatoon et al. 2006; All About Feed, September 2011).

The more sophisticated products like fish hydrolysates having better functional properties is a focus of attention in Pakistan (Begum et al. 1984). It can further be incorporated into fortified beverages. A variety of fish syrups have been developed by different formulations with the addition of different vitamins, minerals, and antioxidants. The syrups serve the purpose of food supplement/tonic and good remedy for malnutrition, as it has a full range of amino acids and minerals naturally found in fish (Shakir and Bano 1999). Studies have been undertaken on the production of fish silage, fish sauce (Fatima and Qadri 1987; Zuberi et al. 1993, 1995), and ready-to-eat products like fish preserve for marketing (Shakir and Bano 1999).

3.9 Philippines

The catch of trawl fisheries is composed of more than 80 species (Dickson 1997), of which only 10 % are shrimp and the remaining 90 % is bycatch, which is predominantly composed of crab (19 %), *Squilla* spp. (13 %), *Apogon* spp. (11 %), *Dactyloptena orientalis* (11 %), and eel (6 %). The remaining species are in less than 5 % in quantity. In the otter trawl catches, Ordonez (1985) reported 21.6 % bycatch composed of 46 species, of which the top 10 are given in Table 13.2.

In the Philippines, the bycatch is fully utilized in a wide range of products in the form of salted, fermented, dried, or value-added products. (Ordonez 1985) considered this as multispecies trawl fishery with a small amount of trash, though the statement is not justified to any of the definitions of bycatch. The success of the utilization of bycatch can be attributed to the landing of bycatch in ice-chilled condition, which is marketed as fresh or processed into salted, dried, fermented products, fish meal, or used in aquaculture. According to an estimate, more than 50 % of the bycatch and trash fish is consumed by the fresh fish market, where communities belonging to low economic levels buy these small fish. The traditional preparation involves boiling them in water with salt and vinegar or just water with calamansi (a sort of lemon). The utilization of bycatch and trash fish is optimized to such an extent that small crablets entangled in nets and previously discarded are also relieved from the nets and sold to restaurants, where they are prepared as a specialty, i.e., crispy crablets (Legaspy 1999). The most common preservation method for bycatch utilization is salted and sun dried, followed by fermented, cured, and minced fish products. The details of the fish species utilized in specific products are presented in Table 13.3. The Philippines is a ready market for convenience foods (Marfori et al. 1991) like fish balls, noodles, and sausages. The mince of Indian oil sardine (*Sardinella longiceps*) has been used for sausages, as noted by Ravishankar et al. (1993a, b).

3.10 Sri Lanka

Marine fish diversity is very rich in Sri Lanka as it is located in the tropics, and 610 species of coastal fish have been reported, which accounted for about 40 % of the coastal fish catch. In addition, 90 oceanic species of large pelagic and 215 species of demersal fish are found; abundantly found species are *Sardinella* spp., *Amblygaster* spp., *Rastrelliger* spp., *Auxis thazard*, *Anchova commersoni*, and *Hirundichthys coromandelensis*, *Lethrinus* spp., *Trichurus* spp., *Caranx* spp., skates and rays, *Cynoglossus* spp., *Jojnius* spp. and *Tolithus* spp., *Katsuwonus pelamis*, and *Thunnus albacares*. Moreover, about 60 species of sharks live in the oceanic waters of Sri Lanka. Of these, the most common are *Carcharhinus falciformis*, *C. longimanus*, *C. melanopterus*, *Alopias pelagicus*, *Sphyrna zygaena*, and *S. lewini*. Skipjack and yellowfin tuna have dominated the offshore catches within

a 60–70-km range from the coast along the south, west, and east coasts. About 285,000 MT of fish was landed in 2004 (MRAG 2012).

Gillnets account for the majority of catches and are the main contributors of marine turtles and mammals bycatch. Tuna longlines also kill turtles. Sharks also reportedly constitute to the bycatch. A turtle conservation program conducted a study from November to June 2000 and recorded a bycatch that comprised 5,241 turtles, of which 20 % were killed and the remaining 80 % were released alive. This bycatch was composed of 37 % olive ridleys, *Lepidochelys olivacea*, 30 % loggerheads, *Caretta caretta*, green turtles, 20 % *Chelonia mydas*, and the remaining 3 % were unidentified (Kapurusinghe 2006). The turtle bycatch rate has increased from an average of 4,000 in the mid 1970s (Frazier 1980) to 13,000 turtles per year in 2000 (Kapurusinghe and Samana 2001), presenting an increase of 225 % over the last 30 years due to the significant growth in the gillnet fleet in this country.

Traditionally, fish processing in Sri Lanka consisted of activities such as drying and curing. The quality of fish landings in this country is generally poor and wastage is high, especially in catches of multiday boats. About 25–30 % of the catch landed by these boats is of poor quality.

3.11 Thailand

In Thailand, bycatch is divided into two main components, "other food fish" and "trash fish". The "other food fish" consists of both target and non-target species. The specimens of poor quality of the target species are dumped into the bycatch. Out of ~70 species from trash fish, the most abundant is ponyfish, *Leiognathus* spp., and juveniles of economically important demersal species, shrimp, squid, and cuttlefish. The ratio of bycatch and target species determined by Kungsuwan (1999) is as follows: other food fish (6), trash fish (32), commercially important fish (34), shrimp (5), or the shrimp to bycatch ratio is about 1:8.

The species composition of bycatch is presented in Table 13.2. Some of the bycatch species, particularly the white-fleshed fish like threadfin bream, lizardfish, bigeye, and croaker, became the target species later due to the rising demand of minced fish industries (Kungsuwan 1999). In Thailand, the initial efforts for the utilization of bycatch were concentrated on fish balls from minced fish in 1981 and the minced fish products became popular for both the domestic (fish balls) as well as export (surimi-based products) markets. The protein content of noodles has been increased by the inclusion of minced lizardfish at varying proportions in the formulation (Phithakpol et al. 1986). The development of a range of products has been reported (Suwanrangsi 1988), with the assistance of the SEAFDEC. A range of products such as fish pastes, fish biscuits, and fish satay has been developed using different fish species (Table 13.3). The greatest part of the trash fish is utilized for the preparation of fish meal, which is used in aquaculture operations.

Thailand started surimi production in 1978 and reached a level of 65,000 t in 1994, and proceeded to more than double the production to 150,000 t in 2005, from 21 surimi

processing plants. Four species, viz threadfin bream (*Nemipterus* spp.), lizardfish (*Saurida* spp.), bigeye (*Priacanthus* spp.), and croaker, are commonly used in the Thai surimi industry. About 70 % of the total frozen surimi was exported to Japan, Singapore, Taiwan, Korea, Malaysia, Hong Kong, Europe, China, Canada, and the USA in 2005, and another 30 % was used in the surimi-based products within Thailand.

3.12 Vietnam

The annual total amount of trash fish estimated by the Research Institute of Marine Fisheries in Vietnam is 930,000 MT, all of which is used for aquaculture feed or feed ingredients. The entire Vietnamese trawl fleet does not comply with the regulation for minimum net mesh size. The catch from this trawl fleet is unmanaged and, therefore, considered as bycatch in accordance to the criteria defined by Davies et al. (2009). The estimates of Edwards et al. (2004) for the bycatch from the shrimp fleet vary from 60 to 80 %. The previous estimate of bycatch is ~38 % by the Ministry of Fisheries (MOFI), Vietnam, during the period 1991–1995. The main products from 150,000 MT of bycatch (Table 13.3) are dried fish, fresh and chilled fish, fish meal, and fish sauce (Thuc 1999).

Surimi production has shown a many fold increase in Vietnam. Thuc (1999) reported an export figure of 386.85 MT in 1993. The production figure increased from 16,502 MT in 2003 (Vietnam Association of Seafood Exporters and Producers, 2003) to 83,495 MT in 2005 from 17 processing plants (Pangsorn et al. 2007). The total quantity of raw material or fish used was 115,035 MT. Almost 90 % of the total frozen surimi production was exported to many countries. The main importers are Korea and Japan, who imported 69.5 % and 28.2 % of the total production, respectively. The others importer were Singapore, Thailand, Malaysia, China, Taiwan, USA, Europe, Australia, New Zealand, Russia, and Mexico. The fish species King Snapper (*Pristipomoides filamentosus*), lizardfish, bigeye, white croaker, and others are utilized in the surimi industry (Pangsorn et al. 2007).

4 Products from Fish Bycatch

This section presents a comprehensive account of fish products developed from bycatch in Asian countries. These products range from traditional to value-added products (Govindan 1985; Sen 2005; James 1998). The technologies involved in the processing of fish curing, minced fish products, canning, and chilling-freezing have been generally well documented (Redhead and Boelen 1990; Hall 1992; Alasalvar et al. 2010). The processing of these products involves simple methods used at home or at the cottage industry level, as well as high technology being used for large-scale production. The products prepared in different countries largely depend on the cultural, social, religious, and traditional values of the country.

Though the number of products prepared from bycatch in Asia is enormous and varies considerably, these products can be broadly categorized as: (i) cured, (ii) fermented, (iii) hydrolyzed, (iv) minced, (v) dehydrated protein-rich foods, (vi) canned, and (vii) miscellaneous products. The cured products include dried fish, dried salted fish, smoked fish, and pickled fish; the fermented products are categorized as fish sauce, fish silage, and fish paste; among the hydrolyzed products, are fish protein concentrate (FPC) and fish protein hydrolysate (FPH); minced products have a wide range of intermediate products, such as fish surimi and fish mince block, which are further processed into fish balls, fish cakes, fish sausages, fish burgers, etc. The dehydrated protein-rich foods are actually the addition of dried fish powder to increase the protein content of fish noodles, macaroni, fish wafers, fish cutlets, and fish soup powder. The products fish marinades, fish meal, and fish fertilizer are included in the miscellaneous products category.

4.1 Cured Products

4.1.1 Dried Products

Dried fish is the oldest product obtained from bycatch. Drying removes water in order to protect from microorganisms. It reduces the water content to 10–15 % and also inactivates enzymes. Small fish are simply dried in the sun. Large fish take a longer time to dry in sun and, hence, most of the time, they are prone to spoilage before drying. The drying step is a traditional fish preservation method used in most Asian countries (Cutting et al. 1956; Jonsyn 1965; Chakraborty et al. 1976; Balachandran 2001). The product is placed on the ground, mats, racks, etc. in the sun. It depends entirely on the intensity of sunlight, the weather, and moisture removal, which relies on air circulation or the rate of evaporation (Cutting et al. 1956; Cutting 1962). The other method utilizes hot air dryers for drying fresh or salted fish (Chakraborty et al. 1972). The temperature is kept below 45 °C. Hot air drying has the advantage of a controlled environment in order to obtain a hygienic product with no microbial contamination, as well as being free of any extraneous material and infestations. In the accelerated mechanical drying, the temperature of the drying air can be increased without the loss of properties and appearance, i.e., the color of the product. Therefore, a high-quality product is made in much less time (Aitkin et al. 1967; Balachandran and Bose 1964, 1965). The method is being utilized in large operations.

4.1.2 Salted Fish

Salted dried fish is commonly prepared in Asian countries from bycatch (Del Valle and Gonzalez-Inigo 1968). Fish are treated with salt solution in a fixed ratio (15–20 %). For wet curing, fish is washed, eviscerated, split, and mixed with salt and left for 48 h (Sen 2005). The fish are hung or spread over bamboo mats or a cement

platform for drying. In dry curing, the fish is washed, cut dorsoventrally, opened, eviscerated, scored and sprinkled with salt, and put into containers, with a wooden plank placed over them. After 24 h, the fish are turned down to mix thoroughly in brine to degenerate enzymes and kill pathogens; the fish in brine mixture is washed with water and dried in the sun for 2–3 days. Different traditional methods are being used in different countries. However, these methods may fall into one of the three main categories of salting methods:

Curing: The dry salt is applied to the flesh side of the dressed fish and the fish is stacked. The moisture of the fish is drained away with the penetration of the salt. This method is not suitable for fatty fish due to the problem of rancidity, which is the result of fat oxidation.

Pickling: The fish is retained in the brine solution produced as a result of salt penetration. The method is useful for fatty fish.

Brining: This method involves the soaking of fish in saturated salt solution or brine. It is useful for the preservation of fatty fish.

4.1.3 Quick Salting Process

A quick salting process been developed in order to shorten the processing time as well as to mechanize the process for large-scale production. Between 20 and 100 % if salt by weight of fish is added to the skinned fish flesh and ground mechanically (Del Valle and Gonzalez-Inigo 1968; Del Valle and Nickerson 1968). The simultaneous salting during grinding increases the rate of salt penetration; thus, the salting time is reduced. The mix is ground further for homogenization or uniform mixing. The fish is pressed after salting to reduce the moisture by 50 % and then subjected to sun drying. This is a useful method for the preservation of fatty fish because, during salting, the fish remains immersed in brine and, therefore, rancidity is minimized (Del Valle and Nickerson 1968; Hiremath et al. 1985, 1989). Other rapid techniques include boiled salted fish, bagged salted fish, and dehydration injection salted fish (Mendelsohn 1974).

4.1.4 Smoked Fish

The eviscerated, washed, and salted fish is smoked by coconut husk or saw dust or any other wood according to flavor acceptance followed by drying. Smoke impacts on the pleasant flavor and preservative effect (Jonsyn and Lahai 1992; Joseph et al. 1984, 1987).

4.2 Fermented Fish Products

Small marine fish (anchovies, clupeids, lizardfish, etc.) from bycatch are used for the preparation of traditional fish sauce (nuoc-mam, nam-pla), fish silage, and fish marinades.

4.2.1 Fish Sauce

Fish sauce is prepared by stacking the whole fish with salt (with a ratio of 1:1–1:5 w/w) in tubs, which are sealed from air and left at room temperature for 2–18 months (US FDA 1977; Zuberi et al. 1988; Thongthai et al. 1990). Salt acts as a preservative to control microbial spoilage and the autolytic enzymes break down the fish proteins. The fish liquefies and the liquid is drawn and filtered. This filtrate is the first-grade fish sauce having a typical cheesy aroma; 25 % brine is added to the residue and left for a further several days or weeks; the liquid is drawn and filtered to make the second-grade fish sauce. The residue at this stage may be used as a fertilizer or be further extracted with boiling brine. These extracts are mixed with different amounts of first-grade liquid and, thus, different grades are obtained. First-grade sauce contains 2–3 g nitrogen/100 g and has a salt content of 20–25 g/100 g and a pH < 6. The quality of product depends on the raw materials, amount of salt used, fermentation time, and capricious factors.

4.2.2 Fish Silage

Fish of low nutritional quality or commercial value and fish waste from the fish processing plants is hydrolyzed by different acids (50–75 % sulfuric acid or 85–90 % formic acid) to make fish silage. Fish silage is used as an animal/cattle feed. This product is odorless and has high nutrient apparent digestibility, which ranges from 31.9 to 84.4 % (Hertrampf and Piedad-Pascual 2000) and is considered to be superior to fish meal (Stanton and Yeoh 1977; Disney et al. 1978; Disney and James 1980; Srinivasan et al. 1985; Sen 2005). Hertrampf and Piedad-Pascual (2000) concluded that its inclusion level should be restricted to not more than 20 % of the protein content of the diet. Fish silage can also be produced by fermentation using fermentable substrate such as molasses or starch of sweet potato. The pH is adjusted to 5 and microorganisms, mainly lactic acid bacteria, are used for fermentation (Zuberi et al. 1993, 1995).

4.2.3 Fish and Shrimp Paste

Fish and shrimp paste products are common in Indonesia, Malaysia, Myanmar, Thailand, and Vietnam. A large number of fermented products with local names are prepared locally. Fish and shrimp pastes are made either by a fish–salt mixture in various proportions or from salted fish fermented in the presence of cooked, boiled, or roasted flour, bran, rice, wheat, or soybeans. Whole fish or pieces of fermented fish are also marketed (Redhead and Boelen 1990). Fermented fish is broken down into soft paste or puree that has a creamy texture. It is used as a condiment or seasoning to add flavor to food. Fish pastes are hot packed containing fish, potato, butter or lard or hydrogenated oil, and a small amount of flour. The incorporation of starchy material minimizes the fish flavor of the product.

These products are commercially produced in many countries of SE Asia, including Japan. Fresh fish is dressed, washed, and mixed with common salt (1.5 % of the fish weight) and pressure cooked; separated meat is mixed with boiled potato and other ingredients. The blend is solid packed in lacquered cans, which are exhausted and seamed (Sen 2005).

4.3 Hydrolyzed Products

4.3.1 Fish Protein Concentrate (FPC)

FPC processing technology was quite popular during the 1960s and 1970s, and a number of publications mainly from the FAO have been compiled. Later, the technology was transformed mostly to fish protein hydrolysate. The processing of FPC has now almost entirely abandoned. FPC contains about 80 % good-quality protein with a full range of essential and non-essential amino acids in just the right balance for human nutrition. Other foods may contain high amounts of protein but are frequently deficient in one or more of the amino acids that are essential for growth. This is a free-flowing, colorless and odorless powder which can be incorporated in several food preparations at up to 5–10 % for enrichment. It is to be prepared exclusively from the edible portions of the fish, with bone, skin, fat, etc. removed so as to increase the protein content and ensure longer shelf life.

The most common method employed is cooking of the eviscerated fish, separation of the edible meat, and pressing and extracting the press cake with an organic solvent to remove fat and odoriferous compounds. There are a number of processes for the preparation of fish meal. A widely used process is called the "VioBin process" (Govindan 1985). In this process, fish muscle is well ground, suspended in ethylene dichloride (B.P. 181 °C) with water (B.P. 100 °C), and heated externally. The solvent forms an azeotropic mixture with the water in the fish muscle and distills. When all the water is removed, the mixture of residual solvent, which holds all the fat present in the fish meat in the solution, and the dehydrated meat are separated by filtration, and the meat portion is washed repeatedly with the solvent to remove all adhering fat, freed from all the solvent by heating and pulverization.

4.3.2 Fish Protein Hydrolysate (FPH)

FPH is a value-added, protein-rich product obtained by enzymatic protein hydrolysis. The hydrolysis of protein is carried out by the addition of enzymes of animal or vegetable origin, under controlled conditions of pH, temperature, and time (Sen and Lahiry 1962; Halliday and Disney 1974; Sugiyama et al. 1991; Warrier et al. 1996; Sen 2005). Processes for the preparation of FPH for lean and fatty fish are different. In lean fishes, the fish is minced and subjected to enzymatic digestion.

The enzymes are subsequently deactivated by boiling and followed by centrifugation and filtration for clarification. After vacuum concentration, it is dried and packaged.

Fatty fish is cooked and pressed to obtain liquor, which is centrifuged to remove oil. The press cake is dispersed in water and digested with enzymes. After deactivation of the enzyme, the digest may be centrifuged to remove bones and indigestible fractions. Subsequent operations are the same as lean fish. Enzyme should be of food grade and active at 70 °C near neutral pH. If fish has more than 1 % fat, it should be stabilized with an antioxidant. The use of an antifoaming agent like silicone is necessary during vacuum concentration to reduce frothing and foaming. The product is hygroscopic, so it is packed in a humidity-controlled chamber. The recovery rate is 7.5–13.5 % and the color is reddish brown, creamy, or white, with a bitter taste and a fishy smell. Moisture is 1.4–8.5 %, protein 80–93 %, fat 0–5 %, and ash 3.2–8.6 %. It contains nitrogen, proteases, peptones, and also free amino acids.

4.4 Minced Fish Products

Minced fish is a commercial product in many Asian countries prepared from bycatch resources. It is popular because of the ease with which it can be used as a principal or supplementary ingredient in different delicious and nutritious preparations, e.g., kamaboko, sausages, cakes, patties, fish balls, fish sticks, meat extenders, etc. (Pruthiarenum et al. 1985, 1986). The functional properties of mince fish are its succulence, juiciness, fat-absorbing ability, and gel-forming ability, amongst others.

The minced meat is obtained by the deboning of fish; it is prepared by washing with water to remove the water-soluble proteins and a significant amount of fat. Most species of bycatch can be converted to minced fish either mechanically or manually. The yield of mince may vary between 35 and 50 %, depending on the type of fish used. Minced fish cannot be kept unprocessed for long due to its highly perishable nature, particularly where handling practices are not sound during processing and production. It can be kept frozen for 8–9 months without any loss of quality (Revankar et al. 1981; Agarwal et al. 1986). The use of tripolyphosphate and sodium chloride helps in the maintenance of mince quality during frozen storage (Ghadi and Lewis 1977), whereas the addition of benzoate phosphate is reported to have extended the shelf life for 13 days at 0 °C (Jantawat and Yamprayoon 1990). Nonetheless, minced fish has become the most accepted way of utilizing bycatch of low-value and pelagic fish (Steinberg et al. 1977; Sen 2005).

4.4.1 Block-Frozen Fish Mince

Fish mince can be quick frozen at –40 °C after pressing into 500-g and 1-kg blocks. The blocks are glazed with water by dipping in ice-cold water after freezing, as they are very susceptible to freeze dehydration. It is an excellent consumer product when packed properly (Gopakumar 1998).

4.4.2 Texturized or Molded Products

Fish mince can be used as a raw material for the preparation of several products, such as fish balls, fish burgers, fish fingers, etc. (Krishnaswamy et al. 1962; Shenoy et al. 1988; Venugopal 1992; Venugopal et al. 1992; Gopakumar 1998). The mince is washed two to three times with water and mixed with salt, starch, and some form of preservative to make dough, which can be shaped into blocks or other desired shapes, like fingers, cakes, etc. These can be battered and breaded and fried in vegetable oil. Seasoning and spices can also be added to impart desired flavors and tastes. Fish fingers made out of mince may not have the properties of fingers prepared from whole fish fillet, but fingers prepared from minced meat are comparable for most sensory properties, like taste and flavor. However, being much cheaper, they offer better market prospects.

4.4.3 Fish Cutlet

Fish cutlets or cake can be prepared from whole fish or fish mince. Fish cutlet is a highly acceptable consumer product for both urban and rural populations in India. The meat is separated after cooking the fish. Boiled, peeled, and mashed potatoes are added to the cooked fish mince, as well as salt and some spices, and then it is soft fried in vegetable oil. Patties are made, dipped in batter, and rolled over bread crumbs. The battered and breaded fish cutlets are flash fried in vegetable oil, packed in consumer packets, and stored under −20 °C for up to 6 months. Cutlets are to be deep fried prior to consumption (Joseph et al. 1984).

4.4.4 Fish Burger

Fish burgers are included in ready-to-eat convenience products of modern living. Minced fish is used for making various shapes; the product can be battered and breaded and used in fish burgers to produce value-added products. Precooked frozen burgers have been developed using sardine meat. Chopped sardine meat is mixed with 14 % emulsion curd, 8 % bread crumbs, 3 % soy protein, 1.5 % salt, 2 % sugar, 0.4 % bicarbonate, and 0.2 % polyphosphate and spices. The seasoned sardine meat is fried in oil at 165 °C for 3 min (Etoh 1986; Roessink 1989).

4.4.5 Surimi

The process for making surimi was developed in many areas of East Asia over 900 years ago. It is a Japanese term used to denote a semi-processed fish paste material. Surimi is processed from the mechanically deboned fish flesh, which is washed many times to remove lipids, blood, and soluble proteins, leaving behind the myofibrillar protein. These stabilized myofibrillar proteins are

blended with cryoprotectant for long shelf life in frozen storage. A review of the methods has been done by Nopianti et al. (2010) for the improvement of surimi quality and to increase the quantity of myofibrillar protein. The cryoprotectants used by different workers are sorbitol, sucrose, polydextrose, lactitol, Litesse, maltodextrin, trehalose, sodium lactate, and mixtures of the above cryoprotectants. Phosphate is normally added to surimi in combination with cryoprotectants to reduce viscosity, increase moisture retention, and improve the protein's ability to reabsorb liquid when the surimi is thawed or tempered, and to increase the pH slightly, which leads to improved gel-forming ability, gel strength, and cohesiveness. Food additives like egg whites, beef plasma protein, whey protein concentrate, and chitosan are also used to improve physical properties and to prevent the textural degradation of surimi. A more recent process has been developed for surimi production using an acid and alkaline washing method, which has shown a significant increase in the concentration of myofibrillar protein in surimi on an experimental basis (Nopianti et al. 2010). Surimi comprises 17.3 % proteins, 1.4 % fat, 4.1 % carbohydrates, 2.6 % ash, and 75 % moisture. It is an intermediate product made from minced fish meat for further use in various kinds of food products, such as fish balls, fish cakes, fish pastes, fish sausages, imitation crab legs, crab meat, imitation shrimp, and as an ingredient in thick soups.

4.4.6 Surimi-Based Products

Seasoning and salt are added to make seasoned surimi, which is then shaped into various forms, such as fish balls, fish cakes, fish sausages, and many more products in technologically advanced countries like Japan. The development of fish sausages is credited to Japanese scientists, which is based mostly on the surimi technology. The ingredients for fish sausages are fish, any other grounded meat like poultry, pork, beef, and mutton, starch, seasonings, species, phosphates, food colors, and antioxidants, permitted preservatives. Isolated soy protein in a small amount (2 % of the total) may also be included. The product is packed in a casing which is sealed and heat processed to extend the shelf life. Instead of using ground fish meat paste, surimi is almost exclusively used at present in Japan for fish sausage production. Many other value-added products are manufactured in Japan, mostly from walleye pollock, which is not considered bycatch, hence, is not mentioned here. Fish sausages are developed from the mince of Indian oil sardines (Ravishankar et al. 1993a, b). Fish such as trevally and unicorn leatherjacket are also used for battered and breaded products from surimi (Yu and Siah 1996, 1997). Recently, some new surimi-based snacks, locally called seafood samosas, have been developed in India on a commercial scale, which is available in 250-g packs. The snack is a stuffed pastry, consisting of a fried or baked triangular and semi-lunar pastry shell with a savory filling, which may include spices, potatoes, onions, peas, and coriander with fish surimi (Infofish International, 1/2011).

4.5 *Dehydrated Protein-Rich Food*

Protein-rich foods based on fish bycatch in a dehydrated form and starch from different sources have been developed, such as fish macaroni, fish noodles, fish potato flakes, fish flakes, and fish wafers. In addition, various products like cutlets, wafers, spirals, soup powder, etc. are manufactured using fresh *Acetes indicus* (Zynudheen et al. 1998). The following sections detail products which have been developed in India and SE Asia (Gopakumar et al. 1975; Gopakumar 1998; James 1998).

4.5.1 Fish Satay

Fish satay is a very popular and successful product of Malaysia, Thailand, and Indonesia. In Malaysia, fish satay is made from goatfish (Min 1998; Bakar 1987), whereas in Thailand, lizardfish (*Saurida* spp.) is used for the same purpose (Pruthiarenum 1986; Suwanrangsi 1986, 1988). To make fish satay, deboned fish is mixed with salt, sugar, flour, and sesame seeds, spread into sheets, and dried for 4–5 h. Then, the dried sheets are fried. The product has a shelf life of at least 5 months at ambient temperature.

4.5.2 Fish Soup Powder

White flesh of many low-value fish like threadfin bream, sciaenid, perches, etc. can be used to prepare instant fish soup powder with the addition of emulsifier, thickening agent, spices, and preservatives. The fish is used in a partially hydrolyzed form. The product is highly hygroscopic and requires special packaging, such as 12-μm plain polyester laminated with LDPE-HDPE co-extruded film (Alasalvar et al. 2010). This product has gained high consumer acceptability and is produced in India by several manufacturers. It is a high-value item for urban populations due to its short preparation time (Gopakumar 1998; Sen 2005).

4.5.3 Fish Macaroni/Fish Noodles

Fish macaroni is based on a blend of fish flesh and starchy material like wheat semolina or a mixture of wheat semolina and tapioca flour. The starchy component is gelatinized and added to fish muscle. The blend is cooked and pressed in an extrusion press and dried. The protein content of the product is about 15 %. Fish noodles were also developed by Bhatia et al. (1959) and Phitakpol et al. (1986) by the incorporation of 25 and 30 % fish into the noodles mixture, which, in turn, increased the protein content to 28 %.

4.5.4 Fish Potato Flake

Fillets are steam cooked and minced. The bones are removed by rubbing the cooked and minced fish on a 40-mesh stainless sieve. Cooked, peeled, and mashed potatoes are blended with cooked flesh in equal quantities (1:1) and slurry is made. The slurry of fish and potato is dried on a double-drum dryer and flaker. The contact time is 10 s with a drum rotation of 5 rpm, and the steam pressure is 40 psi. The dried product is removed in the form of continuous thin flakes. The product is crisp in texture, with a cream white color and a pleasing mild fish flavor. The product has 7.5 % moisture, 45.5 % total protein, 6.6 % total ash, and 4.5 % ether extractives (Krishnaswamy et al. 1965).

4.5.5 Fish Flake

Some species of fish bycatch are used to prepare fish flakes. The meat is separated from cooked fish and added with orthophosphoric acid, and water is removed by pressing. The deodorized meat is homogenized with corn starch, common salt, and water to obtain slurry, which is poured onto flat aluminum trays to a thickness of 1 mm and steam heated for 20 min. The material is cut to give flakes of appropriate size and dried in a tunnel dryer to a moisture content of about 8 %. In addition to corn starch, wheat flour (*maida*), tapioca starch, and black gram powder could also be used (Moorjani et al. 1962; Venugopal and Govindan 1967).

4.5.6 Fish Wafer

Wafer is a dried product of any shape with small thickness, prepared from a blend of fish meat, starch, and salt. Upon frying, the product swells to two to three times its initial volume. Fried wafer is crisp and highly delicious. Alternatively, fish mince may also be used instead of fish meat. The mince is first cooked in water and is pressed to remove the water; tapioca, corn starch, and, subsequently, common salt are added, followed by grinding after each addition. Homogenized material is poured onto flat aluminum trays to a film thickness of 1–2 mm and dried in drying cabinets or solar dried until the moisture content is below 6 %. The product may be packed in laminated pouches for retail sale. Packed product has a good shelf life. A typical sample has 5–6 % ash, 1–2 % fat, and 60–65 % carbohydrates (Gopakumar 1998).

4.5.7 Protein Powder

Protein powder is made from *Acetes* spp. caught as bycatch, by drying heat-coagulated pulp in the sun and under a vacuum, and can be used as seasoning for various preparations (Garg et al. 1977).

4.6 Canned Fish

A considerable portion of fish bycatch resource is utilized for canning. Sardines are most commonly used for canning. Fresh fish is washed, and the heads, fins, and tails trimmed, scales scraped, and the viscera removed. The dressed fish are cleaned thoroughly to remove blood, slime, and scales with potable water, taking special care of the stomach cavity; the fish are immersed in 15 % salt solution for 15 min. The brined fish are placed in perforated trays and cooked in an autoclave at 0.35 kg/sq cm steam pressure for 35–40 min, allowing the cook drip to drain off. This step assists in bringing about the required shrinkage of the fish, casting off excess water content, inactivating the enzymes, and invoking partial sterilization. The cooked and cooled fish are then packed into 301 × 206 mm, with the head and tail ends alternately facing the bottom, so as to ensure compact packing. Usually, 200 g of the cooked fish is packed into one can; hot refined vegetable oil at 90–95 °C is added to the can, leaving a head space of 5–10 mm. The cans are then passed through an exhaust chamber for 8–10 min in flowing steam. As soon as the cans emerge at the exit, they are double seamed with an automatic seamer. The cans are washed and autoclaved for 70–75 min at 0.84 kg/sq cm steam pressure. Then, they are plunged into chlorinated ice water for rapid cooling (Govindan 1985).

4.7 Miscellaneous Products

4.7.1 Marinated Fish

Salted or fresh fish fillet or small pieces of fish are marinated. Small clupeids are used preferably for marinades after bleaching with hydrogen peroxide and subsequently preserved in acetic acid (5–6 %) and brine (6–8 % salt) for several hours. Fermentation must be avoided; therefore, saccharin or a similar sweetening agent is used instead of sugar (Govindan 1985; Sen 2005). Different spices and lime juice may also be used. The recipe may vary from country to country. The product may be preserved in vinegar in glass jars and stored at refrigeration temperatures. The marinated fillets are rolled and tied with thread or tooth picks before adding the vinegar. The marinated fish (small pieces) can be used with fresh sliced vegetables to make different salads or it can be fried for serving.

4.7.2 Fish Meal

Fish meal is an excellent source of nutrition supplements for land and aquatic animals, as it contains proteins, minerals, B vitamins, and growth factors. The protein content is 50–70 %, fat 5–10 %, ash 2–33 %, and moisture 5–10 %. There are two methods for the preparation of fish meal, wet and dry.

Wet Method: Trash fish is boiled to soften the bones and meat. It is then cooled and filtered. The residue is pressed to obtain press cake, which is dried in the sun or artificially and pulverized in a flour mill. In developed countries, a continuous cooker is used, in which material is moved by a conveyor through the cocker to coagulate the proteins, which are then dehydrated in a continuous press. The press cake is dried to 8 % moisture and the liquor is separated into oil and water.

Dry method: This method is mostly for lean fish. Fish is solar dried to 10 % moisture and pulverized. In one of the latest methods, the raw material is fed into a steal drum containing horizontal plates, which are heated by passing steam under pressure. The drum is rotated and the material is cooked by the heat and dried by the plates.

4.7.3 Fish/Poultry/Shrimp Feed

Fish meal was used mainly as a fertilizer until about 1910, but later, its high nutritional value has been recognized for utilization in animal feeds (Windsor 2001). Fish meal is utilized in supplemental feeds for fish, shrimp, pig, dog, cat, cattle, and poultry. The trash fish is also utilized in fish and shrimp culture directly.

4.7.4 Fertilizers

Fish offal or trash fish can be converted into a fertilizer through acid digestion, especially in organic farming. The process reduces the fish odor, converts fish proteins into ammonium sulfate, and makes the phosphates of the bones absorbable by the plants, thus resulting in better yield and quality of crops, as it increases the mineral content of the soil (Brody 1965).

5 Conclusion

1. The bycatch produced in Asian countries is highly variable; the highest bycatch rate has been found in Bangladesh, whereas the lowest was found in Japan, while for all other countries, the bycatch level is in the range 31–50 %, which demonstrates the uncontrolled state of fisheries management practices, poor compliance to the fisheries regulation, and possible differences in the poverty level or socioeconomic conditions of the coastal communities of different countries.
2. Despite the high value of bycatch, losses have been minimized by the maximum utilization for human food and animal feed. In some countries of SE Asia, like Myanmar, Thailand, Vietnam, etc., all of the bycatch is fully utilized.
3. Technological advances are almost non-existent and the techniques adopted for bycatch utilization are based mainly on simple, traditional, and cost-effective methods in the majority of Asian countries, except for Japan, where technological

advances have been made for the development of value-added fishery products using high-value fish. The quantity of bycatch is quite low, as mentioned above.

4. It is advisable to make efforts to popularize the unpopular edible fish and shellfish as a table dish to provide relief to the already overexploited target fish and shrimp fish species.
5. A system of reliable data collection is lacking for the estimation of bycatch and discards, even in well-developed countries. Concerted efforts should be rationalized for the collection of data and proper methodology be envisaged to achieve the goal of reliable estimates.
6. Some species that are considered as trash in some countries are very popular and are in demand in other countries. Such species can serve as export items if properly processed.
7. The area which should be given due consideration in the supply chain is on-board handling of the bycatch, which, if carried out in a responsible way, can produce large benefits.
8. Sharing of information/data and transfer of technology at the regional, national, and international levels should be encouraged for product development and post-harvest technology for better utilization of bycatch.
9. The vast potential of effective fish bycatch utilization urge for enhancing research on bycatch utilization for safeguarding the fishery resources from the clutches of commercial extinction and augmenting biodiversity conservation.

References

Adams MR, Cooke RD, Ratagool P (1985) Fermented fish products of South East Asia. Trop Sci 25:61–73

Agarwal A, Raghunath MR, Solanki KK (1986) Frozen storage studies of composite fish mince from Dhoma (*Sciaenid* spp.) and *Lactarius* (*Lactarius lactarius*). Fish Technol 23:129–133

Aitkin A, Jason AC, Olley J, Payne PR (1967) Effects of drying, salting and high temperatures on the nutritive value of dried fish. Fish News Int 6(9):42

Alasalvar C, Miyashita K, Shahidi F, Wanasundara W (eds) (2010) Handbook of seafood quality, safety and health applications. Wiley-Blackwell, Oxford

Alverson DL, Freeberg MH, Murawski SA, Pope JG (1994) A global assessment of fisheries bycatch and discards. FAO Fisheries Technical Paper No. 339, FAO, Rome, 233 pp

Armstrong DW, Ferro RST, MacLennan DN, Reeves SA (1990) Gear selectivity and the conservation of fish. J Fish Biol 37:261–262

Aye KM, Pyo A (1998) Bycatch Utilization in Myanmar. In Report and Proceedings of FAO/DFID Expert Consultation on Bycatch Utilization in Tropical Fisheries. Beijing China, 21–28 September 1998. Clucas I and Teutscher F (eds) Natural Resource Institute (1999)

Bakar MMF (1987) Satay fish and other traditional fish products development in Malaysia. In: Proceedings of the 20th anniversary seminar on "Development of fish products in South East Asia", SEAFDEC, Singapore, 27–31 Oct 1987, pp 87–90

Balachandran KK (2001) Post-harvest technology of fish and fish products. Daya Publishing House, Delhi, pp 77–103

Balachandran KK, Bose AN (1964) Dehydration of prawns by rotary dryers. Proc Indo-Pacific Fish Coun 11:272–277

Balachandran KK, Bose AN (1965) Dehydration of prawns in tunnel dryers. Fish Technol 2:126–130
Basu S, Gupta SS, Panduranga RCC (eds) (1987) Proceedings of the symposium on "Diversification of post-harvest technology for low cost fish". Society of Fishery Technologists, Cochin, pp 175–181
Begum A, Shakir S, Ali A, Haq SA (1984) Studies on trash fish (pelagic fishes), a potential source of producing fish protein concentrate (FPC). Proc Pak Acad Sci 22(2):113–124
Bhatia DS, Moorjani MN, Iyengar JR, Visweswaraiah K (1959) Fish macaroni. Food Sci 8(1):169–177, CFTRI, Mysore
Bricklemyer EC Jr, Iudicello S, Hartmann HJ (1989/1990) Discarded catch in U.S. commercial marine fisheries. Aud Wildl Rep 1989/1990:159–295
Brody J (1965) Fishery by-products technology. The AVI Publishing Company, Westport, 232 pp
Burke WT, Freeberg M, Miles EL (1993) United Nations resolutions on driftnet fishing: an unsustainable precedent for high seas and coastal fisheries management. University of Washington, Seattle, Washington, 77 pp
Burt JR, Hardy R, Whittle KJ (eds) (1992) Pelagic fish: the resource and its exploitation. Fishing News Books Ltd./Blackwell Scientific Publications, Oxford
Caddy JF (1989) Species selectivity of fishing gear: an optimal criterion for management of stocks. In: National reports and selected papers presented at the sixth session of the working party on assessment of marine fishery resources, St. George's, Grenada, 15–19 May 1989. Western Central Atlantic Fishery Commission/UN/FAO, Rome
Chakraborty PK (1976) Solar drier for drying fish and fishery products. Res Ind 21:192
Chakraborty PK, Gopala RKR, Kadkol SB, Bhatia BS (1972) Studies on the development of dehydrated curried fish mince. Indian Food Packer, 50 pp
Chan EH, Liew HC (1986) Characteristics of an exploited tropical shallow-water demersal fish community in Malaysia. In: Maclean JL, Dizon LB, Hosillos LV (eds) Proceedings of the 1st Asian fisheries forum, Asian Fisheries Society, Manila, 26–31 May 1986, pp 349–352
Chasana E (1999) Bycatch utilization in Indonesia. In Report and Proceedings of FAO/DFID Expert Consultation on Bycatch Utilization in Tropical Fisheries. Beijing China, 21–28 September 1998. Clucas I and Teutscher F (eds) Natural Resource Institute (1999)
Cheapok C, Pornnareay P (1998) Introducing solar drying in a developing country: the case of Cambodia. Institute of Technology Cambodia. Final report on solar drying, submitted to AIT in the framework of the ITC/AIT collaborative project, Renewable energy technologies in Asia: a regional research and dissemination program, 8 pp
Chee PE (1996) A review of the bycatch and discards in the fisheries of Southeast Asia. In: Clucas IJ, James DG (eds) Report on the technical consultation on reduction of wastage in fisheries. Tokyo, 28 Oct–1 Nov 1996. FAO Fisheries Report No. 547. FAO, Rome
Choorit W, Sophanodora P, Wuttijumnong P, Pooldam A, Buckle K (1991) Quality of fish paste ('Kapi') from sardine. ASEAN Food J 6:28–31
Clucas I (1997) A study of the options for utilization of bycatch and discards from marine capture fisheries. FAO Fisheries Circular No. 928. FAO, Rome, 59 pp
Clucas I, Teutscher F (eds) (1999) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries, Beijing, Sep 1998. Natural Resource Institute, Chatham, 329 pp
Cutting CL (1962) The influence of drying, salting and smoking on the nutritive value of fish. In: Hean E, Kreuzer R (eds) Fish in nutrition. Fishing News Books, London, pp 161–179
Cutting CL, Reay GA, Shewan JM (1956) Dehydration of fish, Food Investigation Special Report No. 62. D.S.I.R., Her Majesty's Stationery Office, London, 66 pp
Davies RWD, Cripps SJ, Nickson A, Porter G (2009) Defining and estimating global marine fisheries bycatch. Mar Policy. doi:10.1016/j.marpol.2009.01.003
Dawson SM (1991a) The potential for reducing entanglement of dolphins and porpoises with acoustic modifications to gillnets. IWC report

Dawson SM (1991b) Modifying gillnets to reduce entanglement of cetaceans. Mar Mamm Sci 7:274–282

Dawson SM (1991c) Incidental catch of Hector's dolphin in inshore gillnets. Mar Mamm Sci 7:283–295

DeAlteris JT, Castro KM, Testaverde SA (1990) Effect of mesh size in the body of a bottom trawl on the catch retained in the cod end. In: Proceedings of the fisheries conservation engineering workshop, Rhode Island, Apr 1990

Del Valle FR, Gonzalez-Inigo JL (1968) A quick salting process for fish, 2. Behaviour of different species of fish with respect to the process. Food Technol 22:1135–1138

Del Valle FR, Nickerson JTR (1968) Studies on salting and drying fish. 3. Diffusion of water. Food Technol 33:499–503

Dickson JO (1997) Selective shrimp trawling in Manila Bay. Paper presented at the cooperative research network workshop in the Asia and Indian Ocean Region on selective shrimp trawling, Darwin

Disney JG, James D (eds) (1980) Fish silage production and its use. FAO Fisheries Report No. 230. FAO, Rome, 105 pp

Disney JG, Hoffman A, Olley J, Clucas IJ, Barranco A, Francis BJ (1978) Development of a fish silage/carbohydrate animal feed for use in the tropics. Trop Sci 20:129–144

Doke SN, Venugopal V, Thomas P (1996) Innovative method for dehydrated laminates from Bombay duck. Infofish Int 6:49–50

Durariaj S, Pichiah P (1985) Production of quick-salted *Lethnuis* (sea bream) fish cakes. In: Proceedings of the harvest and post-harvest technology of fish, Society of Fishery Technologists, Cochin, 556 pp

Edwards P, Tuan LA, Allen GL (2004) A survey of marine trash fish and fish meal as aquaculture feed ingredients in Vietnam. Australian Centre for International Agriculture Research (ACIAR), Canberra, 56 pp

Etoh S (1986) Product development in the use of shrimp by-catch in Bangladesh. Infofish Int 5:14–16

FAO (1995) Code of conduct for responsible fisheries. FAO, Rome, p 41 pp

FAO (1996) Report of the technical consultation on reduction of wastage in fisheries. Tokyo, 28 Oct–1 Nov 1996. FAO Fisheries Report No. 547. FAO, Rome

FAO (2004) The state of world fisheries and aquaculture (SOFIA), 213 pp

FAO (2009) Technical guidelines for responsible fisheries. Number 1: fishing operations. Supplement 2: best practices to reduce incidental catch of seabirds in capture fisheries. FAO, Rome. ISBN 978-92-5-106423-8

FAO (2010) The state of world fisheries and aquaculture 2010. FAO, Rome, 197 pp

Fatima R, Qadri RB (1987) Studies on the preparation of fish silage. Trop Sci 27:1–8

Flick GJ, Barua MA, Enriquez LG (1990) Processing finfish. In: Martin RE, Flick GJ (eds) The seafood industry. Van Nostrand Reinhold, New York, pp 117–164

Frazier J (1980) Exploitation of marine turtles in the Indian Ocean. Hum Ecol 8:329–370

Garg DK, Nair AL, Prabhu PV (1977) Protein from jawla prawn (*Acetes* spp.) and squilla (*Oratosquilla nepa*). Fish Technol 14:53

Ghadi SV, Lewis NF (1977) Preparation of minced muscle blocks from trash fish. Fleischwirtschaft 57:2155–2157

Ghadi SV, Madhavan VN, Kumta US (1976) Expansion in utility of trash fish for preparation of comminuted meat products. In: Proceedings of the symposium on "Fish processing industry in India", Mysore, 13–14 Feb 1975. Association of Food Scientists and Technologists (India), Central Food Technological Research Institute, Mysore, 64 pp

Gopakumar K (1987) Minced fish based products. In: Proceedings of the symposium on diversification of postharvest technology for low cost fish, Society of Fishery Technologists, Cochin, pp 139–148

Gopakumar K (1989) Minced fish based products. In: Balachandran K, others (eds) Recent trends in processing low cost fish. Society of Fisheries Technologists (India), Kochi, 89 pp

Gopakumar K (1991) Utilization of low grade marine fish. In: Sinha VRP, Srivastava HC (eds) Proceedings of the symposium on aquaculture productivity held in Dec 1988, Oxford and IBH Publishing Co., New Delhi, 719 pp
Gopakumar K (1992) Minced fish-based products. In: Proceedings of the symposium on diversification of post harvest technology for low cost fish, Society of Fishery Technologists, Cochin, pp 139–148.
Gopakumar K (1997) Tropical fishery products. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 190 pp
Gopakumar K (1998) Utilization of trawl bycatches in Southeast Asia. In: Proceedings of the APFIC symposium, Beijing, 24–26 Sep 1998. RAP Publication 1998/24, pp 29–48
Gopakumar K, Vasanth Shenoy A, Kutty Ayyappan MP, James MA, Iyer KM (1975) Speciality products from miscellaneous trash fish. In: Proceedings of the symposium on the fish processing industry in India, Mysore. Association of Food Scientists and Technologists (India), Mysore, 68 pp
Gopakumar K, Muraleedharan V, Bhattacharyya SK (1992) Preparation and properties of surimi from tropical fish. Food Control 3(2):109–112
Govindan TK (1985) Salt curing and drying of fish. In: Fish processing technology. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, 252 pp
Grantham GJ (1981) Minced fish technology – a review. FAO Fisheries Technical Paper No. 216, FIIU/T, 72 pp
Gulf of Mexico Fishery Management Council (GMFMC) (2006) Scoping document for amendment 15 to the Shrimp FMP
Hall GM (1992) Fish processing technology. Chapman and Hall, London, 309 pp
Hall MA (1996) On bycatches. Rev Fish Biol Fish 6:319–352
Hall MA, Alverson DL, Metuzals KI (2000) By-catch: problems and solutions. Mar Pollut Bull 41:204–219
Halliday D, Disney JG (1974) Fish protein concentrate: a review. Tropical Products Institute, London. ICMRD. 1988. In: Morrissey MT (ed) Proceedings of the international workshop on "Post harvest fish losses", University of Rhode Island, Kingston, 12–16 Apr 1987. ICMRD
Harris AN, Poiner IR (1990) By-catch of the prawn fishery of Torres Strait; composition and partitioning of the discards into components that float or sink. Aust J Mar Freshw Res 41:37–52
Hertrampf JW, Piedad-Pascual F (2000) Handbook on ingredients for aquaculture feeds. Kluwer, Dordrecht, 624 pp
Hiremath GG, Sudhakara NS, Shetty HPC (1985) A new method for utilization of oil sardine for human consumption. In: Ravindran K, others (eds) Harvest and post-harvest technology of fish. Society of Fisheries Technologists (India), Kochi, 573 pp
Hiremath GG, Serrao AD, Prathapachandra TN, Setty TMR (1989) Salted and pressed sardine processing and storage. In: Balachandran KK et al (eds) Proceedings of "Recent trends in processing low cost fish", Society of Fisheries Technologists (India), Kochi, 48 pp
Huda N, Abdullah A, Babji AS (2001) Functional properties of surimi powder from three Malaysian marine fish. Int J Food Sci Technol 36:401–406
Ihm CW, Kim JS, Joo DS, Lee EH (1992) Processing and quality stability of precooked frozen fish foods: 1, processing of sardine burger. J Korean Agr Chem Soc 35:254–259
James DG (ed) (1998) Fish utilization in Asia Pacific. In: Proceedings of the APFIC symposium. Beijing, 24–26 Sep 1998. RAP Publication 1998/24, 323 pp
Jantawat P, Yamprayoon J (1990) Effect of washing, chemical additives and storage temperature on quality of mechanically deboned by-catch. ASEAN Food J 5:108–113
Jasmine GI, Rathinam AMM, Rathnakumar K, Jeyachandran P (1995) Influence of cryoprotectant on the quality of frozen minced threadfin bream (*Nemipterus bleekeri*) during frozen storage. FAO Fish Report No. 514, pp 211–224
Jonsyn AC (1965) Drying and dehydration. In: Borgström G (ed) Fish as food – processing part 1, vol 3. Academic, New York, p 489

Jonsyn FE, Lahai GP (1992) Mycotoxic flora and mycotoxins in smoke-dried fish from Sierra Leone. Nahrung 36:485–489

Joseph J, Perigreen PA, Thampuran N (1984) Preservation and storage of cutlet from low-priced fish. Fish Technol 21:70–74

Joseph AC, Prabhu PV, Balachandran KK (1987) Studies on dried/smoked barracuda. Fish Technol 24:96–98

Kapurusinghe T (2006) Status and conservation of marine turtles in Sri Lanka. In: Shanker K, Choudhury BC (eds) Marine turtles of the Indian subcontinent. Universities Press, Hyderabad, pp 173–187

Kapurusinghe T, Samana MM (2001) Marine turtle by-catch in Sri Lanka. In: Proceedings of the 21st annual symposium on sea turtle biology and conservation, Philadelphia

Karp WA, Desfosse LL, Brooke SG (2011) U.S. national bycatch report. National Marine Fisheries Service, NOAA Technical Memo NMFS-F/SPO-117E

Kelleher K (2005) Discards in the world's marine fisheries: an update. FAO, Rome, 131 pp

Khatoon S, Hanif NQ, Malik N (2006) Status of fish meal available for poultry rations in Pakistan. Pakistan Vet J 26:97–98

Krishnaswamy MA, Subba Rao KR, Lahiry NL (1962) Manufacture of ready-to-serve/cook fish paste. J Sci Ind Res D 21:303

Krishnaswamy MA, Lahiry NL, Kadkol SB, Venkat Rao S, Revankar GD, Suresh Chandra KC (1965) A nutritional evaluation of fish potato flakes. Food Technol 19:197

Kungsuwan A (1999) Bycatch utilization in Thailand. In: Clucas I, Teutscher F (eds) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries. Natural Resource Institute, Beijing/Chatham, pp 85–103

Legaspy AS (1999) Bycatch utilization in Philippines. In: Clucas I, Teutscher F (eds) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries. Natural Resource Institute, Beijing/Chatham, pp 105–113

Lin Y (1999) A review of by-catch and its utilization for capture fisheries in the South China Sea. In: Clucas I, Teutscher F (eds) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries. Natural Resource Institute, Beijing/Chatham, pp 139–145

Marfori EA, Borja NC, Guevara G (1991) Utilization of low-value fish in the development of convenience foods. In: Hooi KK, Miwa K, Salim MB (eds) Proceedings of the seminar on advances in fishery post harvest technology in Southeast Asia, SEAFDEC, Singapore

Matsuoka T (1997) Discards in Japanese marine capture fisheries and their estimation. In: Clucas IJ, James D (eds) Report on the technical consultation on reduction of wastage in fisheries. Tokyo, 28 Oct–1 Nov 1996. FAO Fisheries Report No. 547 supplement. FAO, Rome

Matsuoka T (2008) A review of bycatch and discard issue toward solution. In: Tsukamoto T, Kawamura T, Takeuchi TD, Beard Jr, Kaiser MJ (eds) Proceedings of the 5th world fisheries congress. Fisheries for global welfare and environment. TERRAPUB 2008, pp 169–180

McCaughran DA (1992) Standardized nomenclature and methods of defining bycatch levels and implications. In: Proceedings of the national industry bycatch workshop, Newport, 4–6 Feb 1992

Mendelsohn JM (1974) Rapid techniques for salt-curing fish: a review. J Food Sci 39:125–127

MFRD (2005) Annual highlights. SEAFDEC Marine Fisheries Research Department, Singapore, p 36

MFRD (2006) Maximizing utilization of pelagic fish resources. SEAFDEC Marine Fisheries Research Department, Singapore, p 124

Min TS (1998) Utilization of trawl bycatches in Southeast Asia. In: Proceedings of the APFIC symposium, Beijing, 24–26 Sep 1998. RAP Publication 1998/24, pp 49–51

MOFI (1998) The final survey report assessment of the living marine resources in Vietnam in the period March 1996–October 1997. Ministry of Fisheries Vietnam/DANIDA

Moorjani MN, Balakrishnan Nair R, Krishnaswamy MA, Lahiry NL (1962) Fish protein enriched corn starch. Res Ind 7:307

MRAG (2012) A review of bycatch in the Indian ocean gillnet tuna fleet focussing on India and Sri Lanka. ISSF Technical Report 2012-05. International Seafood Sustainability Foundation, Washington, DC
Muraleedharan V, Gopakumar K (1997) Suitability of trawl by catch for surimi preparation. FAO Fish Report Suppl. No. 563. FAO, Rome, pp 315–319
Murawski SA (1992) The challenges of finding solutions in multispecies fisheries. In: Schoning RW, Jacobson RW, Alverson DL, Gentle TG, Auyong J (eds) Proceedings of the national industry bycatch workshop, Newport, 4–6 Feb1992. Seattle, Washington, Natural Resources Consultants, Inc., pp 35–45
Nakayama T, Sato N, Kanoh S, Niwa E (1988) Process preparation of restructured sardine [*Sardinops melanostictus*] meat using thoroughly washed pulverized meat. Bull Jpn Soc Sci Fish 54:2127–2134
Nasran S, Irianto BE, Fawzya YN (1986) Urea content reduction in shark meat by mincing and washing for fish meat ball processing. Food Waste Bull 2:144–148
Nopianti R, Huda N, Ismail N (2010) Loss of the functional properties of proteins during frozen storage and the improvement of gel-forming properties of surimi. Asian J Food Agro-Ind 3:535–547
Nowsad AAKM, Uddin MH, Niwa E (1998) Suitability of 3-trash-fish species in the manufacture of surimi in Bangladesh. Indian J Anim Sci 68:284–288
OECD (1997) Towards sustainable fisheries: economic aspects of the management of living marine resources. OECD, Paris
Oh KS, Chung BK, Kim MC, Sung NJ, Lee EH (1988) Processing of smoked, dried and powdered sardines into instant soups. J Korean Soc Food Nutr 17:149–154
Ordoñez J (1985) A study of trash fish caught by otter trawl in the Visayan Sea. Philipp J Fish 18:1–76
Pangsorn S, Laong-Manee P, Siriraksophon S (2007) Status of surimi industry in the Southeast Asia. South East Asian Fisheries Development Center TD/RES 118, SEAFDEC Training Department, Samut Prakan, 28 pp
Perra P (1992) By-catch reduction devices as a conservation measure. Fisheries 17(1):28–30
Phitakpol B, Reungmaneepaitoon S, Phongpipatpong M, Suwansakompul S (1986) Development of fish products from small and underutilized species in Thailand. In: Proceedings of the ASEAN workshop on fish and fish waste processing and utilization, Jakarta, 22–24 Oct 1986
Pitcher TJ (2005) Estimating compliance with FAO code of conduct article, 7. Fisheries management: Pakistan. In: Pitcher TJ, Kalikoski D (eds) Safe conduct: ten years fishing under the UN Code. Fisheries Centre, University of British Columbia
Pruthiarenum R (1986) Appropriate technology for utilization of low cost fish. A review. In: Karossi AT, Surawidjaja TN, Zuddin LZ (eds) Proceedings of the 1st ASEAN workshop on fish and fish waste processing and utilization, Jakarta, 22–24 Oct 1986, pp 185–199,
Pruthiarenun R, Yamprayoon J, Suwansakornkul P, Kiatkungwalkrai P, Suwanrangi S (1985) Utilization of fish by-catch for fish ball manufacture. FAO Fish Report No. 317 supplement. FAO, Rome, pp 428–149
Queirolo LE, Fritz LW, Lingston PA, Loefflad MR, Colpo DA, de Reynier YL (1995) Bycatch utilization and discards in the commercial groundfish fisheries of the Gulf of Alaska, Eastern Bering Sea and Aleutian Islands. NOAA Technical Memorandum NMFS-AFSC-58. US Department of Commerce, NOAA, Nov 1995
Rao R (1981) Preservation of fish cake at low temperature. Seaf Export J 16:25–27
Ravishankar CN, Setty TMR, Setty TS (1993a) Studies on the utilization of Indian oil sardine (*Sardinella longiceps*) for the preparation of fish sausages: 1. Effect of washing in water on meat characteristics and sausage quality. Fish Technol 30:46–51
Ravishankar CN, Setty TMR, Setty TS (1993b) Studies on the utilization of Indian oil sardine (*Sardinella longiceps*) for the preparation of fish sausages: 2. Effect of sodium bicarbonate treatment on meat characteristics and sausage quality. Fish Technol 30:52–56

Reddy L, Shetty TMR, Dora KC (1990) Utilization of low value fish-1. Preparation of fish fingers from croaker and perches. Fish Technol 27:133–137
Redhead JF, Boelen M (1990) Utilization of tropical feeds: animal products. Food and nutrition paper 47/8, FAO, Rome, 56 pp
Regier WL, Raizin MA (1988) Fish mince: its potential for less developed countries and others. In: Morrinsey MT (ed) Proceedings of the international workshop on post harvest fishery losses, University of Rhode Island, Kingston, 202 pp
Revankar GD, Keshava N, Naidu AK, Baliga BL (1981) Fish mince- preparation and composition. Indian Food Pack, July–Aug, pp 20–24
Roessink GL (1989) Battered and breaded products. Infofish Int 4:17–20
Saila S (1983) Importance and assessment of discards in commercial fisheries, vol 765. UN/FAO/FAO Circ, Rome, p 62
Saisithi P (1987) Traditional fermented fish products with special reference to Thai products. ASEAN Food J 3:3–10
Salagrama V (1999) Bycatch the utilization on east coast of India: experiences of the DFID post-harvest fisheries project. In: Clucas I, Teutscher F (eds) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries. Natural Resource Institute, Beijing/Chatham, pp 146–147
Sankar TV, Ramachandran A, Solanki KK (1992) Preparation and shelf life of semi-dried fish cake from dhoma (*Otolithus* spp.). J Food Technol 29:123–124
Sen DP (2005) Traditional salted and dried fish products. In: Advances in fish processing technology. Allied Publishers Pvt. Ltd., New Delhi
Sen DP, Lahiry NL (1962) Salt curing and sun drying of mackerel. In: Proceedings of the 1st international congress, Food Science and Technologists Long
Sengar SH, Khandetod YP, Mohod AG (2009) Low cost solar dryer for fish. Afr J Env Sci Technol 3:265–271
Setty TMR (1987) Fish sausage and paste products. In: Proceedings of the symposium on the development of post harvest technology for low-cost fish. Society of Fishery Technologists, Cochin, pp 129–138
Shakir S, Bano A (1999) Bycatch utilization in Pakistan. In: Clucas I, Teutscher F (eds) Report and proceedings of the FAO/DFID expert consultation on bycatch utilization in tropical fisheries. Natural Resource Institute, Beijing/Chatham, pp 179–189
Shenoy AV, Madhavan P, Thankamma R, Prabhu PV, Gopakumar K (1983) Feasibility report on production of fish wafers. Central Institute of Fisheries Technology, Kochi
Shenoy AV, Thankamma R, Lekshmy NA, Gopakumar K (1988) Textured meat from low cost fish. Fish Technol 25:124
Srinivasan R, Chandrasekaran F, Arokiasamy J (1985) The use of fish ensilage in supplementary feeds for fish culture. In: harvest and post-harvest technology of fish, Society of Fisheries Technologists (India), Cochin, pp 600–602
Stanton WR, Quee LY (1977) Low salt fermentation method for conserving trash fish under SE Asian conditions. In: Proceedings of the conference on the handling, processing and marketing of tropical fish, Tropical Products Institute, London, pp 277–282
Steinberg MA, Spinelli J, Miyauchi D (1977) Minced fish as an ingredient in food combinations. In: Sutcliffe P, Disery J (eds) Proceedings of the conference on the handling, processing and marketing of tropical fish. Tropical Products Institute, London, 245 pp
Sudhakaran R, Sudhakara NS (1985) Studies on the preparation of salted and dried minces from threadfin bream and Indian oil sardine. FAO Fish Report No. 317 supplement, Rome, pp 338–347
Sugiyama K, Egana M, Onzuka H, Oba K (1991) Characteristics of sardine muscle hydrolytes prepared by various enzymic treatments. Bull Jpn Soc Sci Fish 57:475–479, Nippon Suisan Gakkaishi 57
Suwanrangsi S (1986) Improved bycatch utilization in Thailand. In: Maclean JL, Dizon LB, Hosillos LV (eds) Proceedings of the first Asian fisheries forum. Asia Fisheries Society, Manila, pp 467–469

Suwanrangsi S (1987) Development of minced fish industry in Thailand. In: Proceedings of the 20th anniversary seminar on development of fish product in Southeast Asia. SEAFDEC, Singapore, 27–31 Oct 1987, pp 81–86

Suwanrangsi S (1988) By-catch utilization in Thailand. Infofish Int 5:40–42

Thongthai C, Panbangred C, Khoprasert C, Dhaveetiyanond S (1990) Protease activities in the traditional process of fish sauce fermentation. In: Reilly PJA, Parry RWH, Barile LE (eds) Post-harvest technology, preservation and quality of fish in South East Asia. International Foundation of Science, Stockholm, p 61

Thuc NV (1999) Bycatch utilization in Vietnam. In Report and Proceedings of FAO/DFID Expert Consultation on Bycatch Utilization in Tropical Fisheries. Beijing China, 21–28 September 1998. Clucas I and Teutscher F (eds) Natural Resource Institute (1999)

Truelove K (1997) Australia: policy options for fisheries bycatch. In: Towards sustainable fisheries: issue papers. OCDE/GD/OECD, Paris, p 54

US FDA (1977) Definition and standard for food condiments, suces and seasonings standard of identity and quality of patis. Administrative order No. 325, US Food and Drug Administration, Washington, DC

Vannuccini S (1999) Shark utilization, marketing and trade. FAO Fisheries Technical Paper 389, 470 pp

Venkatanarasimha PB, Chidambaram PB (1987) Quick-salted fish cakes from ribbon fish (*Trichiurus* spp.). In: Proceedings of post harvest technology of low-cost fish. Society of Fishery Technologists, Cochin, p 167

Venugopal V (1992) Minced from low-cost fish species. Trends Food Sci Technol 3:2–5

Venugopal V, Govindan TK (1967) Utilization of trash fish 1. Preparation of fish flake. Fish Technol 4(1):35–43

Venugopal V, Ghadi SV, Nair PM (1992) Value added products from low cost fish mince. ASEAN Food J 7:3–12

Venugopal G, Shahidi F, Lee T-C (1995) Value-added products from underutilized fish species. Crit Rev Food Sci Nutr 35:431–453

Warrier SB, Ghadi SV, Ninjoor V (1996) Functional and nutritional properties of fish protein hydrolyzate from Dhoma (*Johnius dussumieri*). Fish Technol 33:101–106

Windsor ML (2001) Fish protein concentrate. Torry advisory note, No. 39. FAO in partnership with support unit for international fisheries and aquatic research, SIFAR

Yean SY (1998) Technological approaches to utilizing bycatch in low cost products for human consumption. In: James DG (ed) Proceedings of the APFIC symposium on fish utilization in Asia and the pacific, 24–26 Sep 1998. RAP publication 1998/24

Yu SY (1993) Utilization of *Decapterus russelli* in fish cakes and surimi. Trop Sci 33:330–337

Yu SY, Kaur R (1992) Development of fish biscuits from round scad (*Decapterus russelli* Rupp.). Trop Sci 32:289–294

Yu SY, Siah WM (1996) Development of fish burgers from some underutilized species of trevally. Infofish Int 2:34–36

Yu SY, Siah WM (1997) Development of fish burgers from *S. leptolepis*, *A. monoceros* and *A. nobilis*. FAO Fish Rep Suppl 563:257–264

Zuberi R, Shamshad SI, Qadri RB (1988) Bacterial flora of fermented fish sauce prepared from sardines in Pakistan. Trop Sci 28:239–246

Zuberi R, Fatima R, Shamshad SI, Qadri RB (1993) Preparation of fish silage by microbial fermentation. Trop Sci 33:171–182

Zuberi R, Fatima R, Shamshad SI, Qadri SRB (1995) Effect of temperature and use of fermented green gram as starter culture on the preparation of fermented fish silage. Pak J Sci Ind Res 38:94–97

Zynudheen AA, Sen A, Ravishankar CN, Kumar KA, Badonia R, Solanki KK (1998) Utilization of Jawla (*Acetes* spp.). In: Balachandran KK, Iyer TSG, Madhavan P, Joseph J, Perigreen PA, Raghunath MR, Varghese MD (eds) Proceedings of the symposium on advances and priorities in fisheries technology, SOFT (I), Cochin, 11–13 Feb 1998, pp 264–266

Internet Resources

All About Feed (2011) http://www.allaboutfeed.net/Home/General/2011/9/Chinese-inspectors-approve-Pakistan-fishmeal-imports-AAF012186W/, Sep 2011

FAO © (2004–2012) Fishery and Aquaculture Country profiles. India. Fishery and aquaculture country profiles. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 5 Aug 2004. [Cited 13 Sep 2012]. http://www.fao.org/fishery/countrysector/FI-CP_IN/en

International Guidelines on Bycatch Management and Reduction of Discards (2010) http://www.ofdc.org.tw/organization/01/fao/13_e.pdf, 14 pp

Chapter 14
Biological, Physical, and Chemical Properties of Fish Oil and Industrial Applications

V. Rizliya and Eresha Mendis

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1 Introduction

Fish oil is a virtually unique source of natural long-chain (LC) omega-3 fatty acids, comprising eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish oils account for about 2 % of the world's consumption of fats and oils and are a by-product of the fish meal industry. Traditionally, the industry focus was on marine oils from fish and also mammals, such as whales and seals. But, at present, greater attention is given to smaller fish with a relatively high fat content, such as anchovies, sardines, pilchards, menhaden, herring, and sand eels, as raw materials in the fish oil industry. Fish liver oil, on the other hand, is produced only from the liver of fish. It has therapeutic value and is an important source of vitamins A and D.

Historically, marine oils have played an important role in human nutrition and there is an increasing demand for fish oil due to its medicinal properties. The major interest in marine oils in these fields is due to their high content of polyunsaturated fatty acids (PUFAs). In recent centuries, the food industry has used PUFAs as a source of fat, either fully or partially hydrogenated, in various foods as substitutes for fats, such as butterfat, lard, and tallow. There is an established market for dietary supplements, as well as a developing market for food ingredients produced from fish oils and concentrates of fish oil. Besides being used as food, fish is also increasingly in demand for use as feed. Nearly one-third of the world's wild-caught fish are reduced to fish meal and fish oil, which are then used in feeds for livestock, like poultry and pigs, and in feeds for farmed carnivorous fish.

2 World Fish Oil Production and Consumption

Worldwide, around 300 dedicated plants produce about 6.3 million tons of fish meal and 1.1 million tons of fish oil annually from about 23–30 million tons (around 25 % of the total) of whole fish and trimmings (FAO FishStat 2009, FIN 2010). The main producing countries in 2008 were Peru (about 275,000 t), followed by Chile (around 167,000 t) and Scandinavia, representing Denmark, Norway, and Iceland (164,000 t) (Fig. 14.1). The USA is also a significant producer of fish oils.

Global fish oil production has remained between 1 and 1.3 million tons for many years, demonstrating the stable nature of the fisheries industry. The production of fish oil in the future is not expected to change. Due to the health benefits provided by fish oils, more oily fish are expected to be processed for direct human use, rather than being reduced into fish meal and fish oil. Thus, raw material available for fish oil production is assumed to decrease in the future. However, growing by-product production from farmed fish is expected to maintain the total production (Pike and Jackson 2010). The production of fish liver oils and fish oils from the main species between 2005 and 2007 is shown in Table 14.1.

In the 1990s, Europe used to dominate regarding the consumption of fish oil, but this has progressively reduced in the following decade, while fish oil usage has

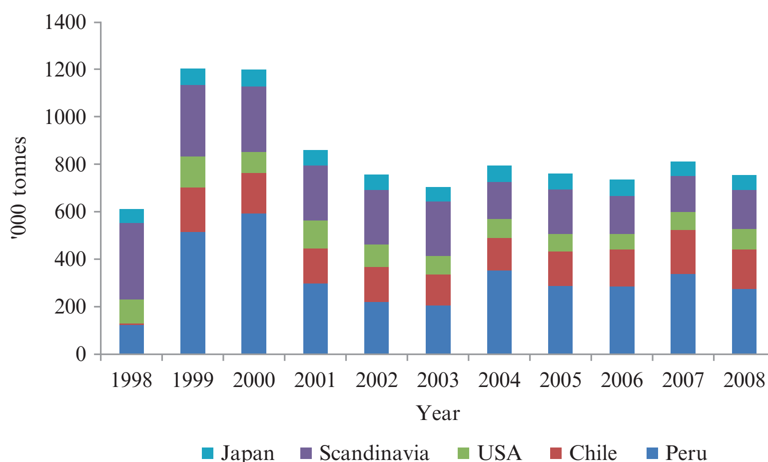

Fig. 14.1 Fish oil production by the major producing countries from 1998 to 2008 (Source: IFFO Fishmeal and Fish Oil Statistical Yearbook 2009)

Table 14.1 Main species used in the production of fish oil

Production (tons)			
	2005	2006	2007
Fish liver oils, total	*22,712*	*18,264*	*25,951*
Halibut liver oil	25	36	8
Cod liver oil	3,172	3,428	4,035
Hake liver oil		23	
Shark liver oil		15	
Other fish liver oils	19,515	14,762	21,908
Fish oils, other than liver oils, total	*690,091*	*765,956*	*839,280*
Herring oil	32,192	49,885	90,292
Menhaden oil	39,665	38,188	45,506
Sardine oil	3	201	3,599
Capelin oil	29,277	16,126	9,042
Shark oil	50	48	35
Other fish oil	588,486	661,508	690,806

Source: FAO 2010; EFSA 2010

increased in the Americas, particularly because of the growth of salmon production in Chile and Canada. Scandinavia has also increased fish oil consumption due to salmon production in Norway. Overall, there has been a significant change in the markets and an interesting change of fish oil usage. The main users are Chile and Norway (236,000 and 213,000 t in 2001 respectively), reflecting the high level of salmonid production in these countries (IFFO 2001).

Even though the world's major producers of fish oil are Peru and Chile, Peru holds a dominant place as the world's number one exporter because Chile became a

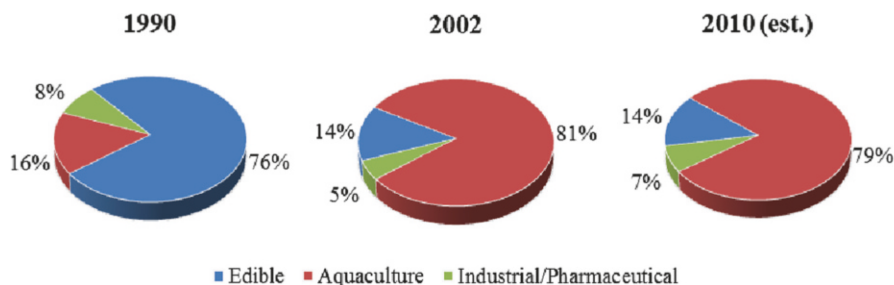

Fig. 14.2 Change in fish oil use for the period 1990–2010 (Source: IFFO)

net importer in order to cater for the increased domestic demand for fish oil to be used as aquaculture feed. Norway and Japan also hold a large share in the import and consumption of fish oil. Denmark exported 98,100 t of fish oil in 2001 and 113,700 t in 2000, and is currently the third main exporter of fish oils after Peru and the USA (FAO 2003). Norway exported 90,126 t of fish oil in 1995, but this was reduced to 44,746 t in 1998 (FAO 2002).

There have been significant changes in the use of fish oil by sector (Fig. 14.2). In 1990, 76 % of fish oil was used for edible food purposes, such as in margarines, shortenings, and bakery fats. The rest was used for aquafeed (16 %) and pharmaceutical and various other industrial uses (8 %) (IFFO 2001). In 2002, there was not much change in the usage of fish oil in the industrial and pharmaceutical sectors. However, the proportion used for aquafeed increased to nearly 81 % and the amount used for edible food purposes decreased to 14 % (Pike 2005). The aquaculture sector consumed about 783,000 t (87 %) of the total fish oil production in 2006, and 117,000 t (13 %) were used for direct human consumption, land animal feeds, and other industrial purposes (Jackson 2007). Thus, there has been a more than threefold growth in the usage of fish oil in aquaculture between 1992 and 2006, from 0.23 million tons to 0.78 million tons. All of the fish oil production was expected to be used in aquafeed in 2010, but according to the IFFO report 2010, there was no reported change in the proportional consumption patterns of fish oil by sectors.

There was an important change in fish oil applications in recent years due to the findings of the health benefits provided by the omega-3 fatty acids present in fish oils. Nowadays, a considerable amount of fish oil is used in the production of nutraceuticals. The production of these products has increased rapidly since 2001 and the world production of refined omega-3 fish oils has increased from 20,000 t in 2001 to 85,000 t in 2009. The majority (around 70 %) of the finished omega-3 fish oils on the world market contain approximately 30 % of DHA and EPA. Approximately 60 % of the omega-3 oils rich in DHA and EPA are used for dietary supplements, 20 % for functional foods, 7 % for animal feed, 6 % for pharmaceuticals, and the remainder for infant and clinical nutrition (EFSA 2010).

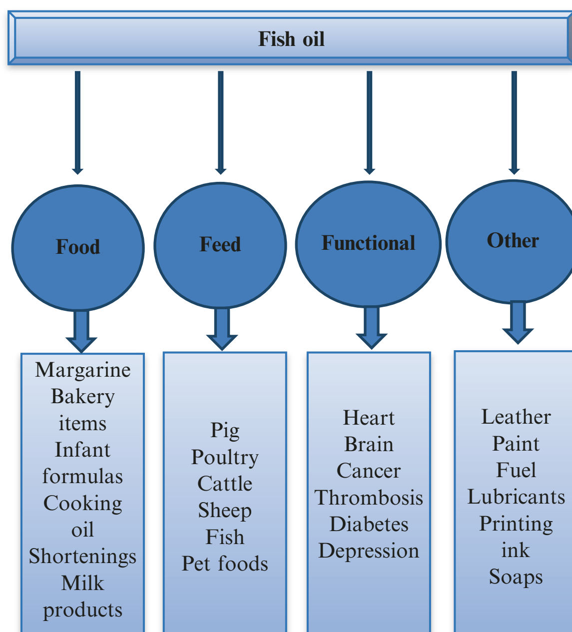

Fig. 14.3 Schematic diagram of fish oil use

3 Fish Oil and Its Uses

The fish oil industry started in the nineteenth century, when surplus catches of herring were processed for their oil for use in tanning, soap production, and other industrial purposes (FAO 1986). For many years, the oil was considered a waste product and was often burnt. Today, fish oil is recognized as a valuable nutrient for both livestock and humans, and is widely used in the food, feed, and other industries (Fig. 14.3).

Fish oil is different from other oils mainly because of the unique variety of fatty acids it contains, including high levels of unsaturated fatty acids. In fish oil, the major free fatty acids present are EPA and DHA. There are indications that EPA and DHA have a positive impact on human health, related to decreasing the risk of cardiovascular disease (atherosclerosis, thrombosis, stroke), cancers, diabetes, depression, immune disorders, proper neural and brain development, and other diseases. Due to these beneficial effects of fish oil, at present, it has been widely used in the food, feed, and pharmaceutical industries.

Edible uses of fish oil can be divided into three major categories: as a pharmaceutical component, as a functional food component, and as a commodity for the food industry. The emphasis on the importance of LC omega-3 fatty acids has led to the commercial availability of purified fish oil supplements that are available in

health food stores (Horrocks and Yeo 1999). Because of its useful effects on human health and nutrition, the quality and stability of the oil has gained more importance (Uauy and Valenzuela 2000). More and more companies are exploring the possibilities to develop dietary supplements and functional foods using fish oil to cater for the needs of health-conscious consumers.

In the food industry, fish oil was mainly used in the production of margarines, salad oil and salad dressing, mayonnaise, and several types of spreads and pastes in bakery products. The unsaturated nature of fish oil provides good creaming properties and, also, the varied chain lengths in these oils contribute to the smoothness and plasticity of margarines and shortenings. However, for the production of margarines, fish oil is hydrogenated, resulting in low unsaturation levels, and trans fatty acids are also of concern. Recently, there has been increasing interest among food manufacturers to start using liquid fish oil instead of hydrogenated oil. The microencapsulation technique seems to be a promising way of incorporating the oil into food products without sacrificing the organoleptic properties.

In the pharmaceutical industry, the demand for LC omega-3 fatty acids has lead to the use of fish oils in dietary supplements and as a therapy for treating some diseases. There are fish oils containing omega-3-enriched capsules available on the market for heart health, to lower blood fat, as supplements for children and adults (plain or flavored), and also cod liver oil, ensuring bone, brain, eye, and skin health. There are also omega-3 fatty acid monoesters and free fatty acids products from fish oils on the market.

At present, the main use of fish oil is for aquaculture, accounting for 81 % of fish oil produced in 2001. It is an important constituent in aquaculture feeds, contributing essential fatty acids needed by fish for normal growth, health, and reproduction. A small amount of fish oil is being also used in the feed of farm-raised animals, such as pigs, poultry, cattle, and sheep, and it is also used in pet foods. The incorporation of fish oil in animal feed has been shown to improve the immunity of the animals against diseases, increase feed appeal, reduce incidences of deformities, and enhance growth. The inclusion of fish oil has changed the fatty acid composition (more unsaturated fatty acids) of the fed animals, making them a healthier food for human consumption.

Over the years, fish oils have been used in many industrial applications. It is used as a raw material in paint production, leather making, as a carrier for pesticides, and in many other applications. In the past, fish oil has been used as fuel for oil lamps and, now, it has been used in modern furnaces and there is an emerging use of fish oil in the production of biodiesel.

4 Sources of Raw Material for Fish Oil

Marine oils consist of mammal oils, fish liver oils, and fish body oils. Fish body oils make up 97 % of the total marine oil supply (Bimbo 1989). The species used in the production of fish oil vary from region to region, but the generally used fish are small, bony, pelagic fish that has little or no demand for human consumption (FAO 1986).

Table 14.2 Species used in the production of fish oil

Country/region of production	Main species used in production
Peru	Anchovy
Chile	Jack mackerel, anchovy, sardines
China	Various species, anchovy
Thailand	Various species, trimmings
USA	Menhaden, Alaska pollock, trimmings
Iceland	Blue whiting, herring, sprat, trimmings
Norway	Blue whiting, capelin, herring, sand eel, trimmings
Denmark	Sand eel, sprat, blue whiting, herring
Japan	Sardine, pilchard, various species

Source: Dawn Purchase, Marine Conservation Society, Seafood Summit, 2009

It is estimated that about 90 % of the fish species used to make fish oil is unmarketable in large quantities as human food (Bose et al. 1991).

Fish oils are derived from either targeted fisheries, that is, from fish caught directly for the purposes of fish meal and fish body oil production, or as by-products (trimmings and cutoffs) from processed fish. About 20–25 % of the raw materials are recycled trimmings from food fish processing (FAO 1986).

There is a wide range of species used in the production of fish oils. However, the biggest part derives mostly from the fatty fish and the liver of lean fish. The commonly used raw materials are menhaden, sardines, anchovies, herring, capelin, mackerel, salmon, tunas, cod liver, etc.

Usually, single fish species are used for the production of fish oil (especially within the Americas and Europe). Only a few countries are being species-specific in their reporting, including Chile (for meals and oils), Canada, Iceland, Peru (for oils), and the USA, while over 58 % of the total fish oil production reported by the FAO is non-species-specific (FAO 2008). In Peru, anchoveta is used for the processing of fish oil and is a by-product of the fish meal manufacturing process (FAO 2008). Moreover, in some Asian countries, fish oil is manufactured from low-value fish (Table 14.2).

The fish oil industry also includes fish liver oils. This industry is considerably smaller than the fish body oil industry. Shark and cod are the two main fish species whose livers are extracted for oil. Whilst cod liver oil is a by-product of the fish sold for human consumption, the squalene oil extracted from the livers of shark are the primary product of this fishery, and shark meat and skins are coproducts (Pineiro et al. 2001).

5 Production and Processing of Fish Oil

The processing techniques involved in the commercial production of fish oils vary according to the type of raw material. There are a number of processes used to convert raw fish and fish trimmings into fish oil. Some of those methods are wet rendering, hydrolysis,

silage production (autolysis), dry rendering, solvent extraction, and supercritical fluid extraction (SCFE). The production of fish oil generally employs the same principles and techniques as the production of other edible fats and oils.

5.1 Wet Reduction Process

The wet reduction process is the primary method for producing crude marine oils. The principal operations in the wet reduction method are cooking, pressing, separation of the oil phase from the water phase, and storage of the recovered oil.

After the fish arrives at the factory, they are stored in holding pits until they are processed. First, the fish are subjected to steam cooking, where the material is steam heated to 90–95 °C for approximately 10–20 min. Steam cooking disrupts the fat cells, coagulates the protein, and releases the oil. In the next step, the cooked material is pressed, usually using a twin-screw press, and the liquid containing water, oil, and fine solids is squeezed out from the solid material. The pressing step takes approximately 30 min. The liquid fraction contains minor amounts of suspended solid protein material, water, and oil. Before centrifugation (separation of oil from water), the decanter removes fine suspended particles from the liquor. The liquor is then separated in three steps involving the following: decanters separate fine solids from the liquid fraction, separators split the liquid fraction into fish oil and water (stick water), and polishing water washes to remove the final traces of moisture and impurities from the fish oil before it is pumped into storage. Finally, the oil is cooled and stored for further processing. The water fraction is evaporated and added back to the solids fraction before drying. The oil from this process is defined as crude fish oil. For crude fish oil that is intended to be used for the omega-3, animal feed, aquaculture, or pet food markets, an optional carbon treatment is carried out to reduce the levels of dioxins, furans, and/or polyaromatic hydrocarbons (PAH) if present in fish oil (Bimbo 2005) (Fig. 14.4).

5.2 Other Production Methods

In addition to the wet reduction process, there are other production methods being or have been used to produce these oils. It should be noted that fish oil is a by-product from fish meal processing. A summary of these methods is covered in this section.

5.2.1 Hydrolysis (Enzymatic)

Hydrolysis is the process of the breaking down of protein into smaller units. Fish proteins are hydrolyzed using proteolytic enzymes naturally present in the fish themselves (autolysis/silage) or from other sources (hydrolysis), like animals,

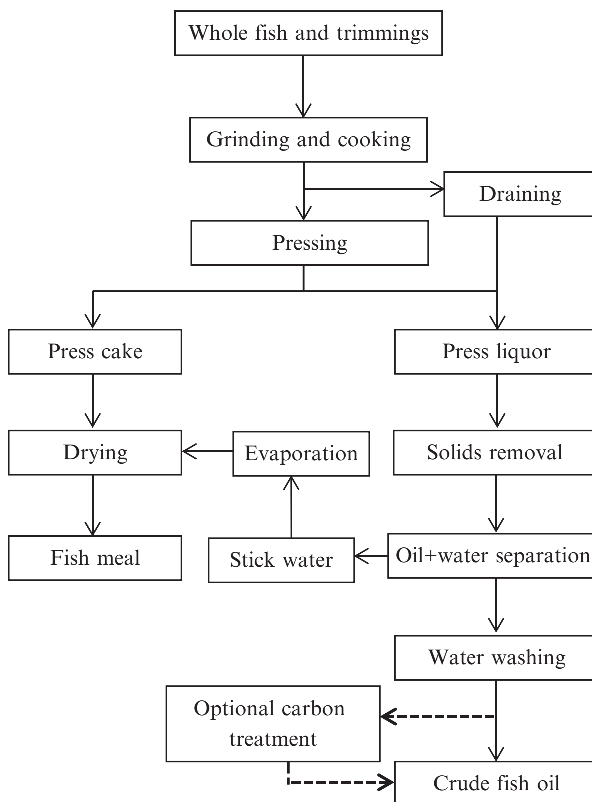

Fig. 14.4 Simplified diagram of fish oil processing by the wet rendering process

plants, or microbials. Chemical hydrolysis can also be done under acidic or alkaline conditions. This method is primarily used to extract oil from fish species or from trimmings that are low in fat, such as in white fish. Though the process can be used with any fish, when oily fish are hydrolyzed, it causes problems in recovering the oil phase without denaturing the proteins. Therefore, when fatty fish is hydrolyzed, it results in a hydrolyzed product with high fat content and reduced functionality.

5.2.2 Silage Production (Autolysis)

Silage production is a simplified, low-cost, hydrolysis process. Fish silage is liquefied fish stabilized against bacterial decomposition by an acid. In this process, the fish is minced and an acid is added for preservation. The addition of acids increases the activity of fish gut enzymes for the breakdown of the fish proteins into smaller soluble units and also prevents bacterial spoilage. Since white fish offal is low in oil, it is not necessary to remove oil during silage production. However, when it is made from fatty fish, it is necessary to remove the oil. If the silage is processed quickly to recover the oil, it is possible to make an acceptable fish oil product.

5.2.3 Dry Rendering

The dry rendering process is not normally used in processing fish meal and fish oil. It is commonly used to prepare animal proteins and fats. However, the process is used with catfish by-products. In this process, the raw material is cooked and dried to remove the water. Then, the dried product is pressed in a hydraulic press to remove any oil. Because the water has been removed, the lipid fraction can contain high levels of phospholipids and it is generally dark in color, and, so, requires refining. In the wet rendering process, the phospholipids normally hydrate and are recovered with the water fraction. In the dry rendering process, they are not hydrated and, therefore, remain dissolved in the oil fraction.

5.2.4 Solvent Extraction

The production of fish protein concentrate by solvent extraction is another process that yields fish oil as a by-product. In this process, most of the water and some or all of the fat are removed using suitable chemical solvents. Normally, the solvent is recovered in the process and reused. The disadvantages of this method are the high cost, limited number of food-grade solvents, and the large volume of solvents needed. However, high-quality oil can be obtained by this method.

5.2.5 Supercritical Fluid Extraction (SCFE)

Supercritical fluid extraction (SCFE) is another extraction method which has been applied to several processes in the food industry. SCFE utilizes the special properties of gas above its supercritical temperature and pressure to extract or fractionate compounds (McHugh and Krukonis 1986). Due to the high cost of processing, this method has been used only to remove undesirable components/impurities from fish oil or to separate EPA and DHA from fish oil. The disadvantages of this process are the use of extremely high pressures and the high capital cost.

6 Processing of Fish Liver Oils

Generally, liver oils are extracted by means of steam cookers. Low-pressure steam is piped into a tank containing the livers and the heat cooks them. When the steam condenses, the oil floats on top. The resulting oil is then separated and pumped into a storage tank.

A diagram for Icelandic processing of liver oil is presented in Fig. 14.5. First, the livers are ground and pumped through magnets to remove any metals present.

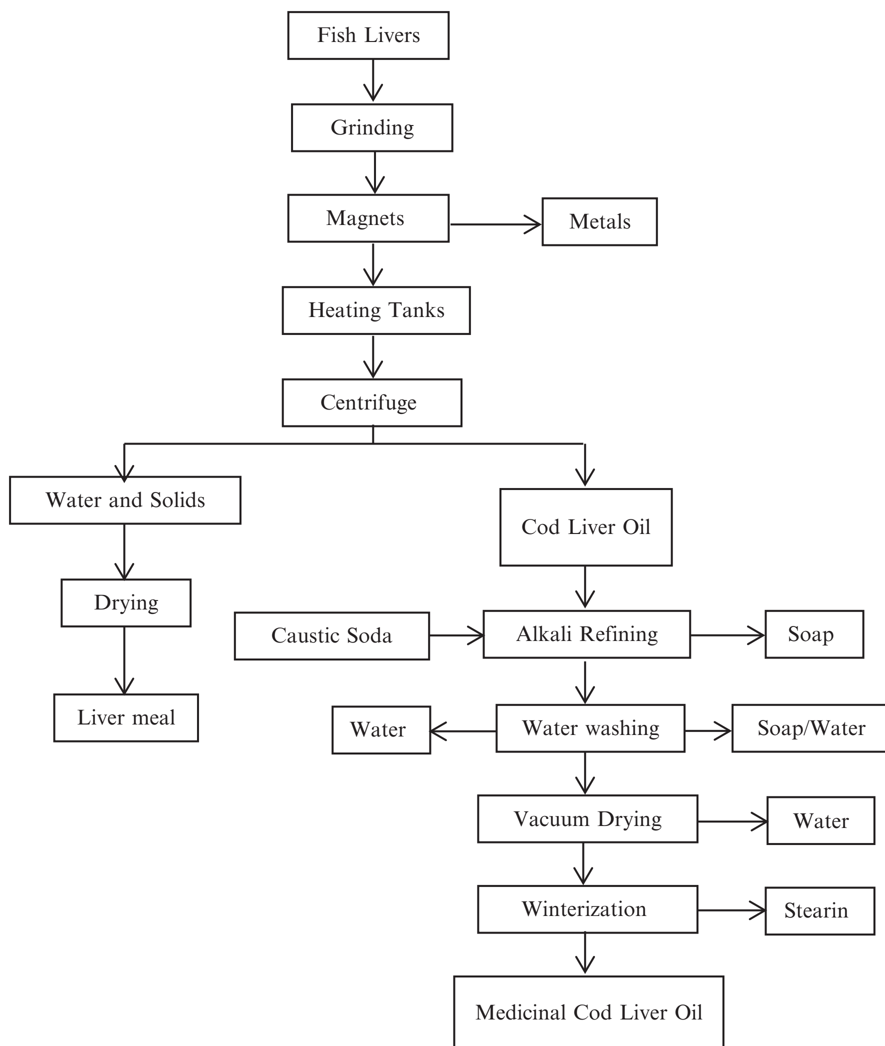

Fig. 14.5 Production of liver oils

Then, the livers are heated and allowed to stand for a period of time to break down the proteins. Next, solids are removed and the liquor is collected and heated to separate the oil. The liquor is then centrifuged and the crude cod liver oil is collected and pumped to the refinery. In the refinery, the oil is alkali refined with caustic soda to remove free fatty acids, washed, and dried in a vacuum tower and then winterized to remove stearin. The resulting oil from this process is medicinal-grade cod liver oil.

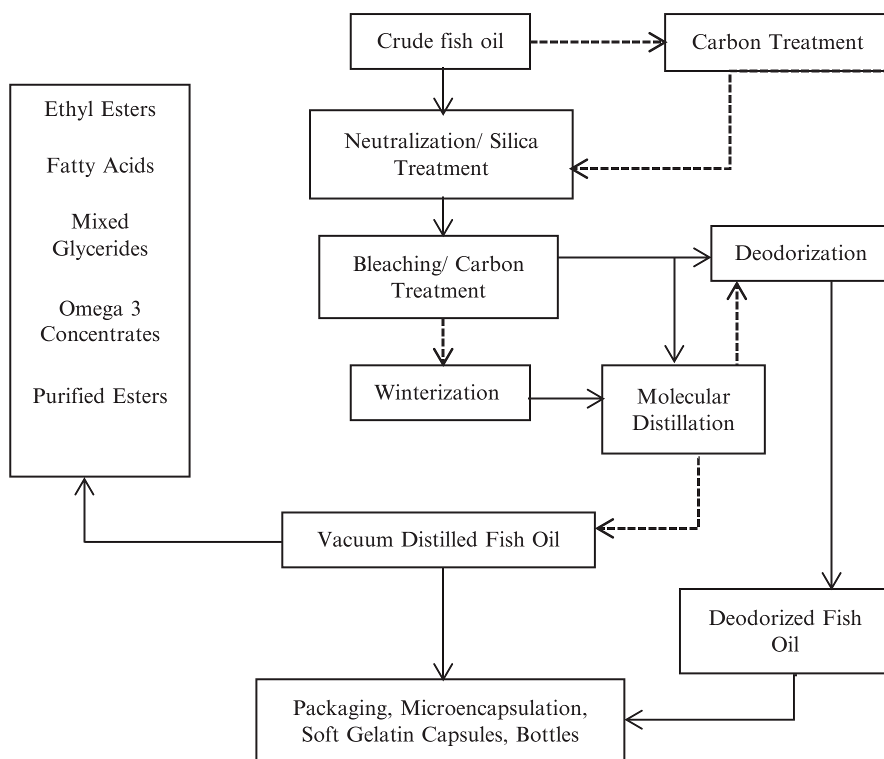

Fig. 14.6 Production of edible and pharmaceutical-grade fish oils and derivatives

6.1 Production of Refined Fish oil for Food and/or Pharmaceutical Purposes

Crude fish oils contain minor amounts of non-triglyceride substances. These impurities reduce the acceptability of the oil by producing off-flavors and odors or by reducing the stability and shelf life of the foods containing the oil. The main aim of refining is to remove undesirable components from the oil for human consumption and further processing. The processing steps involved in purifying fats and oils include degumming, refining, bleaching, deodorizing, and winterization (Fig. 14.6).

6.2 Production of Fish Oil Derivatives and Fractions

A number of fish oil derivatives are on the market today. The refined marine oils should be further processed in order to convert them to concentrates and relatively purified esters or fatty acids. Figure 14.7 illustrates the process flow diagram for the manufacturing of these derivatives and concentrates.

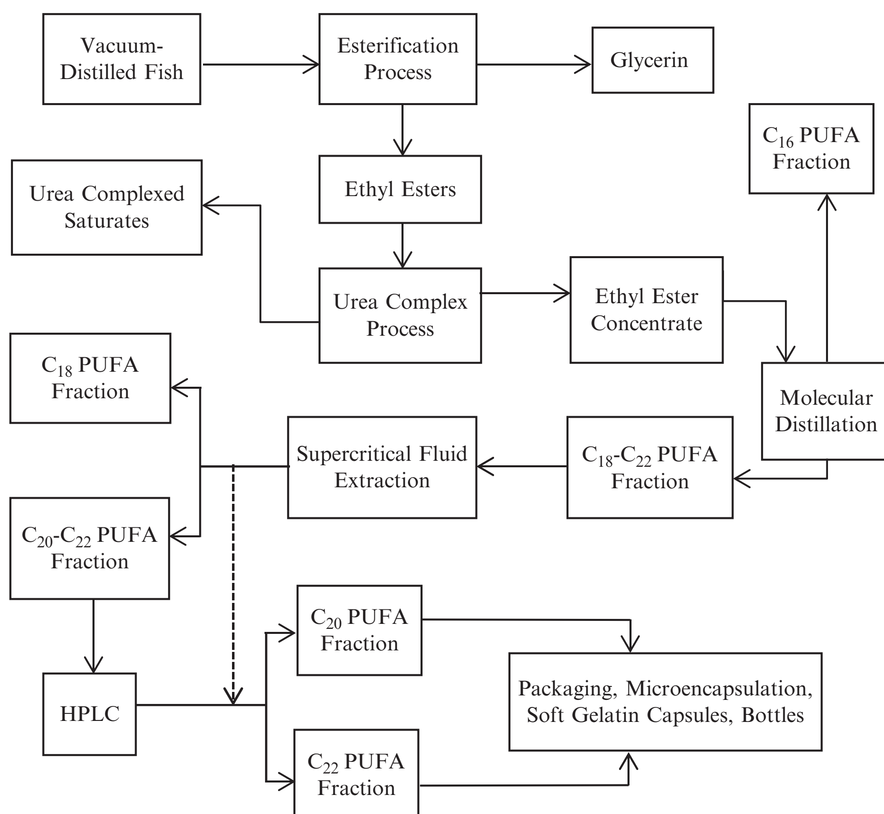

Fig. 14.7 Production of pharmaceutical and food-grade fish oil fractions

7 Properties of Fish Oil

Fish oil is becoming one of the preferred raw materials for the food, feed, and pharmaceutical industries around the world. In the following sections, the physical, chemical, nutritional, and sensorial qualities of fish oil are compiled, in order to understand the properties of the oil in industrial applications.

7.1 Physical

Fish oils are very similar to one another in their physical nature. They are liquid at room temperature and are considered as liquid oils. However, the oil is partially solid at 20 °C due to the presence of intermediate melting point triglycerides. When liquid oil is slowly cooled, solid triglyceride crystals are formed and the crystallization proceeds as cooling is continued. This is the basis of fractionation by crystallization. The solid fraction is known as the stearin and the liquid fraction as the olein. Other physical characteristics of fish oil are presented in Table 14.3.

Table 14.3 Physical characteristics of fish oils

Physical property		Value
Specific heat (cal/g)		0.50–0.55
Heat of fusion (cal/g)		ca. 54
Calorific value (cal/g)		ca. 9,500
Slip melting point (°C)		10–15
Flash point (°C)		
as glycerides		ca. 360
as fatty acids		ca. 220
Boiling point (°C)		>250
Specific gravity at	15 °C	ca. 0.92
	30 °C	ca. 0.91
	45 °C	ca. 0.90
Viscosity (cp) at	20 °C	60–90
	50 °C	20–30
	90 °C	ca. 10
Refractive index (n_D^{30})		1.46–1.48

Source: Bimbo 1998; Young 1986

7.2 Chemical

In most cases, fish oils occur as triglycerides, and less frequently as phospholipids. Fish oils contain a unique variety of unsaturated fatty acids in larger amounts compared to other oils. Most of the fatty acids present are of the LC omega-3 type, with carbon chain lengths of 20 or 22. The long-chain acids (primarily C_{20} and C_{22}) generally make up one-fourth to one-third of all the fatty acids in fish oils. Table 14.4 characterizes the typical chemical properties of fish oil.

The phospholipids occurring in fish are mainly phosphatidylcholines (lecithins) and phosphatidylethanolamines (cephalins), which account for about 60 and 20 % of the total phospholipids, respectively. In small amounts, fish phospholipids occur in the form of inositol phosphatides, cerebrosides, and sphingomyelins (Silk and Koning 1964).

Other substances that occur in or are associated with fish oils are the unsaponifiable matter, which includes hydrocarbons, sterols, glyceryl ethers, and fatty alcohols. It also includes minor quantities of pigments, vitamins, and oxidized matter. Hydrocarbons are a principal component of the liver oils of sharks. For example, Heller et al. (1957) found that squalene made up about 90 % of the shark liver. The body oils of herring have been found to contain around 0.05 % hydrocarbons (Lambersten and Holman 1963). The hydrocarbon content of commercial fish oil is normally less than 0.2 % (Young 1986).

Table 14.4 Chemical properties of crude fish oil

Chemical properties	Value
Moisture and impurities (%)	Usual basis 0.5 up to 1 % maximum
Free fatty acids (% oleic)	Range 1–7 % but usually 2–5 %
Peroxide value (mEq/kg)	3–20
Anisidine number	4–60
Totox value	10–60
Iodine value	
Capelin	95–160
Herring	115–160
Menhaden	120–200
Sardine	160–200
Anchovy	180–200
Jack mackerel	160–190
Sand eel	150–190
Color (Gardner scale)	Up to 14
Iron (ppm)	0.5–7.0
Copper (ppm)	Less than 0.3
Phosphorus (ppm)	5–100

Source: Bimbo 1998; Young 1986

Sterols are another important component of fish oils, and cholesterol is the most abundant group present in the oil of nearly all fish. The ethers found in the unsaponifiable matter are primarily 1,2-diacylglycerol ethers (Mangold and Malins 1960). The fatty alcohols are derived primarily from wax esters.

The pigments responsible for the red and yellow coloration in commercial fish oils are the carotenoids. Alpha- and gamma-carotene, zeaxanthin, violaxanthin, and xanthophyll were identified in menhaden oil, fucoxanthin and xanthophyll in pilchard oil, and astaxanthin is responsible for the occasional pink color of capelin oil. Even though carotenoids are present, the pigments encountered do not possess provitamin A activity (Young 1986).

7.3 Nutritional

The interest in PUFAs of fish oil in human diets had led to the intense use of fish oil in human and animal diets. It is a good source of energy and also possesses many health benefits. Vitamins A and D occur in the oil of most fish, but many species store large amounts of vitamins A and D in their livers (cod, halibut, and tuna). The body oils of fish generally contain vitamins in minute amounts and are not consumed for that purpose. Fish oils contain varying amounts of vitamin E, which also acts as an antioxidant. The tocopherol levels reported in crude fish oils are 30 μg/g in menhaden oil, over 60 μg/g in anchovy oil, and 25 μg/g in capelin oil (Young 1986).

7.4 Sensory

The odor of fish oil is the major limiting factor to its application in food. In principle, fish oil may be used in any food item that contains fat. However, the use of fish oil results in products having a "fishy" taste, and this limits their use in the food industry. Additionally, due to the susceptibility of fish oil to oxidation, the shelf life of the product is also reduced (Aidos 2002).

The seafood industry has been reluctant to manufacture fish oil shortening due to the "fishy" flavor imparted to the baked product and also due to the development of auto-oxidative rancidity. However, a specially processed deodorized fish oil mayonnaise, when properly packaged and stored at 2 °C for 14 weeks, was organoleptically acceptable and not significantly different from soy oil mayonnaise. The hydrogenation process seems to decrease the tendency to oxidize and increase flavor stability (Bimbo and Crowther 1991). The use of fish oil in paint has been hindered by objectionable reversion odors arising during drying of the paint. But, in contrast, in the manufacturing of shoes from leather, the faint odor emitted is considered characteristic of new shoes.

8 Industrial Applications

8.1 Food

In the past, marine oils were used as a low-priced alternative to vegetable oils in the food industry. Hydrogenated marine oils have been used in the manufacturing of margarine and other edible oil products, such as shortenings, salad dressings, mayonnaise, etc. The nutritional and physical properties of fish oil have made hardened fish oils an attractive constituent in the human diet.

Fish and other vegetable oils are subjected to hydrogenation to solidify the fat before use in margarines. Margarines prepared from hardened vegetable oil sometimes recrystallize on storage, making the margarine crumbly and hard. Long-chain PUFAs, in addition to the fatty acids present in vegetable oil and animal fats, are responsible for the excellent plastic consistency of margarines from which they are prepared. When hydrogenating these fatty acids, the oil crystallizes into the beta-prime form. This is the crystalline form required for the characteristic smoothness and plasticity of margarines and shortenings. The physical characteristic of the oil is altered during the hydrogenation process, and this gives it unique functional properties. Unlike most animal and many vegetable oils, it has high crystal stability. Since there is no crystal growth, it retains its smoothness and plasticity (Young 1986).

Fish oils can be hydrogenated to different degrees, and this helps to produce oils having different melting points. A clean-melting domestic margarine is produced using hardened fish oil having a melting point of 30–38 °C. In table-creaming margarines, 5–10 % of highly hydrogenated oils (melting point 40–50 °C) are used and

in puff pastry blends, 30–60 % of it is included (Young 1986). The properties of shortening and bakery margarines are different from those of table margarines. The creaming properties of shortenings prepared from hardened fish oil are better than that of vegetable oil shortening and is resistant to "work softening" during pastry manufacturing (Bimbo 1990). Fat blends containing hydrogenated fish oil have good creaming performance, that is, air-incorporation properties, when used in cake batters (Young 1986).

However, the hydrogenation process reduces the level of unsaturation of fatty acids and ruins the potential health benefits of PUFAs. The introduction of transfatty acids to the product formulation is also a major concern in hydrogenating fish oil. Therefore, the incorporation of LC fatty acids without hydrogenation of fish oil into the diet continues to be a topic of interest for food manufacturers, scientists, and consumers.

Due to the health benefits of LC omega-3 fatty acids, there is increasing interest among food manufacturers to start using liquid fish oil instead of hydrogenated oil. To date, unhardened fish oil has few applications because of its fishy flavor and stability problems. The highly polyunsaturated omega-3 fatty acids are very prone to oxidation. This limits the shelf life of the product and, if fish oil is to be used as a source of omega-3 fatty acids in the food industry, the producers must overcome this problem and guarantee a certain shelf life. The oil must also be able to endure baking and cooking procedures without deterioration.

Fish oil can be used in any food item that contains fat. The main challenge in using fish oil in food products is the introduction of the oil into food products without making them taste fishy. The most promising food applications seems to be in margarine, salad oil and salad dressing, mayonnaise, and several types of spreads and pastes. In 1985, the International Association of Fish Meal Manufacturers (IAFMM) started trials in the United Kingdom using fish oil from different sources at different levels in several foods; French dressing, salad cream, frankfurters, salami, margarine, and mayonnaise. The results showed that it was possible to produce food with acceptable flavor that contains fish oil. In Denmark, a commercial margarine product was launched containing 20–25 % unhydrogenated fish oil and 75–80 % vegetable oil (Barlow and Young 1988).

In order to solve the stability problem of the oil, processors have been looking into the possibility of using microencapsulated fish oil to be included in infant formulas and baked products. The microencapsulation technique is a very promising solution and is based on forming subminiature capsules or microcapsules consisting of a shell and some fill material. Typical microcapsules are small enough to be used as a free-flowing powder or suspended in water. Microencapsulation by emulsion spray-drying has been used successfully to increase the shelf life of omega-3 PUFAs to more than 2 years, and has led to the incorporation of omega-3 fish oils into a variety of food products: infant formulas, baked goods, tortillas, fortified dairy products, beverages, low-fat meat products, liquid egg products, chews, and even chocolate (Bimbo 1989; Newton 1996; Bimbo 1997; Patterson 2008). The disadvantage is that the volume of coating material is very large compared to the fill material. This is now a commercial product and intensive research work is in progress in the field of fortification of fish oil in different food products.

8.2 Feed

Nearly one-third of the world's wild-caught fish are not consumed directly by humans, but, rather, are reduced to fish meal and fish oil, and consumed in feed by farm-raised animals, such as chickens, pigs, cattle, and other carnivorous fish. The contribution of fish oil to animal diets started during the latter half of the twentieth century due to the advances in animal and nutritional sciences throughout the 1940s and 1950s. It was discovered that fish meal and fish oil were excellent supplements for animal feeds and key sources of both energy and essential fatty acids such as EPA and DHA. With the rapid rise in aquaculture production since the 1970s, an increasing proportion of fish oil is now diverted away from terrestrial animal feeds to aquatic feeds (Pike and Barlow 2003; FAO 1986).

The aquaculture industry is by far the biggest consumer of fish oil, contributing to around 81 % of the fish oil produced in 2001. The fat sources for the first successful complete fish diets were tallow and it is only in the past 20 years that fish oils have been used as the preferred energy source. Fish oil and fish meal are better feed ingredients for rearing fish than for poultry, swine, and cattle. Although birds and mammals can thrive on fish oil and fish meal, they are not very efficient in conserving the inherent biological value of these materials compared to fish. The conversion efficiency based on the edible protein and energy recovery basis in fish is about twice that of poultry and many more times efficient than cattle (Shepherd et al. 2005; FAO 1986).

Fish oil is preferred in the diet of farmed animals and aquaculture due to the presence of LC omega-3 fatty acids, which has been reported to offer major benefits to animal health, including improved immunity against disease (Ashton et al. 1994), higher survival and growth, and reduced incidences of deformities (FAO 1986). It is highly digestible, which leads to increased growth and less wastage of food, and is also considered to increase feed appeal.

Another reason for the use of fish oil in animal feeds is that it results in a change in the fatty acid composition of raised animals delivering "healthier" foods for use in the human food chain. In farm-raised animals such as chicken, pigs, and cattle, the omega-3 levels have been increased by feeding these animals with feeds rich in omega-3. For example, feeding dairy cattle with spray-dried *Schizochytrium* species microorganisms containing about 20 % DHA by weight increased the level of DHA by 100 % in their milk, and feeding it to chickens has led to the production of high DHA eggs (Garcia 1998).

8.3 Pharmaceutical

It has been proven that fish oil positively affects the biological activities in the body. More and more companies are exploring the possibilities to incorporate fish oils in dietary supplements and functional foods to cater for the needs of the growing group

of more health-conscious consumers. Many clinical studies are ongoing, and the pharmaceutical industry has shown interest in the therapeutic application of fish oil concentrates. The drug companies demand the most purified, highest quality fish oil from the fish oil industry, and, therefore, have set standards for the fish oil processing industry.

Several fish oil products are now on the market and, typically, these products are promoted and advertised for their omega-3 and vitamin content. This ranges from traditional types of fish oil, such as cod liver oil with vitamin D_3(plain or with added flavor) and fish oil encapsulated in gelatin capsules, concentrated fish oil with elevated levels of EPA and DHA, and flavored gummies for children to formulated products such as emulsions with added flavor and vitamins. On the market are also omega-3 fatty acid monoesters and free fatty acids products from fish oil. Cod liver oil has been sold for many years as a supplement for vitamins A and D. A great demand has developed in the last few years for fish oil containing high amounts of omega-3 fatty acids, especially EPA and DHA. Although antioxidants and vitamin E have been added, these oils are highly unstable, and, therefore, most of these products are encapsulated in a soft gelatin capsule that protects the oil.

Some companies have succeeded in marketing combinations of plant and fish omega-3 fatty acids targeting heart or prostate health. In addition, they provide highly purified pharmaceutical-grade fish oils for heart, joint, brain, eye, and inflammatory response health. A company in Canada has developed a high DHA oil (5:1 ratio of DHA:EPA) for the formulation of products aimed at mother and infant nutrition (Patterson 2008).

During the early 1960s, one fish oil manufacturing company tested on a trial basis capsules of fish oil and fish oil fatty acid concentrates for use by heart patients. Due to the lack of information on the activity of fish oil in treating heart problems, there was no market established for this product. However, today, with the increasing scientific evidence, there are fish oil-containing omega-3-enriched capsules available on the market for heart health, to lower blood fat, as supplements for children and adults, and, also, cod liver oil ensuring bone, brain, and eye and skin health. There has been tremendous growth in the use of fish oil in the pharmaceutical industry and it is expected to play a dominant role in the future.

8.4 Other

For centuries, fish have been harvested for the purpose of producing fish oil and yielding food. In the past, fish oil was used as a fuel for heating and lighting lamps. Initially, fish was reduced primarily for the value of their oil. It was not a by-product of the fish meal industry. The meal or cake was a by-product of oil production and was used as a fertilizer. Table 14.5 summarizes some of the non-edible uses of menhaden fish oil.

Oleo chemicals (fatty acids, fatty alcohols, esters of methyl and other alcohols, nitrogen derivatives) derived from marine oils have found a wide range of industrial

Table 14.5 Industrial uses of menhaden fish oil

Printing inks	Soaps	Refractory compounds
Leather tanning	Rubber compounds	Cutting oils
Lubricants and greases	Caulking compounds	Plasticizers
Illuminating oils	Glazing compounds	Linoleum
Fuel oils	Automotive gaskets	Pressed wood fiberboards
Mushroom culture	Core oils	Oiled fabrics
Fire retardants	Tin-plating oils	Ceramic deflocculants
Protective coatings	Rust proofing	Attractants
Polyurethane foams	Ore floatation	Insecticidal cds.

Source: Bimbo 1989

applications, including use in lubricants, corrosion inhibitors, plastic and rubber compounding, floatation agents, personal care products, cleaners, textile and paper additives, and asphalt additives. In addition, marine oils have long been used as an alternative fuel to petroleum-based products. Other industrial uses of marine oils are in the manufacturing of polyurethane resins, cutting oils, caulks and sealants, printing ink formulations, insecticides, and buffing compounds (Jangaard 1986). Refined marine oils have also been used in skin and hair care products.

Industrial applications of marine oils are possible because of their highly unsaturated nature. This makes the oils suitable for a number of technical applications, particularly as drying oils and varnishes. The saturated fatty acid fraction is a disadvantage for these purposes and is reduced by specialized processes.

When fish oil is exposed to air, the double bonds of PUFAs react with oxygen and result in relatively soft, durable film. This property is known as “drying” and is responsible for extensive use in manufacturing varnishes, oilcloth, printing inks, and imitation leather, and also as an antispalling and curing agent for concrete surfaces on highways (Rowland et al. 1995). In leather manufacturing, leather is treated with sulfated marine oils to prevent brittleness and dryness.

Fish oils are a significant source for the production of fatty acids with different chain lengths. From these acids, several types of metallic soaps are produced and used in lubricating greases and as waterproofing agents. Along with the use of fish oil as an oil paint carrier, it is also being used as a painting medium, making oil paints more fluid, transparent, and glossy. Fish oil can also be used as a finishing oil for wooden furniture to prevent it from being dented. It does not cover the surface of the wood but soaks into the pores, leaving a shiny surface.

9 Functional Properties of Fish Oil

Functional foods and nutraceuticals are claimed to have health-promoting or disease-prevention properties, contributing to improving the quality of human life. Due to the increasing awareness of the potential health beneficial roles of omega-3 fatty acids in humans, there has been increasing interest in using fish oils for human

consumption. Within the food industry, foods fortified with fish oil are emerging as a novel food category promoted as containing omega-3. These products include margarine, milk, bread, cheese, yoghurt, etc. Fish oils utilized in these products should be of high quality, fulfilling very specific demands for fatty acid composition, very low oxidation parameters (peroxide and anisidine values), high oxidative stability, and neutral taste and flavor (no fish taste or odor).

Many health claims have been made for fish oil, but the most thoroughly documented effects concern the cardiovascular system (Psota et al. 2006; Iso et al. 2006; Mozaffarian and Rimm 2006). The consumption of fish oil increases the levels of EPA and DHA in blood, thereby reducing the rate of coronary heart disease (CHD) via differential actions; improving the elasticity of artery walls, preventing blood clotting, lowering blood pressure, and decreasing inflammation (Kris-Etherton et al. 2003). Arachidonic acid derived from silver carp has pronounced effects on blood pressure, serum lipid, and platelet function (Wirth et al. 1992). A role of fish oil in preventing thrombosis by the antiatherogenic and antithrombotic effects of omega-3 fatty acids has been reported in a study by Von Schacky (2000). Fish oil prevents the accumulation of triglycerides and further reduces the serum triglyceride levels, which could play an important role in the prevention of CHD. Stark et al. (2000), in their study of postmenopausal women (either receiving or not receiving hormone replacement therapy), found fish oil supplements to reduce serum triglyceride levels significantly, by an average of 26 % in both groups. It was estimated to decrease CHD risk by 27 % in postmenopausal women. Mori et al. (1997) found that omega-3 in fish oil prevented atherosclerosis in coronary patients and was also effective in treating heart strokes that can lead to sudden cardiac death. Moreover, fish oils prevent arrhythmias, generate prostanoids and leukotrienes with anti-inflammatory actions, and inhibit the synthesis of cytokines and mitogens that augment inflammation and promote plaque formation (Uauy and Valenzuela 2000).

The intake of fish oil has been reported to exert beneficial effects against diabetes mellitus. Fish oil enhances insulin secretion by the incorporation of omega-3 fatty acids into the plasma membrane to compete with arachidonic acid production. This reduces the concentration of arachidonic acid in the plasma membrane, decreasing the production of PGE2, which, in turn, suppresses the production of cAMP, a well-known enhancer of glucose-induced insulin secretion. Consequently, fish oil enhances insulin secretion from β-cells, regulating blood sugar (Ajiro et al. 2000). A study by Storlien et al. (1987) found that providing 5–10 % of dietary energy from fish oil accelerates glucose uptake and maintains normal glucose metabolism.

Many studies have shown that fish oil has important roles in the prevention of some types of cancer, including colon (Moyad 2005), breast (Barascu et al. 2006), renal (Moyad 2005), prostate, pancreatic cell, and liver (Jiang et al. 1998). Several in vitro and animal experiments have clearly shown that the LC omega-3 PUFAs, EPA and DHA, are responsible for the inhibition of promotion and progression of cancer (Jiang et al. 1998; Moyad 2005; Horrocks and Yeo 1999; Mahéo et al. 2005). Epidemiological, experimental, and mechanistic data implicate LC omega-3 PUFAs (specifically, fish oil) as inhibitors and omega-6 PUFAs as stimulators of the development and progression of a range of human cancers (Bougnoux 1999).

The antitumor effect of EPA has been attributed to its suppression of cell proliferation and the effect of DHA appears to be related to its ability to induce apoptosis (Calviello et al. 1998; Lai et al. 1996).

Fish oil is effective in reducing inflammation in blood and tissues due to its anti-inflammatory properties. EPA and DHA are successful for this because they can be converted into natural anti-inflammatory substances, called prostaglandins and leukotrienes, which help to decrease inflammation and pain (Belch and Muir 1998). Prostaglandins, prostacyclins, thromboxanes, and leukotrienes derived from LC omega-3 PUFAs play a key role in modulating inflammation, cytokine release, immune response, platelet aggregation, vascular reactivity, thrombosis, and allergic phenomenon (Uauy and Valenzuela 2000).

The main PUFAs in the brain are DHA, alpha-linolenic acid, arachidonic acid, and docosatetraenoic acid, and DHA is particularly abundant in the membranes of retinal photoreceptors, in neural tissue, and, especially, in the grey matter of the brain (Das et al. 2009; Arterburn et al. 2000). The brain developmental functions of omega-3 fatty acids have been linked to their ability to prevent mental health problems (Severus et al. 1999). Experimental studies in animals have shown that diets lacking omega-3 PUFAs lead to substantial disturbances in neural function (Sinclair et al. 2007). DHA play a role in brain development and retina formation of a child during pregnancy (Navarro-García et al. 2004).

Moreover, studies have reported that fish oil is useful in treating rheumatoid arthritis (Connor 2000), depression (Holman 1998), psoriasis (Dewsbury et al. 1989), multiple sclerosis (Gallai et al. 1995), dysmenorrhea (Deutch 1995), and is also effective in treating acute respiratory syndrome (Kumar et al. 2000). Investigation of the functionality of fish oil is an exciting field of research that holds promise for additional fish oil products and health benefits.

9.1 Incorporation of Fish Oil to Develop Functional Foods

Omega-3 has emerged as a very important element in the international functional foods market. The use of LC omega-3 oil as a functional food ingredient is currently more common in Japan and North America. In Japan, LC omega-3 oil is used in several baked goods, margarines, and infant formulas (Yazawa 2001). In North America, LC omega-3 oil is beginning to be incorporated into food products and there is increasing demand for these products. For example, according to market research in the USA, in 2002, the market for foods and drinks fortified with omega-3 fatty acids was $100 million, which has increased to $2 billion in 2006. Sales of fish oil supplements in the USA are reported to have doubled between 1995 and 2007. Martek has obtained "generally recognized as safe" (GRAS) approval for the use of marine algae-derived DHA in infant formula. Also, Ocean Nutrition Canada has obtained self-affirmed GRAS for a variety of LC omega-3 concentrates for use in functional foods.

The instability of EPA and DHA is a major problem in the incorporation of LC omega-3 oils into food products. These molecules are susceptible to oxidation,

Table 14.6 Organizational recommendations for omega-3 intake

Organization	Recommended level
Health and Welfare Canada	1.0–1.8 g omega-3 PUFAs/day
International Society for the Study of Fatty Acids and Lipids (ISSFAL)	0.22 g DHA and EPA/day
British Nutrition Foundation (BNF)	1.4 g DHA and EPA/day (males)
	1.1 g DHA and EPA/day (females)
Institute of Medicine (IOM)	0.5 g omega-3 PUFAs/day (for infants)
United States Food and Drug Administration (US FDA)	3 g DHA and EPA/day

Source: Kroes et al. 2003

which leads to unpleasant odor and taste in the oil and in any food product in which it is contained. Therefore, new technologies need to be developed in order to incorporate LC omega-3 into foods successfully. The microencapsulation of oil to form a stable powdered ingredient seems to be promising in this field and has been used in processed foods, such as battered products, baked goods, and drinks. Microencapsulation by emulsion spray drying has been used to enable the enrichment of infant formulas and breads (Schrooyen et al. 2001). Companies such as Roche, Basel, Switzerland (ROPUFA), BASF, Ludwigshafen, Germany (Dry n-3), and Loders Croklaan, Wormerveer, the Netherlands (Marinol) are marketing microencapsulated LC omega-3 ingredients. Several other companies, including Ocean Nutrition Canada, Halifax, Canada and Clover Corporation Melbourne, Australia, are working on novel technologies to deliver LC omega-3 cost-effectively to a broad range of food products. Although there are several problems, it is likely that, within the next few years, a broad range of LC omega-3-containing foods will be available on the world market.

9.2 Recommended Intake of Omega-3

Several international scientific authorities have published recommendations for the daily intake of omega-3 PUFAs (Kroes et al. 2003). The UK Government has recommended that people eat fish twice a week, including oily fish, to provide 3 g of LC omega-3 weekly. A similar recommendation has been made by the US Heart Association (Table 14.6).

10 Products Available on the Market

A large range of food products containing fish oils have been launched over the past decade. These include refined white bread, margarine, cookies, ice cream, yoghurt, milk, pasta, and fruit juices all fortified with omega-3. A factor that is critical to the

success of the production and marketing of these types of products is the control of oxidation and, thus, the development of fishy or rancid taste or smell.

For 15 years, the food manufacturers have been trying to put omega-3 into foods. After the invention of the MEG-3™ brand of omega-3 EPA/DHA food and dietary supplement ingredients by Ocean Nutrition Canada Limited, there has been a huge breakthrough for the omega-3 market. In 2005, three US bakeries successfully introduced bread products containing MEG-3™ ingredients. In January 2005, New York-based Wegmans Food Markets, Inc. introduced three breads containing MEG-3™ ingredients. In February 2005, The Baker™, based in New Jersey, launched healthy whole grain bread containing MEG-3™ ingredients and, in April of the same year, George Weston Bakeries Inc. (Greenwich, Connecticut) launched Arnold Smart & Healthy™ 100 % whole-wheat bread containing the same omega-3 fish oil ingredient.

Woodstock Water Buffalo Yogurt™ introduced yoghurt containing omega-3 from fish oil. The yoghurt contains 100 mg of omega-3 EPA and DHA per 170-g serving. Danone Canada Inc. recently introduced the Cardivia™ brand of yogurt containing MEG-3™ to the Canadian market. Wilmar International Limited, Singapore, launched Arawana 3A+ premium cooking oil containing MEG-3® fish oil. In 2009, this cooking oil was recognized as one of "China's 500 Most Valuable Brands" by the World Brand Laboratory. Another example is eggs enhanced with omega-3. Also, in Spain, a milk product has been successfully supplemented with fish oil.

10.1 Alternatives and Substitutions for Fish Oil

The aquaculture, functional food, and nutraceutical markets are growing rapidly and this continuous growth will increase the demand for fish oil where the production of fish oil seems to be stable. It is likely that the demand for fish oil will exceed supply in the future. Therefore, alternative sources for fish oil will have to be found.

The substitution of fish oil is considerably problematic and, at present, fish oils and cultured phototrophic microalgae are the main commercial sources of omega-3 PUFAs. The development of functional foods and nutraceuticals has fuelled recent research into the molecular biology of omega-3 PUFAs production in prokaryotes (bacteria and algae particularly) (Olsen et al. 2008). DHA is currently produced from the algae *Crypthecodinium cohnii* for use in infant formula to promote brain development (Innis 2007). Major corporations are also working on genetically modified yeast and plants that could serve as factories for the synthesis of LC omega-3 fatty acids, including EPA (Damude and Kinney 2008). In the longer term, biotechnology could be used to create genetically modified plants to produce nutritionally valuable, fatty acid-rich oils (Coutteau et al. 2002).

Vegetable oils such as linseed, rapeseed, canola, and soy oil have received considerable attention as substitutes for marine oils due to their comparative cost and sustainable production levels (Miller et al. 2008). Vegetable oils are rich in omega-6

and omega-9 PUFAs, but are poor sources of omega-3 PUFAs (with the exception of linseed oil). The major problem with substituting fish oil in animal diet is the loss of health-promoting properties of the LC omega-3 PUFAs with the human consumption of fish (Simopoulos 1991), caused by the change in the fatty acid composition of the fish. To overcome this problem, the feed companies substitute fish oils with vegetable oils in the growing period and replace with fish oils in the finishing period of 10–12 weeks (finishing diet), which washes out the omega-6 fatty acids accumulated during growth. This results in a final product that is high in omega-3 fatty acids. Although the fish oils in aquafeeds are substitutable by up to 60–75 % in marine fish diets, a high demand for fish oils is likely to continue (Turchini et al. 2009).

11 Future Prospects

In recent years, the utilization of marine resources for human consumption has increased rapidly worldwide. This has been furthered by recognition of the health benefits of PUFAs, in particular, those of the family that exist in a high proportion in fish species with a high fat content. The use of fish oil in aquaculture feeds and in the food and pharmaceutical industries has increased dramatically where the production seems to be static. If this trend continues, in the not too distant future, there will be a great pressure on the fish oil industry.

One of the future challenges of the fish oil industry should be to optimize the use of marine resources. In some fatty fish processing, after the filleting operation, a substantial amount of fish and fish leftovers are not used. In a time when depletion of the marine resources has become all too real, there is a clear need for more efficient utilization. A 100 % utilization of fish processed for human consumption can be achieved by processing heads, fins, viscera, and so on into fish oil.

Another approach is the improved quality of fish oil production. Fish oil is a by-product of the fish meal industry, thus the amount of EPA and DHA present seems to be very low, while the amount in fish meal seems to be high. The fish oil consists almost exclusively of mono-, di-, and triglycerides (Opstvedt 1985). Therefore, to obtain the highest quality fish oil, preferably with the same composition as that in the fish, emphasis must be placed on the production of fish oil, with fish meal treated as a by-product.

There are indications that genetically engineered crops could provide EPA. So far, genetic engineering of plants does not appear to have achieved DHA production and it seems to be more difficult, and likely to take longer. Docosahexaenoic acid is currently produced from the algae *Crypthecodinium cohnii*. Thus, fish oil seems to be a strategic ingredient for providing DHA for the growing population. It is vital, therefore, that we continue with studies aimed at developing LC omega-3 fatty acids from genetically modified yeast and plants that are sustainable.

Although the emphasis has been on the marketability of the health benefits of fish oil, it is known that fish oil and fish liver oils contain other interesting

compounds. With improved separation techniques and more gentle processing methods, these oils might play an even more important role in the pharmaceutical and health food industries in the near future.

References

Aidos I (2002) Production of high-quality fish oil from herring byproducts. Ph. thesis, Wageningen University, The Netherlands

Ajiro K, Sawamura M, Ikeda K, Nara Y, Nishimura M, Ishida H, Seino Y, Yamori Y (2000) Beneficial effects of fish oil on glucose metabolism in spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 27(5–6):412–415

Arterburn LM, Boswell KD, Koskelo EK, Kassner SL, Kelly C, Kyle DJ (2000) A combined subchronic (90-day) toxicity and neurotoxicity study of a single-cell source of docosahexaenoic acid triglyceride (DHASCO® oil). Food Chem Toxicol 38(1):35–49

Ashton I, Clements K, Barrow SE, Secombes CJ, Rowley AF (1994) Effects of dietary fatty acids on eicosanoid-generating capacity, fatty acid composition and chemotactic activity of rainbow trout (*Oncorhynchus mykiss*) leucocytes. Biochim Biophys Acta 1214(3):253–262

Barascu A, Besson P, Le Floch O, Bougnoux P, Jourdan M-L (2006) CDK1-cyclin B1 mediates the inhibition of proliferation induced by omega-3 fatty acids in MDA-MB-231 breast cancer cells. Int J Biochem Cell Biol 38(2):196–208

Barlow SM, Young FVK (1988) New uses for fish oils. Food manufacture, Oct 1988, 75 pp

Belch JJF, Muir A (1998) n-6 and n-3 essential fatty acids in rheumatoid arthritis and other rheumatic conditions. Proc Nutr Soc 57:563–569

Bimbo AP (1989) Fish oils: past and present food uses. J Am Oil Chem Soc 66:1717–1726

Bimbo AP (1990) In the USA. Oils Fats Int 4:46–49

Bimbo AP (1997) Menhaden oil: the GRAS petition from hell. Inform 8:1069–1075

Bimbo AP (1998) Guidelines for characterizing food-grade fish oils. Inform 9:473–483

Bimbo AP (2005) Rendering. In: Shahidi F (ed) Bailey's industrial oil and fat products, 6th edn. Wiley, Hoboken, pp 57–102

Bimbo AP, Crowther JB (1991) Fish oils: processing beyond crude oil. Infofish Int 6:20–25

Bose AN, Ghosh SN, Yang CT, Mitra A (1991) Coastal aquaculture engineering. Chapman and Hall, New York, 361 pp

Bougnoux P (1999) n-3 polyunsaturated fatty acids and cancer. Curr Opin Clin Nutr Metab Care 2:121–126

Calviello G, Palozza P, Piccioni E, Maggiano N, Frattucci A, Franceschelli P, Bartoli GM (1998) Dietary supplementation with eicosapentaenoic and docosahexaenoic acid inhibits growth of Morris hepatocarcinoma 3924A in rats: effects on proliferation and apoptosis. Int J Cancer 75:699–705

Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71:171S–175S

Coutteau P, Ceulemans S, Halteren AV, Robles R (2002) Fishmeal and fish oil in aquafeeds. Int Aquafeed Dir Buy Guide 2002:20–24

Damude HG, Kinney AJ (2008) Engineering oilseeds to produce nutritional fatty acids. Physiol Plant 132(1):1–10

Das S, Paul BN, Sengupta J, Datta AK (2009) Beneficial effects of fish oil to human health: a review. Agr Rev 30(3):199–205

Deutch B (1995) Menstrual pain in Danish women correlated with low n-3 polyunsaturated fatty acid intake. Eur J Clin Nutr 49:508–516

Dewsbury CE, Graham P, Darley CR (1989) Topical eicosapentaenoic acid (EPA) in the treatment of psoriasis. Br J Dermatol 120(4):581–584

EFSA Panel on Biological Hazards (BIOHAZ) (2010) Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. EFSA J 8(10):1874 (48 pp)

FAO (1986) FAO Fisheries department, fishery industries division, food and agriculture organization of the United Nations. The production of fish meal and oil. FAO Fisheries Technical Paper No. 142, Chapter 1, Via delle Terme di Caracalla, Rome, Italy

FAO (2002) Fisheries statistics, FAO. http://www.fao.org

FAO (2003) Globefish: fishmeal and fish oil update. FAO, Rome

FAO (2008) FAO fisheries department, fishery information, data and statistics unit. FishStat Plus: universal software for fishery statistical time series. Aquaculture production: quantities 1950–2006; aquaculture production: values 1984–2006; capture production: 1950–2006; commodities production and trade: 1950–2006; total production: 1970–2006. Version. 2.30

FAO (2010) Fisheries and aquaculture department, fishery statistical collections, fishery commodities and trade. http://www.fao.org/fishery/statistics/global-commodities-production/en. Accessed 14 Oct 2010

Food and Agriculture Organization (FAO) (2009) FishStat Plus: universal software for fishery statistical time series

Fishmeal Information Network (FIN) (2010) Fishmeal and fish oil figures, p. 4, [http://www.gafta.com/fin/pdfs/FACTSANDFIGURES.pdf]

Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D, Stragliotto E (1995) Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol 56:143–153

Garcia DJ (1998) Omega-3 long chain PUFA nutraceuticals. Food Technol 52:44–49

Heller JH, Heller MS, Springer S, Clarke E (1957) Squalene content of various shark livers. Nature 179:919–920

Holman RT (1998) The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr 128(2S):427S–433S

Horrocks LA, Yeo YK (1999) Health benefits of docosahexaenoic acid (DHA). Pharmacol Res 40:211–225

IFFO (2001) International Fishmeal and Fish Oil Organization. Fishmeal and oil – supplies and markets. Presentation to ground fish forum. http://www.iffo.org.uk/Supplies.pdf. Oct 2001

Innis SM (2007) Dietary (n-3) fatty acids and brain development. J Nutr 137:855–859

Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S (2006) Intake of fish and n-3 fatty acids and risk of coronary heart disease among Japanese. Circulation 113(2):195–202

Jackson AJ (2007) Challenges and opportunities for the fishmeal and fish oil industry. Feed Technol Update 2:3–11

Jangaard PM (1986) Report no. 115, Program development, Fisheries Branch, Halifax, Nova Scotia

Jiang WG, Bryce RP, Horrobin DF (1998) Essential fatty acids: molecular and cellular basis of their anti-cancer action and clinical implications. Crit Rev Oncol Hematol 27(3):179–209

Kris-Etherton PM, Harris WS, Appel LJ (2003) Omega-3 fatty acids and cardiovascular disease: new recommendations from the American Heart Association. Arterioscler Thromb Vasc Biol 23:151–152

Kroes R, Schaefer EJ, Squire RA, Williams GM (2003) A review of the safety of DHA45-oil. Food Chem Toxicol 41(11):1433–1446

Kumar KV, Rao SM, Gayani R, Mohan IK, Naidu MUR (2000) Oxidant stress and essential fatty acids in patients with risk and established ARDS. Clin Chim Acta 298:111–120

Lai PB, Ross JA, Fearon KC, Anderson JD, Carter DC (1996) Cell cycle arrest and induction of apoptosis in pancreatic cancer cells exposed to eicosapentaenoic acid in vitro. Br J Cancer 74(9):1375–1383

Lambertsen G, Holman RT (1963) Partial characterization of the hydrocarbons of herring oil. Acta Chem Scand 17:281–282

Mahéo K, Vibet S, Steghens JP, Dartigeas C, Lehman M, Bougnoux P, Goré J (2005) Differential sensitization of cancer cells to doxorubicin by DHA: a role for lipoperoxidation. Free Radic Biol Med 39:742–751

Mangold HK, Malins DC (1960) Fractionation of fats, oils, and waxes on thin layers of silicic acid. J Am Oil Chem Soc 37(8):383–385
McHugh MA, Krukonis VJ (1986) Supercritical fluid extraction: principles and practice. Butterworth-Heinemann, Stoneham, MA. 1st edn Apr 1986. 2nd edn, Jan 1994, 512 pp
Miller MR, Nichols PD, Carter CG (2008) n-3 Oil sources for use in aquaculture – alternatives to the unsustainable harvest of wild fish. Nutr Res Rev 21(2):85–96
Mori TA, Beilin LJ, Burke V, Morris J, Ritchie J (1997) Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. Arterioscler Thromb Vasc Biol 17:279–286
Moyad MA (2005) An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: part II. Urol Oncol Semin Orig Invest 23:36–48
Mozaffarian D, Rimm EB (2006) Fish intake, contaminants, and human health: evaluating the risks and the benefits. J Am Med Assoc 296(15):1885–1899
Navarro-García G, Pacheco-Aguilar R, Bringas-Alvarado L, Ortega-García J (2004) Characterization of the lipid composition and natural antioxidants in the liver oil of *Dasyatis brevis* and *Gymnura marmorata* rays. Food Chem 87:89–96
Newton IS (1996) Food enrichment with long chain n-3 polyunsaturated fatty acids. Inform 7:169–177
Olsen Y, Otterstad O, Duarte CM (2008) Status and future perspectives of marine aquaculture. In: Holmer M, Black K, Duarte CM, Marbà N, Karakassis I (eds) Aquaculture in the ecosystem. Springer, Berlin
Opstvedt J (1985) Fish lipids in animal nutrition. IAFMM Technical Bulletin 22, International Association of Fish Meal Manufacturers, Hoval House, Mutton Lane, Potters Bar, Hertfordshire EN6, 3AR, UK
Patterson CA (2008) Omega-3 fatty acids (marine derived) essential to human health. Agriculture and Agri-Food Canada. http://www.agr.gc.ca. Aug 2008
Pike IH (2005) Eco-efficiency in aquaculture: global catch of wild fish used in aquaculture. Int Aquafeed 8:38–40
Pike IH, Barlow SM (2003) Impact of fish farming on fish stocks. Fish Farm 26(1):14–16
Pike IH, Jackson A (2010) Fish oil: production and use now and in the future. Lipid Technol 22(3):59–61
Piñeiro CG, Casas M, Bañón R (2001) The deep-water fisheries exploited by Spanish fleets in the Northeast Atlantic: a review of the current status. Fish Res 51:311–320
Psota TL, Gebauer SK, Kris-Etherton P (2006) Dietary omega-3 fatty acid intake and cardiovascular risk. Am J Cardiol 98(4A):3i–18i
Rowland GG, McHughen A, Bhatty RS, MacKenzie SL, Taylor DC (1995) The application of chemical mutagenesis and biotechnology to the modification of linseed (*Linum usitatissimum* L.). Euphytica 85(1–3):317–321
Schrooyen PMM, van der Meer R, De Kruif CG (2001) Microencapsulation: its application in nutrition. Proc Nutr Soc 60:475–479
Severus WE, Ahrens B, Stoll AL, Nemeroff CB (1999) Omega-3 fatty acids – the missing link? Arch Gen Psychiat 56:380–381
Shepherd CJ, Pike IH, Barlow SM (2005) Sustainable feed resources of marine origin. European Aquaculture Society Special Publication no. 35, Aquaculture Europe Jun 2005, pp 59–66
Silk MH, De Koning AJ (1964) Phospholipids of the South African pilchard (*Sardina ocellata* Jenyns). J Am Oil Chem Soc 41:619–622
Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438–463
Sinclair AJ, Begg D, Mathai M, Weisinger RS (2007) Omega 3 fatty acids and the brain: review of studies in depression. Asia Pac J Clin Nutr 16:391–397
Stark KD, Park EJ, Maines VA, Holub BJ (2000) Effect of a fish-oil concentrate on serum lipids in postmenopausal women receiving and not receiving hormone replacement therapy in a placebo-controlled, double-blind trial. Am J Clin Nutr 72(2):389–394

Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. Science 237(4817):885–888

Turchini GM, Torstensen BE, Ng WK (2009) Fish oil replacement in finfish nutrition. Rev Aquac 1:10–57

Uauy R, Valenzuela A (2000) Marine oils: the health benefits of n-3 fatty acids. Nutrition 16:680–684

van der Schacky C (2000) n-3 fatty acids and the prevention of coronary atherosclerosis. Am J Clin Nutr 71:224s–227s

Wirth M et al (1992) Symposium on omega-3 fatty acids: metabolism and biological effects. 15–19 Sept 1992, Oslo, Norway Abst. 158

Yazawa K (2001) Recent development of health foods enriched with DHA, EPA and DPA in Japan. World Rev Nutr Diet 88:249–252

Young FVK (1986) The chemical properties of crude fish oils for refiners and hydrogenators. Fish Oil Bulletin No. 18, June 1986

Chapter 15
Use of Seafood Processing By-products in the Animal Feed Industry

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1 Introduction

The fish industry is a major economic source for a number of countries worldwide. Fish protein is an essential source of nutrients for many people, especially in developing countries. It is estimated that, worldwide, one billion people depend

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on producing, processing, and trading fish for their livelihood (Oosterveer 2008). The total world marine fishery production in 2009 was estimated at 100.0 million tons, of which 75 % was used for direct human consumption and the remainder for other uses, such as meal production and other non-food purposes (FAO 2010). The fish processing industry produces more than 60 % by-products as waste, which includes head, skin, trimmings, fins, frames, viscera, and roes, and only 40 % fish products for human consumption (Dekkers et al. 2011). These large quantities of fish by-product waste from fisheries create serious pollution and disposal problems in both developed and developing countries. These by-product wastes contain satisfactory amounts of protein rich material that are normally processed into low market-value products (Hsu 2010). Considerable studies have been done on the characterization of the processed products as well as the different possible modes of utilization of fish and sea foods (Garcıa-Arias et al. 1994; Espe et al. 2001; Stepnowski et al. 2004). The marine bioprocess industries convert and utilize these discards or by-products as valuable products, such as fish oil, fish meal, fertilizer, pet food, and fish silage (Ward and Singh 2005; Kim and Mendis 2006; Ramírez-Ramírez et al. 2008). The increasing awareness of the potential use of by-products and the recycling of waste materials presents the nutritionist with the new and interesting challenges. There are several reasons for this. Firstly, many waste and by-products, hereafter referred to as waste suitable for animal feeding, are produced in small quantities in differing localities. Secondly, the motive of industrial producers for using organic wastes for animal feeding is more often a desire to obtain a positive return by selling waste rather than paying for their disposal, than real interest in meeting the requirements of the animals (Orskov 1980). The processing of marine fish for filleting, canning, and "surimi" production results in an immense quantity of by-products, which include trimmings, belly flaps, heads, frames, fins, skins, and viscera (Choudhury and Bublitz 1996).

2 Marine By-products and Their Composition

Every year, thousands of tons of marine fish by-products which are high in nutrient content are dumped or discarded by fish processing plants throughout the world. Discarding these by-products creates two major problems. The first is the underutilization of a huge amount of nutrients, such as proteins, minerals, and oil. Second, the disposal of such huge quantities of highly polluting organic matter contributes to major environmental and economic problems. On the other hand, the fish processing industry is faced with the need to develop efficient by-product recovery and utilization methods. Instead of disposing of these fish products as waste, they can supply high-protein feed ingredients and palatability-enhancing agents for use in animal food.

2.1 Fish and Shellfish By-Products

Fishing generates large quantities of waste daily in fish markets and fish processing industries. Fish remains have been discarded usually without putting their wealth of nutritive elements to useful production. Fish offal derived mainly from processing by-products has been carried out in various parts of the world in search of alternative and viable techniques for transforming fish waste into useful agricultural products.

2.1.1 Fish Skin

Processing by-product waste of fish skin is a rich source of collagen and gelatin. There are several studies which demonstrate the potential of fish skin processing by-product waste for conversion into fish protein hydrolysates. North Atlantic lean fish (Picot et al. 2010) and grass carp (Wasswa et al. 2007) fish species have high potential for their fish skin by-products to be used for the production of protein hydrolysates. A study has been carried out on the catfish skin isolated soluble and insoluble protein hydrolysates from hydrolyzed catfish skin and described the rheological and functional properties of the protein hydrolysates Yin et al. (2010). Aleman et al. (2011) attempted to prepare protein hydrolysates from tuna and halibut skin gelatins. Wasswa et al. (2007) carried out a study regarding prepared protein hydrolysates from grass carp skin using alcalase and optimized the hydrolysis conditions by using a response surface methodology.

2.1.2 Fish Muscles

The muscles of different animals are very similar, containing similar protein and similar amino acid profiles. There are slight differences between fish muscle and the muscle of land animals. Fish are supported by a mass of water, thus the muscle fibers require less structural support than those of land animals. Because of this, fish muscle tends to have less connective tissue than muscles from terrestrial animals, resulting in more tender texture. Protein composition in muscles varies by muscle type of the three types (striated, smooth, and cardiac muscle) of muscles; the striated muscles are the predominant form in fish. Fish muscle has “white” and “dark” meat. White meat is generally more abundant, contains fewer lipids than dark meat, and is the most widely consumed type of muscle tissue. Fish dark muscles are have limited uses due to their susceptibility to oxidation and off flavor. The conversion of these high protein content dark muscle into protein hydrolysates for further utilization will lead to producing value-added products (Hsu 2010) with a low cost of production. Nakajima et al. 2009 have carried out a research use for the production of hydrolysates from *Micromesistius australis* dark muscle.

2.1.3 Fish Bones

Fish bone is largely composed of calcium phosphate mineralized cartilage models. Fish backbone waste is another source of protein and minerals. It contains around 30 % protein and Je et al. (2007) conducted an experiment regarding the protein hydrolysates from tuna backbone protein using different proteases, such as papain, pepsin, trypsin, alcalase, α-chymotrypsin, and neutrase. Morimura et al. (2002) found that the hydrolysates derived from fish bone would be suitable as a food additive due its high antiradical activity. For the production of protein hydrolysates and peptides, collagens containing yellowtail fish bone and swine skin wastes were used as raw materials and protein hydrolysates have potential use as food ingredients for many kinds of foods and for animal feed production (Morimura et al. 2002).

2.1.4 Fish Scales and Fins

Fish scale is considered worthless, impracticable, and dismissed as a waste. However, it is known that fish scale contains numerous valuable organic and inorganic components, mainly collagen and hydroxyapatite (Holá et al. 2011), which have commercial value for use in manufacturing functional foods, cosmetics, and biomedical products (Lin et al. 2010; Pati et al. 2010). Okuda et al. (2009) and Chen et al. (2011) studied fish scale waste and focused on isolating and characterizing collagen from fish scales.

2.1.5 Fish Liver, Viscera, and Stomach

Another important fish waste is fish liver and Je et al. (2009) reviewed those protein hydrolysates from the liver of skipjack tuna (*Katsuwonus pelamis*), a fish by-product which is used to produce fish meal and animal feed or is directly discarded as processing waste. These fish liver wastes can mainly be use for the production of commercial proteases such as Flavourzyme, alcalase, Protamex, and Neutrase. Fish viscera waste generated in large amounts during processing is a potential source of protein that can be used as a raw material for the production of protein hydrolysates. The solubilization of cod (*Gadus morhua*) visceral proteins has been studied by Aspmo et al. (2005) at natural substrate pH using endogenous enzymes alone or in combination with one of alcalase, Neutrase, Protamex, papain, bromelain, actinidin, and a plant protease mix of different commercial protease preparations. A study was carried out by Guerard et al. (2002) on protein hydrolysates produced from yellowfin tunas (*Thunnus albacares*) stomach by commercial neutral protease Umamizyme. Ovissipour et al. (2009) and Wasswa et al. (2007) described fish protein hydrolysates exhibiting variation in their amino acid composition.

These protein hydrolysates are produced by the enzymatic hydrolysis of native proteins. Protein hydrolysates are used as readily available sources of protein for humans and animals due to their functional properties (Neklyudov et al. 2000). Raw material, enzyme source, and hydrolysis conditions are the main factors affecting the variation in amino acid composition of different fish protein hydrolysates (Klompong et al. 2009).

2.2 Trash Fish

The overcapitalization of fisheries due to the commissioning of highly efficient vessels and the degradation of aquatic habitats from pollution has increased catches of non-targeted fishes and the resultant practice of discarding large quantities of catch. These discards were large in quantity, with a varying number of species relative to targeted species. Much of the composition of the discards were juveniles, and those that cannot be marketed because they are not considered edible or a delicacy or had been disfigured through poor handling had no market value. The demand for trash fish has steadily increased with the continued expansion of the mariculture industry because, in terms of absolute volume, carnivorous fish consume less feed, but they cannot thrive without fish (or other marine proteins, including squid) as a major component of their diet.

But the disadvantages are an often ignored risk of continued use of trash fish as feeds in the mariculture industry is the likelihood of it being a direct or indirect source of disease in cultured fish. It also constitutes a first-order environmental threat due to the significant risk of spreading diseases to the fish population. Muroga 2001 carried out a study on one of the most serious viral diseases caused by the betanoda virus, which is a major viral pathogen that affects several marine fish species during their seedling and culture process in aquaculture hatcheries. Vertical transmission through the egg may be the most common mode of transmission for these viruses (Mushiake et al. 1994). Also, the direct infection of cultured fish can be via the consumption of trash fish containing high bacterial loads, especially streptococcal types (Austin 1997; Muroga 2001). Furthermore, organic components of trash fish (e.g., bones, muscles, viscera, and scales) contribute to organic loading, and their decomposition rate in water is dependent on their physical and chemical characteristics, as well as conditions of the environment.

The recovery of chemical components from seafood waste materials, which can be used in other segments of the food industry, is a promising area of research and development for the utilization of seafood by-products. Upgrading or recovery of the edible high-grade protein from these wastes will result in renewed interest in the use of fish meal and hydrolysates in animal diets. Chemical and biological methods are the most widely used for protein hydrolysis, with chemical hydrolysis being used more commonly in industrial practices. Biological processes using added enzymes are employed more frequently and enzyme hydrolysis holds the most promise for the future because it results in products of high functionality and nutritive value. The functional properties of fish protein hydrolysates are important, particularly if they are used as ingredients in food products. Enzymatic hydrolysis of fish proteins generates a mixture of free amino acids, di-, tri-, and oligopeptides, increases the number of polar groups and the solubility of the hydrolysate, and, therefore, modifies the functional characteristics of the proteins, improving their functional quality and bioavailability (Gildberg 1993) (Table 15.1).

Table 15.1 Potential uses of fish waste

Final products	Treatment	Physicochemical characteristics	Uses
Fish waste (mainly heads, bones, skin, viscera, and sometimes whole fish and parsley)	Heat treatments at 65, 80, 105, and 150 °C for 12 h for moisture content reduction to 10–12 %	High source of minerals, 58 % protein, 19 % fat, detection of toxic substances (As, Pb, Hg, and Cd) at non-problematic concentrations, decrease of waste digestibility with temperature	Fish waste (mainly heads, bones, skin, viscera, and sometimes whole fish and parsley)
Raw fish oil	Filtration pretreatment with or without the presence of two catalysts (iron oxide and calcium phosphate monobasic) and ozone treatment [5 g/h, 16 g/m^3 (about 8000 ppm)] at room temperature for 1 h and 30 min, respectively	Almost identical HHV (10,700 kcal/kg) and lower flash and pour points (37 °C and −16 °C, respectively) compared to commercial diesel fuel, no production of sulfur oxides, lowered or no soot, polyaromatic, and carbon dioxide emissions	Raw fish oil
Fish skin, bone, and fin	Collagen isolation	36–54 % collagen recovery and denaturation temperatures of skin collagen (25.0 ± 26.5 °C), bone collagen (29.5 ± 30.0 °C), and fin collagen (28.0 ± 29.1 °C)	Fish skin, bone, and fin
Fish bone waste	Heat treatment of raw bone at 600 °C for 24 h or 900 °C for 12 h	Better removal capacity and well-crystallized hydroxyapatite at 600 °C, raw bone showed lower activity and crystallinity, bone sample heated at 900 °C showed similar activity to raw bone and developed crystallinity of hydroxyapatite	Fish bone waste
Fish waste (mainly heads, bones, skin, viscera, and sometimes whole fish and parsley)	Heat treatments at 65, 80, 105, and 150 °C for 12 h for moisture content reduction to 10–12 %	High source of minerals, 58 % protein, 19 % fat, detection of toxic substances (As, Pb, Hg, and Cd) at non-problematic concentrations, decrease of waste digestibility with temperature	Fish waste (mainly heads, bones, skin, viscera, and sometimes whole fish and parsley)

Jayathilakan et al. (2012)

3 Seafood By-product Utilization

Hundreds of different marine species harvested throughout the world are diverse in forms, varying from whales and fish to the shelled forms, such as oysters, crabs, lobsters, and sea urchins. A third to a half of these harvests can be used to produce by-products and further upgrading of these by-products.

Treated seafood waste has found many applications, among which the most important are animal feed, biodiesel/biogas, dietetic products (chitosan), natural pigments, food packaging applications (chitosan), cosmetics (collagen), enzyme isolation, Cr immobilization, soil fertilizer, and moisture maintenance in foods (hydrolysates) (Arvanitoyannis and Kassaveti 2008).

By-products used in agriculture are classified as liquid, dry, and fresh or frozen scraps (Wyatt and McCourty 1990). Fish emulsions and oils generally include liquid by-products and fish silage, fish hydrolysate, and oil by-products, to name a few examples of that category. Fish meal and other meal, such as from crustaceans and composed by-products, are included in the dry by-products. Composting is a relatively new method of treating marine by-products. It consists of mixing waste material with a bulking agent (a carbon source), such as shells, and fish frames (bones) decompose to form an enriched soil amendment or compost (Wyatt and McCourty 1990). The composting process generally requires the addition of some water.

Biodiesel (fatty acid alkyl esters) is a renewable and environmentally friendly energy source. The most commonly used technique to produce biodiesel from fats is transesterification, in which triglycerides are reacted with alcohol, usually methanol, in the presence of a catalyst, usually potassium or sodium hydroxide (KOH or NaOH), to produce mono alkyl esters. A general review on the recent trends in biodiesel production from fatty sources (vegetal or animal) can be found by Demirbas and Karslioglu (2007). Kato et al. (2004) evaluated the ozone-treated fish waste oil as a transportation diesel fuel. El-Mashad et al. (2008) subjected fish oil from salmon to an acid pretreatment, followed by an alkali-catalyzed transesterification in order to improve the biodiesel yield up to 97.6 % (biodiesel/salmon oil ratio).

Fresh and frozen seafood scrap is often considered a separate form of by-product, and it has many uses. Non-agriculture uses include processing for fishing bait and as pet food additives (Wyatt and McCourty 1990). In the early days, much of the scrap material was dumped in landfills because it was the cheapest disposal alternative. As disposal fees increased, finding alternative uses became necessary. Those wastes have been utilized in animal feed ingredients, complete feed, feed supplements such as calcium or phosphate supplements, vitamin supplements, or as other feed additives.

The development of new extraction technologies and research has permitted the identification and isolation of an increasing number of bioactive compounds from remaining fish muscle proteins, collagen and gelatin, fish oil, fish bone, internal organs, and shellfish and crustacean shells. These bioactive compounds

can be extracted and purified with recent technologies (Kim and Mendis 2006) and such compounds may include bioactive peptides, oligosaccharides, fatty acids, enzymes, water-soluble minerals, and biopolymers for biotechnological, nutraceutical, and pharmaceutical applications. Skin, bones, and fins are produced as a consequence of the preparation of different fishery products such as fillets, sashimi (sliced raw fresh fish), etc., representing around 30 % of the fish fillet processing waste. Fish skin, therefore, is an important by-product of the fish-processing industry, causing wastage and pollution. Skin and bones are a rich source of gelatin and collagen; collagen is the major structural protein found in the skin and bones of animals and gelatin are collagen degradation products. Previous studies were found that obtain collagen from skin, bones, scales, and fins of different fish species and invertebrates waste (Morimura et al. 2002; Senaratne et al. 2006). The extraction of gelatin has been reported for cod skin (Gudmundsson and Hafsteinsson 1997), shark and carp skin (Ward and Courts 1977), Alaska pollock skin (Zhou and Regenstein 2005), and cod head skin (Arnesen and Gildberg 2006).

4 Industrial Application of Marine By-product as Animal Feed Industry

The use of seafood wastes as animal feed is an alternative of high interest, because it stands for environmental and public benefit besides reducing the cost of animal production (Samuels et al. 1991; Westendorf et al. 1998; Westendorf 2000). Offal from the fishing industry could be used as a feed ingredient, as it represents a valuable source of high-quality protein and energy (New 1996; Gabrielsen and Austreng 1998).

Feed is important in animal husbandry or farming because it represents 40–50 % of the total variable production costs (Shang 1992). In order to enhance production, improving food security is important and much attention has been focused on a search for cheap and nutritious feedstuffs. Many developing countries are endowed with seafood by-products, which are usually not utilized for human consumption, but may have great potential as fish feed. Seafood by-products selected for feed formulation have low and/or no competition between human and other animal uses, since they are not eaten by humans. This increases their suitability for use in formulating inexpensive animal feeds. Despite the favorable composition, availability, and cost-effectiveness, less experimental work has been done to test their influence on the growth of animals.

Many applications of seafood by-products have been used in aquaculture, poultry, swine, and other animal feed formulation processes. Marine fish waste can be used as alternative feedstuffs in swine diets to partially meet the protein requirements and serve as a substitute for common sources of protein; Esteban et al. (2006) illustrate one such example.

4.1 Fish By-product Application in Animal Feed Ingredient Production

4.1.1 Seafood Processing By-products as Pet Foods

Prior to the commercially prepared pet foods, dogs and cats were table scraps or homemade formulas made from human foods and leftovers. The first commercial pet food to be marketed was in the form of a dry product. The pet population is also increasingly being fed with commercially prepared pet foods. This was continuously developed throughout the last few decades, with products like canned food, biscuits, extruded dry products, pellets, etc.

Commercially prepared pet food products are available in several forms that vary according to the processing methods used, the ingredients included, and the methods of preservation. The major classification of commercial pet foods divides products according to the processing method, methods of preservation, and moisture content, and these categories are the dry, wet, and semi-moist foods. Dry pet foods contain between 6 and 10 % moisture and 90 % or more dry matter. Wet pet foods were developed originally as canned products that had moisture contents of 65–70 % and intermediate moisture products with much less water, being in the range 20–30 % (Crane et al. 2000).

Commercially prepared pet foods are an easy and economical way to fulfill the nutrient requirements among pets. These types of foods provide more than 90 % of the calories consumed by pets. Most manufactured pet foods are formulated to meet specific nutrient goals to support growth, maintenance, or gestation/lactation, as recommended by the Association of American Feed Control Officials (AAFCO 2007). For pet food production, the main ingredients are meat by-products, corn, oil seeds meal (such as soy, cottonseed, peanut, canola, sunflower, etc.), wheat flour by-products, etc. These meats by-products include liquid fat, blood, viscera, etc.

Concerning fish waste, it is an important source of proteins, lipids, minerals, and fats (Rustad 2003). Khan et al. (2003) conducted experiments on the potential utilization of waste fish scraps from white croaker, horse mackerel, flying fish, chub mackerel, and five species of sardine to produce fish protein hydrolysate by enzymatic treatment. Protein hydrolysates are breakdown products of enzymatic conversion of proteins into smaller peptides and they are small fragments of peptides that contain 2–20 amino acids.

The oil content of fish waste by-products is in the range 1.4 and 40.1 %, depending on the species and tissue (Babbitt 1990). Lipids recovered from processing towards sufficing the by-products of commercial fishes are better alternatives to lipids for the feed industry and can effectively meet demands. The major lipid-based compounds that can be recovered from fish by-products are omega-3 (n-3) polyunsaturated fatty acids (PUFAs), phospholipids, squalene, vitamins, and carotenoids (Sen 2005). The main roles of PUFAs in human health concerns have been studied by Mnari et al. (2007) and Connor (2000), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the prevention of cardiac-related disease

conditions, inflammations, cancers, and autoimmune disorders, as well as their beneficial effects in the fatal development and modulating the biosynthesis of eicosanoids. The utilization of these biomolecules with potential biological activities and functionalities provides a means for value addition to the fish processing waste.

4.1.2 Seafood Processing By-products as Fish Meal

Fish meal is a highly nutritious powder, produced by drying and grinding whole fish or processing waste obtained from bycatch and other abundant species, like anchovy (*Engraulis* sp.), menhaden (*Brevoortia* sp.), and capelin (*Mallotus villosus*) (Hevroy et al. 2004). Standard fish meal is blended with other ingredients and used as animal feed, but meal produced from fish of high freshness quality commands a premium price and is sought after as an aquaculture and agricultural weaning feed. Fish meal is a relatively dry product composed mainly of protein (70 %), minerals (10 %), fat (9 %), and water (8 %). It can have different qualities, in terms of amino acid profile, digestibility, and palatability, depending on the raw material used for its production and the type of process employed for obtaining the meal (Gildberg 2002).

Fish meal is a rich source of vitamins and minerals, which play an important role in the nutrition of animals. The ash content in fish meal varies between 12 and 20 %, depending on the species (Babbitt 1990). Fish meal contains pantothenic acid, riboflavin, niacin, B12, calcium, phosphorus, sodium, selenium, and magnesium (Barlow and Windsor 1984). Fish meal is a highly concentrated nutritious feed supplement produced by cooking, pressing, drying, and grinding of the skeletal remains, along with the adhering proteinaceous tissues of fish from filleting or canning operations of the processing of trash fish obtained as bycatch along with other high- valued species (Pike 1999). The production of fish meal is limited in the widespread use of seafood processing waste and bycatch in fish feeds because raw materials are only available during fishing seasons and often in remote areas, making it difficult to justify investment in conventional fish meal reduction plants. Developments in fish meal production technology have resulted in equipment becoming more compact, with a shorter processing time. Sugiura et al. (1998) studied the apparent protein digestibility values of fish meal produced from deboned fillet waste and found that they were as high as those of the highest quality fish meal (Table 15.2).

Fish meal is used in feeds for poultry, pigs, ruminants, fish, crustaceans, pets, and fur-bearing animals because it increases productivity and improves feed efficiency. It provides a unique balance of essential amino acids, energy, vitamins, minerals, and trace elements which complement other feed ingredients. It does this by correcting their deficiencies. Fish meal is also a good source of the amino acid taurine and the fatty acid arachidonic acid (C20:4 n-6), which are essential for cat nutrition and health. In addition to being a major source of energy, the residual fat in the meal is a rich source of omega-3 fatty acids, which represent over 35 % of the total fatty acids present in the fat (referring to the article: http://www.afdf.org/wp/wp-content/uploads/2008_by_product_mkt_study.pdfon 08-09-2012) (Table 15.3), (Alaska Fisheries Development Foundation Anchorage 2008).

Table 15.2 Proximate composition, essential amino acid, and phosphorus and calcium content of commercial anchovy meal (COM), mixed benthic meal (MBM), small pelagic meal (SPM), catfish meal (CAM), and tuna cannery waste meal (TCW)

	Fish meal				
	COM	MBM	SPM	CAM	TCW
Proximate composition (g/kg)					
Crude protein	672	662	629	565	643
Lipids	114	63	68	72	79
Ash	130	161	224	244	173
Moisture	71	82	59	84	70
Essential amino acids (g/100 g) proteins					
Arginine	5.49	6.04	5.5.2	6.38	5.62
Histidine	1.86	2.36	2.37	2.52	2.71
Isoleucine	3.12	3.73	3.32	3.75	3.49
Leucine	6.75	7.70	6.94	8.10	7.01
Lysine	6.66	8.06	7.15	8.44	6.84
Methionine (+cystine)	3.55	4.10	3.75	3.49	3.64
Phenylalanine (+tyrosine)	6.20	7.39	6.70	7.50	6.77
Threonine	4.17	4.73	4.21	4.86	4.39
Tryptophan	1.04	1.29	1.15	1.29	1.17
Valine	3.96	4.32	3.92	4.37	4.05
Mineral composition					
Calcium	1.29	3.61	2.56	2.74	2.94
Phosphorus	0.88	1.85	1.32	1.48	1.54

Goddard et al. (2008)

Table 15.3 Examples for the use of different fish meals as an ingredient for aquaculture feed

Fish meal	Use in	Reference
Anchovy fish meal	Salmon	Anderson et al. (1995)
Anchovy fish meal	Shrimp	Cruz-Suarez et al. (2000)
Alaskan pollock (white) fish meal	Rainbow trout	Satoh et al. (2002)
Groundfish fish meal:	Salmon	Anderson et al. (1997)
Herring fish meal:	Cod	Tibbetts et al. (2006)
	Haddock	Tibbetts et al. (2004)
	Salmon	Anderson et al. (1997)
Mackerel fish meal:	Salmon	Anderson et al. (1997)

4.1.3 Fish Protein Hydrolysates

Viscera wastes, which include digestive tissues (stomachs, pyloric caeca, intestines, liver, pancreas, etc.) and some other organs like spleen and gonads, were used to obtain fish protein hydrolysates and fish silages. Fish protein hydrolysates and silages constitute a nitrogen source for pet foods and aquaculture feed (Martone et al. 2005), reducing the cost of nitrogen supply in feeds. The possibility of fish protein hydrolysate use in the animal feed industry has been studied in the literature (Aksnes et al. 2006; Aguila et al. (2007); Barrias and Oliva-Teles 2000; Gildberg et al. 1995; Hevroy et al. 2005).

4.1.4 Fish Oil

Fish oil is a versatile product and finds many applications in the food, feed, and technical industries of the world. Fish oil is the second major product of rendering the inedible fish and fish by-products. Fish oils contain mainly triglycerides of fatty acids, with variable amounts of phospholipids, glycerol ethers, and wax esters (Ruiter 1995). Moreover, fish oils contain a wide range of long-chain fatty acids (14–22 C), with high degrees of unsaturation. Due to their functional properties, fish oils have been utilized to manufacture food and pharmaceutical products. Hydrogenated fish oils are used to manufacture edible products such as margarine, shortenings, and salad oils (Ruiter 1995). Since fish oils have a widely varied chain length (14–22 C), margarines prepared from them have an excellent plastic consistency (Barlow and Windsor 1984). The highly unsaturated properties of unhydrogenated fish oils make them very beneficial to human health. The fat content of fish varies from 2 to 30 %, depending on the type of species, diet, season, environment, and geographic variations. The composition of fish oil is different from that of other oils and is mainly composed of two types of fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are PUFAs classified as omega-3fatty acids and predominantly found in many marine animals.

Medical and nutritional researchers have found that the long-chain polyunsaturated ω-3 fatty acids found in DHA and EPA are essential to the fetus and young child in order for them to have normal brain and nervous tissue development (Ruiter 1995). Barlow et al. (1990) pointed out that fish oils containing EPA and DHA have positive effects on cardiovascular diseases as they help to reduce blood cholesterol levels. Finally, oil made up from fish livers possesses potential health benefits due to the high contents of vitamins A and D.

Annetta et al. (2009) investigated the effects of manipulating level and source of the fish oil supplement based on herring and salmon oil on forage intake, colostrum production and composition, and lamb output of late gestation ewes. Fish oil in feeds for all male and female pigs changes the muscle fatty acid composition and stability of sensory quality (Hallenstvedt et al. 2010) (Fig. 15.1).

4.1.5 Fish Bone Meal

The dietary Ca to P ratio has a major impact on the efficiency of bone mineralization and maintenance of minerals in the skeleton of warm-blooded animals, while in fish, the results are inconsistent (Vielma and Lall 1998). Lee et al. (2010) evaluated the supplemental fish bone meal made from Alaska seafood processing by-products and dicalcium phosphate in plant protein-based diets for rainbow trout (*Oncorhynchus mykiss*) The results indicated that the fish bone meal made from Alaska seafood processing by-products could be used in fish feed formulations as a supplemental calcium source but not as a primary phosphorus source because of its low bioavailability to fish.

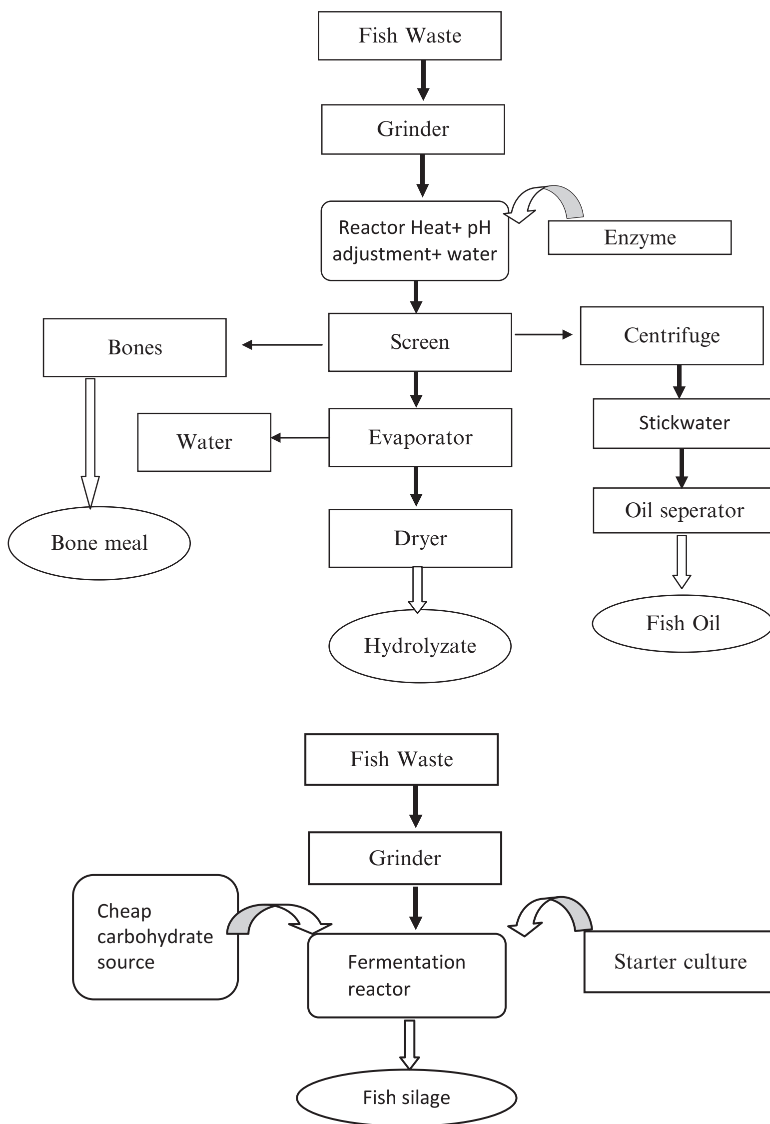

Fig. 15.1 Flow diagram for the production of hydrolysate, fish oil, and bone meal from fish waste

Furthermore, fish bone meal could be used as a calcium and phosphorous source in salmon diet (Nordrum et al. 1997) and trout diet (Vielma et al. 1999). The feed ingredient was tested for the performance of feeding trial using fish bone-rich meal produced by separation of the bone fraction from a commercial fish meal (Toppe et al. 2006).

Bone meal also had phosphorus retention of 60 %, which is lower than that of primary sodium phosphate (Nordrum et al. 1997). Richie and Brown (1996) found the true phosphorus availability for rainbow trout-fed herring meal to be 55.4 %, anchovy meal 35.7 %, and menhaden meal 21.5 %. Watanabe et al. (1980) found that chum salmon balances the calcium/phosphorus ratio in the body by controlling the absorption or excretion of calcium.

4.2 Shellfish By-products Applications in Animal Feed Ingredient Production

4.2.1 Proximate analysis, isolation and identification of amino acids and sugars from raw and cooked commercial shrimp waste

Shrimp waste meal has been identified as an animal protein source that has great potential. The availability of shrimp, in both offshore waters and Lagos Lagoon, amounts to about 300,000 t per year and they form the larger part of the catches of artisan fisherman and commercial shrimp trawlers (Abohweyere 1984). The increase in shrimp farming and production has led to the concomitant production of shrimp waste meal. Larger amounts of shrimps are sold or exported as peeled or unpeeled tail (Lee and Wickins 1992). According to Meyers (1986), shrimp heads alone represent 35–45 % of the total shrimp production. Shrimp waste meal is basically the dried waste of the shrimp industry, consisting of the heads, appendages, and exoskeletons, and is particularly rich in lysine (Fanimo et al. 1996). Furthermore, Fanimo et al. (1996) reported that shrimp waste meal can be used as a substitution for fish meal in broiler feed formulation processes.

Fanimo et al. (2000) assessed the protein quality of shrimp waste meal in a balanced experiment with rats and the results indicated that the shrimp waste meal is inferior to that of fish meal, but that supplemental methionine and lysine in shrimp waste meal diets improved the quality of the protein.

4.2.2 Shell Waste Meal

Crustacean shells were identified as a very good feed ingredient in the animal feed industry. Most of the shells are by-products in the processing of crustaceans such as shrimps and oysters. Cobos et al. (2002) found that shrimp shell waste can be used in an up to 15 % dry matter basis in diets for growing lambs, without a negative effect on animal performance. On the other hand, oyster shells contain 52–55 % calcium oxide and are used in the manufacture of calcium carbide, lime, and cement. The shells crushed to a suitable size are used as poultry grit and dried oyster shell powder is in high demand in animal feed formulation (Sathiadhas and Aswathy 2004).

Table 15.4 Squid by-products based animal feed ingredients

Feed ingredient	References
Squid meal	Lee et al. (1999), Naik et al. (2001), Lim et al. (1997), Millamena, et al. (2000), Sanchez et al. (2005), Vassallo-Agius et al. (2002)
Squid mantle meal	Moksness et al. (1995)
Squid liver meal	Bai and Lee (1998), Sato et al. (2006), Wang et al. (2006)
Squid viscera meal	Mai et al. (2006a)
Squid hydrolysate	Espe et al. (2006), Kolkovski and Tandler (2000), Lian et al. (2008)
Squid offal silage	Wang and Lied (2001)

4.2.3 Crab By-product Meal

By-products from the crab industry are presently underutilized and may constitute more than 50 % of the crab weight (Anon 2004). These types of feed ingredients provide different kinds of minerals; chitin and collagen proteins that are all essential for the structural integrity of crab shell (Toppe et al. 2006). The effect of dietary inclusion of fish bone and crab (*Cancer pagurus*) by-products on growth performance and feed utilization was investigated in a growth trial with Atlantic cod *Gadus morhua* by Toppe et al. (2006). The feed ingredient was prepared using by-product from the crab industry, mainly consisting of shells, viscera, and legs.

Red crab wastes were mainly used in aquacultural feed ingredient production. For example, Spinelli et al. (1974) investigated a method for processing red crab into a dry feed material and found that it was highly suitable for inclusion in salmonid diets. Furthermore, the nutritional value of red crab (*Pleuroncodes planipes*) meal as a protein source and partial replacement for fish meal in diets for juvenile *Litopenaeus vannamei* was evaluated previously by Goytortúa-Bores et al. (2006). The pelagic red crab is not used for human consumption because of the small size of its abdominal muscle. However, its nutrient composition makes it an attractive ingredient for animal feeds. The most abundant nutrients of red crab are protein and minerals. Villarreal et al. (2004), reported good results when using feeds supplemented with red crab meal for the American lobster. Villarreal et al. (2004) found that the growth of *Farfantepenaeus californiensis* post-larvae improved when the diet contained red crab meal.

4.3 Molluscs By-products Applications in Animal Feed Ingredient Production

Molluscs such as squids, clams, cuttlefish, and octopus are also highly demanded seafood in many countries (Mandeville et al. 1992). Therefore, waste from processing gives sources for animal fed ingredients. Table 15.4 shows squid-based feed ingredients, along with their references.

Other than squids, studies have been carried out for the suitability of molluscs by-products as animal feed ingredients, such as blue mussel extract meal tested for

Japanese flounder (Kikuchi et al. 2002), clam meal tested for freshwater prawn (Naik et al. 2001) mussel (green-lipped) flesh meal tested for spiny lobster (Smith et al. 2005), and scallop meal tested for shrimp (Sudaryono et al. 1995).

4.4 Seafood Waste Applications in Animal Feed Additives Production

4.4.1 Natural Pigments

Carotenoids are a group of fat-soluble pigments that can be found in many plants, algae, microorganisms, and animals, and are responsible for the color of many important fish and shellfish products. Most expensive seafood, such as shrimp, lobster, crab, crayfish, trout, salmon, redfish, red snapper, and tuna, have orange-red integument and/or flesh containing carotenoid pigments (Haard 1992).

Whereas in some fish species such as salmonids the fillet color constitutes an important quality parameter (Sigurgisladottir et al. 1997), in others, it is adequate skin pigmentation that greatly improves fish appearance, being associated by the consumer with freshness and product differentiation. The literature indicates that red porgy-like high-valued species is characterized by a natural red-pink skin coloration, which acquires a gray color under culture conditions (Kentouri et al. 1995 and Cejas et al. 2003). Since some fish are unable to synthesize carotenoids (Goodwin 1984), skin color is highly dependent on the carotenoids present in their diet. Therefore, it is interesting to produce color supplements/feed ingredients in feed, especially natural color extractants.

Red crab is an excellent source of β-carotene, two esters of astaxanthin and free astaxanthin (Wilkie 1972), with concentrations estimated to be 8–10 mg/100 g of the whole animal. Red crab has been used previously as a feed ingredient for aquatic organisms. Similarly, Spinelli and Mahnken (1978) used it as a source of pigments in diets for salmonids. Furthermore, extracted pigments from red crab have been used as a feed ingredient for rainbow trout and obtained excellent muscle pigmentation. Also, Spinelli et al. 1974 found highly pigmented muscles in rainbow trout which were fed Oregon moist pellet type diets containing 10 and 25 % red crab.

Shrimp waste is one of the most important natural sources of carotenoids (Shahidi et al. 1998). Shrimp waste, such as head and body carapace, was used for carotenoids extraction with various organic solvents [methanol, ethyl methyl ketone, isopropyl alcohol (IPA), ethyl acetate, ethanol, petroleum ether, and hexane] and solvent mixtures (acetone and hexane, IPA, and hexane) at various extraction conditions (percentage of hexane in the solvent mixture of IPA and hexane, ratio of solvent to waste, and number of extractions) (Sachindra et al. 2001).

Previous studies with cultured *Pagrus pagrus* have shown an improvement in skin coloration by the inclusion of different astaxanthin sources such as shrimp (*Plesionika* sp.) (Cejas et al. 2003), shrimp shell meal (Kalinowski et al. 2005; Kalinowski et al. 2007), or *Haematococcus pluvialis* (Chatzifotis et al. 2005).

4.5 Seafood By-products Application in Silage Production

Fish silage is prepared by combining minced fish or parts of fish with acid or lactic acid bacteria derived from fermentable carbohydrate and hydrolyzable protein substrates. At low pH, the fish waste and parts of fish ingredients liquefied through the action of the digestive tract and proteolytic enzymes. This breakdown is accelerated by the acids, which, in addition to reducing the pH, also break down bones and cartilage and prevent the growth of spoilage bacteria (Tatterson 1982). Fish waste ensilation is an old preserving technique for protecting organic matter against spoilage, and it is friendly to the environment, safer, technologically simpler, and more economical than the manufacture of fish meal (Gildberg 1993), but may lead to the loss of this important potential feed ingredient. The ensilage of fish waste has been practiced, but is not widely used up till now because of the water content, which may render transportation expensive, and fish waste silage is smelly, which may limit its use in a high proportion of feed formulations. To reduce transport costs and to optimize storage, it is desirable to produce dry silage. Suitable carbon sources include carbohydrates, such as glucose, fructose, sucrose, and starch hydrolysate, molasses, and organic acids. As nitrogen sources, various inorganic and organic salts and compounds such as ammonium salts and other similar compounds, urea, natural proteolytic organic substances such as peptone, casein hydrolysate, yeast extract, corn steep liquor, soybean protein hydrolysate, and various other extracts of vegetal and animal tissues may be employed.

Most studies on the use of co-dried mixtures of fish silage and cereals as aquafeed ingredients used drum dryers (Arason 1994), ovens (Vidotti et al. 2002), solar cabinets (Goddard and Al-Yahyai 2001), or sun drying outdoors (Ali et al. 1994). Research has been conducted for the development of fish waste ensilation and identifying nutritional characteristics (Jackson et al. 1984; Haaland and Njaa 1989; Espe et al. 1989). During silage processing, enzymes found in muscles hydrolyze proteins and nitrogen becomes more soluble, which may lead to making silage the most commonly available amino acid source for protein biosynthesis (Espe et al. 1989). Other than that, Ca and P were found in higher concentrations in both fish meal and silage in some fish species studied by Martınez-Valverde et al. (2000).

Vidotti et al. (2003) evaluated the amino acid composition of silages produced from fish as supplement in fish feeds, as the nutritional value of aquaculture fish diet is determined basically by its amino acid composition. Commercial marine fish waste, commercial freshwater fish waste, and tilapia filleting residue were used to produce fish silage by acid digestion (20 ml/kg formic acid and 20 ml/kg sulfuric acid) and anaerobic fermentation (50 g/kg *Lactobacillus plantarum*, 150 g/kg sugar cane molasses). Marine fish waste had higher crude protein content compared to freshwater fish waste and tilapia filleting residue.

Steers were used by Ever and Carroll (1998) to evaluate the ruminal undegradable protein of fresh shrimp waste and shrimp silage (47 % shrimp waste, 17 % molasses), and it was found that ruminal undegradable protein was 57 % for the fresh shrimp waste and 32 % for the shrimp silage. Fish and crab processing waste

were ground and ensiled with low-quality roughages such as corn stover or peanut hulls and Johnson grass in proportions and Samuels et al. (1992) obtained successful results. Biological fermentation using *Lactobacillus plantarum* of fish waste of whole fish, viscera, and heads for potential use in animal and poultry feeds was studied by Hassan and Health (1986).

Chopped pilchard waste, including viscera, heads, and tails, which were mixed with molasses and ensiled with *Saccharomyces* sp. and *Lactobacillus plantarum* produced better quality silage (Faid et al. 1997), and the preservation of crab or shrimp waste as silage for cattle (Evers and Carroll 1996) are another two examples of fish waste silage production experiments.

5 Impact of the Utilization of Marine By-products in Animal Feed

5.1 Safety Impact on Human Health

Animal feed or forage may be the source of a limited number of infections for farm animals that could lead to human illness. Likely organisms include *Salmonella enterica*, *Toxoplasma gondii*, *Trichinella spiralis*, and possibly the agent of bovine spongiform encephalopathy. The risk to human health from other infectious agents which may contaminate either feed or forage appear to be either negligible, e.g., *Bacillus anthracis* and *Mycobacterium bovis*, or non-existent, e.g., *Clostridium botulinum* toxin and *Listeria monocytogenes* (Hinton 2000).

5.2 Environmental and Economical Impact

Fish waste management has been one of the problems with the greatest impact on the environment (Arvanitoyannis and Kassaveti 2008). Fish farming detrimental effects on the marine environment in particular have become an issue of public concern. The intensive farming of marine finfish, commonly practiced in cages or ponds, involves the supply of high-quality artificial feeds and medication with consequent impacts on the environment, mainly because of the release of organic and inorganic nutrients and the release of chemicals used for medication (Arvanitoyannis and Kassaveti 2008). These impacts tend to be the most severe in areas with poor water exchange (Midlen and Redding 1998). Not only can the surrounding area be directly affected by the effluent, but fish waste can also affect a wider coastal zone at different ecosystem levels, thus reducing the biomass, density, and diversity of the benthos, plankton, and nekton, and modifying natural food webs (Gowen 1991; Pillay 1991).

Therefore, most common environmentally friendly and economical methods for the utilization of marine seafood waste are the manufacture of fish meal/oil, the

production of silage, or the use of waste in the manufacture of organic fertilizer. The utilization of by-products is an important cleaner production opportunity for the industry, as it can potentially generate additional revenue as well as reduce disposal costs for these materials.

References

AAFCO (2007) (Association of American Feed Control Officials): Official Publication 2007

Abohweyere PO (1984) Population dynamics of marine shrimp resources. 1984 Annual report of the Nigeria Institute of Oceanography and Marine Research, 33 pp

Aguila J, Cuzon G, Pascual C, Domingues PM, Gaxiola G, Sánchez A, Maldonado T, Rosas C (2007) The effects of fish hydrolysate (CPSP) level on *Octopus maya* (Voss and Solis) diet: digestive enzyme activity, blood metabolites, and energy balance. Aquaculture 273(4):641–655

Aksnes A, Hope B, Høstmark O, Albrektsen S (2006) Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture 261(3):1102–1110

Alaska Fisheries Development Foundation, Anchorage, Alaska (2008) Alaska seafood by-products: potential products, markets and competing products. http://www.afdf.org/wp/wp-content/uploads/2008_by_product_mkt_study.pdf. Accessed 8 Sept 2012

Aleman A, Giménez B, Montero P, Gómez-Guillén MC (2011) Antioxidant activity of several marine skin gelatins. LWT-Food Sci Technol 44:407–413

Ali MZ, Gheyasuddin S, Zaher S, Hossain MA, Islam MN (1994) Evaluation of fish silage from underutilised marine fishes as protein sources in the diet of major carp (*Cirrhinus mrigala*). J Aquac Trop 8:247–254

Anderson GW, McKinley SR, Colavecchia M (1997) The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North Am J Fish Manage 17:301–307

Anderson JS, Lall SP, Anderson MA, McNiven MA (1995) Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*). Aquaculture 138:291–301

Annett RW, Dawson LER, Edgar H, Carson AF (2009) Effects of source and level of fish oil supplementation in late pregnancy on feed intake, colostrum production and lamb output of ewes. Anim Feed Sci Technol 154:169–182

Anonymous (2004) Crab conference, 15–16 Jan 2004. Trondheim, Norway

Arason S (1994) Production of fish silage. In: Martin AM (ed) Fisheries processing: biotechnological applications. Chapman & Hall, London, pp 244–272

Arvanitoyannis IS, Kassaveti A (2008) Fish industry waste: treatments, environmental impacts, current and potential uses. Int J Food Sci Technol 43:726–745

Aspmo SI, Horn SJ, Eijsink VGH (2005) Enzymatic hydrolysis of Atlantic cod (*Gadus morhua* L.) viscera. Process Biochem 40:1957–1966

Austin B (1997) Progress in understanding the fish pathogen *Aeromonas salmonicida*. Trends Biotechnol 15:131–134

Babbitt JK (1990) Intrinsic quality and species of North Pacific fish, in making profits out of seafood wastes. In: Keller S (ed) Proceedings of the international conference on fish by-products, Anchorage, AK, University of Alaska Sea Grant, Fairbanks, 25–27 Apr 1990, pp 39–43

Bai SC, Lee KJ (1998) Different levels of dietary dl-α-tocopheryl acetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. Aquaculture 161:405–414

Barlow SM, Windsor ML (1984) Fishery by-products. International Association of Fish Meal Manufacturers, Potters Bar

Barlow SM, Young FVK, Duthie IF (1990) Nutritional recommendations for n-3 polyunsaturated fatty acids and the challenge to the food industry. Proc Nutr Soc 49:13–21

Barrias C, Oliva-Teles A (2000) The use of locally produced fish meal and other dietary manipulations in practical diets for rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquac Res 31(2):213–218

Cejas JR, Almansa E, Tejera N, Jerez S, Bolaños A, Lorenzo A (2003) Effect of dietary supplementation with shrimp on skin pigmentation and lipid composition of red porgy (*Pagrus pagrus*) alevins. Aquaculture 218:457–469

Chatzifotis S, Pavlidis M, Doñate Jimeno C, Vardanis G, Sterioti A, Divanach P (2005) The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). Aquac Res 36:1517–1525

Chen S, Hirota N, Okuda M, Takeguchi M, Kobayashi H, Hanagata N, Ikoma T (2011) Microstructures and rheological properties of tilapia fish-scale collagen hydrogels with aligned fibrils fabricated under magnetic fields. Acta Biomater 7(2):644–652

Choudhury GS, Bublitz CG (1996) Computer-based controls in fish processing industry. In: Mittal GS (ed) Computerized control systems in the food industry. Marcel Dekker, New York, pp 513–538

Cobos MA, Garcia LE, González SS, Barcena J, Hernández DS, Pérez-Sato M (2002) The effect of shrimp shell waste on ruminal bacteria and performance of lambs. Anim Feed Sci Technol 95(3–4):179–187

Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71:171S–175S

Crane SW, Griffin RW, Messent PR (2000) Introduction to commercial pet foods. In: Hand MS, Thatcher CD, Remillard RL et al (eds) Small animal clinical nutrition, 4th edn. Walsworth Publishing Company, Marceline, pp 111–126

Cruz-Suarez LE, Antimo-Perez JS, Luna-Mendoza N, Tapia-Salazar M, Ricque-Marie D (2000) Relaciones proteína/energía y proteína vegetal/animal optimas en alimentos de engorda para *Litopenaeus vannamei* y *L. stylirostris*. In: Cruz-Suárez LE, Ricque-Marie D, Tapia-Salazar M, Olvera-Novoa MA, Civera-Cerecedo R (eds) Avances en Nutrición Acuícola V—Memorias del Quinto Simposio Internacional de Nutrición Acuícola, Mérida, Yucatán, 19–20 Noviembre de 2000, Universidad Autónoma de Nuevo León, San Nicolas de los Garza, México compact disk in press

Dekkers E, Raghavan S, Kristinsson HG, Marshall MR (2011) Oxidative stability of mahi mahi red muscle dipped in tilapia protein hydrolysates. Food Chem 124:640–645

Demirbas A, Karslioglu S (2007) Biodiesel production facilities from vegetable oils and animal fats. Energy Resour Part A: Recov Util Environ Effects 29:133–141

El-Mashad HM, Zhang R, Avena-Bustillos RJ (2008) A two-step process for biodiesel production from salmon oil. Biosyst Eng 99:220–227

Espe M, Raa J, Njaa LR (1989) Nutritional value of stored fish silage as a protein source for young rats. J Sci Food Agric 49:259–270

Espe M, Nortvedt R, Lie O, Hafsteinsson H (2001) Atlantic salmon (*Salmo salar*, L.) as raw material for the smoking industry. I: effect of different salting methods on the oxidation of lipids. Food Chem 75(4):411–416

Espe M, Lemme A, Petri A, El-Mowafi A (2006) Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? Aquaculture 255(1–4):255–262

Esteban MB, Garcia AJ, Ramos P, Marquez MC (2006) Evaluation of fruit–vegetable and fish wastes as alternative feedstuffs in pig diets. Waste Manag 27:193–200

Evers DJ, Carroll DJ (1996) Preservation of crab or shrimp waste as silage for cattle. Anim Feed Sci Technol 59(4):233–244

Evers DJ, Carroll DJ (1998) Ensiling salt-preserved shrimp waste with grass straw and molasses. Anim Feed Sci Technol 71(3–4):241–249

Faid M, Zouiten A, Elmarrakchi A, Achkari-Begdouri A (1997) Biotransformation of fish waste into a stable feed ingredient. Food Chem 60(1):13–18

Fanimo AO, Mudama E, Umukoro TO, Oduguwa OO (1996) Substitution of shrimp waste meal for fish meal in broiler chicken rations. Trop Agric (Trinidad) 73(3):201–205

Fanimo AO, Oduguwa OO, Onifade AO, Olutunde TO (2000) Protein quality of shrimp-waste meal. Bioresour Technol 72:185–188
FAO (2010) The state of world fisheries and aquaculture 2010. Food and Agriculture Organization, Rome
Gabrielsen BO, Austreng E (1998) Growth, product quality and immune status of Atlantic salmon, *Salmo salar* L., fed wet feed with alginate. Aquac Res 29:397–401
Garcıa-Arias MT, Sánchez-Muniz FJ, Castrillón AM, Pilar NM (1994) White tuna canning, total fat, and fatty acid changes during processing and storage. J Food Compos Anal 7(1–2):119–130
Gildberg A (1993) Enzymic processing of marine raw materials. Process Biochem 28:1–15
Gildberg A (2002) Enhancing returns from greater utilization. In: Bremner HA (ed) Safety and quality issues in fish processing. Woodhead Publishing Ltd., Cambridge, pp 425–449
Gildberg A, Johansen A, Bøgwald J (1995) Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. Aquaculture 138:23–34
Goddard JS, Al-Yahyai DSS (2001) Chemical and nutritional characteristics of dried sardine silage. J Aquat Food Prod Technol 10(4):39–50
Goddard S, Al-Shagaa G, Ali A (2008) Fisheries by-catch and processing waste meals as ingredients in diets for Nile tilapia, *Oreochromis niloticus*. Aquac Res 39:518–525
Goodwin TW (1984) The biochemistry of the carotenoids. In: Animals, vol II, 2nd edn. Chapman & Hall, London, 224 pp
Gowen RJ (1991) Aquaculture and the environment. In: De Pauw N, Joyce J (eds) Aquaculture and the environment. Ghent, European Aquaculture Society Special Publications, No. 16, pp 30–38
Goytortúa-Bores EG, Civera-Cerecedo R, Rocha-Meza S, Green-Yee A (2006) Partial replacement of red crab (*Pleuroncodes planipes*) meal for fish meal in practical diets for the white shrimp *Litopenaeus vannamei*. Effects on growth and in vivo digestibility. Aquaculture 256:414–422
Guerard F, Guimas L, Binet A (2002) Production of tuna waste hydrolysates by a commercial neutral protease preparation. J Mol Catal B: Enzym 19–20:489–498
Gudmundsson M, Hafsteinsson H (1997) Gelatin from cod skins as affected by chemical treatments. J Food Sci 62(1):37–39, 47
Haaland H, Njaa LR (1989) Effect of temperature on the autolysis of capelin silages stored for one year. Fisk Dir Skr Ser Ernoering 2:219–226
Haard NF (1992) Control of chemical composition and food quality attributes of cultured fish. Food Res Int 25:289–307
Hallenstvedt E, Kjos NP, Rehnberg AC, Øverland M, Thomassen M (2010) Fish oil in feeds for entire male and female pigs: changes in muscle fatty acid composition and stability of sensory quality. Meat Sci 85:182–190
Hassan TE, Heath JL (1986) Biological fermentation of fish waste for potential use in animal and poultry feeds. Agric Waste 15(1):1–15
Hevrøy EM, Espe M, Waagbø R, Sandnes K, Ruud M, Hemre GI (2005) Nutrient utilization in Atlantic salmon (*Salmo salar* L.) fed increased levels of fish protein hydrolysate during a period of fast growth. Aquac Nutr 11(4):301–313
Hevroy EM, Sandnes K, Hemre GI (2004) Growth, feed utilisation, appetite and health in Atlantic salmon (*Salmo salar* L.) fed a new type of high lipid fish meal, Sea Grain®, processed from various pelagic marine fish species. Aquaculture 235:371–392
Hinton MH (2000) Infections and intoxications associated with animal feed and forage which may present a hazard to human health. Vet J 159:124–138
Holá M, Kalvoda J, Novákova H, Škoda R, Kanický V (2011) Possibilities of LA-ICP-MS technique for the spatial elemental analysis of the recent fish scales: line scan vs. depth profiling. Appl Surf Sci 257(6):1932–1940
Hsu KC (2010) Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product. Food Chem 122:42–48
Jackson AJ, Kerr AK, Bullock AM (1984) Fish silage as a dietary ingredient for salmon. II. Preliminary growth findings and nutritional pathology. Aquaculture 40:283–291

Jayathilakan K, Sultana K, Radhakrishna K, Bawa AS (2012) Utilization of byproducts and waste materials from meat, poultry and fish processing industries. J Food Sci Technol 49(3):278–293

Je J-Y, Qian Z-J, Byun H-G, Kim S-K (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42:840–846

Je J-Y, Lee K-H, Lee MH, Ahn C-B (2009) Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. Food Res Int 42:1266–1272

Kalinowski CT, Robaina LE, Fernández-Palacios H, Schuchardt D, Izquierdo MS (2005) Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244:223–231

Kalinowski CT, Izquierdo MS, Schuchardt D, Robaina LE (2007) Dietary supplementation time with shrimp shell meal on red porgy (*Pagrus pagrus*) skin colour and carotenoid concentration. Aquaculture 272(1–4):451–457

Kato S, Kunisawa K, Kojima T, Murakami S (2004) Evaluation of ozone treated fish waste oil as a fuel for transportation. J Chem Eng Jpn 37:863–870

Kentouri M, Pavlidis M, Papandroulakis N, Divanach P (1995) Culture of red porgy, Pagrus pagrus, in Crete. Present knowledge, problems and perspectives. Cah Options Méditérr 16:65–78

Khan MAA, Hossain MA, Hara K, Osatomi K, Ishihara T, Nozaki Y (2003) Effect of enzymatic fish-scrap protein hydrolysate on gel-forming ability and denaturation of lizard fish *Saurida wanieso* surimi during frozen storage. Fish Sci 69(6):1271–1280

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts–a review. Food Res Int 39:383–393

Kikuchi K, Ueda A, Sugita H, Takeda S (2002) Effect of dietary inclusion of blue mussel extracts on growth and body composition of Japanese flounder *Paralichthys olivaceus*. J World Aquacult Soc 33:41–47

Klompong V, Benjakul S, Yachai M, Visessanguan W, Shahidi F, Hayes KD (2009) Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe trevally (*Selaroides leptolepis*). J Food Sci 74:C126–C133

Kolkovski S, Tandler A (2000) The use of squid protein hydrolysate as a protein source in microdiets for gilthead seabream *Sparus aurata* larvae. Aquac Nutr 6(1):11–16

Lee DO, Wickins JF (1992) Crustacean farming. Blackwell Scientific Publication, Oxford

Lee SM, Lim YS, Lee J, Park SR, Myeong JI, Park Y (1999) Effects of supplemental squid meal, attractant, herb or lecithin in the formulated diets on growth performance in juvenile abalone (*haliotis discus hannai*). J Korean Fish Soc 32(3):290–294

Lee K-J, Powell MS, Barrows FT, Smiley S, Bechtel P, Hardy RW (2010) Evaluation of supplemental fish bone meal made from Alaska seafood processing byproducts and dicalcium phosphate in plant protein based diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 302:248–255

Lian PZ, Lee CM, Bengtson DA (2008) Development of a squid-hydrolysate-based larval diet and its feeding performance on summer flounder (*Paralichthys dentatus*) larvae. J World Aquacult Soc 39(2):196–204

Lim C, Beames RM, Eales JG, Prendergast AF, McLeese JM, Shearer KD, Higgs DA (1997) Nutritive values of low and high fibre canola meals for shrimp (*Penaeus vannamei*). Aquac Nutr 3(4):269–279

Lin CC, Ritch R, Lin SM, Ni M-H, Chang Y-C, Lu YL, Lai HJ, Lin F-H (2010) A new fish scale-derived scaffold for corneal regeneration. Eur Cell Mater 19:50–57

Mai K, Li H, Ai Q, Duan Q, Xu W, Zhang C, Zhang L, Tan B, Liufu Z (2006) Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828). Aquac Res 37(11):1063–1069

Mandeville S, Yaylayan V, Simpson BK (1992) Proximate analysis, isolation and identification of amino acids and sugars from raw and cooked commercial shrimp waste. Food Biotechnol 6:51–64

Martınez-Valverde I, Jesús Periago M, Santaella M, Ros G (2000) The content and nutritional significance of minerals on fish flesh in the presence and absence of bone. Food Chem 71:503–509

Martone CB, Borla OP, Sánchez JJ (2005) Fishery by-product as a nutrient source for bacteria and archaea growth media. Bioresour Technol 96:383–387

Meyers SP (1986) Utilization of shrimp processing waste. Infofish Mark Digest 4:18–19

Midlen A, Redding TA (1998) Environmental management for aquaculture. Chapman & Hall, London, 223 pp

Millamena OM, Golez NV, Janssen JAJ, Peschcke-Koedt M (2000) Processed meat solubles, Protamino Aqua, used as an ingredient in juvenile shrimp feeds. Isr J Aquacult Bamidgeh 52(3):91–97

Mnari A, Bouhlel I, Chraief I, Hammami M, Romdhane MS, El Cafsi M, Chaouch A (2007) Fatty acids in muscles and liver of Tunisian wild and farmed gilthead sea bream, *Sparus aurata*. Food Chem 100:1393–1397

Moksness E, Rosenlund G, Lie O (1995) Effect of fish meal quality on growth of juvenile wolffish, *Anarhichas lupus* L. Aquacult Res 26:109–115

Morimura S, Nagata H, Uemura Y, Fahmi A, Shigematsu T, Kida K (2002) Development of an effective process for utilization of collagen from livestock and fish waste. Process Biochem 37(12):1403–1412

Muroga K (2001) Viral and bacterial diseases of marine fish and shellfish in Japanese hatcheries. Aquaculture 202:23–44

Mushiake K, Nishizawa T, Nakai T, Furusawa I, Muroga K (1994) Control of VNN in striped jack: selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). J Fish Pathol 29:177–182

Naik SD, Sahu NP, Jain KK (2001) Use of squilla (*Orato squilla nepa*), squid (*Sepia pharonis*) and clam (*Katelysia opima*) meal alone or in combination as substitute for fish meal in the post larval diet *of Mactrobachium rosenbergii*. Asian Australas J Anim Sci 14(9):1272–1275

Nakajima K, Yoshie-Stark Y, Ogushi M (2009) Comparison of ACE inhibitory and DPPH radical scavenging activities of fish muscle hydrolysates. Food Chem 114:844–851

Neklyudov AD, Ivankin AN, Berdutina AV (2000) Properties and uses of protein hydrolysates. Appl Biochem Microbiol 36:452–459

New MB (1996) Responsible use of aquaculture feeds. Aquac Asia 1:3–15

Nordrum S, Åsgård T, Shearer KD, Arnessen P (1997) Availability of phosphorus in fish bone meal and inorganic salts to Atlantic salmon (*Salmo solar*) as determined by retention. Aquaculture 157:51–61

Okuda M, Takeguchi M, Tagaya M, Tonegawa T, Hashimoto A, Hanagata N, Ikoma T (2009) Elemental distribution analysis of type I collagen fibrils in tilapia fish scale with energy-filtered transmission electron microscope. Micron 40(5–6):665–668

Ørskov ER (1980) Nutritional principles in utilization of waste and by-products. Br Soc Animal Prod 3:13–17

Oosterveer P (2008) Governing global fish provisioning: ownership and management of marine resources. Ocean Coast Manage 51:797–805

Ovissipour M, Abedian Kenari A, Motamedzadegan A, Rasco B, Safari R, Shahiri H (2009) The effect of enzymatic hydrolysis time and temperature on the properties of protein hydrolysates from Persian sturgeon (*Acipenser persicus*) viscera. Food Chem 115:238–242

Pati F, Adhikari B, Dhara S (2010) Isolation and characterization of fish scale collagen of higher thermal stability. Bioresour Technol 101(10):3737–3742

Picot L, Ravallec R, Fouchereau-Péron M, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, Guérard F, Chabeaud A, LeGal Y, Alvarez OM, Bergé J-P, Piot J-M, Batista I, Pires C, Thorkelsson G, Delannoy C, Jakobsen G, Johansson I, Bourseau P (2010) Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties. J Sci Food Agric 90:1819–1826

Pike IH (1999) Future supplies of fishmeal and fish oil: quality requirements for aquaculture. Fish World 7(7&8):8–14
Pillay TVR (1991) Aquaculture and the environment. Blackwell Scientific Publications, London, pp 26–45
Ramirez-Ramírez JC, Huerta S, Arias L, Prado A, Shirai K (2008) Utilization of shrimp by-catch and fish wastes by lactic acid fermentation and evaluation of degree of protein hydrolysis and in vitro digestibility. Revista Mexicana de Ingeniería Química 7(3):195–204
Riche M, Brown PB (1996) Availability of phosphorus from feedstuffs fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 142:269–282
Ruiter A (1995) Fish and fishery products: composition, nutritive properties of stability. CABI Publications, Wallingford
Rustad T (2003) Utilization of marine by-products. Electron J Environ Agric Food Chem 2:458–463
Sachindra NM, Bhaskar N, Sakhare PZ, Mahendrakar NS, Narasimha Rao D (2001) An improved process for recovery of carotenoids from crustacean waste. Indian Patent 95/DEL/2001, 31 Jan 2001
Samuels WA, Fontenot JP, Allen VG, Abazinge MD (1991) Seafood processing wastes ensiled with straw: utilization and intake by sheep. J Anim Sci 69:4983–4992
Samuels WA, Fontenot JP, Allen VG, Flick GJ (1992) Fermentation characteristics of ensiled seafood wastes and low-quality roughages. Anim Feed Sci Technol 38(4):305–317
Sathiadhas R, Aswathy N (2004) Techno-economic analysis of production and marketing of marine by-products in India. J Indian Fish Assoc 31:156–165
Sato N, Fujioka T, Nobuta S (2006) Availability of commercial squid liver meal as diets of fingerling rockfish. Nippon Suisan Gakk 72:401–407
Satoh S, Takanezawa M, Akimoto A, Kiron V, Watanabe T (2002) Changes of phosphorus absorption from several feed ingredients in rainbow trout during growing stages of extrusion of soybean meal. Fish Sci 68:325–331
Sen DP (2005) Advances in fish processing technology. Allied Publishers Private Limited, New Delhi
Senaratne LS, Park P-J, Kim S-K (2006) Isolation and characterization of collagen from brown backed toadfish (*Lagocephalus gloveri*) skin. Bioresour Technol 97:191–197
Shahidi F, Metusalach G, Brown JA (1998) Carotenoid pigments in seafoods and aquaculture. Crit Rev Food Sci 38:1–67
Shang YC (1992) The role of aquaculture in the world fisheries. Presented at the world fisheries congress, Athens, Greece, 3–8 May 1992, 30 pp
Smith DM, Williams KC, Irvin SJ (2005) Response of the tropical spiny lobster *Panulirus ornatus* to protein content of pelleted feed and to diet of mussel flesh. Aquacult Nutr 11:209–217
Sigurgisladottir S, ØTorrissen O, Lie Ø, Thomassen M, Hafsteinsson H (1997) Salmon quality: methods to determine the quality parameters. Rev Fish Sci 5:223–252
Spinelli J, Mahnken C (1978) Carotenoid deposition in pen-reared salmonids fed diets containing oil extracts of red crab (*Pleuroncodes planipes*). Aquaculture 13:213–223
Spinelli J, Lehman L, Wieg D (1974) Composition, processing, and utilization of Red crab (*Pleuroncodes planipes*) as an aquacultural feed ingredient. J Fish Res Board Can 31(6):1025–1029
Stepnowski P, Olafsson G, Helgason H, Jastorff B (2004) Recovery of astaxanthin from seafood wastewater utilizing fish scales waste. Chemosphere 54(3):413–417
Sudaryono A, Hoxey MJ, Kailis SG, Evans LH (1995) Investigation of alternative protein sources in practical diets for juvenile shrimp *Penaeus monodon*. Aquaculture 134:313–323
Sugiura SH, Dong FM, Rathbone CK, Hardy RW (1998) Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159:177–202
Tatterson IN (1982) Fish silage—preparation, properties and uses. Anim Feed Sci Technol 7:153–159
Tibbetts SM, Lall SP, Milley JE (2004) Apparent digestibility of common feed ingredients by juvenile haddock, *Melanogrammus aeglefinus L.* Aquacult Res 35:643–651

Tibbetts SM, Milley JE, Lall SP (2006) Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, *Gadus morhua (Linnaeus, 1758)*. Aquaculture 261:1314–1327

Toppe J, Aksnes A, Hope B, Albrektsen S (2006) Inclusion of fish bone and crab by-products in diets for Atlantic cod, *Gadus morhua*. Aquaculture 253:636–645

Vassallo-Agius R, Watanabe T, Imaizumi H, Yamazaki T (2002) Spawning performance of yellowtail fed dry pellets containing paprika and squid meal. Fish Sci 68:230–232

Vidotti RM, Carneiro DJ, Viegas EMM (2002) Acid and fermented silage characterization and determination of apparent digestibility coefficient of crude protein for pacu *Piaractus mesopotamicus*. J World Aqua Soc 33(1):57–62

Vidotti RM, Viegas EMM, Carneiro DJ (2003) Amino acid composition of processed fish silage using different raw materials. Animal Feed Sci Technol 105(1–5):199–204

Vielma J, Ruohonen K, Lall SP (1999) Supplemental citric acid and particle size of fish bone-meal influence the availability of minerals in rainbow trout *Oncorhynchus mykiss* (Walbaum). Aquacult Nutr 5:65–71

Vielma J, Lall SP (1998) Phosphorus utilization by Atlantic salmon (*Salmo salar*) reared in freshwater is not influenced by higher dietary calcium intake. Aquaculture 160:117–128

Villarreal H, Hernandez-Llamas A, Rivera MC, Millán A, Rocha S (2004) Effect of substitution of shrimp meal, fish meal and soy meal with red crab *Pleuroncodes planipes* (Stimpson) meal in pelleted diets for postlarvae and juvenile *Farfantepenaeus californiensis* (Holmes). Aquac Res 35:178–183

Ward AG, Courts A (1977) The science and technology of gelatin. Academic Press, London

Ward OP, Singh A (2005) Omega-3/6 fatty acids: alternative sources of production. Process Biochem 40:3627–3652

Wang J, Lied E (2001) Two types of fish silage and its use in fish feed. Mar Fish Res 22(4):1–8

Wang Y, Kong LJ, Li C, Bureau DP (2006) Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). Aquaculture 261:1307–1313

Wasswa J, Tang J, Gu X-H, Yuan X-Q (2007) Influence of the extent of enzymatic hydrolysis on the functional properties of protein hydrolysate from grass carp (*Ctenopharyngodon idella*) skin. Food Chem 104:1698–1704

Watanabe T, Murakami A, Takeuchi L, Nose T, Ogino C (1980) Requirement of chum salmon held in freshwater for dietary phosphorus. Bull Jpn Sot Sci Fish 46:361–367

Westendorf ML (2000) Food waste as animal feed: an introduction. In: Westendorf ML (ed) Food waste to animal feed. Iowa State University Press, Ames, pp 3–16, 69–90

Westendorf ML, Dong ZC, Schoknecht PA (1998) Recycled cafeteria food waste as a feed for swine: nutrient content digestibility, growth, and meat quality. J Anim Sci 76:2976–2983

Wilkie DW (1972) The carotenoid pigmentation of *Pleuroncodes planipes* Stimpson (Crustacea: Decapoda: Galatheidae). Comp Biochem Physiol 42B:731–734

Wyatt B, McCourty G (1990) Use of marine by-product on agricultural crops. In: International by-products conference, Anchorage, Alaska, pp 187–217

Yin H, Pu J, Wan Y, Xiang B, Bechtel PJ, Sathivel S (2010) Rheological and functional properties of Catfish skin protein hydrolysates. J Food Sci 75:E11–E17

Zhou P, Regenstein JM (2005) Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. J Food Sci 70(6):c392–c396

Chapter 16
Potential Uses of Lactic Acid Bacteria in Seafood Products

Panchanathan Manivasagan, Jayachandran Venkatesan, and Se-Kwon Kim

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1 Introduction

The lactic acid bacteria (LAB) are a group of Gram-positive rod- and coccus-shaped organisms that have less than 55 % mol G+C content in their DNA. They are non-spore-forming, non-motile, and produce lactic acid as their major end product during the fermentation of carbohydrates. The taxonomy of LAB has changed considerably during the last few years and, at present, this group comprises the following genera: *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*, *Tetragenococcus*, *Vagococcus*, *Weissella*, and *Bifidobacterium*. They are widespread in nature and commonly found in many food products (dairy, meat, fruit, vegetables, etc.), as well as in the genital regions, intestinal regions, and oral cavities of animals and humans. For thousands of years, humans have empirically used LAB for the natural fermentation of milk, meat, vegetables, and fruits that led to a new stabilized product. The acidification process due to lactic acid production as the major end metabolite of the carbohydrate fermentation is one of the most desirable side effects of their growth, inhibiting microorganisms, including the most common human pathogens. Since the scientific basis of the mechanisms have been set up, the LAB species traditionally used have been selected and produced as lyophilized starters that can be added into the food and allow better control of the fermentation.

LAB in fish flesh have long been disregarded because they are not currently present in seafood. However, significant changes in culinary habits in the last several decades of the past century and current consumer demands have led the food industry to produce a great variety of convenient foods, many of them ready-to-eat, such as lightly preserved fish products (LPFP), in which certain ingredients (e.g., salt or sugar) are usually added and a mild processing (e.g., cold-smoked) is frequently applied. These approaches give rise to changes of the normal characteristics of fresh fish (e.g., reduced aw), inhibiting the growth of organisms responsible for spoilage and enhancing that of other microbiota, such as LAB. Given the great increase in the production of these foods, LAB can achieve a great interest in LPFP packaged under different conditions. The potential uses of LAB in seafood products are discussed in this chapter.

2 Microbial Ecology of Living Fish

The muscle of living marine fish is sterile. However, the skin, mucus, gills, and gut contain significant bacteria, whose composition and quantity vary according to the fish species, temperature and salinity of the water, level of dissolved oxygen, degree of pollution, feed, stress, etc.

The microbiota of marine fish from temperate waters is usually composed of Gram-negative psychrotrophic bacteria, whose growth is possible at 0 °C and optimal at around 25 °C. The majority belongs to the class γ of proteobacteria: *Pseudomonas*, *Shewanella*, *Acinetobacter*, *Aeromonas*, *Vibrio*, *Moraxella*, *Psychrobacter*,

Photobacterium, etc., and, to a lesser extent, to the CFB group (*Cytophaga–Flavobacterium–Bacteroides*). Nevertheless, Gram-positive bacteria, such as *Micrococcus*, *Corynebacterium*, *Bacillus*, *Lactobacillus*, and *Clostridium*, may also be present in variable proportions (Shewan 1971; Mudarris and Austin 1988; Gram and Huss 1996; Gennari et al. 1999; Huber et al. 2004; Wilson et al. 2008). The same bacterial genus can be found in tropical marine fish, but Gram-positive bacteria, Enterobacteriaceae, and Vibrionaceae are often dominant (Liston 1980). The indigenous microbiota of the gastrointestinal tract have been studied in much more detail than those of the skin or mucus due to their importance in digestion, nutrition, and disease in aquaculture (Ringø et al. 1995; Spanggaard et al. 2000). Although this environment is partially anaerobic, most researchers have observed a predominance of aerobic bacteria, which are also present in the surrounding water. This could be due to the collecting techniques, which are not always suitable for strict anaerobes (Burr et al. 2005). Nevertheless, Huber et al. (2004) have shown, using molecular methods, that the aerobic microbiota of rainbow trout intestine usually represents 50–90 % of the total microbiota (Huber et al. 2004). In general, Gram-negative bacteria (*Vibrio*, *Acinetobacter*, and *Enterobacteriaceae*) dominate the microbiota (Ringø et al. 1995; Ringo and Birkbeck 1999). These are fermentative bacteria that develop rapidly in the gastrointestinal tract due to the low pH, lack of oxygen, and abundance of nutrients. Sometimes, staphylococci have also been found to be the dominant microbiota in the fish intestine. However, both the number and diversity of the microbiota are probably widely underestimated due to the classical microbiological methods involving growth on agar media that, so far, have been used. The bacterial cultivability has been estimated to be less than 2 % of intestinal microbiota rainbow trout (Huber et al. 2004) and less than 0.01 % of the skin microbiota (Bernadsky and Rosenberg 1992).

3 Lactic Acid Bacteria

Although not the most common, it is generally accepted that LAB occur among the normal intestinal microbiota of fish from the first few days and towards (Yang et al. 2007; Ringø 2008). Lactobacilli, notably *Lactobacillus plantarum*, have been found in Atlantic salmon (Ringø et al. 1997), pollock (Schrøder et al. 1980), Arctic char (Ringø and Gatesoupe 1998), and cod (Strøm and Olafsen 1990). Carnobacteria, including *Carnobacterium maltaromaticum* (previously *piscicola*), *divergens*, *gallinarum*, and *inhibens*, have been isolated from all these species, as well as from rainbow trout (Jöborn et al. 1999; Ringø et al. 2001; Huber et al. 2004). Carnobacteria have even been quoted as being the dominant genus in the gastrointestinal tract of juvenile Atlantic salmon (Ringø et al. 1997) and cod (Seppola et al. 2006). Other authors have also reported the presence of *Leuconostoc mesenteroides*, *Lactococcus piscium*, *Vagococcus salmoninarum*, *Lactobacillus fuchuensis*, *Streptococcus* spp., and *Weissella* spp. (Wallbanks et al. 1990; Williams et al. 1990; Ringø and Gatesoupe 1998; Liu et al. 2009; Matamoros et al. 2009). Various factors, such as the salinity of the water or stress, can affect the presence of LAB (Ringø and Strøm 1994).

For instance, the number of lactobacilli in the gastrointestinal tract of Arctic char was smaller among those reared in seawater than those reared in freshwater, while the number of *Leuconostoc* and enterococci remained the same (Ringø and Strøm 1994). Atlantic salmon stressed by daily manipulations experienced a drop in the number of Gram-negative bacteria in their intestinal microbiota and an increase in the carnobacteria count (Ringø and Gatesoupe 1998).

Even though most LAB are generally recognized as safe by the US Food and Drug Administration, their implication in fish disease has been reported. In marine-farmed fish, numerous epizootics linked to streptococci have been notified, beginning in Japan and North America, then spreading worldwide (Eldar et al. 1996). These bacteria, reclassified as *Lactococcus garvieae*, are responsible for septicemias, opthalmias, and hemorrhages. *C. maltaromaticum* has been isolated from different diseased fish and its virulence has been clearly established in rainbow trout and striped bass that were experimentally infected (Baya et al. 1991). Recently, a novel *Weissella* species has been described as an opportunistic pathogen for rainbow trout (Liu et al. 2009).

4 Lactic Acid Bacteria in the Gastrointestinal Tract of Fish

4.1 Larval Stages

The gut is sterile until hatching, but soon after hatching, the fish comes into contact with the environment and live food, which leads to successive colonization by a variety of microbes (Munro et al. 1994; Ringø et al. 1996). The balance of this microbiota is influenced by a variety of factors, including food, animal physiology, and immunological factors. The establishment of a normal gut flora may be regarded as complementary to the establishment of digestive enzymes, and, under normal conditions, it serves as a barrier against invading pathogens.

The digestive tract of fish develops as a simple tube, first adjacent to the yolk material and then enveloping it. During the first few weeks, the gut lengthens, twists, and develops pouches for specific functions. Intestinal cells proliferate widely for the first few days, but then continue to proliferate only in the areas that will form the bases of the mucosal folds (Rombout et al. 1984). The anatomy and functions may eventually stabilize during larval development, but then change again significantly at metamorphosis, especially if there is a major change in diet.

It is generally considered that Gram-positive bacteria, including LAB, are numerically dominant members of the normal microbiota in the gastrointestinal tract of endothermic animals during their early stages of life. However, only three investigations have isolated LAB from the gastrointestinal tract of larval and juvenile fish (Strøm and Olafsen 1990). The important question arises as to why LAB are seldom isolated from larval fish. The limiting factors may have been the incubation temperature, incubation time, and the absence of glucose in the medium. The incubation time and the absence of glucose may be the most critical factors, since it is generally accepted that LAB, like *Lactobacillus* and *Carnobacterium*

genera, are regarded as slow-growing, demanding in their nutritional requirements, and their growth is usually restricted to habitats in which sugars are present (Brock and Madigan 1991). In addition to carbohydrate as an energy and carbon source, a variety of nucleotides, amino acids, and vitamins are required for growth. Based on the observation that LAB are seldom isolated from larvae, it is suggested that future investigations on larval fish should carefully consider if LAB could inhabit the digestive tract and act as a probiotic.

4.2 Juveniles and Ongrowing Fish

A short background on the general gross anatomy is first provided here to help the understanding of the fish digestive tract. The fish gastrointestinal tract consists of the esophagus, stomach, pyloric caeca, and small and large intestines. The gastrointestinal system is essentially a muscular tract lined by a mucous membrane that exhibits regional variations in structure reflecting the changing functions of the system from the mouth to the anus. The esophagus is generally short and distensible, so that relatively large objects can be swallowed. The bacterial population of the esophagus has been investigated in one study, but only in one fish (rainbow trout, *Oncorhynchus mykiss* Walbaum) (Austin and Al-Zahrani 1988), and the authors reported a bacterial population level of 3×10^5 CFU/g wet weight. Microphagous fishes have less distensible tubes than those of predatory fishes.

Not all fishes have a stomach, which is lacking in lampreys, hagfish, chimaeras, and some bony fishes, e.g., minnows, carp (Cyprinidae), sauries (Scomberesocidae), and parrotfishes (Scaridae). In most fishes where a stomach is present, it may vary in shape, size, and structure according to the diet of the various species. Usually, the stomach is shaped like a ‘U’ or a ‘J’ (*Salmo*, *Coregonus*, and *Clupea*), ‘Y’ (*Alosa*, *Anguilla*, and *Gadidae*) (Suyehiro and Kaigi 1941). In salmonid fish, with a pH between 3.5 and 4.5 in the stomach (Ash 1980), the bacterial population level is in the range from 2×10^4 to 10^5 CFU/g (Austin and Al-Zahrani 1988). Ringø (1993) isolated a wide variety of bacteria from the stomach of Arctic charr, *SalÍelinus alpinus* (L.), but LAB were only a minor part of the microbiota (approximately 10 %). The destruction of ingested bacteria in the digestive tract is mainly due to acid in the stomach and bile in the intestine (Ringø 1993).

5 Bacteriocins of LAB

As already stated, numerous bacteriocins have been isolated from LAB over the last three decades, varying in size from small (<3 kDa), heavily posttranslationally modified peptides to large heat-labile proteins. The continual emergence of new bacteriocins has necessitated a continual updating of the classification of bacteriocins.

Bacteriocins produced by LAB are commonly divided into three main groups (Kelly et al. 1996; Nes et al. 1996). Class I consists of small, posttranslationally

modified peptides which are characterized by the presence of modified thioether amino acids such as lanthionine, β-methyllanthionine, and α,β-unsaturated amino acids such as dehydroalanine and dehydrobutyrine, and are usually referred to as lantibiotics. Based on the alignment of mature peptides, lantibiotic peptides from LAB can be subdivided into six subgroups (Twomey et al. 2002). Nisin A and nisin Z make up a single group, while lacticin 481 and lacticin J49 belong to the lacticin 481 group; plantaricin C, LtnA1 (one component of the two-peptide lacticin 3147), and Plwα belong to the mersacidin group; LtnA2 (the second component of lacticin 3147) and Plwβ belong to the LtnA2 group; the two peptides $CylL_l$ and $CylL_s$ from the two-peptide cytolysin form a group of their own; while lactocin S is also grouped separately. Such criteria as the stability, inhibition spectrum, and mode of action are important when considering lantibiotic peptides for particular applications, as these will influence the efficacy of the bacteriocin in different environments.

Class II comprises a very large group of heat-stable unmodified peptide bacteriocins, which can also be further subdivided. Class IIa includes pediocin-like *Listeria-active* peptides with a conserved N-terminal sequence YGNGV-C and two cysteines forming a disulfide bridge in the N-terminal half of the peptide. Bacteriocins that fit into this category include pediocin PA-1(AcH), leucocin A, sakacins A and P, and enterocin A. These bacteriocins and their producer organisms are of considerable interest as biopreservatives due to their high antilisterial activity. Indeed, recent reports have suggested that class IIa bacteriocins are more interesting antilisterial agents than class I bacteriocins such as nisin, because they do not have as broad an inhibitory spectrum as nisin and, thus, do not kill many starter cultures, while they are more effective in killing *Listeria* strains. However, there are limitations to the usefulness of class IIa bacteriocins as antilisterial agents, since full suppression of the pathogen is rarely achieved in food systems (Ennahar et al. 1999). Class IIb comprises two-component bacteriocins, including lactococcins G and F and lactacin F, which require both peptides for full activity.

A third group consists of large heat-labile bacteriocins, of which helveticin J (Joerger and Klaenhammer 1990) and enterolysin A are examples. The examples in this review focus mainly on bacteriocin representatives from classes I and IIa, as these bacteriocins are the most effective in killing pathogens such as *Listeria monocytogenes* and/or *Clostridium botulinum*.

6 Seafood Consumption

Because of its nutritional value, seafood is increasingly recognized as a healthy dietary component by consumers worldwide, offering high-quality protein, omega-3 fatty acids, and essential micronutrients and minerals. Seafood includes molluscs (e.g., oysters, clams, and mussels), finfish (e.g., salmon and tuna), marine mammals (e.g., seals and whales), fish eggs (roe), and crustaceans (e.g., shrimps, crabs, and lobsters) (Iwamoto et al. 2010).

Seafood consumption levels and trends have recently been estimated by the Food and Agriculture Organization (FAO) of the United Nations and are available in the

FAOSTAT Database (http://faostat.fao.org/). According to these data, 24.05 kg/capita/year were consumed in the USA and 22.03 kg/capita/year in the EU. Japan had one of the highest global per-capita levels of fish consumption (60.78 kg/capita/year), and China showed a consumption level of 26.46 kg/capita/year. In addition, fish provides at least 50 % of the total animal protein intake in some small developing island states (Laurenti 2007), and it is estimated that future demand will grow (Failler et al. 2007).

Aquaculture, mainly based in the Asia-Pacific region, accounts for 46 % of the total world seafood supply (FAO 2010). The contribution of aquaculture to global supplies of fish, crustaceans, molluscs, and other aquatic animals has grown considerably over the past four decades. It increased from 3.9 % of the total production by weight in 1970 to 36 % in 2006, and is expected to continue growing in the future (Failler et al. 2007). This trend could potentially lead to an increase in health issues related to seafood consumption because of the greater risk of biological and chemical contamination in coastal areas and freshwaters, compared to open seas, due to the proximity to urbanized areas (Feldhusen 2000).

As a highly perishable commodity, seafood has significant processing requirements, and can be consumed in a great variety of ways and product forms. It is generally distributed as live, fresh, chilled, frozen, heat-treated, fermented, dried, smoked, salted, pickled, boiled, fried, freeze-dried, minced, powdered, or canned, or a combination of these methods may be employed. Several preparations are also based on traditions. Processed fishery products, ranging from ready-to-cook, partly cooked, or even ready-to-eat dishes, are increasingly popular among consumers, who have less time for preparing meals (Failler et al. 2007). The most important fish products destined for direct human consumption are fresh fish (40 %), frozen fish (32 %), canned fish (16 %), and cured fish (12 %) (Ababouch 2006).

7 Microbiological Risk Associated with Seafood

The biological agents involved in seafood contamination consist of bacteria, viruses, and parasites, which can cause illnesses ranging from mild gastroenteritis to life-threatening diseases. Some of these pathogens are naturally present in the aquatic environment, while others can be introduced through animal or human fecal shedding and sewage pollution (Table 16.1). Bacteria naturally present in seawater can be found in limited numbers in live and raw fish, although they can be concentrated by filter-feeding molluscs, which are often eaten raw.

Huss et al. (2000) classified seafood into risk categories. According to this ranking system, the highest risk category includes molluscs (fresh and frozen mussels, clams, oysters) and fish that are served raw. The next highest risk category includes crustaceans and fish, fresh or frozen, to be eaten after cooking. Finally, low-risk categories include lightly preserved fish products (salted, marinated, fermented, cold-smoked, and gravad fish); semi-preserved fish (caviar); mildly heat-processed (pasteurized, hot-smoked); and heat-processed (sterilized, packed in sealed containers) (Huss et al. 2000).

Table 16.1 Microbiological risks associated with seafood products

S. no.	Origin	Species
1	Naturally present in the aquatic environment (indigenous)	*Vibrio*, *Aeromonas*, *Plesiomonas*, *Clostridium botulinum* type E, helminths, *Amoeba*
2	Human and animal origin	*Salmonella*, *Shigella*, *Escherichia coli*, *Legionella*, *Campylobacter*, *Staphylococcus*
		Enteric viruses: enteroviruses, adenoviruses, HAV, noroviruses, rotaviruses
3	General environment	*Listeria*, proteolytic *C. botulinum*, *Staphylococcus*

8 Specific Spoilage Organism (SSO) Concept

While early studies of seafood microbiology acknowledged that only part of the spoilage microflora participated in the spoilage process (Huss et al. 2000; Castell and Anderson 1948; Lerke et al. 1967), the recent establishment of the specific spoilage organism (SSO) concept (Dalgaard 1995) has contributed significantly to our understanding of seafood spoilage. The SSOs are typically present in low numbers and constitute only a very small fraction of the microflora on newly processed seafood. Different SSOs are found in different seafoods and may be a single species (Table 16.2). The identification of an SSO relies on comparison of the sensory and chemical characteristics of spoiled products with those of isolates from the spoilage microflora. The qualitative ability to produce off-odors (spoilage potential) and the quantitative ability to produce spoilage metabolites (spoilage activity) are essential in the identification of an SSO. Quantitative comparison of the yield factor for the production of trimethylamine (TMA), biogenic amines, or volatile amines in products and by bacterial isolates grown in model substrates has been useful for the identification of SSOs (Jørgensen et al. 2000b; Dalgaard 1995; Koutsoumanis and Nychas 2000). Comparison of the chemical profiles of spoiled seafoods and of the metabolites produced by potential spoilage organisms has only been used to a limited extent in the identification of SSOs. This approach, which should involve the use of multivariate statistical methods for pattern matching, deserves further study. The spoilage domain has been defined as the range of conditions (pH, temperature, water activity, and atmosphere) under which an SSO can grow and produce spoilage metabolites. The identification of SSOs and their spoilage domains has substantially facilitated the development of methods to determine, predict, and extend the shelf life of seafood. This concept is also applicable to other foods where spoilage is caused by microbial activity.

9 Microbial Metabolites and Seafood Spoilage

Fish contains little carbohydrate but typically has a high content of free amino acids. Many fish species contain trimethylamine oxide (TMAO). The seafood SSOs produce ammonia, biogenic amines, organic acids, and sulfur compounds from amino

Table 16.2 Examples of specific spoilage organisms (SSO) of seafood products

S. no.	Product	Specific spoilage organisms
1	Iced marine fish	*Shewanella putrefaciens*
2	Iced freshwater fish	*Pseudomonas* spp.
3	CO_2-packed chilled fish	*Photobacterium phosphoreum*

acids, hypoxanthine from ATP degradation products, and acetate from lactate. TMA is produced by some bacteria capable of using TMAO in anaerobic respiration (Gram et al. 1990; Chinivasagam et al. 1998; Olafsdottir et al. 1997; Koutsoumanis and Nychas 1999; Joffraud et al. 2001; Dainty and Mackey 1992). Many microbial metabolites produced in seafood are similar to those observed in meat and poultry products (Dainty and Mackey 1992; Nychas et al. 1998); however, in seafood spoilage, TMA in particular contributes to the characteristic ammonia-like and 'fishy' off-flavors. *Aeromonas* spp., psychrotolerant Enterobacteriaceae, *P. phosphoreum*, *Shewanella putrefaciens*-like organisms, and *Vibrio* spp. can all reduce TMAO to TMA.

Some spoilage metabolites can be used as quality indices. Compared with microbiological methods, which are slow, chemical analyses may be significantly faster; however, for some compounds, measurable concentrations are not present until close to spoilage. The classical single-compound quality index (SCQI) for seafood includes measurements of total volatile nitrogen (TVN), TMA, and hypoxanthine. Ratios between ATP degradation products (K values) and biogenic amines have also been used for some time as quality indices (Dalgaard 1995). Multiple-compound quality indices (MCQI), in which combinations of several metabolites are identified by statistical methods, have recently been introduced and correlate better with sensory properties and/or shelf life in some products (Leroi et al. 2001; Jørgensen et al. 2000a).

10 Bacteriocin-Producing LAB for Improvements in Seafood

In spite of modern technologies and safety concepts such as Hazard Analysis and Critical Control Points (HACCP), the reported numbers of food-borne illnesses and intoxications are still on the increase. According to the Council for Agricultural Science and Technology (CAST), microbial pathogens in food cause an estimated 6.5–33 million cases of human illness and up to 9,000 deaths annually, with the main foods implicated including meat, poultry, eggs, seafood, and dairy products. The bacterial pathogens that account for many of these cases include *Salmonella*, *Campylobacter jejuni*, *Escherichia coli* 0157:H7, *L. monocytogenes*, *S. aureus*, and *Clostridium botulinum* (Buzby et al. 1996). With regard to the use of bacteriocin-producing LAB as biopreservatives for food improvement, much work has focused on the use of bacteriocin-producing cultures with inhibitory activity against Gram-positive pathogens such as *L. monocytogenes*, while no bacteriocin produced by a Gram-positive organism with activity against Gram negatives has been characterized in detail to date.

The potential of bacteriocin-producing starters or starter adjuncts, especially lactococci, pediococci, and lactobacilli, to control undesirable microbes in food has

been evaluated by a number of research groups in recent years. This section will review examples where bacteriocin-producing LAB have successfully been employed, at the laboratory scale, as 'protective cultures' to inhibit pathogenic microorganisms in a variety of food systems and also those bacteriocin-producing cultures which show potential for future applications.

The deterioration of fish is generally caused by Gram-negative microorganisms and, as such, few attempts have focused on evaluating the potential of LAB bacteriocins in such products. However, in vacuum-packed fish and seafood, spoilage organisms such as *C. Botulinum* and *L. monocytogenes* can cause problems. The inhibition of these spoilage organisms by bacteriocins has been studied (Degnan et al. 1994; Einarsson and Lauzon 1995; Nilsson et al. 1997). However, up until recently, little work had focused on incorporating live bacteriocin-producing cultures into the product but, rather, on addition of the bacteriocin as a concentrated preparation. In the last several years, different groups tested the growth and bacteriocin production of *Carnobacterium divergens* V41 and *Carnobacterium piscicola* V1 in a simulated cold-smoked fish system at 4 °C. In coculture, these strains were very effective, inhibiting *L. monocytogenes* as early as day 4 (Duffes et al. 1999b). The same group later demonstrated the inhibition of *L. monocytogenes* by *Carnobacterium* strains on sterile and commercial vacuum-packed, cold-smoked salmon stored at 4 °C and 8 °C. *C. piscicola* V1 was bactericidal against *L. monocytogenes* at both temperatures, whereas *C. divergens* V41 had a bacteriostatic effect. *C. piscicola* SF668 delayed *L. monocytogenes* growth at 8 °C and had a bacteriostatic effect at 4 °C (Duffes et al. 1999a). Importantly, the use of these bacteriocin-producing strains did not have any effect on the quality of the end-product. Recently, Nilsson et al. (1999) also demonstrated that a bacteriocin-producing *Carnobacterium* could be used as a biopreservative in vacuum-packed, cold-smoked salmon stored at 5 °C. *C. piscicola* (A9b) initially caused a 7-day lag phase of *L. monocytogenes*, followed by a significant reduction in numbers from 10^3 CFU/ml to below 10 CFU/ml after 32 days of incubation (Nilsson et al. 1999). More recently, *C. divergens* V41 has been shown to have potential as a biopreservative for refrigerated cold-smoked salmon, given that the bacteriocin divercin V41 was produced under harsh culture conditions, which included variations in temperature, NaCl, and glucose concentration (Connil et al. 2002).

11 Important Role of Lactic Acid Bacteria in Seafood Products

11.1 New Preservation Strategies Targeted at the Inhibition of Spoilage Bacteria

The spoilage of some seafoods is well understood and this understanding has enabled the development of preservation techniques targeted at the SSO. An example of this is the CO_2-packaging of fresh, marine, iced fish. This inhibits the

respiratory spoilage bacteria (*Shewanella* and *Pseudomonas*) and should, in principle, result in a dramatic extension of shelf life. However, because of the presence of the CO_2-resistant, TMAO-reducing *P. phosphoreum*, the product spoils almost at the same rate as non-CO_2-packed fillets. Targeted inhibition of *P. phosphoreum* (e.g., by freezing or the addition of spices) reduces its growth and results in a significant extension of shelf life (Guldager et al. 1998; Mejlholm and Dalgaard 2002). Non-spoilage LAB or pure bacteriocins have been used to extend the shelf life of brined shrimp, which, if unpreserved, spoil due to the growth of spoilage LAB (Einarsson and Lauzon 1995). The possible involvement of AHL regulation in the spoilage of some foods also opens up a new field of food preservation. Although, until recently, preservation has relied on the elimination (killing) or growth inhibition of spoilage organisms, AHL-regulated traits can be specifically blocked (e.g., by the *Delisea pulchra* halogenated furanones (Givskov et al. 1996; Kjelleberg et al. 1997)). This 'quorum-sensing interference' will not necessarily inhibit growth but will, in principle, only block the unwanted spoilage reactions; for example, the export of enzymes involved in the spoilage process.

11.2 Seafood Safety

Food safety is defined by the World Health Organization (WHO) as the assurance that food will not cause harm to the consumer.

The safety of seafood products varies considerably and is influenced by a number of factors; therefore, it is important to determine whether the hazard is significant for a particular product, and how it should be controlled.

Currently, the major risk associated with seafood safety originates from the environment; contamination of seafood can occur before harvest or at any point from harvest through final preparation. However, the survival of food-borne pathogens is more likely to occur in foods that are consumed undercooked or raw, particularly bivalve molluscs, as well as in those that experience time and temperature abuse, such as during delays between harvest and refrigeration (Iwamoto et al. 2010).

Every seafood harvester and processor is required to use an HACCP-based system, able to identify sources and points of process, from harvest to consumption, at high risk of contamination, so that strategies aimed at decreasing these risks can be implemented and monitored.

In this context, the FDA plays an important role in establishing guidelines and providing oversight to ensure safer fish and fishery products. HACCP, GMP, GHP, and SSOPs are major components of the safety management systems in the food supply chain (Aruoma 2006). HACCP has also been endorsed worldwide by Codex Alimentarius, the EU, and by several countries, including Canada, Australia, New Zealand, and Japan. Additional control strategies, such as the National Shellfish Sanitation Program (NSSP) guidelines, that regulate the harvesting, processing, and shipping of shellfish for interstate commerce in the USA, are aimed at promoting the safety of molluscan shellfish (Iwamoto et al. 2010).

Control strategies to prevent seafood-associated illnesses include monitoring harvest waters, identification and implementation of process controls, and consumer education (Iwamoto et al. 2010).

The most common factors contributing to salmonellosis outbreaks are improper cooking, inadequate storage, cross-contamination, and use of raw ingredients in the preparation of seafood. The main postharvest CCPs for *Salmonella* control in seafood, irrespective of whether the primary source is a marine or an aquaculture product, include: primary chilling immediately in an ice-water slurry on vessels and at the harvest site; in cooked products, applying time–temperature regimes to give log reductions of contamination levels at sites of microbiological concern; rapid chilling after cooking; plate freezing, followed by frozen storage.

Studies have shown that there is an additional risk of cross-contamination or recontamination between raw and cooked products at processing plants (Norhana et al. 2010). A number of fish products receive heat treatment during processing. Examples of such products include: pasteurized or cooked and breaded fish fillets, cooked shrimp and crabmeat, cook–chill products, and hot-smoked fish. After the heat-treatment, the various products may pass through further processing steps before being packed and stored/distributed as chilled or frozen products. Some of these products may receive additional heat treatment before consumption or they may be eaten without further treatment (ready-to-eat). The last category includes products that are extremely sensitive to secondary contaminations. Hence, in the application of the HACCP system, the heat treatment is a very critical processing step.

A further important aspect of quality and safety assurance is the ability to trace products, ingredients, suppliers, retailers, processing operations, or storage procedures throughout the food production chain (McKean 2001). Many food (fish) processing companies already have effective internal traceability systems as part of their HACCP-based quality assurance systems. This is especially relevant when failures occur. Traceability is important in the fresh fish chain, since it may guarantee freshness, which is almost exclusively a function of time and temperature. Moreover, it may trace fish from polluted waters.

Achieving food safety in the global marketplace is a fundamental human right and a global responsibility, in order to protect both the public and the economic health of a nation.

Federal agencies, state governments, and private industry all bear responsibility for reducing seafood-associated infections (Iwamoto et al. 2010).

Effective alternatives are now offered by the so-called rapid methods, like membrane filtration, automated electrical techniques, and immunological assays (Martinez-Urtaza and Liebana 2005), but the most promising are those based on polymerase chain reaction (PCR) and real-time PCR (Amagliani et al. 2010; DePaola et al. 2010; Kumar et al. 2008; Minami et al. 2010; Shabarinath et al. 2007), thanks to their high sensitivity and specificity.

One of the main limitations imputable to DNA-based diagnostic methods concerns the possibility of the detection of nucleic acids from non-living (thus, non-infecting) microorganisms, leading to false-positive results. This problem is usually circumvented

by adding a culture-enrichment step of the food under inspection, before DNA extraction, which ensures that positive results are obtained only from viable cells.

In addition, epidemiological surveillance takes advantage of the application of molecular typing [i.e., PFGE, (Martinez-Urtaza and Liebana 2005; Ponce et al. 2008); PCR ribotyping and ERIC-PCR (Kumar et al. 2008)], which makes it possible to trace related strains and vehicles, and prevent the spread of infection.

11.3 Predicting Spoilage and Shelf Life of Seafood

An exciting area for the use of the SSO concept is its ability to use mathematical models that quantitatively describe the growth of SSOs to predict the shelf life of seafood. Models for the growth of *Brochothrix thermosphacta*, *P. phosphoreum*, *Pseudomonas* spp., and *S. putrefaciens* have been successfully validated for shelf-life prediction of different aerobically stored and CO_2-packed fresh fish (Koutsoumanis and Nychas 2000; Dalgaard et al. 1997; Koutsoumanis 2001). In addition, stochastic models that take into account the distribution of spoilage bacteria on products and the storage temperature have been developed for shelf-life prediction of fresh aerobically stored fish (Giannakourou et al. 2001; Rasmussen et al. 2002). Several successfully validated models for the growth of SSOs have been included in application software, and this has facilitated prediction of seafood shelf life under constant and dynamic temperature storage conditions (Dalgaard et al. 2002). A simple model to predict interactions between groups of bacteria growing on seafood was also recently suggested (Gimenez and Dalgaard 2003). The construction of models to predict the development of microbial spoilage associations in new formulations of lightly preserved seafood remains an important challenge in the field of seafood microbiology.

11.4 Probiotics

Probiotics are living microorganisms, generally LAB, which, when ingested in sufficient quantities, exert a positive effect on health, such as regulation of transit (diarrhea or constipation), stimulation of the immune system, and reduction of the digestive inflammatory diseases. Most of the studies concerning probiotics for humans have been performed on LAB isolated from dairy products (Ebringer et al. 2008), probably because they have been ingested for thousands of years by humans through traditional products like yogurt, cheese, koumiss, kefir, etc. A few examples of probiotics available in the form of fermented milk products, dietary supplements, or medicinal products are strains of *Bifidobacterium lactis*, *B. animalis*, *Lactobacillus casei*, *L. acidophilus*, *L. rhamnosus*, *L. johnsonii*, *L. reuteri*, and *Enterococcus faecium*. The yeast *Saccharomyces boulardii* is also available as a commercial preparation on the probiotics market.

LPFP, which are the main category of marine products containing viable LAB, have never been considered as probiotics for humans, because they are not eaten in sufficient quantities. For this reason, incorporating new strains into seafood does not seem a realistic approach. However, the efficacy of some strains isolated from those products could be investigated to develop new dietary supplements. In case of strong evidence of beneficial effects for humans, evaluation as to whether those strains share the safety status of traditional food-grade organisms should be carefully assessed.

In the last several years, some work has been carried out on the use of LAB as probiotics for living fish. Many studies still remain at an in vitro level (Ringø 2008; Ma et al. 2009) and some promising strains did not confirm their effect in living fish (Spanggaard et al. 2000). However, some authors have demonstrated the in vivo potential of LAB, and Lauzon et al. (2008) have emphasized the importance and potential of LAB in aquaculture (Lauzon et al. 2008). A strain of *Carnobacterium* spp. isolated from the intestines of Atlantic salmon has been successfully implanted in salmonids, which reduced the diseases due to *Aeromonas salmonicida*, *Vibrio ordalii*, and *Yersinia ruckeri* (Robertson et al. 2000). *Lactobacillus fructivorans* isolated from the seabream intestinal microbiota significantly improved the survival of seabream larvae and fry, and stimulated the immune system (Picchietti et al. 2007). Merrifield et al. (2010) and Ringø et al. (2010) have reviewed the application of favorable LAB for salmonids as pre- and probiotics. Despite the successful proof of these concepts, it is still difficult to find a strategy applicable at the industrial level of farming (Merrifield et al. 2010; Ringø et al. 2010). The science of probiotics in aquaculture is in its infancy (Azad and Al-Marzouk 2008) and there is a real issue in developing feed with beneficial effects for fish.

12 Conclusions

The characteristics of fish flesh favor the growth of psychrotrophic Gram-negative bacteria that are much more competitive than LAB in this matrix. Change in consumers' habits has led to an increase of ready-to-eat and convenient food, a concept that includes both the easy-to-use aspect and an extended shelf life of the products. The nutritional aspects are also taken more and more into consideration by consumers who want natural products, with technological treatment and level of preservatives being as low as possible. LPFP, like VP or MAP fish fillets, carpaccio, cold-smoked fish, peeled and mildly cooked shrimp, etc., meet those requirements and their production has increased dramatically in the last several years. In those products, the environmental conditions favor the development LAB, explaining the high interest for this bacterial group in the last decade. Whether the presence of LAB in the final product is from fish origin or postcontamination during processing is not yet established and more detailed studies, as have been performed for *L. monocytogenes* in some fish industries, should be done in order to better understand and control the route of contamination. The role of LAB in marine products is

complex, depending on the fish species, treatment and storage conditions, bacterial species and strains, and interaction between the bacteria. Sometimes, LAB have no particular negative effect, but in certain cases, they are responsible for strong sensory degradation, leading to rejection of the products. The use of LAB in the fish industry is not extensively developed, except in Asia for the preparation of fish sauces and traditional food with a fermented mixture of fish and vegetables. In most cases, fermentation is due to LAB naturally present in the fish or in the carbohydrate-added sources (vegetables, garlic etc.), and no selected starter is added to control the fermentation. In the last several years, the bioprotective potential of endogenous LAB in relation to pathogens has been highlighted. Increasing numbers of studies are aiming to exploit this ability to control the quality and safety of marine products and some industries producing LAB starters are currently testing several bacteria for application in fish. However, this technology is still in its infancy compared to dairy products. The LAB have not been traditionally used in seafood for technological applications, so the strains now available have still not received the Qualified Presumption of Safety (QPS) status. Moreover, combining the bioprotection of seafood with no modification of the sensory characteristics of the product remains a challenge. An application of marine LAB as probiotics for humans does not seem realistic in the next few years, as many strains are psychrotrophic bacteria that do not support temperatures higher than 30 °C. Moreover, seafood products are not consumed in a high enough quantity to naturally observe a positive effect. However, some *Lactobacillus* and *Carnobacterium* strains that are easy to cultivate and resistant to various conditions could be studied. Their production on fish protein hydrolysates and ingestion as dietary supplements may combine the beneficial effects of fish and LAB. Finally, very encouraging results have been obtained with the use of marine LAB as probiotics for fish, and this is probably an approach to rapid development for the marine LAB market.

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References

Ababouch L (2006) Assuring fish safety and quality in international fish trade. Mar Pollut Bull 53(10):561–568

Amagliani G, Omiccioli E, Brandi G, Bruce IJ, Magnani M (2010) A multiplex magnetic capture hybridisation and multiplex real-time PCR protocol for pathogen detection in seafood. Food Microbiol 27(5):580–585

Aruoma OI (2006) The impact of food regulation on the food supply chain. Toxicology 221(1):119–127

Ash R (1980) Hydrolytic capacity of the trout (*Salmo gairdneri*) intestinal mucosa with respect to three specific dipeptides. Comp Biochem Physiol Part B: Comp Biochem 65(1):173–176

Austin B, Al-Zahrani AMJ (1988) The effect of antimicrobial compounds on the gastrointestinal microflora of rainbow trout, *Salmo gairdneri* Richardson. J Fish Biol 33(1):1–14

Azad IS, Ai-Marzouk A (2008) Autochthonous aquaculture probiotics—a critical analysis. Res J Biotechnol 3:171–177

Baya AM, Toranzo AE, Lupiani B, Li T, Roberson BS, Hetrick FM (1991) Biochemical and serological characterization of *Carnobacterium* spp. isolated from farmed and natural populations of striped bass and catfish. Appl Environ Microbiol 57(11):3114–3120

Bernadsky G, Rosenberg E (1992) Drag-reducing properties of bacteria from the skin mucus of the cornetfish (*Fistularia commersonii*). Microbial Ecol 24(1):63–76

Brock TD, Madigan MT (1991) Biology of microorganisms. Prentice Hall, Englewood Cliffs

Burr G, Gatlin D, Ricke S (2005) Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. J World Aqua Soc 36(4):425–436

Buzby JC, Roberts T, Jordan Lin C-T, MacDonald JM (1996) Bacterial foodborne disease: medical costs and productivity losses. Agricultural Economics Reports No. AER-741

Castell CH, Anderson GW (1948) Bacteria associated with spoilage of cod fillets. J Fish Board Canada 7(6):370–377

Chinivasagam HN, Bremner HA, Wood AF, Nottingham SM (1998) Volatile components associated with bacterial spoilage of tropical prawns. Int J Food Microbiol 42(1):45–55

Connil N, Plissoneau L, Onno B, Pilet M-F, Prevost H, Dousset X (2002) Growth of *Carnobacterium divergens* V41 and production of biogenic amines and divercin V41 in sterile cold-smoked salmon extract at varying temperatures, NaCl levels, and glucose concentrations. J Food Prot 65(2):333–338

Dainty RH, Mackey BM (1992) The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J Appl Microbiol 73:103s–114s

Dalgaard P (1995) Qualitative and quantitative characterization of spoilage bacteria from packed fish. Int J Food Microbiol 26(3):319–333

Dalgaard P, Mejlholm O, Huss HH (1997) Application of an iterative approach for development of a microbial model predicting the shelf-life of packed fish. Int J Food Microbiol 38(2): 169–179

Dalgaard P, Buch P, Silberg S (2002) Seafood spoilage predictor—development and distribution of a product specific application software. Int J Food Microbiol 73(2):343–349

Degnan AJ, Kaspar CW, Otwell WS, Tamplin ML, Luchansky JB (1994) Evaluation of lactic acid bacterium fermentation products and food-grade chemicals to control Listeria monocytogenes in blue crab (*Callinectes sapidus*) meat. Appl Environ Microbiol 60(9):3198–3203

DePaola A, Jones JL, Woods J, Burkhardt W III, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D, Nabe K (2010) Bacterial and viral pathogens in live oysters: 2007 United States market survey. Appl Environ Microbiol 76(9):2754–2768

Duffes F, Corre C, Leroi F, Dousset X, Boyaval P (1999a) Inhibition of *Listeria monocytogenes* by in situ produced and semipurified bacteriocins of *Carnobacterium* spp. on vacuum-packed, refrigerated cold-smoked salmon. J Food Prot 62:1394–1403

Duffes F, Leroi F, Boyaval P, Dousset X (1999b) Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4 °C. Int J Food Microbiol 47(1):33–42

Ebringer L, Ferenčík M, Krajčovič J (2008) Beneficial health effects of milk and fermented dairy products—review. Folia Microbiol 53(5):378–394

Einarsson H, Lauzon HL (1995) Biopreservation of brined shrimp (*Pandalus borealis*) by bacteriocins from lactic acid bacteria. Appl Environ Microbiol 61(2):669–676

Eldar A, Ghittino C, Asanta L, Bozzetta E, Goria M, Prearo M, Bercovier H (1996) *Enterococcus seriolicida* is a junior synonym of *Lactococcus garvieae*, a causative agent of septicemia and meningoencephalitis in fish. Cur Microbiol 32(2):85–88

Ennahar S, Sonomoto K, Ishizaki A (1999) Class IIa bacteriocins from lactic acid bacteria: antibacterial activity and food preservation. J Biosci Bioeng 87(6):705–716

Failler P, Van de Walle G, Lecrivain N, Himbes A, Lewins R (2007) Future prospects for fish and fishery products. 4. Fish consumption in the European Union in 2015 and 2030. Part 1. European overview. FAO Fisheries Circular, Rome

FAO (2010) Fisheries and Aquaculture Department. The State of World Fisheries and Aquaculture 2010. A report of the Food and Agriculture Organization of the United Nations. FAO, Rome

Feldhusen F (2000) The role of seafood in bacterial foodborne diseases. Microbes Infect 2(13): 1651–1660

Gennari M, Tomaselli S, Cotrona V (1999) The microflora of fresh and spoiled sardines (*Sardina pilchardus*) caught in Adriatic (Mediterranean) Sea and stored in ice. Food Microbiol 16(1):15–28

Giannakourou MC, Koutsoumanis K, Nychas GJE, Taoukis PS (2001) Development and assessment of an intelligent shelf life decision system for quality optimization of the food chill chain. J Food Prot 64(7):1051–1057

Giménez B, Dalgaard P (2003) Modelling and predicting the simultaneous growth of *Listeria monocytogenes* and spoilage micro-organisms in cold-smoked salmon. J Appl Microbiol 96(1):96–109

Givskov M, de Nys R, Manefield M, Gram L, Maximilien RIA, Eberl LEO, Molin S, Steinberg PD, Kjelleberg S (1996) Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. J Bacteriol 178(22):6618–6622

Gram L, Huss HH (1996) Microbiological spoilage of fish and fish products. Int J Food Microbiol 33(1):121–137

Gram L, Wedell-Neergaard C, Huss HH (1990) The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lates niloticus*). Int J Food Microbiol 10(3):303–316

Guldager HS, Boknaes N, Osterberg C, Nielsen J, Dalgaard P (1998) Thawed cod fillets spoil less rapidly than unfrozen fillets when stored under modified atmosphere at 2C. J Food Prot 61(9):1129–1136

Huber I, Spanggaard B, Appel FK, Rossen L, Nielsen T, Gram L (2004) Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (*Oncorhynchus mykiss*, Walbaum). J Appl Microbiol 96(1):117–132

Huss HH, Reilly A, Karim Ben Embarek P (2000) Prevention and control of hazards in seafood. Food Control 11(2):149–156

Iwamoto M, Ayers T, Mahon BE, Swerdlow DL (2010) Epidemiology of seafood-associated infections in the United States. Clin Microbiol Rev 23(2):399–411

Jöborn A, Dorsch M, Olsson JC, Westerdahl A, Kjelleberg S (1999) *Carnobacterium inhibens* sp. nov., isolated from the intestine of Atlantic salmon (*Salmo salar*). Int J Syst Evol Microbiol 49(4):1891–1898

Joerger MC, Klaenhammer TR (1990) Cloning, expression, and nucleotide sequence of the *Lactobacillus helveticus* 481 gene encoding the bacteriocin helveticin J. J Bacteriol 172(11):6339–6347

Joffraud J-J, Leroi F, Roy C, Berdague JL (2001) Characterisation of volatile compounds produced by bacteria isolated from the spoilage flora of cold-smoked salmon. Int J Food Microbiol 66(3):175–184

Jørgensen LV, Dalgaard P, Huss HH (2000a) Multiple compound quality index for cold-smoked salmon (*Salmo salar*) developed by multivariate regression of biogenic amines and pH. J Agric Food Chem 48(6):2448–2453

Jørgensen LV, Huss HH, Dalgaard P (2000b) The effect of biogenic amine production by single bacterial cultures and metabiosis on cold-smoked salmon. J Appl Microbiol 89(6):920–934

Kelly WJ, Asmundson RV, Huang CM (1996) Isolation and characterization of bacteriocin-producing lactic acid bacteria from ready-to-eat food products. Int J Food Microbiol 33(2):209–218

Kjelleberg S, Steinberg P, Givskov MC, Gram L, Manefield M, De Nys R (1997) Do marine natural products interfere with prokaryotic AHL regulatory systems? Aquat Microbial Ecol 13: 85–93

Koutsoumanis K (2001) Predictive modeling of the shelf life of fish under nonisothermal conditions. Appl Environ Microbiol 67(4):1821–1829

Koutsoumanis K, Nychas G-JE (1999) Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7, and 10 C. Appl Environ Microbiol 65(2):698–706

Koutsoumanis K, Nychas G-JE (2000) Application of a systematic experimental procedure to develop a microbial model for rapid fish shelf life predictions. Int J Food Microbiol 60(2):171–184

Kumar R, Surendran PK, Thampuran N (2008) Evaluation of culture, ELISA and PCR assays for the detection of *Salmonella* in seafood. Lett Appl Microbiol 46(2):221–226

Laurenti E (2007) Fish and fishery products: world apparent consumption statistics based on food balance sheets (1961–1995), vol 821. FAO, Rome

Lauzon HL, Gudmundsdottir S, Pedersen MH, Budde BB, Gudmundsdottir BK (2008) Isolation of putative probionts from cod rearing environment. Vet Microbiol 132(3):328–339

Lerke P, Farber L, Adams R (1967) Bacteriology of spoilage of fish muscle IV. Role of protein. Appl Microbiol 15(4):770–776

Leroi F, Joffraud J-J, Chevalier F, Cardinal M (2001) Research of quality indices for cold-smoked salmon using a stepwise multiple regression of microbiological counts and physico-chemical parameters. J Appl Microbiol 90(4):578–587

Liston J (1980) Microbiology in fishery science. In: Advances in fish science and technology: papers presented at the Jubilee conference of the Torry Research Station, Aberdeen, 23–27 July 1979, pp 138–157

Liu JY, Li AH, Ji C, Yang WM (2009) First description of a novel *Weissella* species as an opportunistic pathogen for rainbow trout *Oncorhynchus mykiss* (Walbaum) in China. Vet Microbiol 136(3–4):314–320

Ma C-W, Cho Y-S, Oh K-H (2009) Removal of pathogenic bacteria and nitrogens by *Lactobacillus* spp. JK-8 and JK-11. Aquaculture 287(3–4):266–270

Martinez-Urtaza J, Liebana E (2005) Use of pulsed-field gel electrophoresis to characterize the genetic diversity and clonal persistence of *Salmonella senftenberg* in mussel processing facilities. Int J Food Microbiol 105(2):153–163

Matamoros S, Leroi F, Cardinal M, Gigout F, Kasbi Chadli F, Cornet J, Prevost H, Pilet MF (2009) Psychrotrophic lactic acid bacteria used to improve the safety and quality of vacuum-packaged cooked and peeled tropical shrimp and cold-smoked salmon. J Food Prot 72(2):365–374

McKean JD (2001) The importance of traceability for public health and consumer protection. Revue Scientifique et Technique (Int Off Epizoot) 20(2):363

Mejlholm O, Dalgaard P (2002) Antimicrobial effect of essential oils on the seafood spoilage micro-organism *Photobacterium phosphoreum* in liquid media and fish products. Lett Appl Microbiol 34(1):27–31

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker R, Bøgwald J, Castex M, Ringø E (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302(1):1–18

Minami A, Chaicumpa W, Chongsa-Nguan M, Samosornsuk S, Monden S, Takeshi K, Makino S-i, Kawamoto K (2010) Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control 21(3):221–226

Mudarris M, Austin B (1988) Quantitative and qualitative studies of the bacterial microflora of turbot, *Scophthalmus maximus* L., gills. J Fish Biol 32(2):223–229

Munro PO, Barbour A, Birkbeck TH (1994) Comparison of the gut bacterial flora of start-feeding larval turbot reared under different conditions. J Appl Microbiol 77(5):560–566

Nes IF, Diep DB, Håvarstein LS, Brurberg MB, Eijsink V, Holo H (1996) Biosynthesis of bacteriocins in lactic acid bacteria. Antonie Van Leeuwenhoek 70(2–4):113–128

Nilsson L, Huss HH, Gram L (1997) Inhibition of *Listeria monocytogenes* on cold-smoked salmon by nisin and carbon dioxide atmosphere. Int J Food Microbiol 38(2–3):217–227

Nilsson L, Gram L, Huss HH (1999) Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora. J Food Prot 62(4):336–342

Norhana MNW, Poole SE, Deeth HC, Dykes GA (2010) Prevalence, persistence and control of *Salmonella* and *Listeria* in shrimp and shrimp products: a review. Food Control 21(4): 343–361

Nychas G-JE, Drosinos EH, Board RG (1998) Chemical changes in stored meat. In: Davies AR, Board RG (eds) Microbiology of meat and poultry. Springer, New York, pp 288–327

Olafsdottir G, Martinsdóttir E, Oehlenschläger J, Dalgaard P, Jensen B, Undeland I, Mackie IM, Henehan G, Nielsen J, Nilsen H (1997) Methods to evaluate fish freshness in research and industry. Trends Food Sci Technol 8(8):258–265

Picchietti S, Mazzini M, Taddei AR, Renna R, Fausto AM, Mulero V, Carnevali O, Cresci A, Abelli L (2007) Effects of administration of probiotic strains on GALT of larval gilthead seabream: immunohistochemical and ultrastructural studies. Fish Shellfish Immunol 22(1):57–67

Ponce E, Khan AA, Cheng C-M, Summage-West C, Cerniglia CE (2008) Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiol 25(1):29–35

Rasmussen SKJ, Ross T, Olley J, McMeekin T (2002) A process risk model for the shelf life of Atlantic salmon fillets. Int J Food Microbiol 73(1):47–60

Ringø E (1993) The effect of chromic oxide (Cr_2O_3) on aerobic bacterial populations associated with the intestinal epithelial mucosa of Arctic charr, *Salvelinus alpinus* (L.). Can J Microbiol 39(12):1169–1173

Ringø E (2008) The ability of carnobacteria isolated from fish intestine to inhibit growth of fish pathogenic bacteria: a screening study. Aqua Res 39(2):171–180

Ringo E, Birkbeck TH (1999) Intestinal microflora of fish larvae and fry. Aqua Res 30(2):73–93

Ringø E, Gatesoupe FJ (1998) Lactic acid bacteria in fish: a review. Aquaculture 160(3):177–203

Ringø E, Strøm E (1994) Microflora of Arctic charr, *Salvelinus alpinus* (L.): gastrointestinal microflora of free-living fish and effect of diet and salinity on intestinal microflora. Aqua Res 25(6):623–629

Ringø E, Strøm E, Tabachek J-A (1995) Intestinal microflora of salmonids: a review. Aqua Res 26(10):773–789

Ringø E, Birkbeck TH, Munro PO, Vadstein O, Hjelmeland K (1996) The effect of early exposure to *Vibrio pelagius* on the aerobic bacterial flora of turbot, *Scophthalmus maximus* (L.) larvae. J Appl Microbiol 81(2):207–211

Ringø E, Olsen RE, Øverli Ø, Løvik F (1997) Effect of dominance hierarchy formation on aerobic microbiota associated with epithelial mucosa of subordinate and dominant individuals of Arctic charr, *Salvelinus alpinus* (L.). Aqua Res 28(11):901–904

Ringø E, Wesmajervi MS, Bendiksen HR, Berg A, Olsen RE, Johnsen T, Mikkelsen H, Seppola M, Strøm E, Holzapfel W (2001) Identification and characterization of carnobacteria isolated from fish intestine. Syst Appl Microbiol 24(2):183–191

Ringø E, Løvmo L, Kristiansen M, Bakken Y, Salinas I, Myklebust R, Olsen RE, Mayhew TM (2010) Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: a review. Aqua Res 41(4):451–467

Robertson PAW, O'Dowd C, Burrells C, Williams P, Austin B (2000) Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). Aquaculture 185(3–4):235–243

Rombout JHWM, Stroband HWJ, Taverne-Thiele JJ (1984) Proliferation and differentiation of intestinal epithelial cells during development of *Barbus conchonius* (Teleostei, Cyprinidae). Cell Tissue Res 236(1):207–216

Schrøder K, Clausen E, Sandberg AM, Raa J (1980) Psychrotrophic *Lactobacillus plantarum* from fish and its ability to produce antibiotic substances. Adv Fish Sci Technol 148:480–483

Seppola M, Olsen RE, Sandaker E, Kanapathippillai P, Holzapfel W, Ringø E (2006) Random amplification of polymorphic DNA (RAPD) typing of carnobacteria isolated from hindgut chamber and large intestine of Atlantic cod (*Gadus morhua* L.). Syst Appl Microbiol 29(2):131–137

Shabarinath S, Sanath Kumar H, Khushiramani R, Karunasagar I, Karunasagar I (2007) Detection and characterization of *Salmonella* associated with tropical seafood. Int J Food Microbiol 114(2):227–233

Shewan JM (1971) The microbiology of fish and fishery products—a progress report. J Appl Microbiol 34(2):299–315

Spanggaard B, Huber I, Nielsen J, Nielsen T, Appel KF, Gram L (2000) The microflora of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture 182(1): 1–15

Strøm E, Olafsen JA (1990) The indigenous microflora of wild-captured juvenile cod in net-pen rearing. In: Lesel R (ed) Microbiology of poecilotherms. Elsevier, Amsterdam, pp 181–185

Suyehiro Y, Kaigi GK (1941) A study on the digestive system and feeding habits of fish. National Research Council of Japan, Tokyo

Twomey D, Ross RP, Ryan M, Meaney B, Hill C (2002) Lantibiotics produced by lactic acid bacteria: structure, function and applications. Antonie Van Leeuwenhoek 82(1–4):165–185

Wallbanks S, Martinez-Murcia AJ, Fryer JL, Phillips BA, Collins MD (1990) 16S rRNA sequence determination for members of the genus *Carnobacterium* and related lactic acid bacteria and description of *Vagococcus salmoninarum* sp. nov. Int J Syst Bacteriol 40(3):224–230

Williams AM, Fryer JL, Collins MD (1990) *Lactococcus piscium* sp. nov. a new *Lactococcus* species from salmonid fish. FEMS Microbiol Lett 68(1–2):109–113

Wilson B, Danilowicz BS, Meijer WG (2008) The diversity of bacterial communities associated with Atlantic cod *Gadus morhua*. Microbial Ecol 55(3):425–434

Yang G, Bao B, Peatman E, Li H, Huang L, Ren D (2007) Analysis of the composition of the bacterial community in puffer fish *Takifugu obscurus*. Aquaculture 262(2–4):183–191

Chapter 17
Chitin and Its Beneficial Activity as an Immunomodulator in Allergic Reactions

Thanh-Sang Vo and Se-Kwon Kim

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1 Introduction

Chitin is a linear polysaccharide consisting of β-(1-4)-N-acetyl D-glucosamine residues. It is widely distributed in nature and is the second most abundant polysaccharide in nature after cellulose. It may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamino group. Chitin is a white, hard, inelastic,

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nitrogenous polysaccharide found in the cell walls of bacteria and fungi, mushrooms, exoskeletons of crustaceans and insects, the microfilarial sheath of parasitic nematodes, and the lining of the digestive tracts of many insects. These organisms use chitin to protect the invader from the harsh conditions inside the animal or plant host (Elias et al. 2005). It can be degraded by chitinase. Chitin is highly hydrophobic and it is insoluble in water and most organic solvents. It exists mainly in two forms: α-chitin and β-chitin. α-chitin consists of sheets of tightly packed alternating parallel and antiparallel chains (Minke and Blackwell 1978). Meanwhile, β-chitin is arranged in parallel (Gardner and Blackwell 1975), which occurs less frequently in nature than α-chitin. Being non-toxic and environmentally safe, chitin has become of great interest not only as a utilized resource, but also as a new functional biomaterial of high potential in many fields, such as medical, agricultural, and cosmetic applications. It is readily obtained for commercial use from crustacean shell waste products generated by the seafood industry (Kurita 2006; Shahidi and Synowiecki 1991; Ravi Kumar 2000; Pillai et al. 2009). Chitosan, a partially deacetylated polymer of N-acetylglucosamine, is produced commercially by the deacetylation of chitin (Aranaz et al. 2009; Dutta et al. 2004). During the past several decades, chitosan has received considerable attention due to its biodegradable, non-toxic, and non-allergenic properties, which made it possible to be used in many fields, including food, cosmetics, biomedicine, agriculture, and environmental protection (Shahidi et al. 1999; Kim and Rajapaksea 2005). Recent studies have focused on the conversion of chitosan to chitooligosaccharides (COS), since COS are not only water-soluble (Yang et al. 2010) and possess higher oral absorption (Chae et al. 2005), but they also have various biological effects, including antimicrobial, antitumor, anticancer, antioxidant, anti-inflammatory, and anti-angiotensin I-converting enzyme activities (Kim and Rajapaksea 2005; Park and Kim 2010; Xia et al. 2011). Especially, chitin and its derivatives have been determined to be therapeutic agents against allergic diseases (Catalli and Kulka 2010).

2 Source of Chitin

2.1 Shellfish Waste

Crustaceans are known to be the major source of chitin for industrial processing. It is estimated that about 600 and 1,600 million tons of this polysaccharide is synthesized annually from freshwater and marine ecosystems (Cauchie 1997). Shellfish (including shrimp, crab, lobster, and krill), oyster, and squid are the best sources of chitin, with quantities of about 29.9, 1.4, and 0.7 million tons per year (Synowiecki and Al-Khateeb 2000). The crustacean shell consists of 13–42 % chitin, along with 30–40 % protein and 30–50 % mineral salts (Ikeda et al. 1996). Among crustacean shell wastes, crab and shrimp offal are the most exploited sources of chitin.

The shell discards from crab (*Cancer magister*) and Pacific shrimp (*Pandalus borealis*) are available in the USA in amounts of up to 39,000 t annually (Knorr 1991). Another promising source of chitin is the Antarctic krill (*Euphausia superba*). It has been estimated that about 100 million tons of this crustacean are obtained annually (Kołakowski et al. 2000). With this amount of krill, a potential source of chitin can be achieved. The clams, oyster shells, and marine diatoms can also be considered as an attractive source of chitin. However, the low availability of these organisms limits its possible utilization.

2.2 Microbial Sources

Various microbial species are also suitable for chitin isolation, such as *Allomyces*, *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, *Rhizopus*, *Choanephora*, *Thamnidium*, *Zygorhynchus*, and *Phycomyces* (Muzzarelli et al. 1994). The development of chitin and chitosan utilization from fungal cell walls is advantageous, as they are readily available. All groups of these microorganisms possess a high growth rate. The cost for the cultivation of fungi is low by using cellulose-containing by-products from the paper and food industries. Moreover, the productivity of chitin from fungal sources can be effectively adjusted through the control of fermentation and processing conditions, and through beneficial genetic manipulation.

3 Production of Chitin

3.1 Chemical Method

The production of chitin is involved in deproteinization and demineralization of the crustacean shells. Shell wastes are deproteinized using aqueous sodium or potassium hydroxide solutions. The effectiveness of alkali deproteinization depends on the temperature of processing, base concentration, and the ratio of its solution to the shells. Crustacean shell wastes are usually treated with dilute sodium hydroxide solution at concentrations ranging from 1 % to 10 % (w/v), an elevated temperature (65–100 °C), and reaction time from 0.5 to 6 h.

The removal of calcium carbonate and calcium phosphate is accomplished by dissolution with dilute acids. Hydrochloric acid is used most commonly (1–8 %) in 1–3 h of extraction at room temperature (Hackman and Goldberg 1965; No et al. 1989). The demineralization of the shells using acetic acid or sulfuric acid has also been reported (No and Meyers 1997). The use of EDTA for decalcification of the shells can protect chitin chains from hydrolysis and deacetylation (Austin et al. 1981; Roberts 1997).

3.2 Enzymatic Methods

Proteases can be used for the deproteinization of crustacean shells for the production of chitin. Bacterial protease from *Pseudomonas maltophilia* was used in a culture medium with crustacean shells, and it was observed that, after 24 h, the protein content remaining in the shells was only about 1 % (Shimahara and Takiguchi 1988). Moreover, proteolytic enzymes were also used to deproteinize crustaceans, such as tuna proteinase (pH 8.6 and 37.5 °C), papain (pH 5.6–6.0 and 37.5 °C), and bacterial proteinase (pH 7 and 60 °C), for over 60 h (Takeda and Abe 1962; Takeda and Katsuura 1964; Broussignac 1968). After treatment with the enzymes, the amount of protein still associated with chitin was about 5 %. The use of papain, pepsin, or trypsin was recommended for the deproteinization of crustacean shells during chitin preparation (Broussignac 1968).

4 Chitin Receptor

Chitin is able to modulate the innate immune system by binding to its receptors, such as mannose receptor, toll-like receptor 2, or Dectin-1. These receptors have been identified to be expressed on cells of the innate immune system, such as neutrophils, macrophages, and dendritic cells, which play an important role in modulating and maintaining inflammatory immune responses (Catalli and Kulka 2010; Mogensen 2009; Rasmussen et al. 2009). According to Shibata et al. (1997b), chitin has been shown to bind to mannose receptors on mouse spleen-derived macrophages, which facilitates their uptake via phagocytosis. Moreover, Da Silva and colleagues have reported that smaller chitin fragments (<40 μm) preferentially bind to Dectin-1, while larger particles (40–70 μm) activate TLR2 receptors on mouse peritoneal macrophages (Da Silva et al. 2009).

5 The Modulatory Effect of Chitin on Allergic Responses

5.1 Allergic Responses

Allergic diseases are one of the major public health problems in the developed world. It was estimated that approximately one-third of the general population and one-fifth of the population in Western countries were affected by allergic diseases (Ono 2000; Ouwehand 2007). Especially, allergic rhinitis, asthma, and atopic eczema are among the most common causes of chronic ill health. The prevalence, severity, and complexity of these allergic diseases are rapidly rising and considerably adding to the burden of healthcare costs (Kay 2000). Therefore, knowledge about the pathophysiology of allergic diseases has increased, offering new opportunities for therapeutic intervention. Substantially, allergy is caused by an

exaggerated reaction of the immune system to harmless environmental substances, such as animal dander, house dust mites, foods, pollen, insects, and chemical agents (Arshad 2010; Milián and Díaz 2004). The initial event responsible for the development of allergic diseases is the generation of allergen-specific $CD4^+$ Th2 cells. Once generated, effector Th2 cells produce IL-4, IL-5, IL-9, and IL-13, which cause the production of allergen-specific IgE by B cells (Akdis et al. 2005). Subsequently, allergic reactions are induced upon the binding of allergen to IgE, which is tethered to the high-affinity IgE receptor on the surface of mast cells and basophils. Following the aggregation of cell-surface receptors is a cascade of intracellular events, including the increase of the intracellular Ca^{2+} level, the release of preformed inflammatory mediators from secretory granules such as histamine and β-hexosaminidase, and the generation and secretion of the newly synthesized substances such as leukotrienes, prostaglandins, and cytokines. These mediators cause allergic inflammatory responses due to airway constriction, mucous production, and the recruitment of inflammatory cells (Galli et al. 2008). According to this mechanism, the control of Th2-type cytokine expression and IgE levels are especially important for the regulation of type I allergic reaction, thus allergic diseases may be managed.

5.2 Chitin Modulates Th2 and Th1 Immunity

As is known, Th1 cells are characterized by the prevalent production of IL-12 and IFN-γ, while Th-2 cells are characterized by the prevalent production of IL-4, IL-5, IL-9, and IL-13 (Romagnani 2004). Evidently, Th2 cytokines play a crucial role in allergic inflammatory responses. Thus, immune modulation due to the suppression of Th2 responses has been proposed as a promising concept for the treatment of allergic diseases (Nguyen and Casale 2011; Araujo et al. 2010). Especially, type I cytokines are produced by innate immune cells and it has been determined that they downregulate type II allergic immune responses (Shibata et al. 2000). Notably, chitin has been evidenced as a potent innate immune stimulator of macrophages and other innate immune cells, and, thus, chitin is able to suppress allergen-induced type II allergic responses. Indeed, Shibata and colleagues have determined the immunological effects of chitin in vivo and in vitro using phagocytosable, small-sized chitin particles. It has shown that the intravenous administration of fractionated chitin particles into the lung activated alveolar macrophages to express cytokines such as IL-12, tumor necrosis factor (TNF)-α, and IL-18, leading to INF-γ production, mainly by NK cells (Shibata et al. 1997a). The production of cytokines induced by chitin is identified to be mediated by a mannose receptor (Shibata et al. 1997b). In another study, Lee and colleagues determined that chitin stimulates macrophages by interacting with different cell surface receptors such as macrophage mannose receptor, toll-like receptor 2 (TLR-2), C-type lectin receptor Dectin-1, and leukotriene 134 receptor (BLT1) (Lee 2009). These studies have shown the direct interactions between chitin and its cell surface receptors and, thus, chitin regulates the specific signaling pathways in immune responses.

In the further study of Shibata and colleagues, the suppressive effect of Th2 responses has been confirmed when chitin was given orally in BALB/c and C57BL/6 mice (Shibata et al. 2000). It was observed that chitin treatment resulted in decreases of serum IgE levels and lung eosinophil numbers in both strains. The inhibitory mechanisms of Th2 responses by chitin was found to be due to decreases of Th2 cytokines, including IL-4, IL-5, and IL-10 levels, and the production of Th1 cytokine IFN-γ in spleen cells isolated from the ragweed-immunized mice. These results indicated that the immune responses were redirected toward a Th1 response by chitin treatment, and, thus, downregulating Th2-facilitated IgE production and lung eosinophilia in the allergic mouse. Moreover, the Th1 adjuvant role of chitin has been determined via upregulating Th1 immunity induced by heat-killed *Mycobacterium bovis* and downregulating Th2 immunity induced by mycobacterial protein (Shibata et al. 2001). Likewise, Hamajima et al. (2003) also reported the Th1 adjuvant effect of chitin microparticles in inducing viral-specific immunity.

Notably, the effectiveness of chitin microparticles when given intranasally as a treatment for the symptoms of respiratory allergy and allergy asthma has been tested in two different mouse models of allergy, namely, *Dermatophagoids pteronyssinus* and *Aspergillus fumigatus* (Strong et al. 2002). The intranasal application of microgram doses of chitin microparticles substantially reduced the allergen-induced serum IgE levels, peripheral eosinophilia, airway hyperresponsiveness, and lung inflammation in both allergy models. This effectiveness was found to be due to the increase in Th1 cytokines IL-12, IFN-γ, and TNF-α and decrease in IL-4 production during allergen challenge. The immunostimulatory properties of chitin microparticles could offer a novel and natural approach to treating allergic disease in humans.

6 Conclusion

Numerous assessments have concerned biological activities and the application of chitin. In this regard, chitin has been shown to be an immunomodulator of allergic reaction via increase in Th1 cytokine production and decrease in Th2 cytokine production, and, thus, suppressing the generation of serum IgE in vivo. Accordingly, chitin is considered a potential agent in the control of allergic diseases. The development of new technologies for the utilization of chitin as antiallergic agents will play a significant role in the treatment of allergic diseases in the future.

References

Akdis M, Blaser K, Akdis CA (2005) T regulatory cells in allergy: novel concepts in the pathogenesis, prevention, and treatment of allergic diseases. J Allergy Clin Immunol 116:961–968

Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B, Acosta N, Galed G, Heras Á (2009) Functional characterization of chitin and chitosan. Curr Chem Biol 3:203–230

Araujo MI, Campos RA, Cardoso LS, Oliveira SC, Carvalho EM (2010) Immunomodulation of the allergic inflammatory response: new developments. Inflamm Allergy Drug Targets 9:73–82

Arshad SH (2010) Does exposure to indoor allergens contribute to the development of asthma and allergy? Curr Allergy Asthma Rep 10:49–55

Austin PR, Brine CJ, Castle JE, Zikakis JP (1981) Chitin: new facets of research. Science 212:749–753

Broussignac P (1968) Chitosan, a natural polymer not well known by the industry. Chim Ind Genie Chim 99:1241–1247

Catalli A, Kulka M (2010) Chitin and β-glucan polysaccharides as immunomodulators of airway inflammation and atopic disease. Recent Patents Endocr Metab Immune Drug Discov 4:175–189

Cauchie HM (1997) An attempt to estimate crustacean chitin production in the hydrosphere. In: Domard A, Roberts GAF, Varum KM (eds) Advances in chitin science. Jacques André Publisher, Lyon, pp 32–39

Chae SY, Jang MK, Nah JW (2005) Influence of molecular weight on oral absorption of water soluble chitosans. J Control Release 102:383–394

Da Silva CA, Chalouni C, Williams A, Hartl D, Lee CG, Elias JA (2009) Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. J Immunol 182:3573–3582

Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. J Sci Ind Res 63:20–31

Elias JA, Homer RJ, Hamid Q, Lee CG (2005) Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. J Allergy Clin Immunol 116:497–500

Galli SJ, Tsai M, Piliponsky AM (2008) The development of allergic inflammation. Nature 454:445–454

Gardner KH, Blackwell J (1975) Refinement of the structure of beta-chitin. Biopolymers 14:1581–1595

Hackman RH, Goldberg M (1965) Studies on chitin. VI. Nature of α- and β-chitins. Aust J Biol Sci 18:941–965

Hamajima K, Kojima Y, Matsui K, Toda Y, Jounai N, Ozaki T, Xin K-Q, Strong P, Okuda K (2003) Chitin micro-particles (CMP): a useful adjuvant for inducing viral specific immunity when delivered intranasally with an HIV-DNA vaccine. Viral Immunol 16:541–547

Ikeda I, Toyoda H, Matsuda Y, Ouchi S (1996) A molecular strategy for biological control of powdery mildew pathogens by a chitinase gene chiSH1 cloned from a Gram-positive bacterium *Kurthia zopfii*. In: Muzzarelli RAA (ed) Chitin enzymology. Atec Edizioni, Grottammare, pp 349–358

Kay AB (2000) Overview of 'allergy and allergic diseases: with a view to the future'. Br Med Bull 56:843–864

Kim S-K, Rajapakse N (2005) Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review. Carbohydr Polym 62:357–368

Knorr D (1991) Recovery and utilization of chitin and chitosan in food processing waste management. Food Technol 45:114–122

Kołakowski E, Haard NF, Simpson BK (2000) Endogenous enzymes in Antarctic krill: control of activity during storage and utilization. In: Sikorski ZE (ed) Seafood enzymes. Marcel Dekker, New York, pp 505–529

Kurita K (2006) Chitin and chitosan: functional biopolymers from marine crustaceans. Marine Biotechnol 8:203–226

Lee CG (2009) Chitin, chitinases and chitinase-like proteins in allergic inflammation and tissue remodeling. Yonsei Med J 50:22–30

Milián E, Díaz AM (2004) Allergy to house dust mites and asthma. P R Health Sci J 23:47–57

Minke R, Blackwell J (1978) The structure of alpha-chitin. J Mol Biol 120:167–181

Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22:240–273

Muzzarelli RAA, Ilari P, Tarsi R, Dubini B, Xia W (1994) Chitosan from *Absidia coerulea*. Carbohydr Polym 25:45–50

Nguyen THT, Casale TB (2011) Immune modulation for treatment of allergic disease. Immunol Rev 242:258–271

No HK, Meyers SP (1997) Preparation of chitin and chitosan. In: Muzzarelli RAA, Peter MG (eds) Chitin handbook. European Chitin Society, Atec, Grottammare, pp 475–489

No HK, Meyers SP, Lee KS (1989) Isolation and characterization of chitin from crawfish shell waste. J Agric Food Chem 37:138–144

Ono SJ (2000) Molecular genetics of allergic diseases. Annu Rev Immunol 18:347–366

Ouwehand AC (2007) Antiallergic effects of probiotics. J Nutr 137:794S–797S

Park BK, Kim MM (2010) Applications of chitin and its derivatives in biological medicine. Int J Mol Sci 11:5152–5164

Pillai CKS, Paul W, Sharma CP (2009) Chitin and chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci 34:641–678

Rasmussen SB, Reinert LS, Paludan SR (2009) Innate recognition of intracellular pathogens: detection and activation of the first line of defense. APMIS 117:323–337

Ravi Kumar MNV (2000) A review of chitin and chitosan applications. React Funct Polym 46:1–27

Roberts GAF (1997) Chitosan production routes and their role in determining the structure and properties of the product. In: Domard A, Roberts GAF, Varum KM (eds) Advances in chitin science. Jacques André Publisher, Lyon, pp 22–31

Romagnani S (2004) Immunologic influences on allergy and the TH1/TH2 balance. J Allergy Clin Immunol 113:395–400

Shahidi F, Synowiecki J (1991) Isolation and characterization of nutrients and value-added products from snow crab (*Chionoecetes opilio*) and shrimp (*Pandalus borealis*) processing discards. J Agric Food Chem 39:1527–1532

Shahidi F, Arachchi JKV, Jeon Y-J (1999) Food applications of chitin and chitosans. Trends Food Sci Technol 10:37–51

Shibata Y, Foster LA, Metzger WJ, Myrvik QN (1997a) Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. Infect Immun 65:1734–1741

Shibata Y, Metzger WJ, Myrvik QN (1997b) Chitin particle-induced cell-mediated immunity is inhibited by soluble mannan: mannose receptor-mediated phagocytosis initiates IL-12 production. J Immunol 159:2462–2467

Shibata Y, Foster LA, Bradfield JF, Myrvik QN (2000) Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. J Immunol 164:1314–1321

Shibata Y, Honda I, Justice JP, Van Scott MR, Nakamura RM, Myrvik QN (2001) Th1 adjuvant N-acetyl-D-glucosamine polymer up-regulates Th1 immunity but down-regulates Th2 immunity against a mycobacterial protein (MPB-59) in interleukin-10-knockout and wild-type mice. Infect Immun 69:6123–6130

Shimahara K, Takiguchi Y (1988) Preparation of crustacean chitin. Methods Enzymol 161:417–423

Strong P, Clark H, Reid K (2002) Intranasal application of chitin microparticles down-regulates symptoms of allergic hypersensitivity to *Dermatophagoides pteronyssinus* and *Aspergillus fumigatus* in murine models of allergy. Clin Exp Allergy 32:1794–1800

Synowiecki J, Al-Khateeb NAAQ (2000) The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp *Crangon crangon* processing discards. Food Chem 68:147–152

Takeda M, Abe E (1962) Isolation of crustacean chitin. Decalcification by disodium ethylenediaminetetraacetate and enzymic hydrolysis of incidental proteins. Norisho Suisan Koshusho Kenkyu Hokohu 11:339–406

Takeda M, Katsuura H (1964) Purification of king crab chitin. Suisan Daigaku Kenkyu Hokoku 13:109–116
Xia W, Liu P, Zhang J, Chen J (2011) Biological activities of chitosan and chitooligosaccharides. Food Hydrocoll 25:170–179
Yang EJ, Kim JG, Kim JY, Kim SC, Lee NH, Hyun CG (2010) Anti-inflammatory effect of chitosan oligosaccharides in RAW 264.7 cells. Cent Eur J Biol 5:95–102

Chapter 18
Chitosan Nanoparticles: Preparation, Characterization, and Applications

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1 Introduction

Chitosan has a wide range of applications, as it is economical and easily available. Especially, chitosan has many medical applications, including accelerated wound healing by wound dressing (Dutta et al. 2004) and as effective drug delivery systems (Bansal et al. 2011). It is used in agriculture for fertilizer production (Corradini et al. 2010) and is often used in food processing as an ingredient, food-coating material (Değírmencíoğlu et al. 2009). It is also promoted in the United States and some other countries as a dietary fiber to reduce fat absorption (Anonymous 2010). Chitosan has recently been used in the cosmetics industry as a skin moisturizer (Dutta et al. 2004). The derivatives of chitosan, produced by modifying its basic structure to obtain polymers with a wide range of properties (Bansal et al. 2011), are the key for the versatility of applications. Modern technological advancement has lead towards the development of chitosan microparticles followed by chitosan nanoparticles, ensuring the effective use of chitosan in various industries.

This chapter focuses on chitosan nanoparticles to identify their preparation methods, physicochemical characteristics, applications, and potential risks.

2 Sources and Availability of Chitosan

Over the past two decades, the interest in chitosan has increased, as it appeared to be an excellent solution for many challenges faced by the commercial industries across the globe. The unique physical and chemical characteristics of chitosan opened the path to a wide array of avenues, which lead this remarkable material to be used in different products and applications, ranging from pharmaceutical and cosmetic products to water treatment and plant protection (Dutta et al. 2004). Chitosan is a cationic polysaccharide obtained by partial deacetylation of chitin, the major component of crustacean shells (López-León et al. 2005). It is a linear polymer of α (1-4)-linked 2-amino-2-deoxy-β-D-glucopyranose, derived by N-deacetylation (Dutta et al. 2004). In general, when the nitrogen content is more than 7 % and the degree of deacetylation reaches over 60 %, the term “chitosan” is used instead of chitin (Peter et al. 1986). However, the degree of deacetylation is varied depending upon the purpose of use and 66–95 % deacetylated chitosan is available commercially, with an average molecular weight ranging from 3,800 to 20,000 Daltons (Bansal et al. 2011). If the chemical composition of the chitosan taken into account, it is clear that chitosan is not a single chemical entity, but, instead, varies in composition depending on the source and method of preparation, and also on the physiological conditions (Bansal et al. 2011). In fact, the remarkable properties of chitosan, such as biodegradability, biocompatibility, biorenewability, non-toxicity, and antimicrobial activity (VandeVord et al. 2001; Chung et al. 2004; Keong and Halim 2009; Lim et al. 2010), along with modern technological advancements, gave rise to the extensive applications in commercial industries during the recent past.

After cellulose, chitin is the second most ubiquitous natural polysaccharide on this planet (Dutta et al. 2004). Chitin, as well as chitosan, can be found as supporting materials in many aquatic organisms, terrestrial organisms, and some microorganisms (Tokura and Tamura 2007). Commercially, chitins and chitosans are produced from biowastes generated from the processing of marine organisms, such as shrimps, crabs, lobsters, krill, and squid (Teng et al. 2001; Nwe et al. 2010). The industrial-scale production of chitosan from biowaste from aquatic organisms has encountered limitations due to the seasonal and variable supply of raw materials, as well as variability and difficulties in the processing conditions (Crestini et al. 1996; Nwe and Stevens 2008). Therefore, the potential use of terrestrial organisms like insects, terrestrial crustaceans, and mushrooms for the production of chitosan has been discussed as an alternative source (Brück et al. 2010).

Among the terrestrial organisms, silkworms and honeybees have been given a major focus, as the waste materials of these two industries appeared to be a good source for the large-scale production of chitin and chitosan (Nwe et al. 2010). In the microbial world, both chitin and chitosan can be found in molds, yeasts, some ciliates, chrysophyte algae, and in several bacteria, including the spores of streptomycete and the stalks of some prosthecate bacteria (Gooday 1990). The production of chitin and chitosan from microbial sources appears promising because the process can be manipulated to obtain a pure, rather uniform product with specific characteristics (Knezevic-Jugovic et al. 2010). Though chitin and chitosan can be extracted from a wide range of aquatic and terrestrial macro- and microorganisms, commercial exploitation has focused on a limited number of organisms (Hayes et al. 2008).

3 Chitosan Nanoparticles

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range 1–1,000 nm (Zhao et al. 2011). Simply, the nanoparticles prepared using chitosan or its derivatives can be called chitosan nanoparticles. The *N*-deacetylated product of chitin is an interesting biopolymer from which to prepare nanoparticles, owing to its unique polymeric cationic character, good biocompatibility, non-toxicity, biodegradability, mucoadhesivity, and absorption-enhancing effects (Kunjachan et al. 2010). Being a linear polyamine containing a number of free amine groups that are readily available for cross-linking and the cationic nature allowing for ionic cross-linking with multivalent anions (Agnihotri et al. 2004) can be considered as the key factors which make chitosan significant in producing nanoparticles. The unique characteristics of chitosan nanoparticles could provide a higher affinity for negatively charged biological membranes and site-specific targeting in vivo (Qi et al. 2004). Hence, it can be successfully used in the encapsulation of drugs, enzymes, and DNA (Bowman and Leong 2006; Colonna et al. 2007). These nanoparticles are ideal materials to be used in controlled release systems (Corradini et al. 2010), with a wide range of applications in a variety of industries. Chitosan nanoparticles do possess their own physical, chemical, and morphological characteristics that are

responsible for their bioactivity and those characteristics are influenced by the method of preparation and the conditions existing within the system. It is clear that the morphology of the particles is not similar. Chitosan nanoparticles exhibit regular assemblage shapes such as snowflakes, round shape, and spherical shape.

4 Methods of Preparation of Chitosan Nanoparticles

Several methods have been described in order to prepare chitosan nanoparticles, and the main methods of preparation include emulsion cross-linking, emulsion-droplet coalescence, coacervation/precipitation, ionotropic gelation, reverse micelles, template polymerization, and molecular self-assembly (Shi et al. 2011). The selection of any of the methods depends upon factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic profiles, stability of the final product, and residual toxicity associated with the final product (Agnihotri et al. 2004).

4.1 Emulsion Cross-linking

This method is commonly used to produce both chitosan nanoparticles and chitosan microparticles (Agnihotri et al. 2004; Kunjachan et al. 2010). The emulsion cross-linking method utilizes the reactive functional groups of chitosan to cross-link with a cross-linking agent (Bodnar et al. 2005). As described by Shi et al. (2011), this method employs the development of a water-in-oil emulsion by emulsifying chitosan solution in an oil phase. The aqueous droplets are stabilized using a suitable surfactant. The emulsion is then reacted with an appropriate cross-linking agent, such as glutaraldehyde, to stabilize the polysaccharide droplets. Finally, the nanoparticles are washed and dried. In this method, the size of the particles can be controlled by varying the size of the aqueous droplets. However, the amount of cross-linking agent used for hardening and the speed of stirring during the formation of the emulsion determine the particle size of the final product (Agnihotri et al. 2004). However, this method has some drawbacks as well. The major ones are the use of organic solvents and harsh cross-linking agents, which ultimately have adverse effects on the stability of proteins and cell viability. Also, complete removal of unreacted cross-linking agent may be difficult in this process (Agnihotri et al. 2004; Shi et al. 2011).

4.2 Emulsion-Droplet Coalescence

The principles of this method involve both emulsion cross-linking and precipitation. The method was initially developed by Tokumitsu et al. (1999) in order to develop

gadopentetic acid (Gd-DTPA)-loaded chitosan nanoparticles (Gd-nanoCPs) for gadolinium neutron-capture therapy (Gd-NCT). In this method, two emulsions are prepared in a similar manner. A stable emulsion containing aqueous chitosan solution along with the drug in liquid paraffin oil is prepared first. Then, the second stable emulsion containing chitosan aqueous solution of NaOH is prepared. Following that, both emulsions are mixed under high-speed stirring, droplets of each emulsion collide at random, coalesce, and, finally, precipitate as small-sized particles. Nanoparticles are obtained within the emulsion droplets.

4.3 Coacervation/Precipitation

As illustrated by Nishimura et al. (1986), the nanoparticles were formed by spraying the chitosan solution into sodium hydroxide, NaOH methanol, or ethanediamine alkaline solutions, using compressed air to originate coacervated droplets. Afterwards, the separation and purification of the particles is done by either filtration or centrifugation, followed by successive washing with hot and cold water. In addition, Berthod and Kreuter (1996) demonstrated a quite different method to obtain nanoparticles. According to their method, sodium sulfate solution is added dropwise to an acidic solution of chitosan containing surfactant while undergoing stirring and continuous sonication for 30 min.

4.4 Ionotropic Gelation

Since chitosan contains a number of ammine groups in its backbone and they are subjected to protonization to form NH_3^+ in acidic solutions, it can be chemically and physically cross-linked in order to prepare nanoparticles (Kafshgari et al. 2011). Physical cross-linking has gained considerable attention, as it possesses many advantages over chemical cross-linking, such as avoiding the use of toxic reagents, reduced undesirable effects, and improved biocompatibility (Agnihotri et al. 2004; Rayment and Butler 2008). Also, the very simple and mild process supports physical cross-linking (Shi et al. 2011). Physical cross-linking is based on the complexation of positively charged chitosan with negatively charged multivalent ions, derived from sodium tripolyphosphate (TPP) (Bodmeier et al. 1989), citrate, and sulfate (Shu and Zhu 2001). The ionic gelation method was first introduced by Bodmeier et al. (1989), who prepared a chitosan–tripolyphosphate complex by adding acidic chitosan to tripolyphosphate solution. Briefly, chitosan is dissolved in an aqueous acidic solution such as acetic, malic, tartaric, or citric acid (Bodnar et al. 2005; Kafshgari et al. 2011) in order to obtain cations of chitosan. Then, the solution is added to tripolyphosphate solution drop-wise under constant stirring. Due to the complexation between oppositely charged species, chitosan undergoes ionic gelation and precipitates to form spherical particles (Agnihotri et al. 2004).

4.5 Reverse Micelles

Reverse micellar medium is used to prepare ultrafine polymeric nanoparticles with a narrow size distribution (Bodnar et al. 2005). Reverse micelles are thermodynamically stable liquid mixtures of water, oil, and surfactant. The remarkable property of a reverse micelles hosted system is that it has a dynamic behavior compared to the other conventional methods of emulsion polymerization due to the production of ultrafine particles with a narrow size range (Agnihotri et al. 2004). In this technique, the surfactant was dissolved in organic solvent to prepare reverse micelles. The micellar droplets are displaced randomly and subjected to Brownian motion. They exchange their water content and reform into two distinct micelles (Pileni 2006). Then, the aqueous phase containing the chitosan and drugs are added to this emulsion with constant vortexing and the nanoparticles form in the core of the reverse micelles. The aqueous phase is regulated in such a way as to keep the entire mixture in an optically transparent microemulsion phase (Agnihotri et al. 2004). An additional amount of water may be added to produce larger sized nanoparticles (Shi et al. 2011), as the size of the water-in-oil droplets increase linearly, i.e., the micellar concentration decreases with increasing water content (Pileni 2006). To this transparent solution, a cross-linking agent is added with constant stirring, and cross-linking is achieved by stirring overnight. The maximum amount of drug that can be dissolved in reverse micelles varies from drug to drug and has to be determined by gradually increasing the amount of drug until the clear microemulsion is transformed into a translucent solution. Ultimately, the solvent is evaporated and then the dry mass is purified in order to obtain the nanoparticles (Agnihotri et al. 2004).

4.6 Molecular Self-Assembly

Molecular self-assembly to prepare nanostructured materials has been a great interest in extensive studies in the agriculture, food, and pharmaceutical arenas, as it is considered a powerful strategy to create such materials (Gonçalves et al. 2010; Wang and Zhang 2012). Since this method is cost-effective, versatile, and facile, it opens up a path for diverse applications. The self-assembly process, defined as the autonomous organization of components into structurally well-defined aggregates, is characterized by diffusion followed by specific association of molecules through non-covalent interactions, including electrostatic and/or hydrophobic associations. The hydrophobic association with hydrophilic polymer to produce chitosan nanoparticles can be achieved in three different ways: the first is hydrophobic chains grafted to a hydrophilic backbone, the second is hydrophilic chains grafted to a hydrophobic backbone (grafted polymers), and the last is alternating hydrophilic and hydrophobic segments (block polymers). Upon contact with an aqueous environment, amphiphilic polymers spontaneously form self-aggregated nanoparticles, via intra- or intermolecular associations between the hydrophobic moieties, primarily to minimize the interfacial free energy process (Gonçalves et al. 2010).

4.7 Large-Scale Production of Chitosan Nanoparticles: Spinning Disk Processing Technology (SDP)

In industrial perspectives, large-scale production that reliably manufactures chitosan nanoparticles of requisite quality at a reasonable price has been a major concern. As SDP technology appears to be an attractive solution regarding large-scale production, studies have been carried out to evaluate the potential of this technology to be used in mass production and compared the method with the conventional bench-top production of chitosan nanoparticles often used in laboratories (Loh et al. 2010). In this study, the synthesis of nanoparticles was done by ionotropic gelation using a spinning disk processor operating at ambient temperature. Then, the chitosan and tripolyphosphate solutions were feed onto the spinning disk and the effluent was collected. The processing conditions were moderated by changing the solvents [acetic or L-(+)-tartaric acid] for dissolving chitosan and changing the speed of the spinning disk (1,000–3,000 rpm). The effluent was then filtered and dialyzed. Finally, the resultant dispersion was lyophilized, UV-sterilized overnight, and stored at −20 °C for future use. The blank chitosan nanoparticles were produced in a beaker with agitation using a magnetic stirrer. The conditions of the system were maintained same as the SDP method and the same procedure was carried out for the resultant dispersion.

In this method, the liquid reactants, chitosan solution and TPP solution, are fed through controllable feed jets into the center of a disk, spinning at an adjustable speed in the range 300–3,000 rpm. Then, the high centrifugal force applied on the liquids, causing intense interfering waves which, when combined with the spreading of the liquid into thin film, provide for a high surface area to volume contact, intimate mixing, as well as uniform mass and heat transfer within seconds. A grooved disk can be used when great intensification is required.

Studies have proven the fact that SDP is a robust technology capable of expanding the production of chitosan nanoparticles that are consistent in features with those prepared by bench-top methods. Spherical chitosan nanoparticles are produced from both acetic acid and tartaric acid solvents. The characteristics of the nanoparticles depend upon the type and concentration of the acid used. Post-SDP processing by dialysis and lyophilization can produce comparatively more stable chitosan nanoparticles, which can be reconstituted in acidic media and stimulated biological media. The SDP process has many advantages, such as avoiding harsh processing conditions commonly employed in many conventional methods, minimization of waste produced, improved safety during production, and controlling the particle size through its tunable feed rate, temperature reaction, and spinning rate. More importantly, not requiring changes to the process parameters of production in scaling up the manufacturing and its cost-effectiveness compared to conventional methods make the SDP process significant from an economic point of view.

5 Characterization of Chitosan Nanoparticles

The characteristics of the nanoparticles are greatly affected by the processing method and conditions. The nanoparticles usually change with the time and environment, as they are highly dynamic (Baer et al. 2010). Therefore, it is important to form a comprehensive understanding about the behavior of nanoparticles, prepared by a particular method, in order to make assure the effective use of the intended applications. Hence, the characterization of nanoparticles plays a major role and more focus is directed towards suitable and accurate methods (Cong et al. 2011). The traditional approach of characterization was concerned about characteristics such as composition (molecular structure), melting point, boiling point, pH, vapor pressure, and solubility. On the contrary, the new approach is focused on rather different properties, such as the particle size distribution, morphology, zeta potential, surface area and porosity, aggregation, hydrated surface analysis, and chemical interactions (Corradini et al. 2010).

5.1 Size, Size Distribution, and Morphology of Nanoparticles

Size, size distribution, and morphology are important characteristics commonly being evaluated. The size of the particles formed is not the same at all times. It varies widely depending on the method of preparation and various other extrinsic and intrinsic factors. Generally, either narrow or comparatively broad distributions can be observed. When the distribution is narrow, greater homogeneity can be achieved.

Generally, morphology refers to the shape of the nanoparticles, which is also highly variable. The characterization of theses aspects of nanoparticles is usually done using complex technologies. The size distribution is determined by dynamic light scattering (DLS) using a zetasizer, at a scattering angle of 90° at 25 °C using nanoparticles dispersed in deionized distilled water (Qi et al. 2004; Kafshgari et al. 2011). The size and morphology of the nanoparticles are mainly determined by atomic force microscopy (AFM), transmittance electron microscopy (TEM), and scanning electron microscopy (SEM).

5.2 Zeta Potential

The zeta potential, i.e., surface charge, is another important physical property exhibited by any particle in a suspension. The knowledge about the zeta potential can be used to optimize the formation of suspensions and emulsions. It has long been recognized that the zeta potential is a very good index of the magnitude of the interaction among colloidal particles and it is widely used to assess the stability of colloidal systems. The surrounded liquid layer of the particle exists as two layers, the inner

stern layer and outer diffused layer. The ions are strongly bound to the stern layer, whereas ions are less firmly bound to the outer layer. Within the diffused layer, there is a distinct boundary in which the particles and the ions form a stable entity. When a particle moves due to gravity or other forces, ions within the boundary move it. The ions existing beyond the boundary stay with the bulk dispersant. The potential at this boundary (surface of hydrodynamic shear) is called the zeta potential. The general dividing line between stable and unstable suspensions is generally taken at either +30 or −30 mV. Particles with zeta potentials more positive than +30 mV or more negative than −30 mV are normally considered stable. The zeta potential usually depends on the pH, conductivity, and concentration of the formulation. The zeta potential of nanoparticles is also measured using the same method as for the particle size distribution.

5.3 Chemical Interactions

When a particular active compound is incorporated into chitosan nanoparticles, it is important to evaluate and confirm their chemical interactions (Kafshgari et al. 2011). In order to achieve this, Fourier transform infrared spectroscopy (FT-IR) is used. In this method, infrared radiation is passed through a sample and some of the IR radiation is absorbed, with the rest being transmitted by the sample. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Similar to a fingerprint, no two unique molecular structures produce the same infrared spectrum.

When nanoparticles are used in industrial applications, they are sometimes purchased from other manufacturers, so characterization of the nanoparticles is essential prior to use. Cong et al. (2011) conducted a study to characterize commercially available silver particles to find out whether they possess those exact initial characteristics indicated by the manufacturers. The results revealed clear differences between the test results and the manufacturers' information with respect to particle size. Batch-to-batch variation during production, changes in material properties between synthesis and initial characterization, and particular experimental conditions (e.g., pH, ionic strength, and temperature, salinity, competing natural cations) are the possible causes for such variation. Therefore, it is important to perform the characterization under conditions as close as possible to the exposure medium. In fact, the characterization of nanostructured materials is increasingly important. Even though there are many methods and technologies for the characterization of nanoparticles, many challenging studies need to be carried out on surface and interface contaminations. Also, the needs for understanding and improving sample handling protocols and using complimentary analysis tools and theory are often essential. In some cases, new concepts and tools are needed to understand and characterize the fundamental nature of nanostructured materials (Baer et al. 2010).

6 Applications of Chitosan Nanoparticles

6.1 *Biomedical Applications*

Chitosan nanoparticles have been extensively used in biomedical applications. During the past decade, chitosan nanoparticles have been widely studied, as they possess the ability to control the drug release rate, prolong the duration of therapeutic effectiveness, and deliver drugs, proteins, DNA, and antigens to the targeted specific site inside the body (Ritthidej et al. 2007; Papadimitriou et al. 2008). It is reported that such drug or other active agents-loaded chitosan nanoparticles have been successively used in drug delivery applications such as pulmonary inhalations, mucoadhesive systems, oral delivery systems for treating peptic ulcers, and, finally, for intratumoral drug targeting (Kunjachan et al. 2010). As far as the biomedical applications are concerned, it is interesting to understand the mechanisms of drug loading and release.

Drugs loading can be done by either physical entrapment into the polymeric matrix or covalently bound to the polymer backbone. However, physical entrapment is considered to be more efficient and commonly used in drug delivery applications. The physical drug loading can be performed in two ways, i.e., during the preparation of nanoparticles, which is known as incorporation, and after the formation of nanoparticles, which is known as incubation. In these systems, the drug is physically embedded into the matrix or adsorbed onto the surface. Various methods have been developed to improve the efficiency of loading, which largely depends upon the method of preparation, as well as the physicochemical properties of the drug. Considering the two systems, incorporation provides maximum drug loading capacity over incubation. Both water-soluble and -insoluble drugs can be loaded by these methods. However, the objective of any method is focused on the incorporation of biomolecules without compromising its bioactivity (Agnihotri et al. 2004; Gonçalves et al. 2010). The drug delivery mediated by nanoparticles can be either an active or a passive process. Passive delivery refers to the transport through leaky capillary fenestrations, into, e.g., tumor interstitium and cells, by passive diffusion. Active targeting involves the use of peripherally conjugated targeting moieties, for enhanced delivery to a specific site, based on molecular recognition. One such approach is to surface-coat nanoparticles with an antibody, which can interact with its specific antigenic target cell site (Zambaux et al. 1999; Gonçalves et al. 2010).

In vitro drug release studies are also important in obtaining a proper understanding of the behavior of the drug-loaded nanoparticles. Drug release from chitosan-based particulate systems depends upon the extent of cross-linking, morphology, size and density of the particulate system, physicochemical properties of the drug, as well as the presence of adjuvant. In vitro release also depends upon the pH, polarity, and presence of enzymes in the dissolution media. Three basic mechanisms that are generally employed in releasing drugs are release from the surface of particles, diffusion through the swollen rubbery matrix, and release due to polymer erosion. Most of the time, drug release follows more than one mechanism at any particular time (Agnihotri et al. 2004).

The method described by Kaloti and Bohidar (2010) can be taken as a good example for drug loading and release for chitosan nanoparticles. In this study, cycloheximide, which is a hydrophobic drug that is soluble in chloroform, was used and incubation was done. Briefly, a known amount (5 mg/100 μl) of drug dissolved in chloroform was added to a known amount (4 ml) of nanoparticles in a solution using a sonication bath until the turbid solution turned clear. Once the free drug was entrapped inside the hydrophobic core of the nanoparticles, absorbance of the free drug was determined using a UV-visible spectrophotometer at 249 nm and the entrapment efficiency was calculated. Then, in order to study the release kinetics, two physiologically important media, phosphate buffer (pH 7.4), which mimics the blood pH, and simulated intestinal fluid (pH 6.8), which mimics the intestinal pH without pancreatic enzyme, were used in vitro at 37 °C. Chitosan nanoparticles were then dialyzed against the medium. After regular time intervals, a known amount was tested spectrometrically.

6.2 Applications in the Food Industry

The versatility of chitosan nanoparticles has generated a wide range of applications in the food industry. The main focus was to develop chitosan-based films to be used as packaging material to act against bacteria and fungi, like microorganisms, to improve shelf life by preserving the food and maintaining food quality in the meantime. Although conventional packaging materials such as plastics are effective for food preservation, they have created many serious environmental problems. The use of environmentally friendly, cost-effective packaging materials has gained the considerable attention of scientists and the use of bio-based active films appeared to be a better solution in respect to these requirements (Aider 2009). The use of chitosan nanoparticle-based edible films has been reported on many occasions. De Moura et al. (2009) investigated the possibility of producing N,N,N-trimethyl chitosan (TMC) nanoparticles for application in the food industry as intelligent packaging. TMC was obtained by quaternization reaction and then the TMC tripolyphosphate (TPP) nanoparticles were produced using ionic gelation of chitosan with TPP anions. Then, the effect of certain preparation conditions and properties of TMC nanoparticles were investigated. The results suggest that the possibility of producing TMC-TPP nanoparticles with controlled sizes is of key importance to optimize its use in the food industry, because nanotechnology can improve the functionality of edible films for food applications.

In a previous study, De Moura et al. (2009) developed a hydroxypropyl methylcellulose-based edible film with chitosan nanoparticles. In their study, chitosan/tripolyphosphate nanoparticles were incorporated into hydroxypropyl methylcellulose. Then, the mechanical properties, such as water vapor permeability, thermal stability, and other physicochemical properties, of the film were analyzed. It has been shown that the incorporation of chitosan nanoparticles into the film has significantly improved the mechanical and film barrier properties and thermal stability. Further, chitosan nanoparticles tend to occupy the empty spaces in the pores of the matrix, increasing the collapse of the pores and, thereby, improving the film's

tensile properties and water vapor permeability. Also, the use of carboxymethylcellulose film containing chitosan nanoparticles as potential material for food and beverage packaging applications has been discussed due their increased mechanical properties and high stability (De Moura et al. 2011).

It is accepted that chitosan nanoparticle-based films can be effectively used in the food industry, as they provide various benefits, including good edibility, biocompatibility with human tissues, aesthetically pleasing appearance, barrier properties against pathogenic microorganisms, non-toxicity, non-polluting, and low cost (Vasconez et al. 2009). The use of chitosan active bio-based film as a food coating has been reported with respect to a variety of food, including cheese and meat products, such as fermented sausages (Wang et al. 2004).

Moreover, chitosan nanoparticles have opened pathways to minimize the loss of important food constituents during processing. According to Jang and Lee (2008), chitosan nanoparticles can be used to enhance the stability of L-ascorbic acid during heat processing. The study was conducted by preparing ascorbic acid-loaded chitosan using ionic gelation with tripolyphosphate anions. Various concentrations of ascorbic acid-loaded nanoparticles were subjected to various different temperatures and the release profile was evaluated. The results showed that, at the beginning of the release profiles, the burst release-related stability of the surface increased with increase of the temperature. Then, the release of the internal ascorbic acid was constantly higher with a longer release time. Consequently, it was confirmed that the stability of ascorbic acid-loaded chitosan nanoparticles was affected by temperature and the internal stability was greater than the surface stability. Ascorbic acid-loaded chitosan nanoparticles were reported to enhance antioxidant effects due to the continuous release of ascorbic acid from chitosan nanoparticles. Also, Sato et al. (2012) demonstrated the possibility of producing food-grade stable nanoparticles with simple processing techniques, using lecithin and sodium caseinate, which could be further used as base systems for the production of nanocapsules.

The United States Department of Agriculture (USDA 2011) has implemented a project to develop green nanotechnology for eliminating foodborne pathogens. In this study, the USDA envisage the development of a nanoparticle wash treatment with the capability of significantly reducing or eliminating pathogenic bacteria associated with fresh or fresh-cut fruits and vegetables, to be used in minimal processing. The specific tasks involve the design, synthesis, and characterization of ultrapotent chitosan nanoparticles coated by antimicrobial peptides, evaluation of peptide-enhanced nanoparticles as a lysis agent in realistic food processing environments, and the development of a postharvest nanoparticle electric field treatment for decreasing the bacterial load of fresh fruits and vegetables.

6.3 Other Applications

In addition to biomedical and food applications, chitosan nanoparticles have many other applications, as described previously. More importantly, they have various

applications in the field of agriculture. It is reported that chitosan nanoparticles are used to encapsulate 1-naphthalene acetic acid (NAA), which is a plant growth regulator, for controlled release as a way to achieve efficient use of harmful agrochemicals (Gill 2003). Corradini et al. (2010) tried to use chitosan nanoparticles as a controlled release agent for nitrogen (N), phosphorus (P), and potassium (K) fertilizers for the first time. In this work, chitosan nanoparticles were obtained by polymerizing methacrylic acid. The interaction and stability of chitosan nanoparticle suspensions containing N, P, and K in terms of particle size analysis and zeta potential were evaluated by FT-IR spectroscopy. The results confirmed the positive potential of chitosan nanoparticle incorporation in NPK fertilizers. However, further studies are needed in order to understand the mechanism and to optimize the incorporation of N, P, and K elements into the nanoparticles. A study conducted by Hettiarachchi et al. (2012) evaluated the potential use of silver nanochitosan composites against banana pathogen *Colletotrichum musae* and confirmed the fungicidal effects of the chitosan silver nanocomposite application. These studies open up new dimensions for chitosan nanoparticles to be used in various disciplines of agriculture.

The use of chitosan-based particulate systems in wastewater treatment and the cosmetics industry has attracted much attention recently. It is reported that chitosan could carry out efficient removal of metal ions and humic acids, and act as a good absorbent to absorb impurities present in wastewater (Yang et al. 2009). In the cosmetics industry, chitosan is used in several applications, including hair care, skin care, and oral care (Dutta et al. 2004). Lee et al. (2011) developed hair dye incorporating poly-γ-glutamic acid chitosan nanoparticles to be used as a solution for the problematic allergic reactions that occur due to *p*-phenylenediamine or its related chemicals that are commonly used in hair dye formulations. Also, there are evidences of the potential use of chitosan nanoparticles in anti-aging skin care products (Leonida et al. 2011), proving the inimitable uses of chitosan nanoparticles.

7 Possible Risks and Regulations

Even though the chitosan nanoparticles appear to be safe in laboratory-scale studies, the knowledge of the risks involved in real-world applications leaves much to be desired. There are some cases in which the use of chitosan nanoparticles has been questionable (Kuzma et al. 2008). As described earlier, 1-naphthalene acetic acid (NAA)- incorporated chitosan nanoparticles were used for controlled release, as they increase precision and potency per volume that would result in the delivery of a smaller amount of potentially harmful agrichemicals (Gill 2003). However, the use of these particles on the macroscale is questionable, as nanomaterials exhibit novel properties due to their extremely small size, high surface area, and reactivity (Oberdorster et al. 2005; Maynard 2006). Therefore, the consequences of releasing them into the environment need to be assessed. In another study, poly lactic-co-glycolic acid (PLGA) chitosan nanospheres have been used to determine where

non-point sources pollution originates, travels, and resides in agro ecosystems. Chitosan capsules have been used in drug delivery systems, which showed that they are generally non-toxic and biodegradable, and considered non-problematic to release into the environment (Vila et al. 2002; Olivier 2005). However, previous toxicity studies were conducted using only small amounts of capsules consistent with their use for drug delivery and studies on the effects of the environmental release of PLGA-chitosan capsules have not yet been conducted. It is important to assess the extent and timing of degradation of PLGA-chitosan under various environmental conditions, as well as the potential impacts of the particles and their breakdown products on biota and ecosystems. As described by the authors, no ecological or environmental analyses for chitosan nanoparticles have been conducted. The early findings of these particles in favor of human use at the nanolevel are promising signs for their possible safe environmental applications. In addition, some doubts have been aroused with respect to the use of nanoencapsulated food additives and nanocoated films in food packaging. However, complete toxicity effects have not yet been studied and more exposure assessments are required in order to obtain a better picture of the relationship between applications of nanoparticles and their health risks. Most of the time, the risks of nanoparticles are assessed with their chemical composition and, to date, no widely accepted or well-defined risk assessment methods or test strategies exist explicitly designed for nanoparticles (Cong et al. 2011). It is important to gather more information regarding health and environmental risks associated with applications of nanoparticles, identify the proper risk assessment strategies, and implement regulatory policies, in order to ensure the safety of these nanomaterials to mankind.

References

Agnihotri SA, Mallikarjuna NN, Aminabhavi TM (2004) Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release 100:5–28

Aider M (2009) Chitosan application for active bio-based films production and potential in the food industry: review. http://www.ebah.com.br/content/ABAAAA4rUAD/chitosan-application-for-active-bio-based-films-production-and-potentialin-the-food-industry-review. Accessed Aug 2012

Anonymous (2010) Chitosan (deacetylated chitin biopolymer). Natural Standard professional monograph. http://www.livingnaturally.com/ns/DisplayMonograph.asp?storeID=E32FA6C399AB4C99897032581851D45D&DocID=bottomline-chitosan. Accessed July 2012

Baer DR (2010) Surface characterization of nanoparticles: challenges and opportunities. http://www.greennano.org/webfm_send/49. Accessed Aug 2012

Bansal V, Sharma PK, Sharma N, Pal OP, Malviya R (2011) Applications of chitosan and chitosan derivatives in drug delivery. Adv Biol Res 5:28–37

Berthod A, Kreuter J (1996) Chitosan microspheres-improved acid stability and change in physicochemical properties by cross-linking. Proc Int Symp Control Release Bioact Mater 23:369–370

Bodmeier R, Oh K-H, Pramar Y (1989) Preparation and evaluation of drug-containing chitosan beads. Drug Dev Ind Pharm 15:1475–1494

Bodnar M, Hartmann JF, Borbely J (2005) Preparation and characterization of chitosan-based nanoparticles. Biomacromolecules 6:2521–2527

Bowman K, Leong KW (2006) Chitosan nanoparticles for oral drug and gene delivery. Int J Nanomedicine 1:117–128

Brück MW, Slater JW, Carney BF (2010) Chitin and chitosan from marine organisms. In: Kim S-K (ed) Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications. Taylor & Francis, Boca Raton, pp 11–19

Chung Y-C, Su Y-P, Chen C-C, Jia G, Wang H-I, Wu JCG, Lin J-J (2004) Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 25:932–936

Colonna C, Conti B, Perugini P, Pavanetto F, Modena T, Dorati R, Genta I (2007) Chitosan glutamate nanoparticles for protein delivery: development and effect on prolidase stability. J Microencapsul 24:553–564

Cong Y, Pang CF, Dai L, Banta GT, Selck H, Forbes VE (2011) Importance of characterizing nanoparticles before conducting toxicity tests. Integr Environ Assess Manag 7:502–503

Corradini E, De Moura MR, Mattoso LHC (2010) A preliminary study of the incorporation of NPK fertilizer into chitosan nanoparticles. Express Polym Lett 4:509–515

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

De Moura MR, Aouada FA, Avena-Bustillos RJ, McHugh TH, Krochta JM, Mattoso LHC (2009) Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. J Food Eng 92:448–453

De Moura MR, Lorevice MV, Mattoso LHC, Zucolotto V (2011) Highly stable, edible cellulose films incorporating chitosan nanoparticles. J Food Sci 76:N25–N29

Değirmencioğlu N, İrkin R, Değirmencioğlu A, Kabasakal A (2009) Chitosan and its application in food industry. Akademik Gida 7:35–41

Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. J Sci Ind Res 63:20–31

Gill R (2003) Nano- and micro-encapsulation of food additives and agrochemicals. USDA Grant Propos 2003–00331

Gonçalves C, Pereira P, Gama M (2010) Self-assembled hydrogel nanoparticles for drug delivery applications. Materials 3:1420–1460

Gooday GW (1990) The ecology of chitin degradation. In: Marschall KC (ed) Advances in microbial ecology. Plenum Press, New York, pp 387–440

Hayes M, Carney B, Slater J, Brück W (2008) Mining marine shellfish wastes for bioactive molecules: chitin and chitosan – Part A: extraction methods. Biotechnol J 3:871–877

Hettiarachchi, MA, Wickramarachchi PASR (2012) Activity of silver nano chitosan composites against the banana pathogen *Colletotrichum musae*. Abstracts. In: International conference on chemical sciences, Colombo, p 12

Jang K-I, Lee HG (2008) Stability of chitosan nanoparticles for L-ascorbic acid during heat treatment in aqueous solution. J Agric Food Chem 56:1936–1941

Kafshgari MH, Khorram M, Khodadoost M, Khavari S (2011) Reinforcement of chitosan nanoparticles obtained by an ionic cross-linking process. Iran Polym J 20:445–456

Kaloti M, Bohidar HB (2010) Kinetics of coacervation transition versus nanoparticle formation in chitosan–sodium tripolyphosphate solutions. Colloids Surf B Biointerfaces 81:165–173

Knezevic-Jugovic Z, Petronijevic Z, Smelcerovic A (2010) Chitin and chitosan from microorganisms. In: Kim S-K (ed) Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications. Taylor & Francis, Boca Raton, pp 25–34

Keong LC, Halim AS (2009) In vitro models in biocompatibility assessment for biomedical-grade chitosan derivatives in wound management. Int J Mol Sci 10:1300–1313

Kunjachan S, Jose S, Lammers T (2010) Understanding the mechanism of ionic gelation for synthesis of chitosan nanoparticles using qualitative techniques. Asian J Pharm 4:148–153

Kuzma J, Romanchek J, Kokotovich A (2008) Upstream oversight assessment for agrifood nanotechnology: a case studies approach. https://www2.hhh.umn.edu/publications/6269/document.pdf. Accessed Aug 2012

Lee H-Y, Jeong Y-L, Choi K-C (2011) Hair dye-incorporated poly-γ-glutamic acid/glycol chitosan nanoparticles based on ion-complex formation. Int J Nanomedicine 6:2879–2888

Leonida MD, Banjade S, Vo T, Anderle G, Haas GJ, Philips N (2011) Nanocomposite materials with antimicrobial activity based on chitosan. Int J Nano Biomater 3:316–334

Lim CK, Yaacob NS, Ismail Z, Halim AS (2010) In vitro biocompatibility of chitosan porous skin regenerating templates (PSRTs) using primary human skin keratinocytes. Toxicol In Vitro 24:721–727

Loh JW, Schneider J, Carter M, Saunders M, Lim LY (2010) Spinning disc processing technology: potential for large-scale manufacture of chitosan nanoparticles. J Pharm Sci 99:4326–4336

López-León T, Carvalho ELS, Seijo B, Ortega-Vinuesa JL, Bastos-González D (2005) Physicochemical characterization of chitosan nanoparticles: electrokinetic and stability behavior. J Colloid Interface Sci 283:344–351

Maynard AD (2006) Nanotechnology: a research strategy for addressing risk, vol 3, PEN. Project on Emerging Nanotechnologies, Washington, DC

Nishimura K, Nishimura SI, Seo H, Nishi N, Tokura S, Azuma I (1986) Macrophage activation with multi-porous beads prepared from partially deacetylated chitin. J Biomed Mater Res 20:1359–1372

Nwe N, Stevens WF (2008) Production of chitin and chitosan and their applications in the medical and biological sector. In: Tamura H (ed) Recent research in biomedical aspects of chitin and chitosan. Research Signpost, Kerala, pp 161–176

Nwe N, Furuike T, Tamura H (2010) Chitin and chitosan from terrestrial organisms. In: Kim S-K (ed) Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications. Taylor & Francis, Boca Raton, pp 3–10

Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839

Olivier J-C (2005) Drug transport to brain with targeted nanoparticles. NeuroRx 2:108–119

Papadimitriou S, Bikiaris D, Avgoustakis K, Karavas E, Georgarakis M (2008) Chitosan nanoparticles loaded with dorzolamide and pramipexole. Carbohydr Polym 73:44–54

Peter MG, Kegel G, Keller R (1986) Structural studies on sclerotized insect cuticle. In: Muzzarelli RAA, Jeuniaux C, Gooday GW (eds) Chitin in nature and technology. Plenum Press, New York, pp 21–28

Pileni MP (2006) Reverse micelles used as templates: a new understanding in nanocrystal growth. J Exp Nanosci 1:13–27

Qi LF, Xu ZR, Jiang X, Hu CH, Zou XF (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res 339:2693–2700

Qi L-F, Xu Z-R, Li Y, Jiang X, Han X-Y (2005) In vitro effects of chitosan nanoparticles on proliferation of human gastric carcinoma cell line MGC803 cells. World J Gastroenterol 11:5136–5141

Rayment P, Butler MF (2008) Investigation of ionically crosslinked chitosan and chitosan–bovine serum albumin beads for novel gastrointestinal functionality. J Appl Polym Sci 108:2876–2885

Ritthidej GC, Pichayakorn W, Kusonwiriyawong N, Lipipun V (2007) Preparation of chitosan nanoparticles for protein delivery by w/o/w emulsion solvent evaporation and simple ionotropic gelation techniques. Solid State Phenom 121:751–754

Sato ACK, Quintas MAC, Vicente AA, Cunha RL (2012) Food grade nanoparticles obtained from natural source ingredients. http://www.icef11.org/content/papers/fms/FMS1083.pdf. Accessed Aug 2012

Shi L-E, Fang X-J, Xing L-Y, Chen M, Zhu D-S, Guo X-F, Zhao L-M, Tang Z-X (2011) Chitosan nanoparticles as drug delivery carriers for biomedical engineering. J Chem Soc Pak 33:929–934

Shu XZ, Zhu KJ (2001) Chitosan/gelatin microspheres prepared by modified emulsification and ionotropic gelation. J Microencapsul 18:237–245

Teng WL, Khor E, Tan TK, Lim LY, Tan SC (2001) Concurrent production of chitin from shrimp shells and fungi. Carbohydr Res 332:305–316

Tokumitsu H, Ichikawa H, Fukumori Y (1999) Chitosan–gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsion-droplet coalescence technique and characterization. Pharm Res 16:1830–1835

Tokura S, Tamura H (2007) Chitin and chitosan. In: Kamerling H, Boons G, Lee YC, Suzuki A, Taniguchi N, Voragen AGJ (eds) Comprehensive glycoscience. Elsevier, Boston, pp 449–475

USDA (2011) Developing green nanotechnology for eliminating foodborne pathogens. http://fsrio.nal.usda.gov/nal_web/fsrio/printresults.php?ID=6762. Accessed Aug 2012

VandeVord PJ, Matthew HWT, DeSilva SP, Mayton L, Wu B, Wooley PH (2001) Evaluation of the biocompatibility of a chitosan scaffold in mice. http://www.eng.wayne.edu/user_files/372/file/Quick_Upload/vandevord%20et%20al%20evaluation%20of%20biocompatibility.pdf. Accessed Jul 2012

Vasconez MB, Flores SK, Campos CA, Alvarado J, Gershenson LN (2009) United States Patent 5,919,574

Vila A, Sánchez A, Tobıo M, Calvo P, Alonso MJ (2002) Design of biodegradable particles for protein delivery. J Control Release 78:15–24

Wang Q, Zhang B (2012) Self-assembled nanostructures. In: Padua GW, Wang Q (eds) Nanotechnology research methods for foods and bioproducts. Wiley-Blackwell, Oxford

Wang X, Du Y, Liu H (2004) Preparation, characterization and antimicrobial activity of chitosan–Zn complex. Carbohydr Polym 56:21–26

Yang H, Yuan B, Lu YB, Cheng RS (2009) Preparation of magnetic chitosan microspheres and its applications in wastewater treatment. Sci Chin Ser B Chem 52:249–256

Zambaux MF, Bonneaux F, Gref R, Dellacherie E, Vigneron C (1999) Preparation and characterization of protein C-loaded PLA nanoparticles. J Control Release 60:179–188

Zhao L-M, Shi L-E, Zhang Z-L, Chen J-M, Shi D-D, Yang J, Tang Z-X (2011) Preparation and application of chitosan nanoparticles and nanofibers. Braz J Chem Eng 28:353–362

Chapter 19
Chitin, Chitosan, and Their Derivatives Against Oxidative Stress and Inflammation, and Some Applications

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1 Introduction

Chitin and chitosan, which are naturally occurring biopolymers, have attracted a great deal of attention during the past several decades, due to their wide range of applications in various fields. Chitin, (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan, is widely distributed among invertebrates and crustaceans as structural material in their exoskeletons (Jeon and Kim 2002; Muzzareli 2002). Chitin is a polysaccharide, which is a white, hard, inelastic, nitrogenous compound, and a water-insoluble material resembling cellulose in its solubility. It may be regarded as

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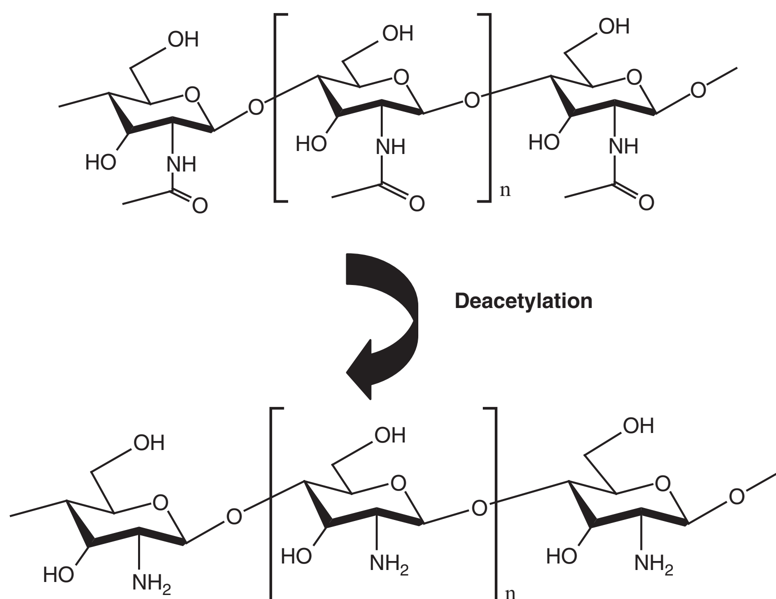

Fig. 19.1 Structures of chitin and chitosan

cellulose with a hydroxyl group at position C-2 replaced by an acetamido group. In addition, it has similar function to cellulose in plants, serving as a supportive and protective material.

Chitosan, a partially deacetylated polymer of *N*-acetylglucosamine, is prepared with alkaline deacetylation of chitin in the presence of alkali at high temperature. It consists of β-(1→4)-2-amino-D-glucose residues and is water soluble by making salt with various acids on the amino groups of D-glucosamine units. Furthermore, partially acetylated chitosan having about 50 % D-glucosamine units is only able to dissolve in water (Aiba 1989; Rinaudo 2006). The structures of chitin and chitosan are shown in Fig. 19.1.

Chitin and chitosan have been applied in a broad range of fields, such as agriculture, water and waste treatment, and food processing. Furthermore, new applications are concentrated on producing more and more valuable products by means of cosmetics, drug carriers, gene carriers, food additives, semipermeable membranes, and biopharmaceuticals.

Chitin and its derivatives have a number of biological functions that serve as useful and safe bioactive substances in various applications because they have specific bioactivities, such as antimicrobial activity, immune-enhancing effects (Nishimura and Azuma 1992; Suzuki 1998), hypocholesterolemic activity (Ngo et al. 2008a, b), antitumor, antioxidant) Prashanth and Tharanathan 2005; Liang et al. 2007; Ngo et al. 2008a, b), anticoagulant activity (Huang et al. 2003), inhibitor of enzymes (e.g., β-secretase causing Alzheimer's disease, angiostensin I-converting enzyme causing hypertension, prolyl endopeptidase) (Byun et al. 2005; Je and Kim 2005; Je et al. 2006, 2007; Ngo et al. 2007, 2008a, b), and stimulation of wound healing

(Usami et al. 1997; Okamura et al. 2005). Especially, biocompatibility, biodegradability, polyelectrolyte properties, presence of reactive functional groups, and the ability to modify the structure via chemical modifications have attracted applicability in the biomedical and pharmaceutical industries (Kim and Park 2001; Huang et al. 2003; Sashiwa and Aiba 2004).

Even though chitin and chitosan were known to have very interesting functional properties in many areas, their high molecular weights (MWs), high viscosity, and water insolubility are the major limiting factors for their utilization in vivo. Furthermore, the actions of chitin and chitosan in vivo still remain ambiguous as the physiological functional properties because most animal intestines, including the human gastrointestinal tract, do not possess enzymes which digest the β-glycosidic linkage in chitin and chitosan, with the result of unchanged feces without any degradation of significant absorption (Weiner 1992). In recent researches, therefore, more interest has been directed to converting chitin and chitosan to their oligomers.

Chitin, chitosan, and their derivatives are known to have immunoenhancing effects, such as macrophage activation and the activation of polymorphonuclear cells. Seferian and Martinez (2000) reported on the immunostimulating activity of zinc-chitosan particles in adjuvant formulations and an emulsion formulation containing chitosan. These chitosan-based adjuvant formulations were effective in sensitizing mice and guinea pigs for antigen-specific delayed-type hypersensitivity responses, supporting the fact that chitosan can stimulate both B and T lymphocytes. Chitooligosaccharides (COS) were effective in stimulating the release of TNF-α and IL-1β in macrophages (Han and Yu 2005). In addition, Seo et al. (2000) reported that water-soluble COS in combination with IFN-γ could exhibit antitumor activity, and it was shown to be correlated with nitric oxide (NO) synthesis in activated macrophages.

Furthermore, Tamai et al. (2002, 2003) demonstrated that *N*-acetylglucosamine and glucosamine are effective for the prevention and treatment of degenerative joint disease. *N*-acetylglucosamine prepared with the hydrolysis of chitin has been used as a material for therapeutic activity in osteoarthritis and the regeneration of cartilaginous tissues (Sashiwa et al. 2002, 2003). Oligomers and monomers of chitin and chitosan stimulated acceleration of the mechanisms of wound healing (Okamura et al. 2005). This chapter will focus on researches investigating oxidative stress and inflammatory diseases.

2 Antioxidant Activities Assays in Cell Systems

2.1 Myeloperoxidase Activity Assay

For the determination of myeloperoxidase (MPO) released to cells, cells were treated with different concentrations of samples and blank (no inhibitor) in an assay mixture containing 2 mM H_2O_2 with 1.6 mM 3,3′5,5′-tetramethybenzidine (TMB) added, and the amount of MPO released was measured spectrophotometrically at 655 nm (Naito et al. 1998).

2.2 Membrane Protein Oxidation Assay

The oxidation degree of cell membrane proteins was assessed by determining the content of protein by carbonyl group (Levine et al. 1990). Cultured cells were washed three times with PBS and lysed in lysis buffer without reducing agents. Aliquots of cell lysate were transferred into microtubes and treated with different concentrations of NA-COSs or their derivatives. After incubation for 30 min at 37 °C, 0.1 mM $FeSO_4$ and 2 mM H_2O_2 were added to the mixture and it was allowed to continue to incubate for 1 h. Solubilized protein was precipitated by centrifugation after the addition of 400 μL of 20 % trichloroacetic acid. The pellet was resuspended in 0.2 % 2,4-dinitrophenylhydrazine in 2 N HCl and allowed to stand at 25 °C for 40 min. The protein was precipitated again with 20 % trichloroacetic acid and the pellet was washed three times with ethanol:ethyl acetate (1:1 v/v) solution. It was then dissolved in 200 μL 6 N guanidine hydrochloride and incubated for 15 min at 37 °C. After centrifugation at 1,500 × g for 5 min, the absorbance of the supernatant was recorded against a complementary blank at 370 nm using a UV/Vis microplate reader. A blank was prepared with a parallel procedure using 2 N HCl alone instead of 2,4-dinitrophenylhydrazine reagent. The carbonyl group of protein was expressed by comparing with the control group (Ngo et al. 2009).

2.3 Membrane Lipid Peroxidation Assay

Cells were analyzed for the generation of lipid peroxidation products by the DPPP method. A sensitive fluorescence probe (DPPP) was used to measure the lipid hydroperoxide level of cells produced by a strong carbon-centered radical generating agent (AAPH). DPPP penetrated into the cell membranes and hydroperoxides, an oxidative product of lipids, preferably react with DPPP to produce DPPP-oxide. DPPP itself is not fluorescent, but DPPP-oxide is, with high fluorescence (Takahashi et al. 2001). Briefly, cells growing in culture dishes were washed three times with PBS and labeled with 13 μM DPPP for 30 min at 37 °C, followed by washing three times with PBS and seeded into fluorescence microtiter 96-well plates. After complete attachment of the cells, they were treated with predetermined concentrations of samples and incubated for 1 h. AAPH (3 mM) was added and the DPPP oxide fluorescence intensity was measured after 3 h ($\lambda_{excitation} = 361$ nm, $\lambda_{emission} = 380$ nm) (Ngo et al. 2012a, b).

2.4 Determination of Radical-Mediated DNA Damage

Genomic DNA, which was extracted from the cells using a standard phenol/proteinase K procedure with minor modification (Sambrook and Russell 2001), was pretreated with various concentrations of samples exposed to •OH using Fenton chemistry,

as described by Milne et al. (1993). For that, 40 μL of DNA reaction mixture was prepared by adding predetermined concentrations of test sample (or the same volume of distilled water as the control), 100 μM final concentration of $FeSO_4$, 0.1 mM final concentration of H_2O_2, and genomic DNA in the same order. Then, the mixture was incubated at room temperature for 10 min and the reaction was terminated by adding 10 mM final concentration of EDTA. The reaction mixture containing DNA was electrophoresed on a 1 % agarose gel for 40 min at 100 V. Gels were then stained with 1 mg/mL ethidium bromide and visualized by UV light (Ngo et al. 2010).

2.5 Cellular ROS Determination by DCFH-DA

Oxidation-sensitive dye DCFH-DA was used to detect the formation of intracellular reactive oxygen species (ROS), as described previously (Engelmann et al. 2005). For that, cells growing in fluorescence microtiter 96-well plates were labeled with 20 μM DCFH-DA in HBSS (Hanks balanced salt solution) for 20 min in the dark. The cells were then treated with different concentrations of samples and incubated for another 1 h. After washing the cells with PBS three times, 500 μM H_2O_2 was added. The intensity of the fluorescence signal emitted by 2′,7′-dichlorofluorescein (DCF) due to the oxidation of DCFH by cellular ROS was detected time dependently ($\lambda_{excitation}=485$ nm, $\lambda_{emission}=528$ nm) using a fluorescence microplate reader. Following the maximum rate of fluorescence increase, each well was normalized to cell numbers using the MTT cell viability assay. The effects of treatments were plotted and compared with the fluorescence intensity of the control and blank groups (Ngo et al. 2009).

2.6 Measurement of Intracellular GSH Level

The cellular GSH level was determined using monobromobimane (mBBr) as a thiol-staining reagent (Poot et al. 1986). The cells were seeded into fluorescence microtiter 96-well plates at a density of 1×10^7 cells/mL and, following attachment, treated with different concentrations of sample for 30 min. The cells were then labeled with 40 μM mBBr for 30 min at 37 °C in the dark. After staining, the mBBr-GSH fluorescence intensity was measured ($\lambda_{excitation}=360$ nm, $\lambda_{emission}=465$ nm) using the previously mentioned fluorescence microplate reader. The average fluorescence values of the cell populations were plotted and compared with the blank group, in which cells were grown without treatment of samples (Ngo et al. 2009).

2.7 Assessment of Cellular NO Production

The cells were cultured in 96-well plates using DMEM without phenol red and pretreatment for 1 h following the treatment of test materials. Cellular NO production

was induced by adding 1 μg/mL final concentration of LPS and incubated for 48 h. After incubation, 50 μl of conditioned media containing NO_2^- (primary stable oxidation product of NO) was mixed with the same volume of Griess reagent and incubated for 15 min (Green et al. 1982). The absorbance of the mixture at 550 nm was measured with a microplate reader.

2.8 Antioxidant Activities

In order to protect the cellular biomolecules (lipid, protein, and DNA) in biological systems from oxidation, equilibrium between oxidants formation and the endogenous antioxidant defense mechanisms exists. If this balance is disturbed, it can produce oxidative stress (Kang et al. 2005). This state of oxidative stress can result in injury to all the important cellular components, thus the reactive oxygen species (ROS) generated excessively in tissues can cause cell death. Furthermore, ROS have direct or indirect relationships with the oxidation of cellular biomolecules and play an important role in many diseases, such as cancer, arthritis, neurodegenerative, diabetes, hypertension, inflammation, and aging (Perry et al. 2000; Calabrese et al. 2005; Valko et al. 2007). Therefore, research to find antioxidant compounds from many natural resources has evoked great interest to overcoming the radical-mediated deleterious effects in biological systems. Many biological compounds have been identified as potent radical scavengers, including peptides, carbohydrates, and some phenolic compounds. Especially, many researchers reported the antioxidant activity of marine polysaccharides such as agaro-oligosaccharides, K-carrageenan oligosaccharides, seaweed extracts, and algae extracts (Ahn et al. 2004; Chen and Yan 2005; Yuan et al. 2005).

In recent research, the antioxidant activity of chitin and its derivatives have attracted great attention. Most of the reports indicate that chitin, chitosan, and their oligomers are non-toxic and their derivatives are non- or less toxic. Therefore, they can be applied in living systems. The antioxidant effect of chitosan with different MWs (30, 90, and 120 kDa) in salmon showed that the 30 kDa of chitosan gave rise to the highest antioxidant activity in all tests (Kim and Thomas 2007). Chitooligosaccharides (COSs) have shown that their radical-scavenging properties are dependent on the degree of deacetylation (DD) and MWs. Based on the results obtained from studies carried out using the electron spin trapping technique, COS with MW in the range 1–3 kDa and highly deacetylated (90 %) COSs have been identified to have a higher potential to scavenge different radicals, such as DPPH, hydroxyl, superoxide, and alkyl radicals (Park et al. 2004). Ngo and colleagues (2009) produced two kinds of chitin oligosaccharides or N-acetyl chitooligosaccharides (NA-COSs) with different MWs from crab chitin hydrolysis solution and also determined their effect against oxidative stress in live cells. Two kinds of NA-COSs with MW in the range 1–3 kDa (NA-COS 1–3 kDa) and below 1 kDa (NA-COS < 1 kDa) were obtained using an ultrafiltration membrane system. The results show that NA-COS 1–3 kDa was more effective than NA-COS < 1 kDa

against protein oxidation and the production of intracellular free radicals in live cells. Actually, they exhibited an inhibitory effect against DNA and protein oxidation to protect cells. In addition, in their presence, the intracellular glutathione (GSH) level and direct intracellular radical-scavenging effect were significantly increased in a time-dependent manner in mouse macrophages (RAW 264.7 cells) and rendered an inhibitory effect against cellular oxidative stress. Furthermore, chitin oligosaccharides with a low MW distribution of 229.21–593.12 Da were produced from crab chitin by acid hydrolysis. They also showed reducing power and scavenging effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, and alkyl radicals. It was observed that the radical-scavenging activity of NA-COS increased in a dose-dependent manner. Their IC_{50} values for DPPH, hydroxyl, and alkyl radicals were 0.8, 1.75, and 1.14 mg/mL, respectively (Ngo et al. 2010).

Chen et al. (2003) investigated the antioxidant activity of chitobiose and chitotriose using the inhibition of H_2O_2-induced hydroxylation of benzoate and free radical-scavenging effects. The results showed that chitobiose is more effective as an antioxidant than chitotriose and even glucosamine hydrochloride displayed an antioxidant effect. Moreover, the derivatives of chitin, chitosan, and their oligomers, such as acylated chitosan, N,O-carboxymethyl chitosan (NOCC), hydroxypropylated chitosan (HPC), hexanoyl chitin (HCH), N-benzoylhexanoyl chitosan (NBHC), sulfated chitosan, low MW carboxymethyl chitosan (LMWCMC), chitosan gallic acid, aminoderivatized chitosan, quaternary chitosan, carboxylated chitooligosaccharides, and sulfated glucosamine, were prepared by chemical modification, and have been assayed by their antioxidant capacity (Matsugo et al. 1998; Xue et al. 1998; Xie et al. 2001; Xing et al. 2005a, b; Sun et al. 2007; Pasanphan and Chirachanchai 2008; Je and Kim 2006; Guo et al. 2006; Rajapakse et al. 2007; Mendis et al. 2008; Ngo et al. 2011a, 2012). Acylated chitosan inhibited thiobarbituric acid reactive substrate formation in tert-butyl hydroperoxide and benzoyl peroxide induced lipid peroxidation. However, they did not show any scavenging activity toward superoxide (Matsugo et al. 1998). NOCC and HPC inhibited the peroxidation of phosphatidylcholine, HCH and NBHC, liposoluble derivatives, and also retarded the hydroperoxide accumulation of methyl linoleate by effectively trapping peroxide radicals in organic solvents (Xue et al. 1998). Xie et al. (2001) prepared hydroxypropyl chitosan maleic acid sodium and carboxymethyl chitosan maleic acid sodium by graft copolymerization, and investigated hydroxyl radical-scavenging effects. Different MWs of sulfated chitosan were synthesized and tested for their antioxidant activity. The results suggested that their antioxidant activity depended on the MW, and low MWs showed a stronger effect (Xing et al. 2005a).

Sun et al. (2007) synthesized LMWCMC, which had superoxide anion scavenging activity. In addition, a novel synthetic chitosan derivative, chitosan gallic acid, has a wide range of antioxidant activity, including alkyl and hydroxyl radicals (Pasanphan and Chirachanchai 2008). Je and Kim (2006) also synthesized novel aminoderivatized chitosans (aminoethyl-chitosan, AEC, dimethylaminoethyl-chitosan, DMAEC, and diethylaminoethyl-chitosan, DEAEC) with different DDs (50 % and 90 %). AEC with 90 % DD showed the highest scavenging effects against hydroxyl and superoxide anion radicals, being 91.67 % and 65.34 % at 0.25 and

5 mg/mL, respectively. The results also showed that their antioxidant effect depends on the substituted group. Furthermore, when comparing the scavenging effect of quaternized COS with carboxylated COS, Huang et al. (2006) found that their scavenging effects against hydroxyl and alkyl radicals depend not only on the substituted group but also the special structure of the group.

However, there are some discrepancies about the hydroxyl radical-scavenging activities of COSs and some of their derivatives (Xie et al. 2001). Rajapakse et al. (2007) and Mendis et al. (2007) investigated carboxylated COS and sulfated glucosamine, which are non-toxic and exhibited inhibition of free radical-mediated oxidation of cellular biomolecules in live cells. Even though the precise mechanism of the radical-scavenging activity of chitin, chitosan, and their oligomers are not yet clear, it is attributed that the amino and hydroxyl groups attached to the C-2, C-3, and C-6 positions of the pyranose ring react with unstable free radicals to form stable macromolecule radicals. In the recent studies of Ngo et al. (2011a, b), gallyl-COS was synthesized and its inhibition of oxidative damage to lipids, proteins, and DNA in cells was demonstrated. In addition, gallate-COS could decrease the activation and expression of NF-κB and increase the level of intracellular antioxidant enzymes (SOD and GSH) in oxidative stress-induced cells. Furthermore, Ngo et al. (2012a, b) carried out a study on the inhibitory effect of oxidation of aminoethyl-COS to protect cells against DNA, lipid, and protein damage. Moreover, it stimulated an increase in the intracellular glutathione (GSH) level and inhibited direct intracellular free radicals in a time-dependent manner in cells (Ngo et al. 2012).

3 Anti-inflammatory Activities

Inflammation is the reaction of a living tissue against damage and is a part of the host response to either internal or external environmental stimuli. It is a defensive mechanism to return to a normal state by localizing the damage when a biologic tissue is damaged for some reason. The reactions of blood vessels, nerves, body fluid, and cells are related to inflammation. Causes of inflammation include biological agents (bacteria), physical agents (injury), chemical agents (endogenous or exogenous chemical substances), and immunological agents.

Inflammation reactions are characterized by the change in blood vessels, exudation of liquid, and invasion of leukocytes, and typical symptoms are swelling, redness, fever, pain, and, sometimes, loss of function. The natural cell control carrier of the present invention is particularly superior in the restoration of leukocyte invasion (Gao et al. 2006; Goswami et al. 2008). In addition, inflammation is one phase in four phases of normal wound healing (hemostasis, inflammation, proliferation, and remodeling). However, a chronic wound can be defined as a wound in which the normal process of healing has been disrupted at one or more points in the four phases, especially the inflammatory or proliferation phases, which can last a long time and will slow down the process of wound healing (Enoch and Harding 2003; Diegelmann and Evans 2004).

Furthermore, if acute inflammation is manifested for a short period of time, it is a part of the host defense response, whereas for inflammation that lasts too long and becomes chronic, it can prove harmful and may lead to disease. Chronic inflammation has been found to mediate a wide variety of diseases, including cardiovascular diseases, diabetes, arthritis, cancer, Alzheimer's disease, pulmonary diseases, and autoimmune diseases (Alexandrova and Bochev 2005; Canonica 2006).

For inflammation exhibited through cell signaling pathways, among them, nuclear factor-kappa B (NF-κB) and activator protein 1 (AP-1) are the pivotal transcription factors that orchestrate the expression of many genes involved in inflammation as well as other important events, such as embryonic development, lymphoid differentiation, oncogenesis, and apoptosis (Li and Verma 2002; Prashanth and Tharanathan 2005).

NF-κB and AP-1 activities are induced by a plethora of physiological and environmental stimuli through different cellular signal transduction cascades. The NF-κB transcription family proteins consists of c-Rel, p65 (RelA), RelB, p50 (NF-κB1), and p52 (NF-κB2). These factors are found in a variety of active homo- and heterodimers. A heterodimer consisting of a p50 or p52 subunit and p65, which contains transactivation domains necessary for gene induction, are usually in the inactivated form of NF-κB (Baeuerele and Henkel 1994). The activated or inactivated forms of NF-κB are regulated by the inhibitor of NF-κB proteins, known as IκB. When IκB interacts with NF-κB, it forms the inactive NF-κB/IκB complexes in the cytoplasm (Prashanth and Tharanathan 2005). In the activated form, NF-κB enters the nucleus, where it binds to cognate DNA binding sites within the promoters of target genes, thereby enhancing transcription.

As a transcription factor, NF-κB is central to driving the expression of many mediators of inflammation, including proinflammatory cytokines, such as interleukins (IL-1α, IL-1β, IL-6), and tumor necrosis factor alpha (TNF-α), as well as numerous chemokines, including IL-8. The full induction of IL-2 and IFN-γ production by T cells is also mediated by NF-κB. In addition, NF-κB is responsible for increasing the cell surface expression of various cell adhesion molecules required for leukocyte transendothelial migration, as well as cyclooxygenases (COXs such as COX-1, COX-2, and COX-3; enzymes required for prostaglandin synthesis) and NO synthases (NOSs such as endothelial NOS, eNOS, neuronal NOS, nNOS, and inducible NOS, iNOS, an enzyme required for NO production). NF-κB is not the only transcription factor involved in the regulation of these genes, and it frequently functions together with other transcription factors, most notably, activator protein 1 (AP-1). The impacts of the activation of NF-κB and AP-1 during the inflammatory sequence directly correlated with the expression and activation of inflammation-related genes and their protein products (Ngo et al. 2012).

That is because the above transcription factors are essential for the transcription of genes that encode a number of proinflammatory molecules which participate in the acute inflammatory response. Transcriptional upregulation of this battery of genes constitutes a phenotypic switch that might be propagated to other cells in a feed-forward manner (Peng et al. 1995). Especially, the immune cells, macrophages, and monocytes play a crucial role in eliciting the inflammatory response cascades

via NF-κB and AP-1 in the acute phase of inflammation (Baumann and Gauldie 1994). After being stimulated, they produce a number of chemokines, enzymes such as cytokine, iNOS, and COX-2, for the primary protection of the host (Kim et al. 2003). However, the accumulation of these defense molecules is excessive and uncontrollable, leading to a more severe and acute level of inflammation.

NO and prostaglandins, the end products of major inflammatory enzymes, iNOS and COX-2, have been extensively studied for their activation through NF-κB and AP-1 (Bengmark 2006). Both iNOS and COX-2 genes have putative binding sites for NF-κB and/or AP-1 at their promoter sites to activate gene expressions. The activation and expression of NF-κB and AP-1 during inflammation has a remarkable link with the production of NO and a number of other inflammatory prostaglandins, including prostaglandin E_2 (PGE_2). In addition, they also attend in matrix metalloproteinases (MMPs) of inflammation, a group of zinc-dependent gelatinases and collagenases that are activated through MAPK pathways, promoting the activation of NF-κB and AP-1 (Huang et al. 2005). As major sources of MMPs, immune cells rely heavily on these enzymes to mediate extravasation into tissues during the inflammatory process and, if there is overproduction of MMPs during chronic inflammation, this may lead to cancer. Therefore, aside from non-steroidal anti-inflammatory drugs (NSAIDs), which share the therapeutic action of aspirin, a well-known non-specific NSAID to inhibit NF-κB-induced COX-2 activation and expression (Hawkey et al. 2001; Arellano et al. 2006), in recent studies, researchers have been interested in natural compounds which can inhibit inflammation; one of them is chitin and its derivatives. Until now, there have been few reports on their anti-inflammatory and stimulatory effects accelerating the wound-healing process.

Okamura et al. (2005) investigated the effects of chitin, chitosan, and their oligomers/monomers on the release of type I collagenase (MMP-1) from fibroblasts. The results showed that they influenced the release of MMP-1, which plays a central role in the final phase of wound healing; most of them tend to increase MMP-1 release from adult human fibroblasts (Okamura et al. 2005). In addition, glucosamine hydrochloride (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc) were of benefit for cartilage repair (Tamai et al. 2002, 2003), GlcNAc inhibited IL-1β and TNF-α, induced NO production in normal human articular chondrocytes, as well as COX-2 and IL-6 from chondrocytes (Shikhman et al. 2001).

Chitosan activates peritoneal macrophages; this may be the mechanism by which it accelerates wound healing (Mori et al. 2005). Sacchachitin membrane from fungal mycelia is able to promote wound healing by inducing cell proliferation (Su et al. 1999). Furthermore, chitosan inhibited the formation of PGE_2, COX-2 induction accompanied the inhibition of TNF-α and IL-1β formation, and these effects depend on the MW. It was shown that chitosan had a beneficial effect on accelerating wound healing and anti-inflammation (Chou et al. 2003). Rajapakse et al. (2008) investigated the effect of carboxybutyrylated glucosamine (CGlcN) on the expression of iNOS and COX-2 in bacterial lipopolysaccharide (LPS)-induced RAW264.7 cells. The results showed that CGlcN inhibited the production of NO and PGE_2 and gave rise to the possibility of downregulating their respective genes, iNOS and COX-2, via the attenuation of NF-κB signaling by the p38 mitogen-activated

protein kinase (p38 MAPK) and the c-Jun N-terminal kinase (JNK). The authors suggested that CGlcN is a potential anti-inflammatory material. In the latest research, Ngo et al. (2012) investigated the potency of aminoethyl-chitooligosaccharide (AE-COS) against lipopolysaccharide-induced inflammatory responses in BV-2 cells. They found that it reduced the level of NO and PGE_2 production by decreasing the expression of two kinds of enzymes, inducible NO synthase and cyclooxygenase-2, without significant cytotoxicity. The authors also demonstrated the inhibitory effect of AE-COS on the generation of TNF-α and IL-1β. Therefore, chitin, chitosan, and their derivatives could be useful therapeutic agents for the treatment of inflammatory diseases.

4 Some Applications

At present, chitin, chitosan, and their derivatives have recently attracted commercial interest as a potential source for industrial applications. There are many reports on their biological activities and applications as food supplements, nutraceuticals, and cosmeceuticals because of their antioxidant activity. Furthermore, they can also be used as a therapeutic agent in the treatment of inflammatory diseases.

The applications of chitin and chitosan are summarized in Table 19.1 (Shahidi et al. 1999; Jeon et al. 2000; Kim et al. 2006; Rinaudo 2006; Jayakumar et al. 2010, 2011).

In particular, chitin, chitosan, and their derivatives were studied for their application in tissue engineering and wound healing. Tissue engineering is an emerging field that aims to regenerate natural tissues or create new tissues using biomaterials for clinical medicine. Current applications of tissue engineering in regenerative stimulators include the development of various scaffolds for the treatment of skin, cartilage, and bone injuries or diseases. Biomaterials that are selected for scaffold development should possess basic characters such as biocompatibility, biodegradability, mechanical stability, and surface affinity as well as others. A number of studies have demonstrated that chitin, chitosan, and their derivatives can be successfully applied as a promising biomaterial to develop scaffolds for the transplantation and regeneration of tissues with desirable biological properties (Aimin et al. 1999; VandeVord et al. 2002; Li and Zhang 2005; Bhardwaj and Kundu 2011; Chen et al. 2011). The majority of wound healings occur without any complications and with little tissue loss, whereas chronic wounds disrupt phases of the normal healing process and often require external supporting factors to be recovered. Therefore, chronic non-healing wounds involving progressive tissue loss have becomes a major challenge to wound-care product researchers. During the past several years, researchers have discovered chitin, chitosan, and their derivatives as ideal wound covering and grafting materials to treat such wounds, accelerating wound healing and mimicking many properties of natural human skin (Jayakumar et al. 2010). For example, chitosan-LA was soft, flexible, pliable, and bioadhesive, and did not cause inflammation, edema, or systemic toxicity as a successful wound dressing.

Table 19.1 Applications of chitin and chitosan

Field	Applications
Food and beverages	Dietary fiber (reduce cholesterol and lipid-binding agent)
	Food and fruit preservation (antibacterial and antifungal agents and film)
	Food stabilizer
Medicine and pharmaceutics	Wound-healing ointments and dressings
	Contact lenses
	Orthopedic surgery
	Drug and gene carriers
	Antitumor agents
	Immunological stimulant
	Dental implants
	Anticoagulant
Cosmetics and toiletries	Skin care (skin moisture, acne treatment)
	Hair treatment
	Oral care (toothpaste, chewing gum)
Water and waste treatment	Drinking water and pools
	Removal of metal ions
	Ecological polymer
Agriculture	Seed coating
	Foodstuff for animal
Biotechnology	Enzyme and cell immobilization
	Nanoparticles

Moreover, chitin and chitosan have enhanced the wound-healing process because of their stimulation of the attachment and proliferation of keratinocytes and fibroblasts favoring tissue regeneration, especially epithelial restructuring. Chitosan and its oligomers help macrophages and neutrophils to accelerate their innate immunity and healing process by modulating factors released from immune cells in proliferation. Another attractive property of chitin and chitosan is that they can be easily incorporated with other biomaterials, such as collagen, gelatin, alginate, carrageenan, pectin, carboxymethyldextran, and hyaluronic acid in the presence of glutaraldehyde or other common cross-linking agents (Hu et al. 2003) to improve the physicochemical and biological properties of wound dressings. Furthermore, other additives such as growth factors, cytokines, and antibacterial agents play important roles in each stage of the process of wound healing. Therefore, alterations in one or more components of these factors many account for the impaired healing observed in chronic wounds. The incorporation of some materials such as growth factors, extracellular matrix components, and antibacterial agents to chitosan-based wound dressings or scaffolds can improve the wound-healing efficacy in animal models. Such improvements in wound healing were due to the polyelectrolyte property of chitosan materials that allows retainment above biomolecules within their structure and gradual releasing over time (Kim et al. 2006). Many products containing chitin, chitosan, and their derivatives for the treatment of wounds have already been marketed in various forms, including hydrogels, bandages, woven fibers, and wound dressing powders (Jayakumar et al. 2010, 2011).

5 Conclusion

Chitin, chitosan, and their derivatives have important biological properties such as immunostimulatory, antioxidant, anti-inflammatory, antimicrobial, anticancer, anti-cholesteremic, anticoagulant, and wound-healing agents. To date, there have been many reports on the biological activities of derivatives of chitin and chitosan, which are easily soluble in water, helping people to apply them in various scenarios to escape from the limits of chitin, an insoluble polymer in water. In particular, scientists have synthesized many novel derivatives that have activity against oxidative stress and inflammation. Therefore, chitin, chitosan, and their derivatives are being applied more and more often as functional food and wound-healing products.

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References

Ahn C-B, Jeon Y-J, Kang D-S, Shin T-S, Jung B-M (2004) Free radical scavenging activity of enzymatic extracts from a brown seaweed *Scytosiphon lomentaria* by electron spin resonance spectrometry. Food Res Int 37:253–258

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

Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D (1999) Antibiotic loaded chitosan bar. An in vitro, in vivo study of a possible treatment for osteomyelitis. Clin Orthop Relat Res 366:239–247

Alexandrova ML, Bochev PG (2005) Oxidative stress during the chronic phase after stroke. Free Radic Biol Med 39:297–316

Arellano FM, Yood MU, Wentworth CE, Oliveria SA, Rivero E, Verma A, Rothman KJ (2006) Use of cyclo-oxygenase 2 inhibitors (COX-2) and prescription non-steroidal anti-inflammatory drugs (NSAIDS) in UK and USA populations. Implications for COX-2 cardiovascular profile. Pharmacoepidemiol Drug Saf 15:861–872

Baeuerle PA, Henkel T (1994) Function and activation of NF-kappaB in the immune system. Annu Rev Immunol 12:141–179

Baumann H, Gauldie J (1994) The acute phase response. Immunol Today 15:74–80

Bengmark S (2006) Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases. JPEN J Parenter Enteral Nutr 30:45–51

Bhardwaj N, Kundu SC (2011) Silk fibroin protein and chitosan polyelectrolyte complex porous scaffolds for tissue engineering applications. Carbohydr Polym 85:325–333

Byun H-G, Kim Y-T, Park P-J, Lin XL, Kim S-K (2005) Chitooligosaccharides as a novel β-secretase inhibitor. Carbohydr Polym 61:198–202

Calabrese V, Lodi R, Tonon C, D'Agata V, Sapienza M, Scapagnini G, Mangiameli A, Pennisi G, Giuffrida Stella AM, Butterfield DA (2005) Oxidative stress, mitochondrial dysfunction and cellular stress response in Friedreich's ataxia. J Neuro Sci 233:145–162

Canonica GW (2006) Treating asthma as an inflammatory disease Chest. Chest J 130:21S–28S

Chen HM, Yan XJ (2005) Antioxidant activities of agaro-oligosaccharides with different degrees of polymerization in cell-based system. Biochim Biophys Acta 1722:103–111

Chen AS, Taguchi T, Sakai K, Kikuchi K, Wang MW, Miwa I (2003) Antioxidant activities of chitobiose and chitotriose. Biol Pharm Bull 26:1326–1330

Chen H, Huang J, Yu J, Liu S, Gu P (2011) Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering. Int J Biol Macromol 48:13–19

Chou T-C, Fu E, Shen EC (2003) Chitosan inhibits prostaglandin E_2 formation and cyclooxygenase-2 induction in lipopolysaccharide-treated RAW 264.7 macrophages. Biochem Biophys Res Commun 308:403–407

Diegelmann RF, Evans MC (2004) Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 9:283–289

Engelmann J, Volk J, Leyhausen G, Geurtsen W (2005) ROS formation and glutathione levels in human oral fibroblasts exposed to TEGDMA and camphorquinone. J Biomed Mater Res Part B Appl Biomater 75:272–276

Enoch S, Harding K (2003) Wound bed preparation: the science behind the removal of barriers to healing. Wounds 15:213–229

Gao H, Neff T, Ward PA (2006) Regulation of lung inflammation in the model of IgG immune-complex injury. Annu Rev Pathol Mech Dis 1:215–242

Goswami B, Rajappa M, Sharma M, Sharma A (2008) Inflammation: its role and interplay in the development of cancer, with special focus on gynecological malignancies. Int J Gynecol Cancer 18:591–599

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [^{15}N]nitrate in biological fluids. Anal Biochem 126:131–138

Guo Z, Liu H, Chen X, Ji X, Li P (2006) Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan. Bioorg Med Chem Lett 16:6348–6350

Han Y, Zhao L, Yu Z, Feng J, Yu Q (2005) Role of mannose receptor in oligochitosan-mediated stimulation of macrophage function. Int Immunopharmacol 5:1533–1542

Harish Prashanth KV, Tharanathan RN (2005) Depolymerized products of chitosan as potent inhibitors of tumor-induced angiogenesis. Biochim Biophys Acta 1722:22–29

Hawkey CJ, Jackson L, Harper SE, Simon TJ, Mortensen E, Lines CR (2001) The gastrointestinal safety profile of rofecoxib, a highly selective inhibitor of cyclooxygenase-2, in humans. Aliment Pharmacol Ther 15:1–9

Hu SG, Jou CH, Yang MC (2003) Protein adsorption, fibroblast activity and antibacterial properties of poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) grafted with chitosan and chitooligosaccharide after immobilized with hyaluronic acid. Biomaterials 24:2685–2693

Huang R, Du Y, Yang J (2003) Preparation and anticoagulant activity of carboxybutyrylated hydroxyethyl chitosan sulfates. Carbohydr Polym 51:431–438

Huang SC, Ho CT, Lin-Shiau SY, Lin JK (2005) Carnosol inhibits the invasion of B16/F10 mouse melanoma cells by suppressing metalloproteinase-9 through down-regulating nuclear factor-kappa B and c-Jun. Biochem Pharmacol 69:221–232

Huang R, Rajapakse N, Kim S-K (2006) Structural factors affecting radical scavenging activity of chitooligosaccharides (COS) and its derivatives. Carbohydr Polym 63:122–129

Jayakumar R, Prabaharan M, Nair SV, Tokura S, Tamura H, Selvamurugan N (2010) Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Prog Mater Sci 55:675–709

Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotech Adv 29:322–337

Je J-Y, Kim S-K (2005) Water-soluble chitosan derivatives as a BACE1 inhibitor. Bioorg Med Chem 13:6551–6555

Je J-Y, Kim S-K (2006) Reactive oxygen species scavenging activity of aminoderivatized chitosan with different degree of deacetylation. Bioorg Med Chem 14:5989–5994

Je J-Y, Park P-J, Kim B, Kim S-K (2006) Antihypertensive activity of chitin derivatives. Biopolymer 83:250–254

Je J-Y, Kim E-K, Ahn C-B, Moon S-H, Jeon B-T, Kim BK, Park T-K, Park P-J (2007) Sulfated chitooligosaccharides as prolyl endopeptidase inhibitor. Int J Biol Macromol 41:529–533

Jeon Y-J, Kim S-K (2002) Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. J Microbiol Biotechnol 12:503–507

Jeon Y-J, Shahidi F, Kim S-K (2000) Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. Food Rev Int 16:159–176

Kang KA, Lee KH, Chae SW, Zhang R, Jung MS, Lee YK, Kim SY, Kim HS, Joo HG, Park JW, Ham YM, Lee NH, Hyun JW (2005) Eckol isolated from *Ecklonia cava* attenuates oxidative stress induced cell damage in lung fibroblast cells. FEBS Lett 579:6295–6304

Kim KW, Thomas RL (2007) Antioxidative activity of chitosans with varying molecular weights. Food Chem 101:308–313

Kim S-K, Park P-J, Yang H-P, Han S-S (2001) Subacute toxicity of chitosan oligosaccharide in Sprague-Dawley rats. Arzneimittelforschung 51:769–774

Kim SS, Oh O-J, Min H-Y, Park E-J, Kim Y, Park HJ, Ham YN, Lee SK (2003) Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells. Life Sci 73:337–348

Kim S-K, Nghiep ND, Rajapakse N (2006) Therapeutic prospectives of chitin, chitosan and their derivatives. J Chitin Chitosan 11:1–10

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–478

Li Q, Verma IM (2002) NF-kappaB regulation in the immune system. Nat Rev Immunol 2:725–734

Li Z, Zhang M (2005) Chitosan–alginate as scaffolding material for cartilage tissue engineering. J Biomed Mater Res Part A 75A:485–493

Liang T-W, Chen Y-J, Yen Y-H, Wang S-L (2007) The antitumor activity of the hydrolysates of chitinous materials hydrolyzed by crude enzyme from *Bacillus amyloliquefaciens* V656. Process Biochem 42:527–534

Matsugo S, Mizuie M, Matsugo M, Ohwa R, Kitano H, Konishi T (1998) Synthesis and antioxidant activity of water-soluble chitosan derivatives. IUBMB Life 44:939–948

Mendis E, Kim M-M, Rajapakse N, Kim S-K (2007) An in vitro cellular analysis of the radical scavenging efficacy of chitooligosaccharides. Life Sci 80:2118–2127

Mendis E, Kim M-M, Rajapakse N, Kim S-K (2008) Sulfated glucosamine inhibits oxidation of biomolecules in cells via a mechanism involving intracellular free radical scavenging. Eur J Pharmacol 579:74–85

Milne L, Nicotera P, Orrenius S, Burkitt MJ (1993) Effects of glutathione and chelating agents on copper-mediated DNA oxidation: pro-oxidant and antioxidant properties of glutathione. Arch Biochem Biophys 304:102–109

Muzzarelli RAA (2002) The discovery of chitin, a >570 megayear old polymer. In: Chitosan in pharmacy and chemistry. Atec, Italy, pp 1–8

Naito Y, Yoshikawa T, Matsuyama K, Yagi N, Arai M, Nakamura Y, Kaneko T, Yoshida N, Kondo M (1998) Neutrophils, lipid peroxidation, and nitric oxide in gastric reperfusion injury in rats. Free Radic Biol Med 24:494–502

Ngo D-N, Kim M-M, Kim S-K (2007) Effects of chitin oligosaccharides on production of reactive oxygen species and matrix metalloproteinases in live cells. In: Senel S, Varum KM, Murat SM, Atilla HA (eds) Advances in chitin science. Antalya, Turkey, vol X, pp 355–359

Ngo D-N, Kim M-M, Kim S-K (2008a) Chitin oligosaccharides inhibit oxidative stress in live cells. Carbohydr Polym 74:228–234

Ngo D-N, Qian Z-J, Je J-Y, Kim M-M, Kim S-K (2008b) Aminoethyl chitooligosaccharides inhibit the activity of angiotensin converting enzyme. Process Biochem 43:119–123

Ngo D-N, Lee S-H, Kim M-M, Kim S-K (2009) Production of chitin oligosaccharides with different molecular weights and their antioxidant effect in RAW 264.7 cells. J Funct Foods 1:188–198

Ngo D-N, Kim M-M, Qian Z-J, Jung W-K, Lee S-H, Kim S-K (2010) Free radical-scavenging activities of low molecular weight chitin oligosaccharides lead to antioxidant effect in live cells. J Food Biochem 34(s1):161–177

Ngo D-H, Qian Z-J, Ngo D-N, Vo T-S, Wijesekara I, Kim S-K (2011a) Gallyl chitooligosaccharides inhibit intracellular free radical-mediated oxidation. Food Chem 128:974–981

Ngo D-H, Qian Z-J, Vo T-S, Ryu BM, Ngo D-N, Kim S-K (2011b) Antioxidant activity of gallate-chitooligosaccharides in mouse macrophage RAW264.7 cells. Carbohydr Polym 84:1282–1288

Ngo D-H, Ngo D-N, Vo T-S, Ryu BM, Ta Q-V, Kim S-K (2012a) Protective effects of aminoethyl-chitooligosaccharides against oxidative stress and inflammation in murine microglial BV-2 cells. Carbohydr Polym 88:743–747

Ngo D-N, Kim M-M, Kim S-K (2012b) Protective effects of aminoethyl-chitooligosaccharides against oxidative stress in mouse macrophage RAW 264.7 cells. Int J Biol Macromol 50:624–631

Nishimura K, Azuma I (1992) Immunomodulating activities of chitin derivatives. In: Tokura S, Azuma I (eds) Chitin derivatives in life science. Organizing Committee of International Symposium on Chitin Derivatives in Life Sciences and Japanese Society for Chitin/Chitosan, pp 7–11

Okamura Y, Nomura A, Minami S, Okamoto Y (2005) Effects of chitin/chitosan and their oligomers/monomers on release of type I collagenase from fibroblasts. Biomacromolecules 6:2382–2384

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

Pasanphan W, Chirachanchai S (2008) Conjugation of gallic acid onto chitosan: an approach for green and water-based antioxidant. Carbohydr Polym 72:169–177

Peng HB, Libby P, Liao JK (1995) Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. J Biol Chem 270:14214–14219

Perry G, Raina AK, Nunomura A, Wataya T, Sayre LM, Smith MA (2000) How important is oxidative damage? Lessons from Alzheimer's disease. Free Radic Biol Med 28:831–834

Poot M, Verkerk A, Koster JF, Jongkind JF (1986) De novo synthesis of glutathione in human fibroblasts during in vitro ageing and in some metabolic diseases as measured by a flow cytometric method. Biochim Biophys Acta 883:580–584

Rajapakse N, Kim M-M, Mendis E, Kim S-K (2007) Inhibition of free radical-mediated oxidation of cellular biomolecules by carboxylated chitooligosaccharides. Bioorg Med Chem 15:997–1003

Rajapakse N, Kim M-M, Mendis E, Kim S-K (2008) Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 in lipopolysaccharide-stimulated RAW264.7 cells by carboxybutyrylated glucosamine takes place via down-regulation of mitogen-activated protein kinase-mediated nuclear factor-κB signaling. Immunology 123:348–357

Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31:603–632

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, pp 84–87

Sashiwa H, Aiba S-i (2004) Chemically modified chitin and chitosan as biomaterials. Prog Polym Sci 29:887–908

Sashiwa H, Fujishima S, Yamano N, Kawasaki N, Nakayama A, Muraki E, Hiraga K, Oda K, Aiba S-i (2002) Production of *N*-acetyl-D-glucosamine from α-chitin by crude enzymes from *Aeromonas hydrophila* H-2330. Carbohydr Res 337:761–763

Sashiwa H, Fujishima S, Yamano N, Kawasaki N, Nakayama A, Murakia E, Sukwattanasinitt M, Pichyangkura R, Aiba S-i (2003) Enzymatic production of *N*-acetyl-D-glucosamine from chitin. Degradation study of *N*-acetylchitooligosaccharide and the effect of mixing of crude enzymes. Carbohydr Polym 51:391–395

Seferian PG, Martinez ML (2000) Immune stimulating activity of two new chitosan containing adjuvant formulations. Vaccine 19:661–668

Seo W-G, Pae H-O, Kim N-Y, Oh G-S, Park I-S, Kim Y-H, Kim Y-M, Lee Y-H, Jun C-D, Chung H-T (2000) Synergistic cooperation between water-soluble chitosan oligomers and interferon-γ for induction of nitric oxide synthesis and tumoricidal activity in murine peritoneal macrophages. Cancer Lett 159:189–195

Shahidi F, Arachchi JKV, Jeon Y-J (1999) Food applications of chitin and chitosans. Trends Food Sci Technol 10:37–51

Shikhman AR, Kuhn K, Alaaeddine N, Lotz M (2001) N-acetylglucosamine prevents IL-1 beta-mediated activation of human chondrocytes. J Immunol 166:5155–5160

Su C-H, Sun C-S, Juan S-W, Ho H-O, Hu C-H, Sheu M-T (1999) Development of fungal mycelia as skin substitutes: effects on wound healing and fibroblast. Biomaterials 20:61–68

Sun T, Zhou D, Mao F, Zhu Y (2007) Preparation of low-molecular-weight carboxymethyl chitosan and their superoxide anion scavenging activity. Eur Polym J 43:652–656

Suzuki S (1998) Trends in application studies on chitic substances as drugs. Chitin Chitosan Res 4:1–11

Takahashi M, Shibata M, Niki E (2001) Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. Free Radic Biol Med 31:164–174

Tamai Y, Miyatake K, Okamoto Y, Takamori Y, Sakamoto K, Minami S (2002) Enhanced healing of cartilaginous injuries by glucosamine hydrochloride. Carbohydr Polym 48:369–378

Tamai Y, Miyatake K, Okamoto Y, Takamori Y, Sakamoto K, Minami S (2003) Enhanced healing of cartilaginous injuries by *N*-acetyl-D-glucosamine and glucuronic acid. Carbohydr Polym 54:251–262

Usami Y, Minami S, Okamoto Y, Matsuhashi A, Shigemasa Y (1997) Influence of chain length of *N*-acetyl-D-glucosamine and D-glucosamine residues on direct and complement-mediated chemotactic activities for canine polymorphonuclear cells. Carbohydr Polym 32:115–122

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84

VandeVord PJ, Matthew HWT, DeSilva SP, Mayton L, Wu B, Wooley PH (2002) Evaluation of the biocompatibility of a chitosan scaffold in mice. J Biomed Mater Res 59:585–590

Weiner ML (1992) An overview of the regulatory status and of the safety of chitin and chitosan as food and pharmaceutical ingredients. In: Brine CJ, Sandford PA, Zikakis JP (eds) Advances in chitin and chitosan. Elsevier Applied Science, New York, pp 663–672

Xie W, Xu P, Liu Q (2001) Antioxidant activity of water-soluble chitosan derivatives. Bioorg Med Chem Lett 11:1699–1701

Xing R, Liu S, Guo Z, Yu H, Wang P, Li C, Li Z, Li P (2005a) Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. Bioorg Med Chem 13:1573–1577

Xing R, Liu S, Yu H, Guo Z, Wang P, Li C, Li Z, Li P (2005b) Salt-assisted acid hydrolysis of chitosan to oligomers under microwave irradiation. Carbohydr Res 340:2150–2153

Xue C, Yu G, Hirata T, Terao J, Lin H (1998) Antioxidative activities of several marine polysaccharides evaluated in a phosphatidylcholine-liposomal suspension and organic solvents. Biosci Biotechnol Biochem 62:206–209

Yuan H, Zhang W, Li X, Lü X, Li N, Gao X, Song J (2005) Preparation and in vitro antioxidant activity of κ-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. Carbohydr Res 340:685–692

Chapter 20
Lipid Bodies Isolated Microalgae Residues as a Source for Bioethanol Production

Chamila Jayasinghe and David Gray

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1 Introduction

Microalgae are eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure (Li et al. 2008). Eukaryotic algae are a very diverse group of organisms, which inhabit a huge range of ecosystems, from the Antarctic to deserts (Guschina

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and Harwood 2006). Green algae are the primary food chain source of omega-3 fatty acids in fish, and some species (e.g., *Nannochloropsis*) are being cultivated for their DHA-enriched oil. Microalgae are valuable sources of a multitude of products. They account for over half the primary productivity at the base of the marine food chain (Guschina and Harwood 2006). Depending on the microalgae species, various high-value chemical compounds may be extracted, such as pigments, antioxidants, carotenes, polysaccharides, triacylglycerols, fatty acids, vitamins, and biomass, which are largely used as bulk commodities in different industrial sectors (Barrow and Shahidi 2008), such as pharmaceutical, cosmetics, nutraceutical, functional foods, and biofuel production. The red microalgae are characterized by their accessory pigments, known as phycobiliproteins, which are red or blue. These algal pigments have potential as natural colorants for use in food, cosmetics, and pharmaceuticals, particularly as substitutes for synthetic dyes (Arad and Yaron 1992). Besides, microalgae also act as prebiotic agents that positively affect the health of humans. Today, microalgae, marketed as health food or food supplements, are commonly sold in the form of tablets, capsules, and liquids. They are also incorporated in pasta, snack food, candy bars or gums, in drink mixes and beverages, to name a few, either as a nutritional supplement or as a source of natural colorant (Goh et al. 2009). Lipid bodies are cytoplasmic lipid globules that exist naturally in microalgae and it is believed that lipid bodies are the best vesicle to transport omega-3 fatty acids to several food systems, such as emulsions. This chapter describes lipid bodies in microalgae, the extraction procedure of lipid bodies, and avenues of post-extraction algal residue utilization for bioethanol and methane production.

2 Microalgae

The microalgae cell is generally surrounded by a rigid cell wall, which consists of two components: a microfibrillar framework of cellulose and unstructured mucilaginous material. It has been reported that the cell walls of microalgae in the Haptophyta division (e.g., *Pavlova lutheri*) are typically covered in calcium carbonate scales, which are crystallized onto an organic matrix, while microalgae in the Prasinophyceae class (e.g., *Tetraselmis* sp.) of the Chlorophyta division have a cell wall covered in several polysaccharide scales measuring 50 nm (Barsanti and Gualtieri 2006). Organelles within the cytoplasm of the microalgae cell are: nuclei containing the major part of the genome, chloroplasts (enveloped by a double membrane and comprised of thylakoid membrane stacks), mitochondria, ribosomes, endoplasmic reticulum, vacuoles, centrioles, and Golgi bodies. Storage structures, such as starch granules (SG) and lipid bodies (LB), are common in the cytoplasm of microalgae, although their abundance depends on the function of culturing conditions (Wang et al. 2009). Figure 20.1 illustrates the ultrastructure of the *Isochrysis galbana* cells that consist of LB, SG, plastoglobuli (PG), and thylakoid membranes (TM) of the chloroplasts.

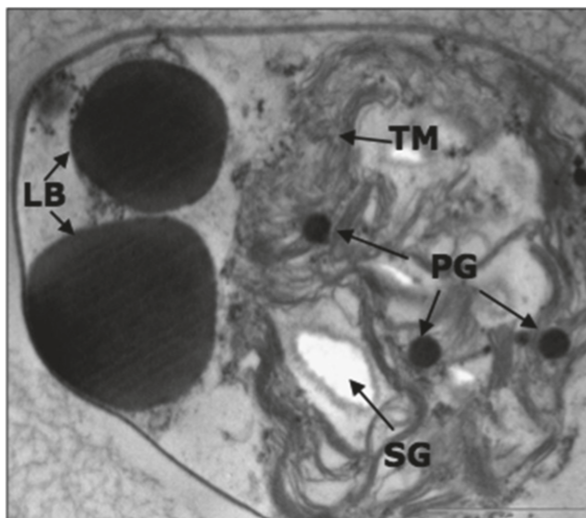

Fig. 20.1 Transmittance electron micrographs of *Isochrysis galbana* cells, showing lipid bodies (*LB*), starch granules (*SG*), plastoglobuli (*PG*), and thylakoid membranes (*TM*) of the chloroplast. Scale bar = 2 μm

3 Culturing Microalgae

There are two modes of cultivation of microalgae cells: batch culture and continuous culture. Batch culture is the most common method due to the simplicity of the culture system and operational ease. It is widely used for the cultivation of microalgae in commercial settings (Lee and Shen 2004). Carbon dioxide, water, nutrients, and trace elements are the essential biochemical components, and the growth can be synthesized by means of photosynthesis (Barsanti and Gualtieri 2006).

3.1 Growth Phases

Changes in microalgae biomass and the surrounding environment of a batch culture medium can be divided into phases according to growth. There are six well-defined growth phases in microalgae culturing; Lag, Acceleration, Exponential, Retardation, Stationary, and Negative, as demonstrated in Fig. 20.2.

During the lag phase, physical adaptations of the inoculum take place. The growth rate of the algae in this phase is zero. Following the acceleration phase, which is characterized by an increasing rate of growth, the exponential growth phase is achieved. During this short-lasting phase, there is a constant increasing rate of growth. However, the environment of the cells is altered by increased culture concentration. Decreasing growth during the retardation phase is due to limiting factors such as light, nutrients, pH, or carbon dioxide. Cell concentration during the stationary phase is maintained at its maximum value (Barsanti and Gualtieri 2006).

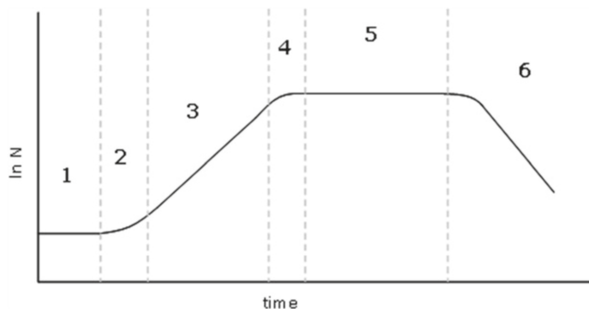

Fig. 20.2 Population growth curve of algae under conditions for batch culture (*1* Lag, *2* Acceleration, *3* Exponential, *4* Retardation, *5* Stationary, *6* Negative) (Adapted from Barsanti and Gualtieri 2006)

Since the stationary phase is associated with limiting factors restricting the growth rate, cells are experiencing stress. Nurturing the highest percentage of lipids within the microalgae cells also happens through the stationary phase (Bigogno et al. 2002). During the early stationary phase, saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are most abundant. As the culture progresses to the late stationary phase, a maximal value of n-3 and n-6 polyunsaturated fatty acids (PUFAs) begin to build in the lipid bodies (Lin et al. 2007). A negative growth rate during the final phase is due to catalyzer accumulation and nutrient depletion (Barsanti and Gualtieri 2006). Most green microalgae produce starch under normal conditions. Upon exposure to nutritional stress, such as nitrogen or phosphate starvation, they start to synthesize triacylglycerol, which are stored in their oil bodies (Shifrin and Chisholm 1981).

4 Chemical Composition of Microalgae

As a general rule, protein is the major macromolecular constituent of the cell, followed by lipids and carbohydrates (Becker 2004). The composition of microalgae grown in the late exponential growth phase consists of 30–40 % protein, 10–20 % lipids, and 5–15 % carbohydrates. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites, and sources of energy. The chemical compositions of various microalgae are shown in Table 20.1. Algal oils have a range of potential applications in food and feed formulations, cosmetics, and biodiesel production (Chu 2012), which are discussed in a later section.

5 Lipid Bodies in Microalgae

Lipid bodies or oil bodies are cytoplasmic lipid globules, which exist in nature in heterogeneous forms: their size and composition vary according to the organisms and environmental conditions (Murphy 2001). These macromolecular proteolipid assemblies consist of a hydrophilic phospholipid monolayer membrane interspersed

Table 20.1 Chemical composition of oleaginous microalgae. Data are given as % of dry matter

Microalgae	Protein (%)	Carbohydrate (%)	Lipid (%)	Reference
Scenedesmus obliquus	50–56	10–17	12–14	Becker (1993)
Scenedesmus dimorphus	8–18	21–52	16–40	
Chlamydomonas rheinhardii	48	17	21	
Chlorella vulgaris	51–58	12–17	14–22	
Chlorella pyrenoidosa	57	26	2	
Spirogyra sp.	6–20	33–64	11–21	
Dunaliella salina	57	32	6	
Euglena gracilis	39–61	14–18	14–20	
Porphyridium cruentum	29–39	440–57	9–14	
Prymnesium parvum	28–45	25–33	22–38	
Phaeodactylum sp.	55	10.5	19.8	Thomas et al. (1984)
Monallanthus sp.	41.3	13.9	20.7	
Tetraselmis sp.	64.4	10.7	23.4	
Isochrysis sp.	44.9	23.1	28.5	
Botryococcus sp.	24	11	29	
Pavlova lutheri	51.6	22–24	19.56	Reed Mariculture products™, http://www.reed-mariculture.com
Isochrysis galbana	46.7	24.2	17.1	
Nannochloropsis sp.	52.11	16	16.7	
Tetraselmis sp.	54.7	18.3	14.3	

with amphiphilic oleosin proteins and associated phytochemicals (Gray et al. 2010). This structure surrounds a hydrophobic core of neutral lipids. Figure 20.3 shows the typical structure of a lipid body (oil body).

Triacylglycerol (TAG), diacylglycerol, and sterol esters are among some of the lipids and lipid derivatives stored in lipid bodies (Nguyen et al. 2012). The size of such lipid bodies in microalgae varies between 0.1 and 8.0 μm, and have round shapes. The size and lipid composition depend on different growth phases, growth conditions (stress), and species. The Zeiss Axioskop epifluorescence microscope photograph in Fig. 20.4 illustrates several lipid bodies (yellowish green) accumulated in the algal cell (Cooper et al. 2010). Generally, microalgae lipid bodies have the ability to concentrate SFAs and MUFAs. Some microalgae have the capacity to accumulate high levels of long-chain PUFAs as TAG in their lipid bodies (Bigogno et al. 2002; Alonso et al. 1998). During periods of normal growth, lipid bodies may disappear completely, or exist as tiny organelles, but they are abundant under conditions not optimal for growth.

5.1 Lipid Body Formation According to Growth Phase

Lipid body formation commences during the exponential stage (Lin et al. 2007), whilst cytoplasmic lipid globules are produced most abundantly during the

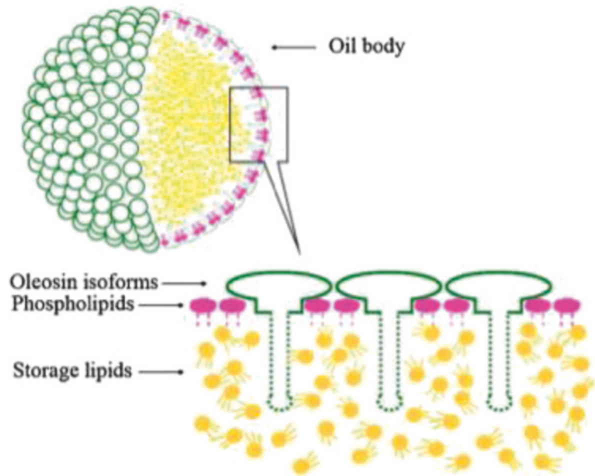

Fig. 20.3 Structure of a lipid body (oil body) (Adapted from Qu et al. 2013)

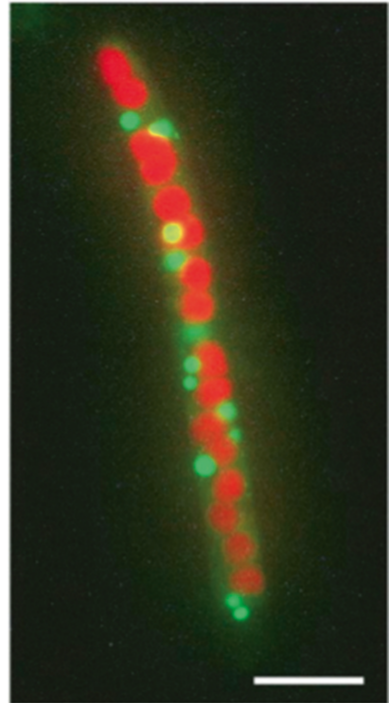

Fig. 20.4 Oil-containing stained lipid bodies (*green*) are easily distinguished from chloroplasts (*red*) in *O. maius Naegeli* freshwater filamentous algal cells. Scale bar = 10 μm (Adapted from Cooper et al. 2010)

stationary phase (Wang et al. 2009). The lipid body number varies between growth phases, with the maximum number being produced during the stationary phase (Liu and Lin 2005).

Many researchers have documented that nitrogen starvation greatly induces lipid body formation in microalgae (Wang et al. 2011; Solovchenko et al. 2008).

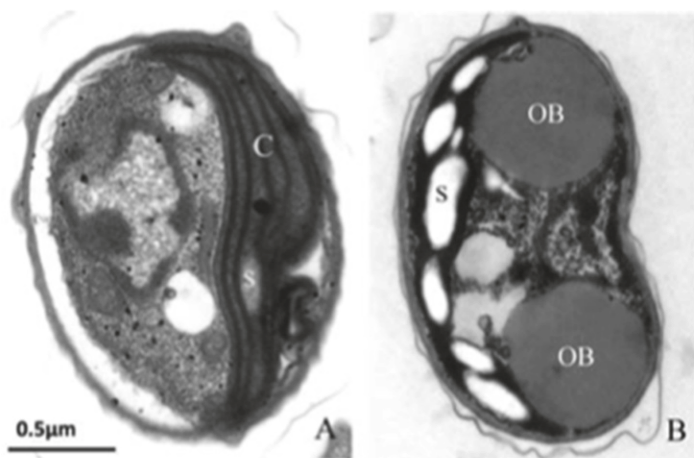

Fig. 20.5 TEM of UTEX 2219-4 before and after nitrogen starvation. (**a**) Before nitrogen starvation. (**b**) After 3 days of nitrogen starvation. *C* chloroplast, *S* starch granule, *OB* oil body (Adapted from Wang et al. 2011)

Figure 20.5 shows the increase of the quantity of starch granules, and its co-existence with lipid bodies in the cells due to nitrogen starvation. This suggests that carbon sources in these cells during nitrogen starvation are not only allocated to storage lipid production, but also to starch biosynthesis (Wang et al. 2011). This information implies that, when microalgae consist of lipid bodies, an appropriate quantity of starch is also contained in microalgae cells.

5.2 Nutritional Importance of Lipid Bodies

There is a large potential for microalgae to enhance the nutritional content of conventional food preparations, as microalgae represent a valuable source of all essential vitamins (e.g., A, B1, B2, B3, B5, B6, B12, C, E, biotin, and folic acid), enzymes, fatty acids, and carotenoids that benefit humans (Mendes et al.1995; Becker 2004; Goh et al. 2009). Moreover, algal lipid bodies are a rich source of α-tocopherols (Durmaz 2007).

The bulk of lipid bodies typically consist of triglycerides of at least 80–90 wt%. Phospholipids are normally present in the oil bodies in a concentration of 0.2–5 wt%. The structural proteins usually represent 0.2–7 wt% of the oil bodies. The triglycerides contained in the lipid matrix preferably contain at least 10 %, most preferably at least 20 %, ω-3 long-chain polyunsaturated fatty acids (LC-PUFAs) by weight of the lipid bodies (Michael et al. 2008). The fatty acid compositions of several marine microalgae oils are given in Table 20.2.

The ω-3 LC-PUFAs in the lipid bodies help to reduce or prevent serum oxidative stress leading to physiological disorders. Moreover, lipid bodies are also

Table 20.2 The fatty acid compositions of several marine microalgae (% total fatty acids) (Adapted from Liu and Lin 2001)

Fatty acid	*Pavlova salina*	*Isochrysis* sp. CCMP 463	*Isochrysis* sp. CCMP 1324	*Isochrysis galbana*	*Isochrysis galbana* TK1	*Pavlova lutheri* CCMP 1325 TK2	*Nannochloropsis oculata*	*Chlorella minutissima* UTEX2341
14:0	10.1 ± 0.2	10.4 ± 0.9	10.2 ± 0.5	17.5 ± 1.0	16.3 ± 0.9	10.3 ± 0.5	5.1 ± 0.4	4.5 ± 0.4
16:0	23.4 ± 0.4	14.9 ± 0.7	17.6 ± 0.6	14.3 ± 0.4	12.9 ± 0.7	20.8 ± 1.2	32.1 ± 1.4	33.9 ± 1.6
16:1n-7	6.2 ± 0.1	4.5 ± 0.2	3.9 ± 0.1	6.3 ± 0.5	4.0 ± 0.1	18.4 ± 0.4	24.9 ± 1.7	23.2 ± 1.0
18:0	0.9 ± 0.1	N.D.	N.D.	N.D.	N.D.	0.4 ± 0.1	2.7 ± 0.2	2.9 ± 0.1
18:1n-9	16.9 ± 0.3	29.8 ± 1.2	32.0 ± 1.4	15.1 ± 0.7	28.1 ± 1.0	3.3 ± 0.5	16.5 ± 0.9	20.4 ± 1.1
18:2n-6	7.8 ± 0.4	5.7 ± 0.4	4.1 ± 0.3	8.8 ± 0.4	3.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.3	3.4 ± 0.1
18:3n-3	3.1 ± 0.1	6.4 ± 0.5	6.4 ± 0.3	8.2 ± 0.3	5.5 ± 0.2	1.5 ± 0.2	N.D.	N.D.
18:4n-3	5.9 ± 0.1	17.5 ± 0.9	15.1 ± 0.6	24.9 ± 1.4	18.9 ± 0.3	6.8 ± 0.5	N.D.	N.D.
20:4n-6	1.6 ± 0.1	N.D.	N.D.	N.D.	N.D.	N.D.	2.8 ± 0.2	1.9 ± 0.1
20:5n-3	11.8 ± 0.3	N.D.	N.D.	N.D.	N.D.	21.0 ± 0.5	9.4 ± 0.7	8.7 ± 0.2
22:6n-3	4.4 ± 0.4	10.7 ± 0.5	10.9 ± 0.3	8.2 ± 0.6	11.1 ± 0.4	6.2 ± 0.3	N.D.	N.D.
ΣUn-3	25.2	34.6	32.3	41.4	31.8	35.5	12.2	8.7
Σun-6	9.4	5.7	4.1	8.8	3.0	1.9	4.7	5.2
n-3/n-6	2.7	6.1	7.9	4.7	10.6	18.7	2.6	1.7

N.D. none detected

advantageously used in the improvement or treatment of cognitive and mental conditions and disorders, as well as the maintenance of normal functions of brain-related systems and processes, preferably attention deficit hyperactivity disorder (ADHD), aging, Alzheimer's disease, Parkinson's disease, multiple sclerosis (MS), dyslexia, depression, learning capabilities, stress, anxiety, concentration, and attention. Another utilization aspect of the algal lipid bodies is the treatment or prevention of disorders or of impaired cognitive function. Examples of disorders that may suitably be prevented with the algal oil bodies include atherosclerosis, cardiovascular disorders, and coronary heart disease (Michael et al. 2008).

The European patent 1952695A1 reported that lipid bodies extracted from microalgae edible composition contain added vitamins selected from at least one of the following: vitamin A, vitamin B1, vitamin B2 (riboflavin), vitamin B3 (niacinamide), vitamin B5 (d-Ca pantothenate), vitamin B6, vitamin B11, vitamin B12 (cyanocobalamin), vitamin C (ascorbic acid), vitamin D, vitamin E (tocopherol), vitamin H (biotin), and vitamin K. The composition also preferably comprises added minerals selected from at least one of the following: calcium, magnesium, potassium, zinc, iron, cobalt, nickel, copper, iodine, manganese, molybdenum, phosphorus, selenium, and chromium (Michael et al. 2008).

6 Utilization of Lipid Bodies

More recently, the natural encapsulation of oil has received interest as a potential method for reducing the lipid oxidation of PUFAs (Gray et al. 2010). The encapsulation of lipids in nature occurs in the form of lipid bodies. It has been proposed that PUFAs stored in lipid bodies within cells are less susceptible to lipid oxidation than TAG molecules free in other food systems.

Lipid bodies isolated from plant seeds into aqueous media are, therefore, a natural emulsion that may represent a vehicle to deliver natural, minimally processed, pre-emulsified oil into appropriate food systems (White et al. 2009). Similarly, work carried out by Michael et al. (2008) mentioned that microalgal oil bodies can also be incorporated into food products and beverages (e.g., nutritional beverages and dairy drinks) and spreads, such as: nutrition bars, pasta products, ice cream, desserts, dairy products (e.g., yogurt, quark, cheese), dressings, sauces, soups, instant powders, fillings, dips, and breakfast-type cereal products (e.g., porridge).

Michael et al. (2008) showed that microalgae lipid bodies which accumulate lipids in large spherical lipid-dense bodies have the ability to retard lipid oxidation at a higher rate compared to the algal oil extracted from the same species. Similar observations have been made (Gray et al. 2010) that neutral lipids stored in seed lipid bodies are more stable to oxidation than lipids in an emulsion. The reason for the increased stability is likely to lie in the phytochemical profile of the surface (for example, tocopherols) and the extensive coverage by oleosin and some other proteins.

7 Isolation of Lipid Bodies from Microalgae

Microalgae oils are commonly used for biodiesel production. Many researchers have carried out advanced technologies for extracting and purifying microalgae oil (Mercer and Armenta 2011). Isolating microalgae lipid bodies will help to establish the oxidative stability of naturally encapsulated PUFAs in microalgae; these organelles should also carry many nutrients (vitamins, carotenoids, omega-3 LC-PUFAs, some proteins, etc.). However, few (if any) reports of microalgal oil body isolation and characterization exist. Michael et al. (2008) described a method for isolating lipid bodies from a dry mass of microalgae. For isolating purposes, they used a sugar solution for the floating lipid bodies and attached debris was removed by using NaCl solution. They further reported that these lipid bodies have a volume weighted mean diameter within the range 0.1–100 μm (Michael et al. 2008).

Figure 20.6 presents the method developed by the present authors for isolating microalgae (*Isochrysis galbana*) lipid bodies with algal slurry at a low temperature. In this method, the microalgae cell wall was disrupted by mechanical force (high-speed homogenization). The isolation of crude lipid bodies was done with density

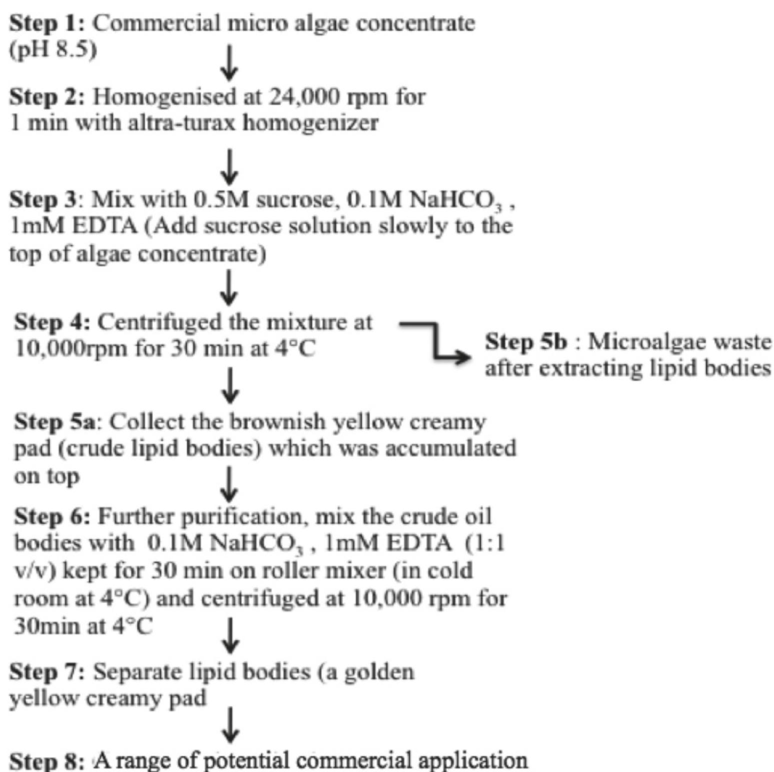

Fig. 20.6 Method of isolating microalgae lipid bodies

gradient centrifugation after mixing with sugar solution. Golden brown or bronze colored microalgae lipid bodies accumulated on top of the solution as a soft pad. The microalgae residue (step 5b in Fig. 20.6) with a liquid/slurry nature is a waste material, which has an opportunity for application in bioethanol production.

8 Production of Bioethanol from Microalgae Biomass

In the twenty-first century, bioethanol has been considered as a candidate of alternative energy to fossil resources. Biofuels (bioethanol, biodiesel, CH_3) are basically obtained from plant-based products, especially waste materials. It refers to renewable fuels from biological sources that can be used for heat, electricity, and fuel. As ethanol is less toxic, readily biodegradable, and produces fewer airborne pollutants than petroleum fuel (Eshaq et al. 2010), many countries, for example, Brazil, the USA, Canada, the UK, etc., are currently utilizing bioethanol for their transportation systems. Since biomass assimilation by algal growth utilize atmospheric carbon dioxide, their biomass for bioethanol production can reduce greenhouse gas levels.

Saccharification and fermentation of starches and sugars in algae biomass is the main step in the production of bioethanol. Saccharification or hydrolysis can be done with the enzymes produced by *Aspergillus niger*, which is cellulolytic and amylolytic in nature, as it produces cellulases and amylases. These enzymes hydrolyze the cellulose and starch present in *Spirogyra* and release free sugars (Miller 1959). Thereafter, fermentation is done by *Saccharomyces cerevisiae* for the production of bioethanol and distillation is carried out to obtain bioethanol.

Microalgae provide carbohydrates (in the form of glucose, starch, and other polysaccharides) and proteins that can be used as carbon sources for fermentation by bacteria, yeast, or fungi (Harun et al. 2010). A study carried out with microalgae (*Chlorococum* sp.) as a substrate for bioethanol production via yeast (*Saccharomyces bayanus*) under different fermentation conditions resulted in a maximum ethanol concentration of 3.83 g L^{-1} obtained from 10 g L^{-1} of lipid-extracted microalgae debris and the productivity level is approximately 38 % w/w (Harun et al. 2010).

Feinberg (1984) reported that conventional ethanol-fermentation yeasts, such as *Saccharomyces cerevisiae*, can convert up to 95 % of the "available" carbohydrate, i.e., glucose, into a 1:l (weight) ratio of ethanol and carbon dioxide. Further, a combined hydrolysis-fermentation yield of 80 % is assumed for this carbohydrate fraction, which is much less homogeneous but more accessible to hydrolysis than, say, lignocellulose. These assumptions result in a net alcohol production of 0.329 L/kg (0.04 gal/lb) and a total energy content of 7.74 MJ/kg (3,330 Btu/lb) of total carbohydrate. Figure 20.7 shows the ethanol yield and energy production as functions of the carbohydrate content. Carbon dioxide (approximately 39 L/L or 5.2 ft/gal of ethanol), a fermentation by-product, is also produced by this method (Feinberg 1984). The production of bioethanol by using microalgae can also be performed via self-fermentation; for example, dark fermentation in the marine green algae *Chlorococcum littorale* was able to produce 450 mol ethanol g^{-1} at 30 °C (Ueno et al. 1998).

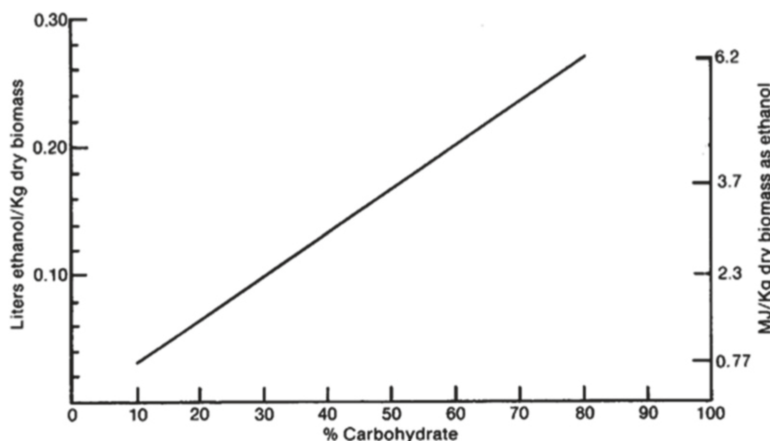

Fig. 20.7 Ethanol yield from microalgae biomass (Adapted from Feinberg 1984)

Some researchers practiced a pretreatment with chemicals before subjecting biomass for ethanol production; however, the research conducted to evaluate the effectiveness of chemical pretreatment damaged the pure cellulose in the cell wall, leading to a lower yield of alcohol when compared with untreated *Spirogyra* biomass. The research concluded that, as the *Spirogyra* cell wall is made up of pure cellulose and simple starch, it does not demand any type of pretreatment (Eshaq et al. 2010).

The production of bioethanol using microalgae-induced glucan (*Schizochytrium* sp.) and *Escherichia coli* KO11 was tested with SSF (simultaneous saccharification and fermentation) (Kim et al. 2012). This is resulted in 11.8 g-ethanol/*l* from 25.7 *g/l* of glucose; i.e., the maximum ethanol yield based on glucan in hydrolyzate was 89.8 % (Kim et al. 2012). This information yielded the opportunity for the production of bioethanol through yeast and bacteria fermentation.

8.1 Utilization of Post-extracted Microalgae Residue

The lipid body isolated "post-extracted microalgae residue" described in the present method is slurry in a sugar solution. When lipid bodies are generated in microalgae cells due to nitrogen starvation, starch granules are also formed. The algal cell walls consisted of polysaccharides and, therefore, this post-extracted microalgae residue contained many components which can be converted into free sugars. As many proteins in algae cells are attached to the surrounding structure of the lipid bodies and separated with the algal lipid bodies, the residue may contain a smaller amount of protein compared to the total proteins present at the initial stage.

The flow chart in Fig. 20.8 illustrates the major steps of free sugar production from post-extracted microalgae residue (or lipid body isolated microalgae slurry). The Novazyme CT-2™ enzyme consists of cellulase, hemicellulase, and β-glucanase.

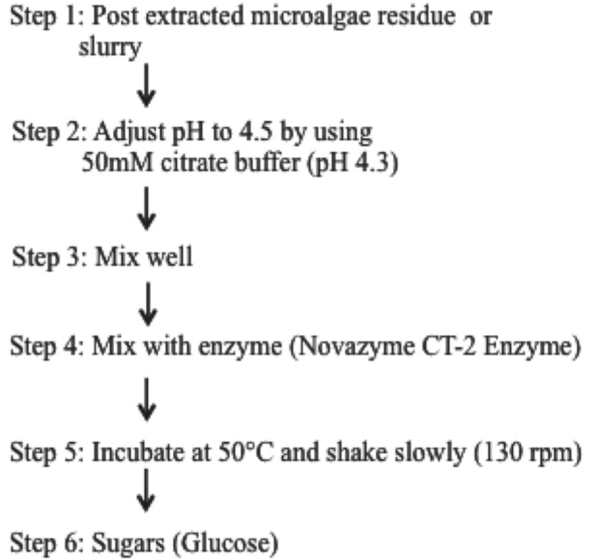

Fig. 20.8 Conversion of post-extracted microalgae slurry to glucose by enzymatic degradation

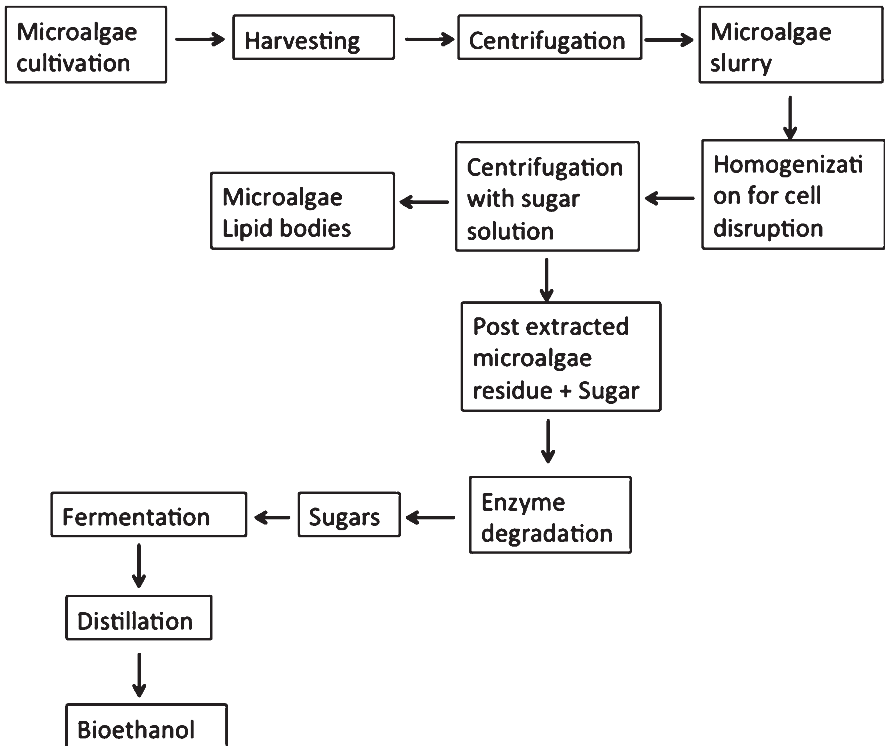

Fig. 20.9 Integrated process for microalgae lipid body isolation and bioethanol production

It confirms the positive effect of converting starches and celluloses in microalgae to free sugars.

Saccharomyces cerevisiae, *Saccharomyces bayanus*, and *Saccharomyces uvarum* are three yeasts that can convert sugars and starches to ethanol efficiently. Figure 20.9 illustrates the integrated process for microalgae lipid body isolation and bioethanol production from the post-extracted microalgae residue. The fermentation process requires lower energy consumption and follows a simplified process compared to biodiesel production system. The CO_2 produced, as a by-product from the fermentation process, can be recycled as carbon sources to microalgae in the cultivation process, thus reducing the greenhouse gases emissions. From this study, it can be concluded that algal biomass is a beneficial raw material for isolating lipid bodies which can be used as a vehicle to transfer omega-3 fatty acids to infant food formulas with high oxidative stability; the post-extracted microalgae residue in the sugar-rich grinding medium then becomes a by-product of the lipid body isolating process, which can be successfully utilized for bioethanol production.

References

Alonso DL, Belarbi EH, Rodríguez-Ruiz J, Segura CI, Giménez A (1998) Acyl lipids of three microalgae. Phytochemistry 47:1473–1481

Arad S, Yaron A (1992) Natural pigments from red microalgae for use in foods and cosmetics. Trends Food Sci Tech 3:92–97

Barrow C, Shahidi F (2008) Marine nutraceuticals and functional foods. CRC Press Taylor & Francis Group, Boca Raton

Barsanti L, Gualtieri P (2006) Algae: anatomy, biochemistry, and biotechnology. Taylor & Francis Group, LLC. CRC Press, London, pp 35–130

Becker EW (1993) Microalgae: biotechnology and microbiology, Cambridge University Press, UK, pp 220–230

Becker EW (2004) Microalgae in human and animal nutrition. In: Richmond A (ed) Handbook of microalgal culture. Blackwell, Oxford, pp 312–351

Bigogno C, Khozin-Goldberg I, Cohen Z (2002) Accumulation of arachidonic acid-rich triacylglycerols in the microalga *Parietochloris incisa* (trebuxiophyceae, chlorophyta). Phytochemistry 60:135–143

Chu W (2012) Biotechnological applications of microalgae. Int J Sci Med 6:24–37

Cooper MS, Hardin WR, Petersen TW, Cattolico RA (2010) Visualizing "green oil" in live algal cells. J Biosci Bioeng 109(2):198–201

Durmaz Y (2007) Vitamin E (α-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. Aquaculture 272:717–722

Eltgroth ML, Watwood RL, Wolfe GV (2005) Production and cellular localization of neutral long-chain lipids in the haptophyte algae *Isochrysis galbana* and *Emiliania huxleyi*. J Phycol 41:1000–1009

Eshaq FS, Ali MN, Mohd MK (2010) Spirogyra biomass a renewable source for biofuel (bioethanol) production. Int J Eng Sci Tech 2(12):7045–7054

Feinberg DA (1984) Fuel options from microalgae with representative chemical compositions. Report SERI/TR-231-2427, US Department of Energy, p3

Goh LP, Loh SP, Fatimah MY, Perumal K (2009) Bioaccessibility of carotenoids and tocopherols in marine microalgae, *Nannochloropsis* sp. and *Chaetoceros* sp. Mal J Nutr 15:77–86

Gray DA, Payne G, McClements DJ, Decker EA, Lad M (2010) Oxidative stability of *Echium plantagineum* seed oil bodies. Eur J Lipid Sci Technol 112:741–749

Guschina IA, Harwood JL (2006) Lipids and lipid metabolism in eukaryotic algae. Prog Lipid Res 45:160–186

Harun R, Danquah MK, Forde GM (2010) Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Tech Biotech 85(2):199–203

Kim JK, Um BH, Kim TH (2012) Bioethanol production from micro-algae, *Schizocytrium* sp., using hydrothermal treatment and biological conversion. Korean J Chem Eng 29(2):209–214

Lee YK, Shen H (2004) Basic culturing techniques. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science, Ames, pp 40–56

Li Y, Horsman M, Wang B, Wu N, Lan CQ (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. Appl Microbiol Biotechnol 81(4):629–636

Lin YH, Chang FL, Tsao CY, Leu JY (2007) Influence of growth phase and nutrient source on fatty acid composition of *Isochrysis galbana* CCMP 1324 in a batch photoreactor. Biochem Eng J 37:166–176

Liu CP, Lin LP (2001) Ultrastructural study and lipid formation of *Isochrysis* sp. CCMP1324. Bot Bull Acad Sin 42:207–214

Liu CP, Lin LP (2005) Morphology and eicosapentaenoic acid production by Monodus subterraneus UTEX 151. Micron 36:545–550

Mendes RL, Fernandes HL, Coelho JP, Reis EC, Cabral JMS, Novais JM, Palavra AF (1995) Supercritical CO_2 extraction of carotenoids and other lipids from *Chlorella vulgaris*. Food Chem 53:99–103

Mercer P, Armenta RE (2011) Developments in oil extraction from microalgae. Eur J Lipid Sci Technol 113(5):539–547

Michael C, Adrianus W, Berrandus R, Chislain CY, Michailovich S (2008) Oil bodies and method of producing such oil bodies. EP 1952695 A1, Unilever N.V.

Miller DL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Murphy DJ (2001) The biogenesis and functions of lipid bodies in animals, plants and microorganisms. Prog Lipid Res 40:325–438

Nguyen HM, Baudet M, Cuiné S, Adriano J-M, Barthe D, Billon E, Bruley C, Beisson F, Peltier G, Ferro M, Li-Beisson Y (2012) Proteomic profiling of oil bodies isolated from the unicellular green microalga *Chlamydomonas reinhardtii*: with focus on proteins involved in lipid metabolism. Proteomics 11:4266–4273

Qu XJ, Fu YJ, Luo M, Zhao CJ, Zu YG, Li CY, Wang W, Li J, Wei ZF (2013) Acidic pH based microwave-assisted aqueous extraction of seed oil from yellow horn (*Xanthoceras sorbifolia* Bunge.). Ind Crops Prod 43:420–426

Shifrin NS, Chisholm SW (1981) Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light–dark cycles. J Phycol 17:374–384

Solovchenko AE, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak MN (2008) Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga *Parietochloris incisa*. J Appl Phycol 20:245–251

Thomas WH, Tornabene TG, Weissman (1984) Screening for lipid yielding microalgae: activities for 1983. Report SERI/STR-231-2207, US Department of Energy, Colarado USA, p 23

Ueno Y, Kurano N, Miyachi S (1998) Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. J Ferment Bioeng 86:38–43

Wang ZT, Ullrich N, Joo S, Waffenschmidt S, Goodenough U (2009) Algal lipid bodies: stress induction, purification, and biochemical characterization in wild-type and starchless *Chlamydomonas reinhardtii*. Eukaryot Cell 8(12):1856–1868

Wang ST, Pan YY, Liu CC, Chuang LT, Chen CN (2011) Characterization of a green microalga UTEX 2219-4: effects of photosynthesis and osmotic stress on oil body formation. Bot Stud 52:305–312

White DA, Fisk ID, Makkhun S, Gray DA (2009) In vitro assessment of the bioaccessibility of tocopherol and fatty acids from sunflower seed oil bodies. J Agric Food Chem 57:5720–5726

Chapter 21
Bioactive Metabolites and Value-Added Products from Marine Macroalgae

Kannan R.R. Rengasamy, Manoj G. Kulkarni, Wendy A. Stirk, and Johannes Van Staden

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1 Introduction

"Let food be thy medicine and medicine be thy food" Hippocrates, 460 BC. In recent years, many studies have shown that diets containing a high content of phytochemicals can provide protection against various diseases. Approximately 90 % of all cancer cases are correlated with environmental factors, including dietary habits. One-third of all cancer deaths in the United States are avoidable by changing

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dietary habits (Willett 1995). These discoveries have rapidly amplified consumer awareness of the potential benefits of naturally occurring compounds from plants in health promotion and maintenance. In this regard, researches in Nutraceuticals and Functional Foods (NFF) and Natural Health Products (NHP) have been hot topics in recent years (Goldberg 1994; Nice 1997). In the search for medicinal foods, increasing attention has been given to marine-based sources.

Marine organisms are potentially productive sources of highly bioactive secondary metabolites that may lead to the development of new pharmaceutical agents (Mayer and Hamann 2002; El Gamal 2012). The trend of eating natural foods has been increased to improve health and vitality and one such natural resource is seaweed. Seaweeds (or marine macroalgae) are one of the most ecologically and economically important resources of the oceans (Dhargalkar and Pereira 2005). Seaweeds have been traditionally used as a human dietary component in Asian countries but are now consumed in North America, South America, and Europe (McHugh 2003). Seaweeds are the lower cryptogams which are classified into three main phyla: Phaeophyceae, or brown seaweeds, which are predominantly brown due to the presence of the xanthophyll pigment fucoxanthin, in addition to chlorophyll 'a' and 'c'; Chlorophyceae, or green seaweeds, which are dominated by chlorophyll 'a' and 'b', along with various characteristic xanthophylls (yellowish or brownish pigments); and Rhodophyceae, or red seaweeds, in which the principal pigments are phycoerythrin and phycocyanin (O'Sullivan et al. 2010). Globally, there are about 900 green, 4,000 red, and 1,500 brown species of seaweeds (Dawes 1998). Red and green seaweeds are found mostly in subtropical and tropical waters, while brown seaweeds are more common in cooler, temperate waters (Khan and Satam 2003). According to the Food and Agriculture Organization (FAO 2012), the global production of aquatic plants rose from 3.2 million tons in 1990 to 19 million tons (wet weight) in 2010. Currently, around 18 million tons (wet weight) of seaweeds and other aquatic plants are cultivated and utilized annually, with an estimated value of 4.4 billion US dollars (FAO 2012).

Around 265 species of seaweeds are harvested commercially and, of these, 145 species are used for food purposes and 110 for phycocolloid production (Zemke-White and Ohno 1999). Edible seaweeds are rich in bioactive compounds, antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids (Gómez-Ordóñez et al. 2010). The most popular commercial food ingredient derived from brown seaweeds is fucoxanthin. Several companies in Korea and Japan are producing standardized phlorotannins from brown seaweeds (Apostolidis and Lee 2012). Seaweeds are staple food (served as salads, soups, jellies, etc.) in some countries, such as Malaysia, Indonesia, Thailand, Korea, Japan, and Australia. Besides vast applications of seaweeds as food, they can also be utilized as feed additives and manure. They are also a proven source of food and pharmacologically important metabolites. Seaweeds are used as a source of vitamin supplements for the treatment of various intestinal disorders, such as vermifuges and hypocholesterolemic and hypoglycemic conditions (El Gamal 2012). In recent years, biological activities, nutritional values, and potential health benefits of marine algae have been intensively investigated and reviewed. This chapter focuses

specifically on the important bioactive metabolites of marine macroalgae and emphasizes their potential application as future pharmaceutical and functional food candidates.

2 Commercial Uses of Marine Macroalgae

Seaweeds are excellent resources of bioactive metabolites that could potentially be exploited as new functional food ingredients in both human and animal feed development. The major uses of seaweeds are discussed here.

2.1 Algal Products for Human Nutrition

Due to their high nutritional value, seaweeds are traditionally used for food, especially in Asian countries (Mabeau and Fleurence 1993; Norziah and Ching 2000; McHugh 2003). These are used in the preparation of salads, soups, jellies, and vinegar. Recently, the utilization of seaweeds as food, particularly as spice and delicacies, has been extended to western countries due to the change in lifestyle and dietary conventions. The most popular algal products are listed in Table 21.1.

2.2 Algal Products for Animal Feed

Seaweed utilization as animal feed was localized in coastal European countries such as Ireland, Iceland, Norway, France, Spain, and Portugal (McHugh 2003; Vinoj Kumar and Kaladharan 2007). The alginate extracted from seaweeds was used in the preparation of seaweed meal in fish farming. In Australia, the brown seaweed *Macrocystis pyrifera* and red seaweed *Gracilaria edulis* have been used. In South Africa, kelp (*Ecklonia maxima* and *Laminaria pallida*), red seaweeds (*Gracilaria*, *Gracilariopsis*, *Gelidium* species, *Plocamium corallorhiza*), and green seaweeds (*Ulva* species) are used as abalone feed alternatives (Troell et al. 2006). Due to their high nutrient content, many of the seaweeds are used for accelerating the growth of oysters, tilapia, salmon, and trout. Therefore, all seaweeds have great commercial importance (Fleming et al. 1996; Vinoj Kumar and Kaladharan 2007).

2.3 Algal Products for Agriculture

Seaweed extracts have been used in agriculture as plant growth stimulants due to their high content of cytokinins (Stirk and Van Staden 1997), auxins (Stirk et al.

Table 21.1 The most popular algal products used as human food

Algal food	Raw material
Hijiki	*Hizikia fusiformis*
Nori or purple laver	*Porphyra* species
Anori or green laver	*Monostroma* species
	Enteromorpha species
Kombu or haidai	*Laminaria japonica*
Sea lettuce	*Ulva lactuca*
Wakame or quandai-cai	*Undaria pinnatifida*
Mozuku	*Cladosiphon okamuranus*
Sea grapes or green caviar	*Caulerpa lentillifera*
Dulse	*Palmaria palmata*
Irish moss or carrageenan moss	*Chondrus crispus*
Winged kelp	*Alaria esculenta*
Ogo, ogonori, or sea moss	*Gracilaria* species
Carola	*Callophyllis variegata*

Source: Hoppe et al. (1979), McHugh (2003)

2004), and polyamines (Papenfus et al. 2013). Seaweed liquid extracts as foliar applications are effective in improving the growth of many crops, including various grasses, cereals, flowers, and vegetables (Crouch and Van Staden 1993). In South Africa, *Ecklonia maxima* is extensively used as a seaweed liquid additive (McHugh 2003).

2.4 Algal Products for Medicine

The uses of marine algae for medicinal purposes is not new, being mentioned in the Chinese "Materia Medica" of Shen-nung 2700 B.C. Seaweeds were used in folk medicines for the treatment of goiter, nephritic diseases, anthelmintic, catarrh, vermifuge, skin diseases, and others (Hoppe et al. 1979). Hoppe (1979) has reported detailed information on the utilization of marine algae in folk medicines.

3 Bioactive Metabolites and Value-Added Products

3.1 Polyunsaturated Fatty Acids (PUFAs)

PUFAs are essential structural components of cell and organelle membranes, contributing to the regulation of membrane properties such as fluidity, structure, phase transitions, and permeability (Yap and Chen 2001). Marine-based long-chain polyunsaturated fatty acids (LC-PUFAs) have 20 or more carbons with two or more double carbon bonds, and are classified by the position of the first double bond from

the methyl (omega) terminus. Of particular interest is the omega-3 fatty acids in which the first double bond is located at the third carbon from the methyl terminus. Omega-3 fatty acids can contain up to six double bonds and cannot be synthesized by animals. The well-studied marine omega-3 fatty acids are eicosapentaenoic acid (EPA), with 20 carbons and five double bonds, and docosahexaenoic acid (DHA), with 22 carbons and six double bonds (Sijtsma and Swaaf 2004). These two omega-3 fatty acids are of increasing interest due to research revealing their beneficial effects on many aspects of human health, such as reducing the risk factors associated with cardiovascular problems, fetal development, Alzheimer's disease, assisting visual and neurodevelopment, and ameliorating diseases such as coronary artery disease, arthritis, and hypertension (Simopoulos 1991; Swanson et al. 2012).

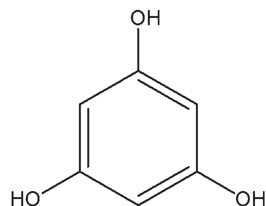
Seaweeds are rich in LC-PUFAs with nutritional implications and are, thus, studied extensively for biotechnological, food, feed, cosmetic, and pharmaceutical applications (Chandini et al. 2008a). The predominant fatty acid in various seaweed products is EPA (C20:5, n-3), occurring at concentrations as high as 50 % of the total fatty acid content (Murata and Nakazoe 2001; Dawczynski et al. 2007). Marine algae also contain the n-3 PUFA 18:4 n-3, which is not found in other organisms. Notably, red seaweed species contain significant quantities of PUFAs such as EPA and DHA. The green algal species are unusual in containing hexadecatetraenoic acid, varying from 4.9 % to 23.1 % of the total fatty acids, and palmitic acid, oleic acid, and linolenic acid have also been found in high amounts. They were predominant in all studied brown seaweeds, whereas saturated fatty acids are predominant in red seaweeds, but both groups are balanced sources of n-3 and n-6 acids. Even when seaweeds are processed (canned and dried), the products are left with substantial nutritional value of protein, ash, and n-3 and n-6 fatty acid contents (Mabeau and Fleurence 1993; Sánchez-Machado et al. 2004). Algae are, therefore, a good source of EPA n-3 and an important source of n-3 PUFAs for the maintenance of health.

3.2 Phlorotannins

Phlorotannins are phenolic compounds formed by the polymerization of phloroglucinol or defined as 1,3,5-trihydroxybenzene monomer units and are biosynthesized through the acetate–malonate pathway. In the brown seaweeds, the only group of tannins present is the phlorotannins. They are polymers of phloroglucinols (1,3,5-trihydroxybenzene) (Fig. 21.1) and may constitute up to 15 % of the dry weight of brown algae (Ragan and Glombitza 1986; Targett and Arnold 1998). The phlorotannins are highly hydrophilic components, with a wide range of molecular sizes ranging between 126 and 650 kDa (Ragan and Glombitza 1986).

The Laminariaceae are reported to be rich resources of phlorotannins compared to other marine algae (Okada et al. 2004). The well-studied phlorotannins from *Eisenia bicyclis* and *Ecklonia cava* are phloroglucinol, phloroglucinol tetramer, eckol, phlorofucofuroeckol A, dieckol, 8,8′-bieckol, and dioxinodehydroeckol

Fig. 21.1 Phloroglucinol (1,3,5-trihydroxybenzene)



(Shibata et al. 2004; Li et al. 2009). Moreover, a few other novel phlorotannins from other edible seaweeds have been reported (Table 21.2). Phlorotannins have several beneficial biological health activities, including antioxidant (Kim et al. 2009; Li et al. 2009), anti-HIV (Artan et al. 2008), antiproliferative (Kong et al. 2009), anti-inflammatory (Lee 2010), anti-Alzheimer's disease (Yoon et al. 2009; Kannan et al. 2013), antimicrobial (Nagayama et al. 2002), anticancer (Parys et al. 2010), antihypersensitive (Jung et al. 2006), anticoagulant (Li et al. 2007), and radioprotective (Moon et al. 2008; Zhang et al. 2008) activities. The nutritional and medicinal importance of biologically active phlorotannins was highlighted by recent reviews (Li et al. 2011; Thomas and Kim 2011; Eom et al. 2012; Lee and Jeon 2013). Detailed information on the biological activities of phlorotannins isolated from various brown seaweeds is presented in Table 21.2.

3.3 Polyamines

Polyamines are a group of bioactive organic compounds having two or more primary amino groups. They were first discovered by van Leeuwenhoek in the 1700s as three-sided crystals in human semen and were named spermine due to their high concentration within human semen. The three most common amines, diamine putrescine (Put, butan-1,4-diamine) and the polyamines spermidine (Spd, [N-(3-aminopropyl) butane-1,4-di-amine]) and spermine (Spm, [N, N′-bis-(3-aminopropyl) butane-1,4-diamine]) (Hopkins and Hüner 1995), are reported to be essential components in all living cells due to their role in cellular growth, normal function, proliferation, the stabilization of negative charges of DNA, RNA transcription, protein synthesis, apoptosis, and the regulation of the immune response (Larqué et al. 2007; Nishibori et al. 2007). In children, high polyamines intake during the first year of life has been significantly correlated to food allergy prevention (Dandrifosse et al. 2000). Spermine and spermidine display a significant antiglycation effect at physiological concentrations, suggesting a new role for polyamines in diabetes (Gugliucci and Menini 2003).

The common polyamines found in plants are cadaverine, and the spermidine- and spermine-related compounds, homospermidine, norspermidine, homospermine, norspermine, and thermospermine (Martin-Tanguy 2001). The polyamines content in plants may vary from micromolar to millimolar levels (Kakkar and Sawhney 2002). Several studies have shown that there are appreciable quantities of polyamines within

Table 21.2 Bioactive phlorotannins isolated from seaweeds

Seaweed species	Phlorotannins	Biological activity	Reference
Ecklonia cava	6,6′-Bieckol, 8,8′-bieckol	Anti-HIV	Ahn et al. (2004), Artan et al. (2008)
	Dioxinodehydroeckol	Anticancer	Kong et al. (2009)
	Eckol	Antibacterial	Choi et al. (2010)
	Dieckol	Antifungal	Lee et al. (2010)
	Phloroglucinol, eckol	Anti-influenza	Ryu et al. (2011)
	7-Phloroeckol, phlorofucofuroeckol, dieckol	Anti-influenza	Ryu et al. (2011)
	Dieckol	Antidiabetic	Kang et al. (2013)
	Dieckol	Tyrosinase inhibition	Kang et al. (2012a)
	2,7-Phloroglucinol, 6,6′-bieckol	Antioxidant	Kang et al. (2012a)
	6,6′-Bieckol	Anti-inflammatory	Yang et al. (2012)
Ecklonia kurome	8,8′-Bieckol, eckol, dieckol, phlorofucofuroeckol A	Antibacterial	Nagayama et al. (2002)
	Phlorofucofuroeckol A	Algicidal	Nagayama et al. (2003)
Ecklonia maxima	Phloroglucinol, dibenzo [1,4] dioxine-2,4,7,9-tetraol, eckol	Anti-Alzheimer's	Kannan et al. (2013)
Ecklonia stolonifera	Phlorofucofuroeckol A	Antihypertension	Jung et al. (2006)
	Dieckol	Antihypertension	Jung et al. (2006)
	Eckol	Antihypertension	Jung et al. (2006)
	Phlorofucofureckol A	Antidiabetic	Jung et al. (2008)
	Dieckol	Antibacterial	Lee et al. (2008)
	Phlorofucofuroeckol A, dieckol, dioxinodehydroeckol	Anti-inflammatory	Kim et al. (2009)
	Phlorofucofuroeckol A, dieckol, dioxinodehydroeckol	Antioxidant	Kim et al. (2009)
Ecklonia stolonifera	Phloroglucinol	Antioxidant	Ahn et al. (2007)
	Dieckol	Immunomodulatory	Park et al. (2010)
	2-Phloroeckol, eckol, phlorofucofuroeckol B, 6,6′-bieckol	Hepatoprotective	Lee et al. (2012a)
	2-Phloroeckol, eckol, phlorofucofuroeckol B, 6,6′-bieckol	Antioxidant	Lee et al. (2012a)

(continued)

Table 21.2 (continued)

Seaweed species	Phlorotannins	Biological activity	Reference
Ecklonia cava and *E. stolonifera*	Dieckol	Antiphotoaging	Joe et al. (2006), Heo et al. (2009a)
	Eckol	Antiphotoaging	Joe et al. (2006), Heo et al. (2009a)
Ecklonia arborea, *E. cava*, and *E. stolonifera*	6,6′-Bieckol, 8,8′-bieckol, phlorofucofuroeckol A, B, eckol, dieckol	Antiallergy	Joe et al. (2006), Sugiura et al. (2006), Le et al. (2009)
Eisenia bicyclis	Eckol	Antidiabetic	Okada et al. (2004)
	Eckol, dieckol, dioxinodehydroeckol, fucofuroeckol A, 7-phloroeckol, phlorofucofuroeckol A	Antibacterial	Eom (2012)
Ecklonia cava and *Eisenia bicyclis*	Dieckol	Antidiabetic	Okada et al. (2004), Lee et al. (2009)
	Dieckol, eckol, fucofuroeckol A	Matrix metalloproteinases inhibition	Ryu et al. (2009), Lee (2010)
Ecklonia cava and *E. kurome* and *Eisenia bicyclis*	Phloroglucinol, eckol	Antioxidant	Ahn et al. (2007)
	Dieckol	Antioxidant	Ahn et al. (2007)
	Triphlorethol A	Antioxidant	Kang et al. (2005, 2007)
	8,8′-Bieckol, phlorofucofuroeckol A, eckol, dieckol	Antioxidant	Shibata et al. (2008)
Fucus vesiculosus	Fucophlorethol A, tetrafucol A, trifucodiphlorethol A	Antioxidant	Liu and Gu (2012)
Ishige okamurae	Diphloroethohydroxycarmalol	Antidiabetic	Heo et al. (2009b, 2010a), Kang et al. (2012b), Lee et al. (2012b)
Ishige okamurae	Diphloroethohydroxycarmalol	Anticancer	Kang et al. (2012b), Park et al. (2012)
		Radioprotective	Ahn et al. (2011)
		Antioxidant	Heo et al. (2008a)
		Photoprotective	Heo and Jeon (2009a), Heo et al. (2010a)
		Neuroprotective	Heo et al. (2012a)
Sargassum patens	2-(4-(3,5-Dihydroxyphenoxy)-3,5-dihydroxyphenoxy) benzene-1,3,5-triol (DDBT)	Antidiabetic	Kawamura-Konishi et al. (2012)

seaweed (Hamana and Matsuzaki 1982; Badini et al. 1994; Lee 1998; Marián et al. 2000; Nishibori et al. 2007; Papenfus et al. 2012). In recent years, there has been considerable interest in the influence of ingested polyamines from plant-based foods on human health (Lima and Vianello 2011). Polyamines are important to human health, particularly because they are involved in an array of specific roles that are essential to cell growth and proliferation (Kalač and Krausová 2005). Therefore, polyamines may be considered especially important in young children. However, it is well established that the capacity for polyamine synthesis decreases with age (Larqué et al. 2007). Polyamines from food are distributed to all organs and tissues, and long-term intake increases polyamine concentration in the blood. As most polyamines are associated with red and white blood cells, they act to suppress the synthesis of proinflammatory cytokines and leukocyte function-associated antigens. Foods with anti-inflammatory properties such as n-3 PUFAs are known to help prevent cardiovascular disease (Soda 2010). However, data on the content of dietary polyamines in foods, particularly in seaweeds, are relatively scarce, and there is a need to focus on the dietary applications of polyamines from seaweeds.

3.4 Fucoxanthin (Carotenoid Pigments)

Carotenoids are tetraterpenoid organic pigments that occur naturally in the chloroplasts and chromoplasts of plants and other photosynthetic organisms, like algae, some types of fungi, and bacteria. These carotenoids are thought to be responsible for the beneficial properties in preventing human diseases, including cardiovascular diseases, cancer, and other chronic diseases. Hence, the consumption of food rich in carotenoids from fruits and vegetables is healthier and lowers mortality due to a number of chronic illnesses (Diplock et al. 1998). The common carotenoids, such as lutein, astaxanthin, and zeaxanthin, are known as xanthophylls. Fucoxanthin and astaxanthin are known to be major constituents of marine algal carotenoids. Fucoxanthin is one of the most abundant marine carotenoids, and contributes more than 10 % of the estimated total production of carotenoids in nature, especially in the marine environment (Dembitsky and Maoka 2007). Fucoxanthin is a characteristic carotenoid present in edible brown seaweeds, such as *Undaria pinnatifida* (Wakame), *Hijikia fusiformis* (Hijiki), *Laminaria japonica* (Ma-Kombu), and *Sargassum fulvellum* (D'Orazio et al. 2012). Fucoxanthin was first isolated by Willstätter and Page (1914) from the brown seaweeds *Fucus*, *Dictyota*, and *Laminaria* species. Fucoxanthin has attracted considerable interest because of its potent bioactivities, including its antioxidant, anti-inflammatory, anticancer, anti-obese, antidiabetic, antiangiogenic, and antimalarial activities, and its protective effects on the liver, blood vessels of the brain, bones, skin, and eyes (Peng et al. 2011). So far, no adverse side effects have been reported in studies using mice (Kadekaru et al. 2008; Zaragozá et al. 2008). Hence, fucoxanthin may be considered a nontoxic, nonallergenic, biocompatible, and bioactive material in food, cosmetic, and pharmacological applications. A summary of fucoxanthin isolated from various brown seaweeds is presented in Table 21.3.

Table 21.3 Fucoxanthin isolated from brown seaweeds and their biological activities

Seaweed species	Biological activity	Reference
Eisenia bicyclis	Antidiabetic	Jung et al. (2012)
Fucus serratus	Antioxidant	Sasaki et al. (2008)
Halocynthia roretzi	Anticancer	Konishi et al. (2006)
Hijikia fusiformis	Neuroprotective	Okuzumi et al. (1990)
	Antioxidant	Nomura et al. (1997)
Laminaria japonica	Photoprotective	Shimoda et al. (2010)
	Prevent osteoporosis	Das et al. (2010)
Myagropsis myagroides	Anti-inflammatory	Heo et al. (2010b)
Padina tetrastromatica	Antioxidant	Yan et al. (1999), Sasaki et al. (2008)
Petalonia binghamiae	Antiobesity	Kang et al. (2011)
Sargassum heterophyllum	Antiplasmodicidal	Afolayan et al. (2008)
Sargassum siliquastrum	Cytoprotective	Heo et al. (2008b)
	Photoproductive	Heo and Jeon (2009b)
	Anti-inflammatory	Heo et al. (2012b)
Undaria pinnatifida	Antioxidant	Sasaki et al. (2008), Fung et al. (2013)
	Anticancer	Kotake-Nara et al. (2005), Nakazawa et al. (2009)
	Antiobesity	Maeda et al. (2005, 2007a, b, 2008), Woo et al. (2009), Hosokawa et al. (2010), Matsumoto et al. (2010), Beppu et al. (2012)
	Antidiabetic	Jung et al. (2012), Nishikawa et al. (2012)
	Antiangiogenic	Sugawara et al. (2006)

3.5 *Polysaccharides*

The high molecular weight sulfated polysaccharides and low molecular weight oligosaccharides are of interest because of their broad range of biological activities and low toxicity. Marine algae are rich sources of polysaccharides (Table 21.4), particularly structural polysaccharides, mucopolysaccharides, and storage polysaccharides (Murata and Nakazoe 2001; Chandini Kumar et al. 2008b). Polysaccharides are polymers of simple sugars linked by glycosidic bonds. They have an enormously wide variety of applications as stabilizers, thickeners, and emulsifiers in food and beverages (Tseng 2001). The major polysaccharides found in seaweeds are classified into fucoidan, alginate, galactans, ulvans, and laminaran (Kraan 2012).

Fucoidans, or fucans, are bioactive polysaccharides which were first isolated by Kylin in 1913. They contain substantial percentages of L-fucose and sulfate ester groups, which have been mainly identified in brown seaweed (Berteau and Mulloy 2003). Fucoidans have pharmacological interest because of their non-animal origin and anti-inflammatory activities with potent modulation of connective tissue proteolysis (Senni et al. 2006). The fucans have received a great deal of attention in biomedical research due to their wide variety of biological activities, including antiadhesive (McCormick et al. 2000), anticoagulant (Mourão 2004; Cumashi et al. 2007), anticomplimentary

Table 21.4 Polysaccharides isolated from different seaweeds and their biological activities

Division	Seaweed species	Biological activity	Reference
Phaeophyceae	*Ascophyllum nodosum*	Anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive	Cumashi et al. (2007)
	Adenocystis utricularis	Antiretroviral	Trinchero et al. (2009)
		Antiviral	Ponce et al. (2003)
	Cladosiphon okamuranus	Anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive	Cumashi et al. (2007)
		Antidengue virus	Hidari et al. (2008)
		Cardioprotective	Thomes et al. (2010)
	Costaria costata	Anticancer	Ermakova et al. (2011)
	Cladosiphon okamuranus	Antitumor	Teruya et al. (2007)
		Gastric protection	Kawamoto et al. (2006), Shibata et al. (2000)
	Cladosiphon okamuranus Tokida	Antiprion	Doh-Ura et al. (2007)
		Anticancer	Haneji et al. (2005)
	Cystoseira indica	Antiviral	Mandal et al. (2007)
	Dictyota mertensii	Anti-HIV	Queiroz et al. (2008)
	Dictyota cervicornis	Anticoagulation and antithrombosis	Costa et al. (2010)
	Dictyota menstrualis	Anticoagulant	Albuquerque et al. (2004)
	Dictyopteris delicatula	Antioxidant	Magalhaes et al. (2011)
		Anticoagulant	
		Antitumor	
	Ecklonia cava	Anticancer	Ermakova et al. (2011)
		Anticoagulation and antithrombosis	Wijesinghe et al. (2011)
	Ecklonia kurome	Anticoagulant	Nishino et al. (1989, 1991)

(continued)

Table 21.4 (continued)

Division	Seaweed species	Biological activity	Reference
Phaeophyceae	*Eisenia bicyclis*	Anticoagulant and antitumor	Usui et al. (1980)
	Fucus distichus	Anti-inflammatory, anticoagulant, antiangiogenic, antiadhesive	Cumashi et al. (2007)
	Fucus evanescens	Anti-inflammatory, anticoagulant, antiangiogenic, antiadhesive activities	Kuznetsova et al. (2003), Cumashi et al. (2007)
		Antitumor and antimetastatic	Alekseyenko et al. (2007)
	Fucus serratus	Anti-inflammatory, anticoagulant, antiangiogenic, antiadhesive	Cumashi et al. (2007)
	Fucus spiralis	Anti-inflammatory, anticoagulant, antiangiogenic, antiadhesive activities	Cumashi et al. (2007)
	Fucus vesiculosus	Anti-HIV	Queiroz et al. (2008)
		Anti-inflammatory	Park et al. (2011a)
		Antiobesity	Park et al. (2011b)
		Against hyperoxaluria	Veena et al. (2007a, b)
		Anticoagulant	Bernardi and Springer (1962)
		Immunostimulatory	Kawashima et al. (2012)
	Hizikia fusiforme	Blood anticoagulant	Dobashi et al. (1989), Li et al. (2008)
	Hydroclathrus clathratus	Antiviral activity	Wang et al. (2007)
	Laminaria digitata	Anticoagulant	Shanmugam and Mody (2000)
	Laminaria hyperborean	Anti-inflammatory	Nasu et al. (1997)
	Laminaria japonica	Antioxidant	Zhang et al. (2003), Zhao et al. (2008), Wang et al. (2008)
		Hypercholesterolemia	Li et al. (1999)
		Hyperlipidemia	Li et al. (2001)
		Antihypertensive	Fu et al. (2004)
		Immunomodulatory	Song et al. (2000)

Phaeophyceae	*Laminaria japonica*	Anti-inflammatory	Kyung et al. (2012)
		Radioprotective	Wu et al. (2004)
		Antitumor	Peng et al. (2012)
	Laminaria saccharina	Anti-inflammatory, anticoagulant, antiangiogenic, antiadhesive	Cumashi et al. (2007)
	Leathesia difformis	Antiviral	Feldman et al. (1999)
	Lessonia vadosa	Anticoagulant and elicitor	Chandía and Matsuhiro (2008)
	Lobophora variegata	Anti-HIV	Queiroz et al. (2008)
	Padina gymnospora	Anticoagulant	Silva et al. (2005)
	Padina tetrastromatica	Antiviral	Karmakar et al. 2010
	Saccharina cichorioides	Anticancer	Vishchuk et al. (2013)
	Saccharina japonica	Antitumor	Vishchuk et al. (2011)
	Sargassum fulvellum	Anticoagulant	De Zoysa et al. (2008)
	Sargassum horneri	Anticancer	Ermakova et al. (2011)
	Sargassum hemiphyllum	Anti-inflammatory	Hwang et al. (2011)
	Sargassum stenophyllum	Antiherpetic	Majczak et al. (2003)
	Sphacelaria indica	Antiviral	Bandyopadhyay et al. (2011)
	Splachnidium rugosum	Anti-HSV	Harden et al. (2009)
	Spatoglossum schroederi	Anti-HIV	Queiroz et al. (2008)
		Hemostatic	Rocha et al. (2005), Farias et al. (2011)
	Stoechospermum marginatum	Antiviral	Adhikari et al. (2006)
	Undaria pinnatifida	Antiviral	Cooper et al. (2002), Lee et al. (2004), Thompson and Dragar (2004), Queiroz et al. (2008), Hemmingson et al. (2006), Hayashi et al. (2008)
		Anti-HSV	Harden et al. (2009)
		Antiplasmodial	Chen et al. (2009)
	Undaria pinnatifida	Antiallergy	Maruyama et al. (2005)
		Antitumor	Maruyama et al. (2006), Costa et al. (2010), Synytsya et al. (2010), Vishchuk et al. (2011)

(continued)

Table 21.4 (continued)

Division	Seaweed species	Biological activity	Reference
Rhodophyceae	*Gigartina atropurpurea*	Anti-HSV	Harden et al. (2009)
	Plocamium cartilagineum	Anti-HSV	Harden et al. (2009)
	Gracilaria corticata	Antiviral	Mazumder et al. (2002)
	Gracilaria lemaneiformis	Antitumor	Fan et al. (2012)
	Gigartina skottsbergii	Antiherpetic and anticoagulant	Carlucci et al. (1997)
		Antioxidant activity	Barahona et al. (2011)
	Gracilaria birdiae	Anti-inflammatory activity	de Sousa Oliveira Vanderlei et al. (2011)
	Gelidium crinale	Anti-inflammation and antinociceptive	de Sousa et al. (2013)
	Porphyra vietnamensis	Immunomodulation effect	Bhatia et al. (2013)
	Champia feldmannii	Antitumor	Lins et al. (2009)
	Solieria filiformis	Anti-inflammation and antinociceptive	de Araújo et al. (2011)
	Sebdenia polydactyla	Antiviral (influenza, herpes, HIV)	Ghosh et al. (2009b)
	Nemalion helminthoides	Antiviral (influenza, herpes, HIV)	Pérez Recalde et al. (2009)
	Sphaerococcus coronopifolius	Antiviral (influenza, herpes, HIV)	Bouhlal et al. (2011)
	Boergeseniella thuyoides	Antiviral (influenza, herpes, HIV)	Bouhlal et al. (2011)
Chlorophyceae	*Enteromorpha prolifera*	Immunostimulatory	Kim et al. (2011)
	Codium fragile	Hemostasis effect	Ciancia et al. (2007)
	Codium vermilara	Hemostasis effect	Ciancia et al. (2007), Fernández et al. (2012)
	Ulva lactuca	Antiperoxidative and antihyperlipidemic	Sathivel et al. (2008)
	Gayralia oxysperma	Antiviral (influenza, herpes, HIV)	Cassolato et al. (2008)

(Tissot et al. 2003), antioxidant (Chew et al. 2008), antiproliferative (Patel et al. 2002), antiplatelet aggregation (Alwayn et al. 2000), antitumor (Alekseyenko et al. 2007), and antiviral properties (Ghosh et al. 2009a, b). Alginates are mainly of linear polymers consisting of ß-D-mannuronic (M) and α-L-guluronic (G) acids with different M/G ratios and linear arrangements. As alginates can absorb water and form viscous gum, they have been used as thickeners, stabilizers, and gelling agents in the food and pharmaceutical industries. The galactanes, such as agarans and carrageenans found in red seaweeds, have great importance as they are widely used in the food industry because of their rheological properties as gelling and thickening agents. Ulvan is the major water-soluble polysaccharide found in green seaweeds of the order Ulvales (*Ulva* and *Enteromorpha* species) that has sulfate, rhamnose, xylose, and iduronic and glucuronic acids as the main constituents (Percival and McDowell 1967; Lahaye and Ray 1996).

3.6 Protein Hydrolysate

Protein hydrolysate, commonly known as peptones or peptides, is a mixture of amino acids prepared from purified protein sources by heating with acid or the addition of proteolytic enzymes, followed by purification procedures. This protein hydrolysate provides the nutritive equivalent of the original material in the form of its constituent amino acids and is used as nutrient and fluid replenishers in special diets or for patients who are unable to take ordinary food proteins (Liu et al. 2013). Apart from the nutritional properties, protein hydrolysates have diverse applications in the biotechnological industry (Pasupuleti and Demain 2010), such as:

- Manufacturing of vaccines by fermentation processes and as vaccine stabilizers
- Use in large-scale industrial fermentations as sources of nitrogen and unknown growth factors, such as certain peptides
- Use in microbiological media and to detect pathogens and perform antibiotic sensitivity tests
- Use in regular diets as well as prescription diets for companion animals
- Plays an important role in animal nutrition, especially for raising healthy animals with increased immune resistance
- Use as plant growth regulators to increase pest resistance to plants (Inagrosa 2002; Figueroa et al. 2008) and to control weeds (Christians et al. 2010)

The red seaweeds contain significant levels of protein and, in some cases, contain higher quantities than conventional protein-rich foods, such as soybean, cereals, eggs, and fish (Kaliaperumal 2003; Fleurence 2004). To date, little attention has been paid to the protein components from this marine source (Harnedy and FitzGerald 2011). The protein hydrolysates reported from various seaweeds are tabulated and presented in Table 21.5. The health effects of protein hydrolysates such as marine proteins and peptides have gained increased scientific interest because in vitro and in vivo animal studies indicate that peptides derived from marine sources have bioactive properties that may promote human health (Kitts and Weiler 2003; Kim and Mendis 2006; Kim and Wijesekara 2010).

Table 21.5 Protein hydrolysates extracted from seaweeds with biological activities

Division	Seaweeds species	Biological activities	Reference
Phaeophyceae	*Costaria costata*	Antihypertensive activity	Lee et al. (2005)
		Antioxidant activity	Lee et al. (2005)
		Antitumor activity	Lee et al. (2005)
		Antityrosinase inhibitory activity	Lee et al. (2005)
	Ecklonia cava	antihypertensive activity	Cha et al. (2006)
		Antioxidant activity	Heo et al. (2003a, b, 2005), Kim et al. (2006)
		Immunomodulatory effects	Ahn et al. (2008)
	Hizikia fusiformis	Antioxidant capacity	Siriwardhana et al. (2004, 2008)
	Ishige okamurae	Antioxidant activity	Heo et al. (2003a, 2005), Heo and Jeon (2008)
		Cytoprotective activity	Heo and Jeon (2008)
	Laminaria japonica	Anticancer activity	Go et al. (2010)
	Sargassum coreanum	Antioxidant activity	Heo et al. (2003b, 2005), Ko et al. (2010)
		Antitumor activity	Ko et al. (2012)
	Sargassum fulvellum	Antioxidant activity	Heo et al. (2003a, 2005)
	Sargassum horneri	Antioxidant activity	Heo et al. (2003a, 2005), Park et al. (2004)
		Anticoagulant activity	Athukorala et al. (2007)
	Sargassum thunbergii	Antioxidant activity	Heo et al. (2003a, 2005)
	Scytosiphon lomentaria	Antioxidant activity	Heo et al. (2003b, 2005)
	Undaria pinnatifida	Antihypertensive activity	Suetsuna and Nakano (2000), Sato et al. (2002a, b)
		Antihypertensive activity	Suetsuna et al. (2004)
Rhodophyceae	*Grateloupia filicina*	Antihypertensive activity	Lee et al. (2005)
		Antioxidant activity	Lee et al. (2005)

Rhodophyceae	*Grateloupia filicina*	Antitumor activity	Lee et al. (2005)
		Antityrosinase activity	Lee et al. (2005)
	Palmaria palmata	antioxidant capacity	Wang et al. (2010)
		Renin inhibitory activities	Fitzgerald et al. (2012)
	Polysiphonia urceolata	Antihypertensive activity	He et al. (2007)
	Porphyra tenera	Antihypertensive activity	Lee et al. (2005)
		Antioxidant activity	Lee et al. (2005)
		Antitumor activity	Lee et al. (2005)
		Antityrosinase inhibitory activity	Lee et al. (2005)
	Porphyra yezoensis	Antihypertensive activity	Suetsuna (1998), Saito and Hagino (2005)
	Porphyra columbina	Antihypertensive activity	Cian et al. (2012a, c)
		Antioxidant capacity	Cian et al. (2012a, c)
		Immunomodulation properties	Cian et al. (2012b, c)
	Porphyra yezoensis	Antihypertensive activity	Qu et al. (2010)
Chlorophyceae	*Caulerpa microphysa*	Antitumor properties	Lin et al. (2012)
		Antihypertensive activity	Lin et al. (2012)
	Codium fragile	Anticoagulant activity	Athukorala et al. (2007)
	Enteromorpha prolifera	Antihypertensive activity	Lee et al. (2005)
		Antioxidant activity	Lee et al. (2005)
		Antitumor activity	Lee et al. (2005)
		Antityrosinase activity	Lee et al. (2005)

4 Concluding Remarks and Future Prospects

Macroalgae or seaweeds are lower cryptogams found at the bottom of the food chain in marine ecosystems. Seaweeds contain several biologically active metabolites, including polysaccharides, phlorotannins, bioactive peptides, terpenoids, PUFAs, carotenoids, and primary and secondary amines. Due to their medicinal properties and high nutrient content, seaweeds are traditionally used as food in Asian countries. This trend of eating seaweeds has now extended to North America, South America, and Europe. The occurrence of these bioactive metabolites in seaweeds have made them a unique source for medicinal and food use. However, among the bioactive metabolites, most of the research has focused on brown algal metabolites such as sulfated polysaccharides, phlorotannins, and fucoxanthins. Seaweeds also contain a reasonable quantity of polyamines, which have been reported as plant growth regulators, but not much information is available on the polyamine content in different seaweeds. Recent studies have shown that the intake of polyamine-rich food prevents age-associated chronic diseases, especially cardiovascular diseases. However, research on the development of seaweed polyamines as dietary ingredients is still in its infancy. There remains vast potential for the discovery of bioactive molecules from marine macroalgae.

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References

Adhikari U, Mateu CG, Chattopadhyay K, Pujol CA, Damonte EB, Ray B (2006) Structure and antiviral activity of sulfated fucans from *Stoechospermum marginatum*. Phytochemistry 67:2474–2482

Afolayan AF, Bolton JJ, Lategan CA, Smith PJ, Beukes DR (2008) Fucoxanthin, tetraprenylated toluquinone and toluhydroquinone metabolites from *Sargassum heterophyllum* inhibit the in vitro growth of the malaria parasite *Plasmodium falciparum*. Z Naturforsch C 63:848–852

Ahn M-J, Yoon K-D, Min S-Y, Lee JS, Kim JH, Kim TG, Kim SH, Kim N-G, Huh H, Kim J (2004) Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. Biol Pharm Bull 27:544–547

Ahn G-N, Kim K-N, Cha S-H, Song C-B, Lee J, Heo M-S, Yeo I-K, Lee N-H, Jee Y-H, Kim J-S, Heu M-S, Jeon Y-J (2007) Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Eur Food Res Technol 226:71–79

Ahn G, Hwang I, Park E, Kim J, Jeon Y-J, Lee J, Park JW, Jee Y (2008) Immunomodulatory effects of an enzymatic extract from *Ecklonia cava* on murine splenocytes. Mar Biotechnol (NY) 10:278–289

Ahn M, Moon C, Yang W, Ko E-J, Hyun JW, Joo HG, Jee Y, Lee NH, Park JW, Ko RK, Kim GO, Shin T (2011) Diphlorethohydroxycarmalol, isolated from the brown algae *Ishige okamurae,* protects against radiation-induced cell damage in mice. Food Chem Toxicol 49:864–870

Albuquerque IR, Queiroz KC, Alves LG, Santos EA, Leite EL, Rocha HA (2004) Heterofucans from *Dictyota menstrualis* have anticoagulant activity. Braz J Med Biol Res 37:167–171

Alekseyenko TV, Zhanayeva SY, Venediktova AA, Zvyagintseva TN, Kuznetsova TA, Besednova NN, Korolenko TA (2007) Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk Sea *Fucus evanescens* brown alga. Bull Exp Biol Med 143:730–732

Alwayn IP, Appel JZ, Goepfert C, Buhler L, Cooper DK, Robson SC (2000) Inhibition of platelet aggregation in baboons: therapeutic implications for xenotransplantation. Xenotransplantation 7:247–257

Apostolidis E, Lee CM (2012) Brown seaweed-derived phenolic phytochemicals and their biological activities for functional food ingredients with focus on *Ascophyllum nodosum*. In: Kim S-K (ed) Handbook of marine macroalgae: biotechnology and applied phycology. Wiley Online Library, Chichester, UK, pp 356–370

Artan M, Li Y, Karadeniz F, Lee S-H, Kim M-M, Kim S-K (2008) Anti-HIV-1 activity of phloroglucinol derivative, 6, 6′-bieckol, from *Ecklonia cava*. Bioorg Med Chem 16:7921–7926

Athukorala Y, Lee K-W, Kim S-K, Jeon Y-J (2007) Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour Technol 98:1711–1716

Badini L, Pistocchi R, Bagni N (1994) Polyamine transport in the seaweed *Ulva rigida* (Chlorophyta). J Phycol 30:599–605

Bandyopadhyay SS, Navid MH, Ghosh T, Schnitzler P, Ray B (2011) Structural features and in vitro antiviral activities of sulfated polysaccharides from *Sphacelaria indica*. Phytochemistry 72:276–283

Barahona T, Chandía NP, Encinas MV, Matsuhiro B, Zúñiga EA (2011) Antioxidant capacity of sulfated polysaccharides from seaweeds. A kinetic approach. Food Hydrocoll 25:529–535

Beppu F, Hosokawa M, Niwano Y, Miyashita K (2012) Effects of dietary fucoxanthin on cholesterol metabolism in diabetic/obese KK-A(y) mice. Lipids Health Dis 11:112

Bernardi G, Springer GF (1962) Properties of highly purified fucan. J Biol Chem 237:75–80

Berteau O, Mulloy B (2003) Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. Glycobiology 13:29R–40R

Bhatia S, Rathee P, Sharma K, Chaugule BB, Kar N, Bera T (2013) Immuno-modulation effect of sulphated polysaccharide (porphyran) from *Porphyra vietnamensis*. Int J Biol Macromol. doi:10.1016/j.ijbiomac.2013.03.012

Bouhlal R, Haslin C, Chermann J-C, Colliec-Jouault S, Sinquin C, Simon G, Cerantola S, Riadi H, Bourgougnon N (2011) Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophytha, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales). Mar Drugs 9:1187–1209

Carlucci MJ, Pujol CA, Ciancia M, Noseda MD, Matulewicz MC, Damonte EB, Cerezo AS (1997) Antiherpetic and anticoagulant properties of carrageenans from the red seaweed *Gigartina skottsbergii* and their cyclized derivatives: correlation between structure and biological activity. Int J Biol Macromol 20:97–105

Cassolato JE, Noseda MD, Pujol CA, Pellizzari FM, Damonte EB, Duarte ME (2008) Chemical structure and antiviral activity of the sulfated heterorhamnan isolated from the green seaweed *Gayralia oxysperma*. Carbohydr Res 343:3085–3095

Cha S, Lee K, Jeon Y (2006) Screening of extracts from red algae in Jeju for potentials marine angiotensin-I converting enzyme (ACE) inhibitory activity. Algae 21:343

Chandía NP, Matsuhiro B (2008) Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. Int J Biol Macromol 42:235–240

Chandini SK, Ganesan P, Suresh PV, Bhaskar N (2008a) Seaweeds as a source of nutritionally beneficial compounds – a review. J Food Sci Technol 45:1–13

Chandini SK, Ganesan P, Bhaskar N (2008b) In vitro antioxidant activities of three selected brown seaweeds of India. Food Chem 107:707–713

Chen J-H, Lim J-D, Sohn E-H, Choi Y-S, Han E-T (2009) Growth-inhibitory effect of a fucoidan from brown seaweed *Undaria pinnatifida* on *Plasmodium* parasites. Parasitol Res 104:245–250

Chew YL, Lim YY, Omar M, Khoo KS (2008) Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol 41:1067–1072

Choi J-G, Kang O-H, Brice O-O, Lee Y-S, Chae H-S, Oh Y-C, Sohn D-H, Park H, Choi H-G, Kim S-G, Shin DW, Kwon DY (2010) Antibacterial activity of *Ecklonia cav*a against methicillin-resistant *Staphylococcus aureus* and *Salmonella* spp. Foodborne Pathog Dis 7:435–441

Christians N, Liu D, Unruh JB (2010) The use of protein hydrolysates for weed control. In: Pasupuleti VK, Demain AL (eds) Protein hydrolysates in biotechnology. Springer, New York, pp 127–133

Cian RE, Alaiz M, Vioque J, Drago SR (2012a) Enzyme proteolysis enhanced extraction of ACE inhibitory and antioxidant compounds (peptides and polyphenols) from *Porphyra columbina residual cake*. J Appl Phycol: 1–10. doi:10.1007/s10811-012-9913-2

Cian RE, Martínez-Augustin O, Drago SR (2012b) Bioactive properties of peptides obtained by enzymatic hydrolysis from protein byproducts of *Porphyra columbina*. Food Res Int 49:364–372

Cian RE, López-Posadas R, Drago SR, Sánchez de Medina F, Martínez-Augustin O (2012c) A *Porphyra columbina* hydrolysate upregulates IL-10 production in rat macrophages and lymphocytes through an NF-κB, and p38 and JNK dependent mechanism. Food Chem 134:1982–1990

Ciancia M, Quintana I, Vizcargüénaga MI, Kasulin L, de Dios A, Estevez JM, Cerezo AS (2007) Polysaccharides from the green seaweeds *Codium fragile* and *C. vermilara* with controversial effects on hemostasis. Int J Biol Macromol 41:641–649

Cooper R, Dragar C, Elliot K, Fitton JH, Godwin J, Thompson K (2002) GFS, a preparation of Tasmanian *Undaria pinnatifida* is associated with healing and inhibition of reactivation of Herpes. BMC Complement Altern Med 2:11

Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Câmara RB, Nobre LT, Costa MS, Almeida-Lima J, Farias EH, Leite EL, Rocha HA (2010) Biological activities of sulfated polysaccharides from tropical seaweeds. Biomed Pharmacother 64:21–28

Crouch IJ, Van Staden J (1993) Commercial seaweed products as biostimulants in horticulture. J Home Consum Hort 1:19–76

Cumashi A, Ushakova NA, Preobrazhenskaya ME, D'Incecco A, Piccoli A, Totani L, Tinari N, Morozevich GE, Berman AE, Bilan MI, Usov AI, Ustyuzhanina NE, Grachev AA, Sanderson CJ, Kelly M, Rabinovich GA, Iacobelli S, Nifantiev NE (2007) A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology 17:541–552

D'Orazio N, Gemello E, Gammone M, de Girolamo M, Ficoneri C, Riccioni G (2012) Fucoxantin: a treasure from the sea. Mar Drugs 10:604–616

Dandrifosse G, Peulen O, El Khefif N, Deloyer P, Dandrifosse A-C, Grandfils C (2000) Are milk polyamines preventine agents against food allergy? In: Proceedings in nutrition society of London, Cambridge University Press, Cambridge, pp 81–86

Das SK, Ren R, Hashimoto T, Kanazawa K (2010) Fucoxanthin induces apoptosis in osteoclast-like cells differentiated from RAW264. 7 cells. J Agric Food Chem 58:6090–6095

Dawczynski C, Schubert R, Jahreis G (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem 103:891–899

Dawes CJ (1998) Marine botany, 2nd edn. Wiley, New York, 496 pp

de Araújo IWF, de Sousa Oliveira Vanderlei E, Rodrigues JAG, Coura CO, Quinderé ALG, Fontes BP, de Queiroz INL, Jorge RJB, Bezerra MM, Rodrigues e Silva AA, Chaves HV, Monteiro HSA, de Paula RCM, Benevides NMB (2011) Effects of a sulfated polysaccharide isolated from the red seaweed *Solieria filiformis* on models of nociception and inflammation. Carbohydr Polym 86:1207–1215

de Sousa Oliveira Vanderlei E, de Araújo IWF, Quinderé ALG, Fontes BP, Eloy YRG, Rodrigues JAG, Rodrigues e Silva AA, Chaves HV, Jorge RJB, de Menezes DB, Evangelista JSAM, Bezerra MM, Benevides NMB (2011) The involvement of the HO-1 pathway in the anti-inflammatory action of a sulfated polysaccharide isolated from the red seaweed *Gracilaria birdiae*. Inflamm Res 60:1121–1130

de Sousa AA, Benevides NM, de Freitas Pires A, Fiúza FP, Queiroz MG, Morais TM, Pereira MG, Assreuy AM (2013) A report of a galactan from marine alga *Gelidium crinale* with in vivo anti-inflammatory and antinociceptive effects. Fundam Clin Pharmacol 27:173–180

De Zoysa M, Nikapitiya C, Jeon Y-J, Jee Y, Lee J (2008) Anticoagulant activity of sulfated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*. J Appl Phycol 20:67–74

Dembitsky V, Maoka T (2007) Allenic and cumulenic lipids. Prog Lipid Res 46:328–375

Dhargalkar VK, Pereira N (2005) Seaweed: promising plant of the millennium. Sci Cult 71:60–66

Diplock AT, Charleux J-L, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Viña-Ribes J (1998) Functional food science and defence against reactive oxidative species. Br J Nutr 80:S77–S112

Dobashi K, Nishino T, Fujihara M, Nagumo T (1989) Isolation and preliminary characterization of fucose-containing sulfated polysaccharides with blood-anticoagulant activity from the brown seaweed *Hizikia fusiforme*. Carbohydr Res 194:315–320

Doh-Ura K, Kuge T, Uomoto M, Nishizawa K, Kawasaki Y, Iha M (2007) Prophylactic effect of dietary seaweed Fucoidan against enteral prion infection. Antimicrob Agents Chemother 51:2274–2277

El Gamal AA (2012) Biological importance of marine algae. In: Handbook of marine macroalgae. Wiley, New York, pp 1–35. doi:10.1002/9781119977087

Eom SH (2012) Anti-MRSA (methicillin-resistant *Staphylococcus aureus*) substance isolated from *Eisenia bicyclis* and its action mechanism. Ph.D. dissertation. Pukyong National University, Busan, 45 pp

Eom S-H, Kim Y-M, Kim S-K (2012) Antimicrobial effect of phlorotannins from marine brown algae. Food Chem Toxicol 50:3251–3255

Ermakova S, Sokolova R, Kim S-M, Um B-H, Isakov V, Zvyagintseva T (2011) Fucoidans from brown seaweeds *Sargassum hornery*, *Eclonia cava*, *Costaria costata*: structural characteristics and anticancer activity. Appl Biochem Biotechnol 164(6):841–850. doi:10.1007/s12010-011-9178-2

Fan Y, Wang W, Song W, Chen H, Teng A, Liu A (2012) Partial characterization and anti-tumor activity of an acidic polysaccharide from *Gracilaria lemaneiformis*. Carbohydr Polym 88:1313–1318

FAO (2012) The State of World Fisheries and Aquaculture 2012 (SOFIA). FAO Fisheries and Aquaculture Department, Rome

Farias WR, Lima PC, Rodrigues NV, Siqueira RC, Amorim RM, Pereira MG, Assreuy AM (2011) A novel antinociceptive sulphated polysaccharide of the brown marine alga *Spatoglossum schroederi*. Nat Prod Commun 6:863–866

Feldman SC, Reynaldi S, Stortz CA, Cerezo AS, Damonte EB (1999) Antiviral properties of fucoidan fractions from *Leathesia difformis*. Phytomedicine 6:335–340

Fernández PV, Estevez JM, Cerezo AS, Ciancia M (2012) Sulfated β-d-mannan from green seaweed *Codium vermilara*. Carbohydr Polym 87:916–919

Figueroa JA, Kimball BA, Perry KR (2008) Lagomorph and rodent responses to two protein hydrolysates. Crop Prot 27:851–854

Fitzgerald C, Mora-Soler L, Gallagher E, O'Connor P, Prieto JA, Soler-Vila A, Hayes M (2012) Isolation and characterization of bioactive pro-peptides with in vitro renin inhibitory activities from the macroalga *Palmaria palmata*. J Agric Food Chem 60:7421–7427

Fleming AE, Van Barneveld RV, Hone PW (1996) The development of artificial diets for abalone: a review and future directions. Aquaculture 140:5–53

Fleurence J (2004) Seaweed proteins. In: Yada RY (ed) Proteins in food processing. Woodhead Publishing, Cambridge, pp 197–213

Fu XY, Xue CH, Ning Y, Li ZJ, Xu JC (2004) Acute antihypertensive effects of fucoidan oligosaccharides prepared from *Laminaria japonica* on renovascular hypertensive rat. J Ocean Univ Qingdao 34:560–564

Fung A, Hamid N, Lu J (2013) Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. Food Chem 136:1055–1062

Ghosh T, Chattopadhyay K, Marschall M, Karmakar P, Mandal P, Ray B (2009a) Focus on antivirally active sulfated polysaccharides: from structure–activity analysis to clinical evaluation. Glycobiology 19:2–15

Ghosh T, Pujol CA, Damonte EB, Sinha S, Ray B (2009b) Sulfated xylomannans from the red seaweed *Sebdenia polydactyla*: structural features, chemical modification and antiviral activity. Antivir Chem Chemother 19:235–242

Go H, Hwang H-J, Nam T-J (2010) A glycoprotein from *Laminaria japonica* induces apoptosis in HT-29 colon cancer cells. Toxicol In Vitro 24:1546–1553

Goldberg I (1994) Functional foods: designer foods, pharmafoods, nutraceuticals. Springer, Chapman & Hall, New York

Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P (2010) Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Res Int 43:2289–2294

Gugliucci A, Menini T (2003) The polyamines spermine and spermidine protect proteins from structural and functional damage by AGE precursors: a new role for old molecules? Life Sci 72:2603–2616

Hamana K, Matsuzaki S (1982) Widespread occurrence of norspermidine and norspermine in eukaryotic algae. J Biochem 91:1321–1328

Haneji K, Matsuda T, Tomita M, Kawakami H, Ohshiro K, Uchihara J-N, Masuda M, Takasu N, Tanaka Y, Ohta T, Mori N (2005) Fucoidan extracted from *Cladosiphon okamuranus* Tokida induces apoptosis of human T-cell leukemia virus type 1-infected T-cell lines and primary adult T-cell leukemia cells. Nutr Cancer 52:189–201

Harden EA, Falshaw R, Carnachan SM, Kern ER, Prichard MN (2009) Virucidal activity of polysaccharide extracts from four algal species against herpes simplex virus. Antiviral Res 83:282–289

Harnedy PA, FitzGerald RJ (2011) Bioactive proteins, peptides, and amino acids from macroalgae. J Phycol 47:218–232

Hayashi K, Nakano T, Hashimoto M, Kanekiyo K, Hayashi T (2008) Defensive effects of a fucoidan from brown alga *Undaria pinnatifida* against herpes simplex virus infection. Int Immunopharmacol 8:109–116

He H-L, Chen X-L, Wu H, Sun C-Y, Zhang Y-Z, Zhou B-C (2007) High throughput and rapid screening of marine protein hydrolysates enriched in peptides with angiotensin-I-converting enzyme inhibitory activity by capillary electrophoresis. Bioresour Technol 98:3499–3505

Hemmingson JA, Falshaw R, Furneaux RH, Thompson K (2006) Structure and antiviral activity of the galactofucan sulfates extracted from *Undaria pinnatifida* (Phaeophyta). J Appl Phycol 18:185–193

Heo S-J, Jeon Y-J (2008) Radical scavenging capacity and cytoprotective effect of enzymatic digests of *Ishige okamurae*. J Appl Phycol 20:1087–1095

Heo S-J, Jeon Y-J (2009a) Evaluation of diphlorethohydroxycarmalol isolated from *Ishige okamurae* for radical scavenging activity and its protective effect against H_2O_2-induced cell damage. Process Biochem 44:412–418

Heo S-J, Jeon Y-J (2009b) Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage. J Photochem Photobiol B 95:101–107

Heo S-J, Lee K-W, Song CB, Jeon Y-J (2003a) Antioxidant activity of enzymatic extracts from brown seaweeds. Algae 18:71–82

Heo S-J, Jeon Y-J, Lee J, Kim HT, Lee K-W (2003b) Antioxidant effect of enzymatic hydrolyzate from a Kelp, *Ecklonia cava*. Algae 18:341–348

Heo S-J, Park E-J, Lee K-W, Jeon Y-J (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 96:1613–1623

Heo SJ, Kim JP, Jung WK, Lee NH, Kang HS, Jun EM, Park SH, Kang SM, Lee YJ, Park PJ, Jeon YJ (2008a) Identification of chemical structure and free radical scavenging activity of diphlor-

ethohydroxycarmalol isolated from a brown alga, *Ishige okamurae*. J Microbiol Biotechnol 18:676–681

Heo S-J, Ko S-C, Kang S-M, Kang H-S, Kim J-P, Kim S-H, Lee K-W, Cho M-G, Jeon Y-J (2008b) Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H_2O_2-induced cell damage. Eur Food Res Technol 228:145–151

Heo S-J, Ko S-C, Cha S-H, Kang D-H, Park H-S, Choi Y-U, Kim D, Jung W-K, Jeon Y-J (2009a) Effect of phlorotannins isolated from *Ecklonia cava* on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation. Toxicol In Vitro 23:1123–1130

Heo S-J, Hwang J-Y, Choi J-I, Han J-S, Kim H-J, Jeon Y-J (2009b) Diphlorethohydroxycarmalol isolated from *Ishige okamurae*, a brown algae, a potent α-glucosidase and α-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. Eur J Pharmacol 615:252–256

Heo S-J, Ko S-C, Kang S-M, Cha S-H, Lee S-H, Kang D-H, Jung W-K, Affan A, Oh C, Jeon Y-J (2010a) Inhibitory effect of diphlorethohydroxycarmalol on melanogenesis and its protective effect against UV-B radiation-induced cell damage. Food Chem Toxicol 48:1355–1361

Heo S-J, Yoon W-J, Kim K-N, Ahn G-N, Kang S-M, Kang D-H, Affan A, Oh C, Jung W-K, Jeon Y-J (2010b) Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. Food Chem Toxicol 48:2045–2051

Heo S-J, Cha S-H, Kim K-N, Lee S-H, Ahn G, Kang D-H, Oh C, Choi Y-U, Affan A, Kim D, Jeon Y-J (2012a) Neuroprotective effect of phlorotannin isolated from *Ishige okamurae* against H_2O_2-induced oxidative stress in murine hippocampal neuronal cells, HT22. Appl Biochem Biotechnol 166:1520–1532

Heo S-J, Yoon W-J, Kim K-N, Oh C, Choi Y-U, Yoon K-T, Kang D-H, Qian Z-J, Choi I-W, Jung W-K (2012b) Anti-inflammatory effect of fucoxanthin derivatives isolated from *Sargassum siliquastrum* in lipopolysaccharide-stimulated RAW 264.7 macrophage. Food Chem Toxicol 50:3336–3342

Hidari KI, Takahashi N, Arihara M, Nagaoka M, Morita K, Suzuki T (2008) Structure and anti-dengue virus activity of sulfated polysaccharide from a marine alga. Biochem Biophys Res Commun 376:91–95

Hopkins WG, Hüner NPA (1995) Introduction to plant physiology, vol 355. Wiley, New York

Hoppe HA (1979) Marine algae and their products and constituents in pharmacy. In: Hoppe HA, Levring T, Tanaka Y (eds) Marine algae in pharmaceutical science. Walter de Gruyter, New York, p 807

Hoppe HA, Levring T, Tanaka Y (1979) Marine algae in pharmaceutical science. Walter de Gruyter, New York, pp 25–119

Hosokawa M, Miyashita T, Nishikawa S, Emi S, Tsukui T, Beppu F, Okada T, Miyashita K (2010) Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. Arch Biochem Biophys 504:17–25

Hwang P-A, Chien S-Y, Chan Y-L, Lu M-K, Wu C-H, Kong Z-L, Wu C-J (2011) Inhibition of lipopolysaccharide (LPS)-induced inflammatory responses by *Sargassum hemiphyllum* sulfated polysaccharide extract in RAW 264.7 macrophage cells. J Agric Food Chem 59:2062–2068

Inagrosa (2002) http://www.inagrosa.es/biblioteca_i1.html

Joe M-J, Kim S-N, Choi H-Y, Shin W-S, Park G-M, Kang D-W, Kim YK (2006) The inhibitory effects of eckol and dieckol from *Ecklonia stolonifera* on the expression of matrix metalloproteinase-1 in human dermal fibroblasts. Biol Pharm Bull 29:1735–1739

Jung HA, Hyun SK, Kim HR, Choi JS (2006) Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. Fisheries Sci 72:1292–1299

Jung HA, Yoon NY, Woo M-H, Choi JS (2008) Inhibitory activities of extracts from several kinds of seaweeds and phlorotannins from the brown alga *Ecklonia stolonifera* on glucose-mediated protein damage and rat lens aldose reductase. Fisheries Sci 74:1363–1365

Jung HA, Islam MN, Lee CM, Jeong HO, Chung HY, Woo HC, Choi JS (2012) Promising antidiabetic potential of fucoxanthin isolated from the edible brown algae *Eisenia bicyclis* and *Undaria pinnatifida*. Fisheries Sci 78:1321–1329

Kadekaru T, Toyama H, Yasumoto T (2008) Safety evaluation of fucoxanthin purified from *Undaria pinnatifida*. J Jpn Soc Food Sci 55:304–308

Kakkar RK, Sawhney VK (2002) Polyamine research in plants – a changing perspective. Physiol Plantarum 116:281–292

Kalač P, Krausová P (2005) A review of dietary polyamines: formation, implications for growth and health and occurrence in foods. Food Chem 90:219–230

Kaliaperumal N (2003) Products from seaweeds. SDMRI Res Publ 3:33–42

Kang KA, Lee KH, Chae S, Koh YS, Yoo B-S, Kim JH, Ham YM, Baik JS, Lee NH, Hyun JW (2005) Triphlorethol-A from *Ecklonia cava* protects V79-4 lung fibroblast against hydrogen peroxide induced cell damage. Free Rad Res 39:883–892

Kang KA, Lee KH, Park JW, Lee NH, Na HK, Surh YJ, You HJ, Chung MH, Hyun JW (2007) Triphlorethol-A induces heme oxygenase-1 via activation of ERK and NF-E2 related factor 2 transcription factor. FEBS Lett 581:2000–2008

Kang S-I, Ko H-C, Shin H-S, Kim H-M, Hong Y-S, Lee N-H, Kim S-J (2011) Fucoxanthin exerts differing effects on 3T3-L1 cells according to differentiation stage and inhibits glucose uptake in mature adipocytes. Biochem Biophy Res Commun 409:769–774

Kang S-M, Heo S-J, Kim K-N, Lee S-H, Yang H-M, Kim A-D, Jeon Y-J (2012a) Molecular docking studies of a phlorotannin, dieckol isolated from *Ecklonia cava* with tyrosinase inhibitory activity. Bioorg Med Chem 20:311–316

Kang S-M, Kim A-D, Heo S-J, Kim K-N, Lee S-H, Ko S-C, Jeon Y-J (2012b) Induction of apoptosis by diphlorethohydroxycarmalol isolated from brown alga, *Ishige okamurae*. J Funct Food 4:433–439

Kang M-C, Wijesinghe WA, Lee S-H, Kang S-M, Ko S-C, Yang X, Kang N, Jeon B-T, Kim J, Lee D-H, Jeon Y-J (2013) Dieckol isolated from brown seaweed *Ecklonia cava* attenuates type II diabetes in *db/db* mouse model. Food Chem Toxicol 53:294–298

Kannan RRR, Aderogba MA, Ndhlala AR, Stirk WA, Van Staden J (2013) Acetylcholinesterase inhibitory activity of phlorotannins isolated from the brown alga, *Ecklonia maxima* (Osbeck) Papenfuss. Food Res Int. doi:10.1016/j.foodres.2012.11.017

Karmakar P, Pujol CA, Damonte EB, Ghosh T, Ray B (2010) Polysaccharides from *Padina tetrastromatica*: structural features, chemical modification and antiviral activity. Carbohydr Polym 80:513–520

Kawamoto H, Miki Y, Kimura T, Tanaka K, Nakagawa T, Kawamukai M, Matsuda H (2006) Effects of fucoidan from Mozuku on human stomach cell lines. Food Sci Technol Res 12:218–222

Kawamura-Konishi Y, Watanabe N, Saito M, Nakajima N, Sakaki T, Katayama T, Enomoto T (2012) Isolation of a new phlorotannin, a potent inhibitor of carbohydrate-hydrolyzing enzymes, from the brown alga *Sargassum patens*. J Agric Food Chem 60:5565–5570

Kawashima T, Murakami K, Nishimura I, Nakano T, Obata A (2012) A sulfated polysaccharide, fucoidan, enhances the immunomodulatory effects of lactic acid bacteria. Int J Mol Med 29:447–453

Khan SI, Satam S (2003) Seaweed mariculture: scope and potential in India. Aquac Asia 8:26–29

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Res Int 39:383–393

Kim S-K, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2:1–9

Kim K-N, Heo S-J, Song CB, Lee J, Heo M-S, Yeo I-K, Kang KA, Hyun JW, Jeon Y-J (2006) Protective effect of *Ecklonia cava* enzymatic extracts on hydrogen peroxide-induced cell damage. Process Biochem 41:2393–2401

Kim A-R, Shin T-S, Lee M-S, Park J-Y, Park K-E, Yoon N-Y, Kim J-S, Choi J-S, Jang B-C, Byun D-S, Park N-K, Kim H-R (2009) Isolation and identification of phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. J Agric Food Chem 57:3483–3489

Kim J-K, Cho ML, Karnjanapratum S, Shin I-S, You SG (2011) In vitro and in vivo immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*. Int J Biol Macromol 49:1051–1058

Kitts DD, Weiler K (2003) Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr Pharm Des 9:1309–1323

Ko SC, Kang SM, Ahn GN, Yang HP, Kim KN, Jeon YJ (2010) Antioxidant activity of enzymatic extracts from *Sargassum coreanum*. J Korean Soc Food Sci Nutr 39:494–499

Ko S-C, Lee S-H, Ahn G, Kim K-N, Cha S-H, Kim S-K, Jeon B-T, Park P-J, Lee K-W, Jeon Y-J (2012) Effect of enzyme-assisted extract of *Sargassum coreanum* on induction of apoptosis in HL-60 tumor cells. J Appl Phycol 24:675–684

Kong C-S, Kim J-A, Yoon N-Y, Kim S-K (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. Food Chem Toxicol 47:1653–1658

Konishi I, Hosokawa M, Sashima T, Kobayashi H, Miyashita K (2006) Halocynthiaxanthin and fucoxanthinol isolated from *Halocynthia roretzi* induce apoptosis in human leukemia, breast and colon cancer cells. Comp Biochem Physiol C Toxicol Pharmacol 142:53–59

Kotake-Nara E, Sugawara T, Nagao A (2005) Antiproliferative effect of neoxanthin and fucoxanthin on cultured cells. Fisheries Sci 71:459–461

Kraan S (2012) Algal polysaccharides, novel applications and outlook. In: Chang C-F (ed) Carbohydrates – comprehensive studies on glycobiology and glycotechnology. ISBN: 978-953-51-0864-1. InTech. doi:10.5772/51572. http://www.intechopen.com/books/carbohydrates-comprehensive-studies-on-glycobiology-and-glycotechnology/algal-polysaccharides-novel-applications-and-outlook

Kuznetsova TA, Besednova NN, Mamaev AN, Momot AP, Shevchenko NM, Zvyagintseva TN (2003) Anticoagulant activity of fucoidan from brown algae *Fucus evanescens* of the Okhotsk Sea. Bull Exp Biol Med 136:471–473

Kyung J, Kim D, Park D, Yang Y-H, Choi E-K, Lee S-P, Kim T-S, Lee Y-B, Kim Y-B (2012) Synergistic anti-inflammatory effects of *Laminaria japonica* fucoidan and *Cistanche tubulosa* extract. Lab Animal Res 28:91–97

Lahaye M, Ray B (1996) Cell-wall polysaccharides from the marine green alga *Ulva* “*rigida*” (Ulvales, Chlorophyta) – NMR analysis of ulvan oligosaccharides. Carbohydr Res 283:161–173

Larqué E, Sabater-Molina M, Zamora S (2007) Biological significance of dietary polyamines. Nutrition 23:87–95

Le Q-T, Li Y, Qian Z-J, Kim M-M, Kim S-K (2009) Inhibitory effects of polyphenols isolated from marine alga *Ecklonia cava* on histamine release. Process Biochem 44:168–176

Lee T-M (1998) Investigations of some intertidal green macroalgae to hyposaline stress: detrimental role of putrescine under extreme hyposaline conditions. Plant Sci 138:1–8

Lee SH (2010) Anti-inflammatory mechanisms of phlorotannins derived from *Eisenia bicyclis* and their inhibitory effects on matrix metalloproteinases. Dissertation, Pukyong National University, Busan

Lee S-H, Jeon Y-J (2013) Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. Fitoterapia 86:129–136

Lee J-B, Hayashi K, Hashimoto M, Nakano T, Hayashi T (2004) Novel antiviral fucoidan from sporophyll of *Undaria pinnatifida* (Mekabu). Chem Pharm Bull 52:1091–1094

Lee JM, You SG, Kim SM (2005) Functional activities of low molecular weight peptides purified from enzymatic hydrolysates of seaweeds. J Korean Soc Food Sci Nutr 34:1124–1129

Lee D-S, Kang M-S, Hwang H-J, Eom S-H, Yang J-Y, Lee M-S, Lee W-J, Jeon Y-J, Choi J-S, Kim Y-M (2008) Synergistic effect between dieckol from *Ecklonia stolonifera* and β-lactams against methicillin-resistant *Staphylococcus aureus*. Biotechnol Bioproc Eng 13:758–764

Lee S-H, Karadeniz F, Kim M-M, Kim S-K (2009) α-Glucosidase and α-amylase inhibitory activities of phloroglucinal derivatives from edible marine brown alga, *Ecklonia cava*. J Sci Food Agric 89:1552–1558

Lee MH, Lee KB, Oh SM, Lee BH, Chee HY (2010) Antifungal activities of dieckol isolated from the marine brown alga *Ecklonia cava* against *Trichophyton rubrum*. J Korean Soc Appl Biol Chem 53:504–507

Lee M-S, Shin T, Utsuki T, Choi J-S, Byun D-S, Kim H-R (2012a) Isolation and identification of phlorotannins from *Ecklonia stolonifer*a with antioxidant and hepatoprotective properties in tacrine-treated HepG2 cells. J Agric Food Chem 60:5340–5349

Lee S-H, Choi J-I, Heo S-J, Park M-H, Park P-J, Jeon B-T, Kim S-K, Han J-S, Jeon Y-J (2012b) Diphlorethohydroxycarmalol isolated from Pae (*Ishige okamurae*) protects high glucose-induced damage in RINm5F pancreatic β cells via its antioxidant effects. Food Sci Biotechnol 21:239–246

Li DY, Xu Z, Zhang SH (1999) Prevention and cure of fucoidan of *L. japonica* on mice with hypercholesterolemia. Food Sci 20:45–46

Li DY, Xu Z, Huang LM, Wang HB, Zhang SH (2001) Effect of fucoidan of *L. japonica* on rats with hyperlipidaemia. Food Sci 22:92–95

Li J, Wei YX, Du GC, Hu YF, Li L (2007) Anticoagulant activities of phlorotannins from *Sargassum thunbergii* Kuntze. Trad Chinese Drug Res Clin Pharmacol 18:191–194

Li B, Zhao R-X, Wei X-J (2008) Anticoagulant activity of fucoidan from *Hizikia fusiforme*. Agro Food Ind Hi-Tech 19:22–24

Li Y, Qian Z-J, Ryu B, Lee S-H, Kim M-M, Kim S-K (2009) Chemical components and its antioxidant properties in vitro: an edible marine brown alga, *Ecklonia cava*. Bioorg Med Chem 17:1963–1973

Li Y-X, Wijesekara I, Li Y, Kim S-K (2011) Phlorotannins as bioactive agents from brown algae. Process Biochem 46:2219–2224

Lima GP, Vianello F (2011) Review on the main differences between organic and conventional plant-based foods. Int J Food Sci Technol 46:1–13

Lin H-C, Chou S-T, Chuang M-Y, Liao T-Y, Tsai W-S, Chiu T-H (2012) The effects of *Caulerpa microphysa* enzyme-digested extracts on ACE-inhibitory activity and in vitro anti-tumor properties. Food Chem 134:2235–2241

Lins KO, Bezerra DP, Alves APN, Alencar N, Lima MW, Torres VM, Farias WR, Pessoa C, de Moraes MO, Costa-Lotufo LV (2009) Antitumor properties of a sulfated polysaccharide from the red seaweed *Champia feldmannii* (Diaz-Pifferer). J Appl Toxicol 29:20–26

Liu H, Gu L (2012) Phlorotannins from brown algae (*Fucus vesiculosus*) inhibited the formation of advanced glycation endproducts by scavenging reactive carbonyls. J Agric Food Chem 60:1326–1334

Liu JX, Wang LL, Liu J, Wang JP (2013) Development of an indirect competitive immunoassay for determination of L-hydroxyproline in milk. Food Agric Immunol 1–13. doi:10.1080/09540105.2013.768965

Mabeau S, Fleurence J (1993) Seaweed in food products: biochemical and nutritional aspects. Trends Food Sci Technol 4:103–107

Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K (2005) Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem Biophys Res Commun 332:392–397

Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K (2007a) Effect of medium-chain triacylglycerols on anti-obesity effect of fucoxanthin. J Oleo Sci 56:615–621

Maeda H, Hosokawa M, Sashima T, Miyashita K (2007b) Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. J Agric Food Chem 55:7701–7706

Maeda H, Hosokawa M, Sashima T, Miyashita K (2008) Antiobesity effect of fucoxanthin from edible seaweeds and its multibiological functions. Functional Food and Health 32:376–388

Magalhaes KD, Costa LS, Fidelis GP, Oliveira RM, Nobre LTDB, Dantas-Santos N, Camara RBG, Albuquerque IRL, Cordeiro SL, Sabry DA, Costa MS, Alves LG, Rocha HA (2011) Anticoagulant, antioxidant and antitumor activities of heterofucans from the seaweed *Dictyopteris delicatula*. Int J Mol Sci 12:3352–3365

Majczak G, Richartz R, Duarte M, Noseda M, Chapman A, Anderson R, Vreeland V, Davison I (2003) Antiherpetic activity of heterofucans isolated from *Sargassum stenophyllum* (Fucales, Phaeophyta). In: Proceedings of the 17th international seaweed symposium, Cape Town, South Africa, 28 Jan–2 Feb 2001, Oxford University Press, Oxford, pp 169–174

Mandal P, Mateu CG, Chattopadhyay K, Pujol CA, Damonte EB, Ray B (2007) Structural features and antiviral activity of sulphated fucans from the brown seaweed *Cystoseira indica*. Antivir Chem Chemother 18:153–162

Marián FD, García-Jiménez P, Robaina RR (2000) Polyamines in marine macroalgae: levels of putrescine, spermidine and spermine in the thalli and changes in their concentration during glycerol-induced cell growth in vitro. Physiol Plantarum 110:530–534
Martin-Tanguy J (2001) Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth Regul 34:135–148
Maruyama H, Tamauchi H, Hashimoto M, Nakano T (2005) Suppression of Th2 immune responses by mekabu fucoidan from *Undaria pinnatifida* sporophylls. Int Arch Allergy Immunol 137:289–294
Maruyama H, Tamauchi H, Iizuka M, Nakano T (2006) The role of NK cells in antitumor activity of dietary fucoidan from *Undaria pinnatifida* sporophylls (Mekabu). Planta Med 72:1415–1417
Matsumoto M, Hosokawa M, Matsukawa N, Hagio M, Shinoki A, Nishimukai M, Miyashita K, Yajima T, Hara H (2010) Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. Eur J Nutr 49:243–249
Mayer AM, Hamann MT (2002) Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanisms of action. Comp Biochem Physiol C Toxicol Pharmacol 132:315–339
Mazumder S, Ghosal PK, Pujol CA, Carlucci MJ, Damonte EB, Ray B (2002) Isolation, chemical investigation and antiviral activity of polysaccharides from *Gracilaria corticata* (Gracilariaceae, Rhodophyta). Int J Biol Macromol 31:87–95
McCormick CJ, Newbold CI, Berendt AR (2000) Sulfated glycoconjugates enhance CD36-dependent adhesion of *Plasmodium falciparum*–infected erythrocytes to human microvascular endothelial cells. Blood 96:327–333
McHugh DJ (2003) A guide to the seaweed industry. FAO fisheries technical paper no 441
Moon C, Kim SH, Kim JC, Hyun JW, Lee NH, Park JW, Shin T (2008) Protective effect of phlorotannin components phloroglucinol and eckol on radiation-induced intestinal injury in mice. Phytother Res 22:238–242
Mourão PA (2004) Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. Curr Pharm Des 10:967–981
Murata M, Nakazoe J-I (2001) Production and use of marine algae in Japan. J Agric Res 35:281–290
Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T (2002) Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. J Antimicrob Chemother 50:889–893
Nagayama K, Shibata T, Fujimoto K, Honjo T, Nakamura T (2003) Algicidal effect of phlorotannins from the brown alga *Ecklonia kurome* on red tide microalgae. Aquaculture 218:601–611
Nakazawa Y, Sashima T, Hosokawa M, Miyashita K (2009) Comparative evaluation of growth inhibitory effect of stereoisomers of fucoxanthin in human cancer cell lines. J Funct Food 1:88–97
Nasu T, Fukuda Y, Nagahira K, Kawashima H, Noguchi C, Nakanishi T (1997) Fucoidin, a potent inhibitor of L-selectin function, reduces contact hypersensitivity reaction in mice. Immunol Lett 59:47–51
Nice D (1997) Antioxidant based nutraceuticals. In: Yalpanic M (ed) New technologies for healthy foods and neutraceuticals. Science Publishers, Shrewsbury, pp 105–123
Nishibori N, Fujihara S, Akatuki T (2007) Amounts of polyamines in foods in Japan and intake by Japanese. Food Chem 100:491–497
Nishikawa S, Hosokawa M, Miyashita K (2012) Fucoxanthin promotes translocation and induction of glucose transporter 4 in skeletal muscles of diabetic/obese KK-A(y) mice. Phytomedicine 19:389–394
Nishino T, Yokoyama G, Dobashi K, Fujihara M, Nagumo T (1989) Isolation, purification, and characterization of fucose-containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood-anticoagulant activities. Carbohydr Res 186:119–129
Nishino T, Aizu Y, Nagumo T (1991) The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. Thromb Res 64:723–731

Nomura T, Kikuchi M, Kubodera A, Kawakami Y (1997) Proton-donative antioxidant activity of fucoxanthin with 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Biochem Mol Biol Int 42:361–370

Norziah MH, Ching CY (2000) Nutritional composition of edible seaweed *Gracilaria changgi*. Food Chem 68:69–76

O'Sullivan L, Murphy B, McLoughlin P, Duggan P, Lawlor PG, Hughes H, Gardiner GE (2010) Prebiotics from marine macroalgae for human and animal health applications. Mar Drugs 8:2038–2064

Okada Y, Ishimaru A, Suzuki R, Okuyama T (2004) A new phloroglucinol derivative from the brown alga *Eisenia bicyclis*: potential for the effective treatment of diabetic complications. J Nat Prod 67:103–105

Okuzumi J, Nishino H, Murakoshi M, Iwashima A, Tanaka Y, Yamane T, Fujita Y, Takahashi T (1990) Inhibitory effects of fucoxanthin, a natural carotenoid, on N-*myc* expression and cell cycle progression in human malignant tumor cells. Cancer Lett 55:75–81

Papenfus HB, Stirk WA, Finnie JF, Van Staden J (2012) Seasonal variation in the polyamines of *Ecklonia maxima*. Bot Mar 55:539–546

Papenfus HB, Kulkarni MG, Stirk WA, Finnie J, Van Staden J (2013) Effect of a commercial seaweed extract (Kelpak®) and polyamines on nutrient-deprived (N, P and K) okra seedlings. Scientia Hortic 151:142–146

Park P-J, Shahidi F, Jeon Y-J (2004) Antioxidant activities of enzymatic extracts from an edible seaweed *Sargassum horneri* using ESR spectrometry. J Food Lipids 11:15–27

Park E, Ahn G, Yun J-S, Kim MJ, Bing SJ, Kim DS, Lee J, Lee NH, Park JW, Jee Y (2010) Dieckol rescues mice from lethal irradiation by accelerating hemopoiesis and curtailing immunosuppression. Int J Radiat Biol 86:848–859

Park HY, Han MH, Park C, Jin C-Y, Kim G-Y, Choi I-W, Kim ND, Nam T-J, Kwon TK, Choi YH (2011a) Anti-inflammatory effects of fucoidan through inhibition of NF-κB, MAPK and Akt activation in lipopolysaccharide-induced BV2 microglia cells. Food Chem Toxicol 49:1745–1752

Park M-K, Jung U, Roh C (2011b) Fucoidan from marine brown algae inhibits lipid accumulation. Mar Drugs 9:1359–1367

Park MH, Jeon Y-J, Kim H-J, Han JS (2012) Effect of diphlorethohydroxycarmalol isolated from Ishige okamurae on apoptosis in 3 t3-L1 preadipocytes. Phytother Res 27:931–936. doi:10.1002/ptr.4797

Parys S, Kehraus S, Krick A, Glombitza K-W, Carmeli S, Klimo K, Gerhäuser C, König GM (2010) In vitro chemopreventive potential of fucophlorethols from the brown alga *Fucus vesiculosus* L. by anti-oxidant activity and inhibition of selected cytochrome P450 enzymes. Phytochemistry 71:221–229

Pasupuleti VK, Demain AL (2010) Protein hydrolysates in biotechnology. Springer, New York, 229 pp

Patel MK, Mulloy B, Gallagher KL, O'Brien L, Hughes AD (2002) The antimitogenic action of the sulphated polysaccharide fucoidan differs from heparin in human vascular smooth muscle cells. Thromb Haemost 87:149–154

Peng J, Yuan J-P, Wu C-F, Wang J-H (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. Mar Drugs 9:1806–1828

Peng Z, Liu M, Fang Z, Wu J, Zhang Q (2012) Composition and cytotoxicity of a novel polysaccharide from brown alga (*Laminaria japonica*). Carbohydr Polym 89:1022–1026

Percival E, McDowell RH (1967) Chemistry and enzymology of marine algal polysaccharides. Academic Press, London, 219 pp

Pérez Recalde M, Noseda MD, Pujol CA, Carlucci MJ, Matulewicz MC (2009) Sulfated mannans from the red seaweed *Nemalion helminthoides* of the South Atlantic. Phytochemistry 70:1062–1068

Ponce NM, Pujol CA, Damonte EB, Flores ML, Stortz CA (2003) Fucoidans from the brown seaweed *Adenocystis utricularis*: extraction methods, antiviral activity and structural studies. Carbohydr Res 338:153–165

Qu W, Ma H, Pan Z, Luo L, Wang Z, He R (2010) Preparation and antihypertensive activity of peptides from *Porphyra yezoensis*. Food Chem 123:14–20

Queiroz KC, Medeiros VP, Queiroz LS, Abreu LR, Rocha HA, Ferreira CV, Jucá MB, Aoyama H, Leite EL (2008) Inhibition of reverse transcriptase activity of HIV by polysaccharides of brown algae. Biomed Pharmacother 62:303–307

Ragan MA, Glombitza K-W (1986) Phlorotannins, brown algal polyphenols. Prog Phycol Res 4:129–241

Rocha HA, Moraes FA, Trindade ES, Franco CR, Torquato RJ, Veiga SS, Valente AP, Mourão PA, Leite EL, Nader HB, Dietrich CP (2005) Structural and hemostatic activities of a sulfated galactofucan from the brown alga *Spatoglossum schroederi*. An ideal antithrombotic agent? J Biol Chem 280:41278–41288

Ryu B, Li Y, Qian Z-J, Kim M-M, Kim S-K (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: Implication for chronic articular disease. Chem Biol Interact 179:192–201

Ryu YB, Jeong HJ, Yoon SY, Park J-Y, Kim YM, Park S-J, Rho M-C, Kim S-J, Lee WS (2011) Influenza virus neuraminidase inhibitory activity of phlorotannins from the edible brown alga *Ecklonia cava*. J Agric Food Chem 59:6467–6473

Saito M, Hagino H (2005) Antihypertensive effect of oligopeptides derived from nori (*Porphyra yezoensis*) and Ala-Lys-Tyr-Ser-Tyr in rats. J Jpn Soc Nutr Food Sci 58:177

Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losada P (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem 85:439–444

Sasaki K, Ishihara K, Oyamada C, Sato A, Fukushi A, Arakane T, Motoyama M, Yamazaki M, Mitsumoto M (2008) Effects of fucoxanthin addition to ground chicken breast meat on lipid and colour stability during chilled storage, before and after cooking. Asian Australas J Anim Sci 21:1067–1072

Sathivel A, Raghavendran HRB, Srinivasan P, Devaki T (2008) Anti-peroxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on d-Galactosamine induced hepatitis in rats. Food Chem Toxicol 46:3262–3267

Sato M, Hosokawa T, Yamaguchi T, Nakano T, Muramoto K, Kahara T, Funayama K, Kobayashi A, Nakano T (2002a) Angiotensin I-converting enzyme inhibitory peptides derived from wakame (*Undaria pinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. J Agric Food Chem 50:6245–6252

Sato M, Oba T, Yamaguchi T, Nakano T, Kahara T, Funayama K, Kobayashi A (2002b) Antihypertensive effects of hydrolysates of wakame (*Undaria pinnatifida*) and their angiotensin-I-converting enzyme inhibitory activity. Ann Nutr Metabol 46:259–267

Senni K, Gueniche F, Foucault-Bertaud A, Igondjo-Tchen S, Fioretti F, Colliec-Jouault S, Durand P, Guezennec J, Godeau G, Letourneur D (2006) Fucoidan a sulfated polysaccharide from brown algae is a potent modulator of connective tissue proteolysis. Arch Biochem Biophys 445:56–64

Shanmugam M, Mody KH (2000) Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr Sci 79:1672–1683

Shibata H, Kimura-Takagi I, Nagaoka M, Hashimoto S, Aiyama R, Iha M, Ueyama S, Yokokura T (2000) Properties of fucoidan from *Cladosiphon okamuranus* tokida in gastric mucosal protection. Biofactors 11:235–245

Shibata T, Kawaguchi S, Hama Y, Inagaki M, Yamaguchi K, Nakamura T (2004) Local and chemical distribution of phlorotannins in brown algae. J Appl Phycol 16:291–296

Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, Hama Y (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. J Appl Phycol 20:705–711

Shimoda H, Tanaka J, Shan SJ, Maoka T (2010) Anti-pigmentary activity of fucoxanthin and its influence on skin mRNA expression of melanogenic molecules. J Pharm Pharmacol 62:1137–1145

Sijtsma L, de Swaaf ME (2004) Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol 64:146–153

Silva TM, Alves LG, de Queiroz KC, Santos MG, Marques CT, Chavante SF, Rocha HA, Leite EL (2005) Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. Braz J Med Biol Res 38:523–533

Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54:438–463

Siriwardhana N, Jeon Y-J, Kim S-H, Ha J-H, Heo S-J, Lee K-W (2004) Enzymatic hydrolysis for effective extraction of antioxidative compounds from *Hizikia fusiformis*. Algae 19:59–68

Siriwardhana N, Kim K-N, Lee K-W, Kim S-H, Ha J-H, Song CB, Lee J-B, Jeon Y-J (2008) Optimisation of hydrophilic antioxidant extraction from *Hizikia fusiformis* by integrating treatments of enzymes, heat and pH control. Int J Food Sci Technol 43(4):587–596

Soda K (2010) Polyamine intake, dietary pattern, and cardiovascular disease. Med Hypothes 75:299–301

Song JQ, Xu YT, Zhang HK (2000) Immunomodulation action of sulfate polysaccharide of L*aminaria japonica* on peritoneal macrophages of mice. Chin J Immunol 16:70–70

Stirk WA, Van Staden J (1997) Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L. J Appl Phycol 9:327–330

Stirk WA, Arthur GD, Lourens AF, Novák O, Strnad M, Van Staden J (2004) Changes in cytokinin and auxin concentrations in seaweed concentrates when stored at an elevated temperature. J Appl Phycol 16:31–39

Suetsuna K (1998) Purification and identification of angiotensin I-converting enzyme inhibitors from the red alga *Porphyra yezoensis*. J Mar Biotechnol 6:163–167

Suetsuna K, Nakano T (2000) Identification of an antihypertensive peptide from peptic digest of wakame (*Undaria pinnatifida*). J Nutr Biochem 11:450–454

Suetsuna K, Maekawa K, Chen J-R (2004) Antihypertensive effects of *Undaria pinnatifida* (wakame) peptide on blood pressure in spontaneously hypertensive rats. J Nutr Biochem 15:267–272

Sugawara T, Matsubara K, Akagi R, Mori M, Hirata T (2006) Antiangiogenic activity of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. J Agric Food Chem 54:9805–9810

Sugiura Y, Matsuda K, Yamada Y, Nishikawa M, Shioya K, Katsuzaki H, Imai K, Amano H (2006) Isolation of a new anti-allergic phlorotannin, phlorofucofuroeckol-B, from an edible brown alga, *Eisenia arborea*. Biosci Biotechnol Biochem 70:2807–2811

Swanson D, Block R, Mousa SA (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr 3:1–7

Synytsya A, Kim W-J, Kim S-M, Pohl R, Synytsya A, Kvasnička F, Čopíková J, Park YI (2010) Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed *Undaria pinnatifida*. Carbohydr Polym 81:41–48

Targett NM, Arnold TM (1998) Minireview—predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. J Phycol 34:195–205

Teruya T, Konishi T, Uechi S, Tamaki H, Tako M (2007) Anti-proliferative activity of oversulfated fucoidan from commercially cultured *Cladosiphon okamuranus* Tokida in U937 cells. Int J Biol Macromol 41:221–226

Thomas NV, Kim S-K (2011) Potential pharmacological applications of polyphenolic derivatives from marine brown algae. Environ Toxicol Pharmacol 32:325–335

Thomes P, Rajendran M, Pasanban B, Rengasamy R (2010) Cardioprotective activity of *Cladosiphon okamuranus* fucoidan against isoproterenol induced myocardial infarction in rats. Phytomed 18:52–57

Thompson KD, Dragar C (2004) Antiviral activity of *Undaria pinnatifida* against herpes simplex virus. Phytother Res 18:551–555

Tissot B, Daniel R, Place C (2003) Interaction of the C1 complex of complement with sulfated polysaccharide and DNA probed by single molecule fluorescence microscopy. Eur J Biochem 270:4714–4720

Trinchero J, Ponce N, Córdoba OL, Flores ML, Pampuro S, Stortz CA, Salomón H, Turk G (2009) Antiretroviral activity of fucoidans extracted from the brown seaweed *Adenocystis utricularis*. Phytother Res 23:707–712

Troell M, Robertson-Andersson D, Anderson RJ, Bolton JJ, Maneveldt G, Halling C, Probyn T (2006) Abalone farming in South Africa: an overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture 257:266–281

Tseng CK (2001) Algal biotechnology industries and research activities in China. J Appl Phycol 13:375–380

Usui T, Asari K, Mizuno T (1980) Isolation of highly purified "fucoidan" from *Eisenia bicyclis* and its anticoagulant and antitumor activities. Agric Biol Chem 44:1965–1966

Veena CK, Josephine A, Preetha SP, Varalakshmi P (2007a) Effect of sulphated polysaccharides on erythrocyte changes due to oxidative and nitrosative stress in experimental hyperoxaluria. Human Exp Toxicol 26:923–932

Veena CK, Josephine A, Preetha SP, Varalakshmi P (2007b) Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesiculosus* in experimental hyperoxaluria. Food Chem 100:1552–1559

Vinoj Kumar V, Kaladharan P (2007) Amino acids in the seaweeds as an alternate source of protein for animal feed. J Mar Biol Assoc India 49:35–40

Vishchuk OS, Ermakova SP, Zvyagintseva TN (2011) Sulfated polysaccharides from brown seaweeds *Saccharina japonica* and *Undaria pinnatifida*: isolation, structural characteristics, and antitumor activity. Carbohydr Res 346:2769–2776

Vishchuk OS, Ermakova SP, Zvyagintseva TN (2013) The effect of sulfated (1→3)-α-l-fucan from the brown alga *Saccharina cichorioides* miyabe on resveratrol-induced apoptosis in colon carcinoma cells. Mar Drugs 11:194–212

Wang H, Ooi EV, Ang PO (2007) Antiviral polysaccharides isolated from Hong Kong brown seaweed *Hydroclathrus clathratus*. Sci China Ser C Life Sci 50:611–618

Wang J, Zhang Q, Zhang Z, Li Z (2008) Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. Int J Biol Macromol 42:127–132

Wang T, Jónsdóttir R, Kristinsson HG, Hreggvidsson GO, Jónsson JÓ, Thorkelsson G, Ólafsdóttir G (2010) Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmata*. LWT-Food Sci Technol 43:1387–1393

Wijesinghe WAJP, Athukorala Y, Jeon Y-J (2011) Effect of anticoagulative sulfated polysaccharide purified from enzyme-assistant extract of a brown seaweed *Ecklonia cava* on Wistar rats. Carbohydr Polym 86:917–921

Willett WC (1995) Diet, nutrition, and avoidable cancer. Environ Health Persp 103:165

Willstätter R, Page HJ (1914) Chlorophyll. XXIV. The pigments of the brown algae. Justus Liebigs Ann Chem 404:237–271

Woo MN, Jeon SM, Shin YC, Lee MK, Kang M, Choi MS (2009) Anti-obese property of fucoxanthin is partly mediated by altering lipid-regulating enzymes and uncoupling proteins of visceral adipose tissue in mice. Mol Nutr Food Res 53:1603–1611

Wu X, Yang M, Huang X, Yan J, Luo Q (2004) Effect of *Laminaria japonica* polysaccharides on radioprotection and splenic lymphocyte apoptosis. Med J Wuhan Univ 25:239–241

Yan X, Chuda Y, Suzuki M, Nagata T (1999) Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. Biosci Biotechnol Biochem 63:605–607

Yang Y-I, Shin H-C, Kim SH, Park W-Y, Lee K-T, Choi J-H (2012) 6,6′-Bieckol, isolated from marine alga *Ecklonia cava*, suppressed LPS-induced nitric oxide and PGE_2 production and inflammatory cytokine expression in macrophages: the inhibition of NFκB. Int Immunopharmacol 12:510–517

Yap CY, Chen F (2001) Polyunsaturated fatty acids: biological significance, biosynthesis, and production by microalgae and microalgae-like organisms. In: Chen F, Jiang Y (eds) Algae and their biotechnological potential. Kluwer Academic, The Netherlands, pp 1–32

Yoon NY, Lee S-H, Kim S-K (2009) Phlorotannins from *Ishige okamurae* and their acetyl- and butyrylcholinesterase inhibitory effects. J Funct Foods 1:331–335

Zaragozá MC, López D, Sáiz MP, Poquet M, Pérez J, Puig-Parellada P, Mármol F, Simonetti P, Gardana C, Lerat Y, Burtin P, Inisan C, Rousseau I, Besnard M, Mitjavila MT (2008) Toxicity

and antioxidant activity in vitro and in vivo of two *Fucus vesiculosus* extracts. J Agric Food Chem 56:7773–7780

Zemke-White WL, Ohno M (1999) World seaweed utilisation: an end-of-century summary. J Appl Phycol 11:369–376

Zhang Q-B, Yu P-Z, Zhou G-F, Li Z-E, Xu Z-H (2003) Studies on antioxidant activities of fucoidan from *Laminaria japonica*. Chin Trad Herbal Drugs 34:824–826

Zhang R, Kang KA, Piao MJ, Ko DO, Wang ZH, Lee IK, Kim BJ, Jeong IY, Shin T, Park JW, Lee NH, Hyun JW (2008) Eckol protects V79-4 lung fibroblast cells against γ-ray radiation-induced apoptosis via the scavenging of reactive oxygen species and inhibiting of the c-Jun NH_2-terminal kinase pathway. Eur J Pharmacol 591:114–123

Zhao X, Xue C-H, Li B-F (2008) Study of antioxidant activities of sulfated polysaccharides from *Laminaria japonica*. J Appl Phycol 20:431–436

Chapter 22
The Application of Fish Collagen to Dental and Hard Tissue Regenerative Medicine

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1 Introduction

Twenty-six types of genetically distinct collagen have now been identified. In accordance to their structure and molecular organization, they can be classified into fibril-forming collagens, fibril-associated collagens, network-forming collagens, anchoring fibrils, transmembrane collagens, basement membrane collagens, and others with unique functions (Gelse et al. 2003). There are three major fibrillar collagens in vertebrates (Kawasaki and Weiss 2006): type I collagen as the scaffold for

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bone and dentine; type II collagen found in mineralized cartilage; type III collagen found in most soft tissues. Collagen-based extracellular matrices must have evolved over many millions of years before starting biomineralization (Simmer 2007).

Collagen exists in dental and hard tissues, and is a useful biomaterial. The biocompatibility and safety due to its biological characteristics, such as biodegradability and weak antigenicity, made bovine collagen the primary resource in biomedical applications before the occurrence of bovine spongiform encephalopathy as a zoonosis. It was used for tissue engineering and particular research on bone substitutes, and as a matrix system for the evaluation of tissue mineralization (Lee et al. 2001).

Marine collagen from fish scales, skin, and bone has been widely investigated to apply as a scaffold and a carrier because of its bioactive properties, such as its excellent biocompatibility, low antigenicity, high biodegradability, and cell growth potential (Dillow and Lowman 2002; Yang et al. 2001).

This chapter describes the characteristics and hard tissues applications of fish collagen as a potential source of regenerative medicine.

2 Chemical and Physical Properties of Fish Collagen

Extensive usage of marine collagen sources in the food and cosmetics industries has been in progress (Swatschek et al. 2002). Generally, fish collagen is more sensitive to heat denaturation than bovine collagen because of its low denaturation temperature, which has made it difficult to use as a biomaterial. The lower stability is considered to be due to the lower hydroxyproline content of fish collagen compared to bovine collagen (Winter and Page 2000).

The composition of amino acid is almost identical between bovine and tilapia (Table 22.1), although three amino acids, methionine, isoleucine, and tyrosine, are low level compared to those in bovine collagen. The degree of hydration of proline is also similar between bovine (45.3 %) and tilapia (43 %). These data indicate that the physical properties of tilapia collagen probably meet a clinical demand for regenerative medicine.

Although marine collagen is thought to be a potentially pathogen-free alternative to established collagen sources, its generally low thermal stability (Burjanadze 2000) is a main weak point for clinical application. Therefore, its denaturation temperature (T_d) must be increased to around human body temperature for biomedical usage by cross-linking.

A biologically appropriate method to obtain a more useful T_d is to search for fish having basically high body temperature from around the world. The extracted collagen of originally tropical fish, tilapia (*Oreochromis niloticus*), has a high T_d of 36.1 °C (from the data sheet of Nippi Inc., Tokyo, Japan). Our laboratory recently confirmed a T_d of around 35 °C (unpublished data) for the skin collage of tilapia, which is a similar temperature to that obtained by another research group (Ikoma et al. 2003). This demonstrates that the collagen which originated from tilapia could become a strong candidate as a scaffold for regenerative medicine.

Table 22.1 Amino acid compositions of bovine and tilapia

	Residues/1,000	
Amino acids	Bovine[a]	Tilapia[b]
Hydroxyproline	87	85.5
Asparaginic acid	35	44.0
Threonine	17	25.2
Serine	35	35.6
Glutamic acid	70	72.3
Proline	105	113.4
Glycine	296	332.3
Alanine	122	131.9
Valine	17	17.2
Methionine	17	9.6
Isoleucine	17	8.8
Leucine	35	22.4
Tyrosine	17	1.5
Phenylalanine	17	12.3
Histidine	7	5.6
Hydroxylysine	17	9.0
Lysine	35	23.6
Arginine	52	49.6

[a]Courtesy of Prof. Yamauchi Mitsuo, North Carolina Oral Health Institute, Chapel Hill, NC, USA
[b]Courtesy of Department of Protein Engineering, Nippi Inc., Tokyo, Japan

3 Biocompatibility of Fish Collagen

The main reason for the usage of collagen is its excellent biocompatibility (Pati et al. 2012), low antigenicity (Pati et al. 2012), high direct cell adhesion properties (Lee et al. 2001), and high biodegradability compared to chitin/chitosan and synthetic polymers (Lee et al. 2001). The application of fish collagen as a scaffold has been tested for tissue engineering (Nagai et al. 2008; Sugiura et al. 2009). Our laboratory has also started to evaluate the safety of fish (tilapia) collagen. We have already observed a very mild reaction by tilapia collagen in the rat pulp, even at the initially applied stage (unpublished data).

An in vitro degradation study (using collagenase solution) revealed higher stability of the cross-linked scaffolds originated from tropical freshwater fish scale collagen with only ~50 % reduction of mass in 30 days, while the uncross-linked one degraded completely in 4 days. Furthermore, minimal immunological reaction was observed when collagen solution was injected in mice with or without adjuvant, without significant dilution of sera (Pati et al. 2012). These findings indicate that fish scale collagen are biocompatible in nature and may have potential tissue engineering applications.

4 Application for Regenerative Medicine

Fish type I collagen is an effective material as a biodegradable scaffold or spacer to replicate the natural extracellular matrix, which serves to organize cells spatially, to provide them with environmental signals, and direct site-specific cellular regulation (Wang et al. 2006; Hayashi et al. 2011, 2012).

4.1 *Dental Tissue*

The tooth has a unique characteristic in that soft and hard tissues exist together and the hard tissue covers the soft tissue, known as dental pulp.

An interesting project about dental pulp regeneration is introduced and overviewed in this paragraph. The Japanese Cabinet Office selected 24 projects to stimulate and promote Japanese medical innovation on November 18, 2008. Generally, these projects are named Highly Advanced Medical Treatment Development Fields (so-called Super Special Fields) in Japan. Only one project was selected from dentistry, the title of which is "The application of new treatments for dental caries•pulpitis through dentine•pulp regeneration using pulp stem cells." A representative of this special project is Dr. Misako Nakashima, Director, National Center for Geriatrics and Gerontology, Obu City, Aichi. Contributory facilities are Aichi Gakuin University, Nagasaki University, and Tokyo Medical and Dental University. The basic principle of this research is to investigate recovery using regenerative medicine for once extirpated pulp tissue after pulpectomy and infected root canal treatment (Iohara et al. 2006, 2008, 2009, 2011; Nakashima et al. 2006, 2009; Zheng et al. 2009; Sugiyama et al. 2011; Nakashima and Iohara 2011; Ishizaka et al. 2012; Murakami et al. 2012). In Japan, a super-aging society has already come about, and the proportion of older people having over 20 teeth at 80 years of age is now over 38 %, which means that Japan is the number one country in the world where numerous teeth remain in the oral cavity of elderly people. Generally, non-vital teeth have a high risk of tooth fracture, including root fracture (Fuss et al. 1999; Coppens and De Moor 2003).

Although our department revealed that chitosan had the possibility of numerous biological applications in dental and medical fields (Ikeda et al. 2000, 2002, 2005; Yamada et al. 2003, 2007; Fujiwara et al. 2004; Ohara et al. 2004; Matsunaga et al. 2006; Ganno et al. 2007; Hayashi et al. 2007a, b, 2012; Ishizaki et al. 2009; Hayashi 2010), the weakest point of chitosan is the inflammatory reaction, especially at the initial stage (1–2 weeks) after in vivo application (Yanagiguchi et al. 2001). The role of our department for this project is to evaluate the safety and stability of the alternative, fish collagen. Furthermore, standard operating procedures are established through the transportation of extracted tooth and isolated pulp stem cells to popularize this special treatment for general practitioners of dental clinics (Fig. 22.1).

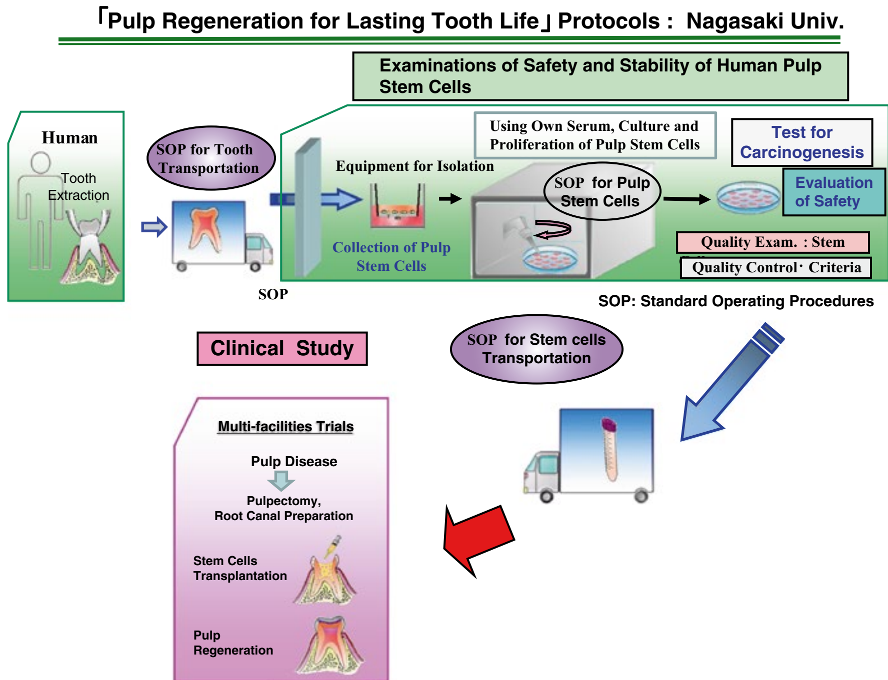

Fig. 22.1 Contributory schedules of our laboratory to conclude the special pulp regeneration therapy (Original schema was drawn by Dr. Nakashima's laboratory.)

4.2 Hard Tissue

Regenerative medicine of hard tissue in the dentistry field is indicated for the defect of alveolar and/or jaw bone originated from diverse etiology. Several procedures have been attempted to achieve periodontal regeneration, including bone graft placement, guided tissue/bone regeneration, and the use of various growth factors and/or host-modulating agents (e.g., Emdogain® and parathyroid hormone) (Zhang et al. 2009; Chen et al. 2009).

The degradation or denaturation of salmon atelocollagen by γ-irradiation affects the proliferation rates of MC3T3-E1 cells (Nagai et al. 2006). Human periodontal ligament fibroblasts could grow and show highly differentiated activity on the salmon collagen gel as well as porcine collagen (Nagai et al. 2007). There is the possibility that including an appropriate functional scaffold (the intricate 3D mesh composed of salmon collagen-coated fibers) would improve the osteogenic potential of cultured periosteal sheets as a graft biomaterial in vitro and in vivo (Kawase et al. 2010). Furthermore, fish collagen peptides promote post-transcriptional modification for collagen maturation in osteoblastic cells (personal communication). These findings indicate that fish collagen application in situ could stimulate hard tissue formation as not only a scaffold for seeded cells but also one of nutritional factors, such as growth factor.

5 Conclusions

Fish collagen is applied as a scaffold and a carrier because of its bioactive properties, such as its excellent biocompatibility, low antigenicity, high biodegradability, and cell growth potential. Tilapia, originally tropical fish, meets the minimum requirements for physicochemical properties. The tilapia collagen is now investigated to be applied as a scaffold for one of the national special regenerative projects. Fish collagen application in situ could stimulate hard tissue formation as not only a scaffold for seeded cells but also one of nutritional factors.

References

Burjanadze TV (2000) New analysis of the phylogenetic change of collagen thermostability. Biopolymers 53:523–528

Chen FM, Shelton RM, Jin Y, Chapple IL (2009) Localized delivery of growth factors for periodontal tissue regeneration: role, strategies, and perspectives. Med Res Rev 29:472–513

Coppens C, De Moor R (2003) Prevalence of vertical root fractures in extracted endodontically treated teeth. Int Endod J 36:926

Dillow AK, Lowman AM (2002) Biomimetic materials and design: biointerfacial strategies, tissue engineering, and targeted drug delivery. Marcel Dekker, New York

Fujiwara M, Hayashi Y, Ohara N (2004) Inhibitory effect of water-soluble chitosan on growth of *Streptococcus mutans*. Microbiologica 27:83–86

Fuss Z, Lustig J, Tamse A (1999) Prevalence of vertical root fractures in extracted endodontically treated teeth. Int Endod J 32:283–286

Ganno T, Yamada S, Ohara N, Matsunaga T, Yanagiguchi K, Ikeda T, Ishizaki H, Hayashi Y (2007) Early gene expression analyzed by cDNA microarray and real-time PCR in osteoblasts cultured with chitosan monomer. J Bomater Res 82A:188–194

Gelse K, Pöschl E, Aigner T (2003) Collagens—structure, function, and biosynthesis. Adv Drug Deliv Rev 55:1531–1546

Hayashi Y (2010) Applications of chitosan oligosaccharide and glucosamine in dentistry. In: Kim S-K (ed) Chitin, chitosan, oligosaccharides and their derivatives. CRC Press, Boca Raton, pp 447–460

Hayashi Y, Ohara N, Ganno T, Yamaguchi K, Ishizaki H, Nakamura T, Sato M (2007a) Chewing chitosan-containing gum effectively inhibits the growth of cariogenic bacteria. Arch Oral Biol 52:290–294

Hayashi Y, Ohara N, Ganno T, Ishizaki H, Yanagiguchi K (2007b) Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. J Dent 35:871–874

Hayashi Y, Yamada S, Ikeda T, Yanagiguchi K (2011) Fish collagen and tissue repair. In: Kim S-K (ed) Marine cosmeceuticals: trends and prospects. CRC Press, Boca Raton, pp 133–141

Hayashi Y, Yamada S, Yanagi K, Ikeda T (2012) Chapter 6. Chitosan and fish collagen as biomaterials for regeneration medicine. In: Kim S-K (ed) Marine medicinal foods, vol 65, AFNR. Academic, UK, pp 107–120

Ikeda T, Yanagiguchi K, Viloria IL, Hayashi Y (2000) Relationship between lysozyme activity and clinical symptoms following the application of chitin/chitosan in endodontic treatment. In: Muzzarelli RAA (ed) Chitosan *per os:* from dietary supplement to drug carrier. Atec Edizioni, Italy, pp 275–292

Ikeda T, Yanagiguchi K, Hayashi Y (2002) Application to dental medicine—in focus on dental caries and alveolar bone healing. Bioindustry 19:22–30 (in Japanese)

Ikeda T, Yanagiguchi K, Mastunaga T, Yamada S, Ohara N, Ganno T, Hayashi Y (2005) Immunohistochemical and electron microscopic study of the biodegradation processes of chitin and chitosan implanted in rat alveolar bone. Oral Med Pathol 10:131–138

Ikoma T, Kobayashi H, Tanaka J, Walsh D, Mann S (2003) Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticas*. Int J Biol Macromol 32:199–204

Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M (2006) Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. Stem Cells 24:2493–2503

Iohara K, Zheng L, Wake H, Ito M, Nabekura J, Wakita H, Nakamura H, Into T, Matsushita K, Nakashima M (2008) A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells 26:2408–2418

Iohara K, Zheng L, Ito M, Ishizaka R, Nakamura H, Into T, Matsushita K, Nakashima M (2009) Regeneration of dental pulp after pulpotomy by transplantation of CD31(−)/CD146(−) side population cells from a canine tooth. Regen Med 4:377–385

Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng Part A 17:1911–1920

Ishizaka R, Iohara K, Murakami M, Fukuta O, Nakashima M (2012) Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials 33:2109–2118

Ishizaki H, Yamada S, Yanagiguchi K, Koyama Z, Ikeda T, Hayashi Y (2009) Pre-treatment with tannic acid inhibits the intracellular IL-8 production by chitosan in a human oral epithelial cancer cell line. Oral Med Pathol 13:135–141

Kawasaki K, Weiss KM (2006) Evolutionary genetics of vertebrate tissue mineralization: the origin and evolution of the secretory calcium-binding phosphoprotein family. J Exp Zool (Mol Dev Evol) 306B:295–316

Kawase T, Okuda K, Kogami H, Nakayama H, Nagata M, Yoshie H (2010) Osteogenic activity of human periosteal sheets cultured on salmon collagen-coated ePTFE meshes. J Mater Sci Mater Med 21:731–739

Lee CH, Singla A, Lee Y (2001) Biomedical applications of collagen. Int J Pharm 221:1–22

Matsunaga T, Yanagiguchi K, Yamada S, Ohara N, Ikeda T, Hayashi Y (2006) Chitosan monomer promotes tissue regeneration on dental pulp wounds. J Biomater Res 76A:711–720

Murakami M, Imabayashi K, Watanabe A, Takeuchi N, Ishizaka R, Iohara K, Yamamoto T, Nakamura H, Nakashima M (2012) Identification of novel function of vimentin for quality standard for regenerated pulp tissue. J Endod 38:920–926

Nagai N, Anzawa T, Satoh Y, Suzuki T, Tajima K, Munekata M (2006) Activities of MC3T3-E1 cells cultured on γ-irradiated salmon atelocollagen scaffold. J Biosci Bioeng 101:511–514

Nagai N, Mori K, Satoh Y, Takahashi N, Yunoki S, Tajima K, Munekata M (2007) *In vitro* growth and differentiated activities of human periodontal ligament fibroblasts cultured on salmon collagen gel. J Biomed Mater Res 82A:395–402

Nagai N, Nakayama Y, Zhou YM, Takamizawa K, Mori K, Munekata M (2008) Development of salmon collagen vascular graft: mechanical and biological properties and preliminary implantation study. J Biomed Mater Res B 87:432–439

Nakashima M, Iohara K (2011) Regeneration of dental pulp by stem cells. Adv Dent Res 23:313–319

Nakashima M, Iohara K, Zheng L (2006) Gene therapy for dentin regeneration with bone morphogenetic proteins. Curr Gene Ther 6:551–560

Nakashima M, Iohara K, Sugiyama M (2009) Human dental pulp stem cells with highly angiogenic and neurogenic potential for possible use in pulp regeneration. Cytokine Growth Factor Rev 20:435–440

Ohara N, Hayashi Y, Yamada S, Kim S-K, Matsunaga T, Yanagiguchi K, Ikeda T (2004) Early gene expression analyzed by cDNA microarray and RT-PCR in osteoblasts cultured with water-soluble and low molecular chitooligosaccharide. Biomaterials 25:1749–1754

Pati F, Datta P, Adhikari B, Dhara S, Ghosh K, Das Mohapatra PK (2012) Collagen scaffolds derived from fresh water fish origin and their biocompatibility. J Biomed Mater Res 100A:1068–1076

Simmer JP (2007) Evolution and genetics of teeth. Cells Tissues Organs 186:4–6

Sugiura H, Yunoki S, Kondo E, Ikoma T, Tanaka J, Yasuda K (2009) *In vivo* biological responses and bioresorption of tilapia scale collagen as a potential biomaterial. J Biomater Sci Poly Ed 20:1353–1368

Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, Matsushita K, Nakashima M (2011) Dental pulp-derived $CD31^-$/$CD146^-$ side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 17:1303–1311

Swatschek D, Schatton W, Kellermann J, Müller WE, Kreuter J (2002) Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. Eur J Pharm Biopharm 53:107–113

Wang J-H, Hung C-H, Young T-H (2006) Proliferation and differentiation of neural stem cells on lysine–alanine sequential polymer substrates. Biomaterials 27:3441–3450

Winter AD, Page AP (2000) Prolyl 4-hydroxylase is an essential procollagen-modifying enzyme required for exoskeleton formation and the maintenance of body shape in the nematode *Caenorhabditis elegans*. Mol Cell Biol 20:4084–4093

Yamada S, Ohara N, Hayashi Y (2003) Mineralization of matrix vesicles isolated from a human osteosarcoma cell line in culture with water-soluble chitosan-containing medium. J Biomed Mater Res 66A:500–506

Yamada S, Ganno T, Ohara N, Hayashi Y (2007) Chitosan monomer accelerates alkaline phosphatase activity on human osteoblastic cells under hypofunctional conditions. J Biomed Mater Res 83A:290–295

Yanagiguchi K, Ikeda T, Takai F, Ogawa K, Hayashi Y (2001) Wound healing following direct pulp capping with chitosan-ascorbic acid complex in rat incisors. In: Uragami T, Kurita K, Fukamizo T (eds) Chitin and chitosan. Kodansha Scientific, Tokyo, pp 240–243

Yang AF, Leong KF, Du ZH, Chua CK (2001) The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng 7:679–689

Zhang W, Abukawa H, Troulis MJ, Kaban LB, Vacanti JP, Yelick PC (2009) Tissue engineered hybrid tooth–bone constructs. Methods 47:122–128

Zheng L, Amano K, Iohara K, Ito M, Imabayashi K, Ito T, Matsushita K, Nakashima M (2009) Matrix metalloproteinase-3 accelerates wound healing following dental pulp injury. Am J Pathol 175:1905–1914

Chapter 23
Applications of Seafood By-products in the Food Industry and Human Nutrition

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1 Introduction

Marine-based food industries are major economic sources for many countries around the world. The seafood processing industry generates more than 60 % as by-products as waste, while only around 40 % of fish products are used for human consumption (Chalamaiah et al. 2012). Recently, seafood-derived by-products have gained much attention due to their potential industrial applications in the food and nutraceutical industries. These by-products mainly include bycatch or discarded body parts of commercial fish, such as heads, gills, skin, trimmings, fins, frames, bones, viscera, blood, and roes. The majority of these by-products are used in the production of animal feed; yet, a considerable amount is discarded without proper utilization (FAO Report 2012). Thus, the proper utilization of seafood by-products is an issue of utmost importance.

Seafood by-products can be directly utilized in many food applications, such as the manufacturing of fish protein concentrate, fish oil, and meat analogues. Furthermore, some by-products could also be further processed into high-value functional and nutraceutical food products that could impart various health-related beneficial effects in humans. For example, fish skin and fins can be utilized to extract collagen and gelatin. Moreover, seafood processing by-products such as viscera and trimmings are rich sources of digestive and other valuable enzymes. Other seafood by-products such as bycatch can also be utilized to extract fish muscle enzymes. Crustaceans produce considerable amounts of by-products and the exoskeletons of these aquatic animals can be used to isolate chitin, chitosan, glucosamine, and carotenoids.

A large amount of antifreeze proteins has been found in the body fluids of coldwater marine fish species. Furthermore, seafood by-products can be considered as valuable sources of many bioactive compounds, such as antioxidants and bioactive peptides.

This chapter focuses on the unique features of important seafood by-product-derived ingredients, extraction procedures, and valuable applications of seafood-derived by-products in the food industry and for human nutrition. Furthermore, possible future applications of seafood by-products in the food industry are also discussed in brief.

2 Seafood By-products Used in the Food Industry

2.1 Fish Oil

Marine animals are reputed for the abundance of essential fatty acids in their tissues (Danielewicz et al. 2011; Robert et al. 2009; Tabarsa et al. 2012; Tsai and Pan 2012). The main reason for this phenomenon is the accumulation of n-3 long-chain fatty acids in marine tissues, which are derived from dietary micro- and macroalgae.

Fish liver contains considerable amounts of fat in certain lean fish species, such as haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*), saithe (*Pollachius virens*), and tusk (*Brosme brosme*). In such species, the level of total lipids stored in the liver varied from 54 % to 69 % in cod, saithe, and haddock and 43 % in tusk (Falch et al. 2006). However, in many fatty species, lipid is stored under the skin and in muscles (Huss 1988). Although the liver of some fish species like cod and shark is commercially used in the production of fish oil, liver from most finfish and shellfish species are discarded as by-products in the seafood industry. Hence, such by-products could be used to extract valuable long-chain fatty acids. For example, Falch et al. (2006) have shown that the production of fillets of gadiform species could generate two-thirds of the round weight as by-products, and an average production of 10,000 kg of fillets could generate by-products with more than 1,000 kg of marine lipids. Furthermore, the same authors have reported that more than 30 % of such lipids are the health-beneficial long-chain n-3 fatty acids with commercial applications.

Fish oil can also be extracted from other marine by-products, such as heads, backbones, viscera, roe, milt, skin, and cutoffs; however, the composition of the oil extracted may differ depending on the tissue. Dumay et al. (2004) reported that fish oil extracted from heads, backbones, viscera, skin, and cutoffs has lower phospholipid contents compared to roes and milt. According to Falch et al. (2006), the viscera from gadiform species contained between 2 % and 9 % as lipids and the trimmings contained approximately 1 % as total lipids. However, when subjected to natural fermentation, the amount of total lipid recovered from the visceral mass obtained from the dressings of freshly harvested Indian major carps was 19–21 % (rohu and catla) (Rai et al. 2010).

2.2 Fish Enzymes

Seafood enzymes are generally problematic to the seafood industry, due to their direct relationship with the deterioration of fresh seafood. However, the same enzymes that cause problems can be utilized as a useful ingredient in the food industry when properly extracted. Seafood processing by-products such as viscera and trimmings are rich sources of digestive and other valuable enzymes. Other seafood by-products such as bycatch can also be used to extract fish muscle enzymes.

Researchers have continuously worked on extracting enzymes from seafood by-products. Reviews on enzymes from fish and aquatic invertebrates by Shahidi and Kamil (2001) and fish-digestive enzymes by Vecchi and Coppes (1996) also compile some valuable information in relation to the types of enzymes extracted from seafood by-products. The main types of enzymes extracted are digestive enzymes, which are very useful in food processing and are discussed in detail later in the chapter.

The digestive enzymes of fish, especially of coldwater fish, are known to be powerful compared to that of land animals because their enzymes have been adapted to carry out metabolic functions efficiently in the cold-body temperatures resulting from the surrounding environment. However, the low thermal stability of coldwater fish enzymes may cause some problems during extraction and hinder potential applications of such enzymes in the food industry (Ásgeirsson and Bjarnason 1993; Gildberg 2004; Klomklao et al. 2005).

2.3 Gelatin and Collagen

Collagen, gelatin's parent molecule, is a fibrous protein found abundantly in all multicellular animals, as the main component of their connective tissues (Swatschek et al. 2002; Zhou and Regenstein 2007; Boran et al. 2010; Zhu et al. 2010). The molecular weight of the collagen molecule is around 330 kDa. Gelatin is the collagen fraction after heat hydrolysis that exceeds an arbitrary minimum molecular weight of 30 kDa (Boran et al. 2010). To date, 27 variants of collagen have been identified (Gómez-Guillén et al. 2011). According to the nature of their aggregated forms or their morphological differences, the majority of collagens can be divided into four major groups: thick striated fibers, non-fibrous networks collagen, non-striated filamentous collagen, and fibril-associated collagen. These collagen molecules consist of closely related but genetically distinct proteins, possessing a basic structure of three polypeptide chains, each with a glycine-X-Y repeat (where X is mostly proline and Y is mostly hydroxyproline), forming tightly bound triple helices (mainly stabilized by intra- and interchain hydrogen bonding), which subsequently aggregate to form various types of supporting structures (Bailey et al. 1998; Gómez-Guillén et al. 2011). Although there are some differences in the amino acid composition of collagens derived from different sources, the composition of

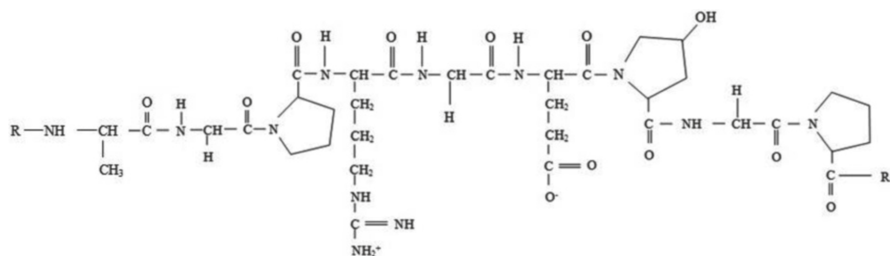

Fig. 23.1 Molecular structure of gelatin. *Source*: Rasika et al. (2013)

collagen encompasses all 20 amino acids (Gómez-Guillén et al. 2002). The amino acid composition of gelatin is closer to that of its precursor gelatin (Karim and Bhat 2009). The molecular structure of gelatin is shown in Fig. 23.1.

2.4 Carotenoids

Carotenoids are a class of pigments that dissolve in neutral fat (Armstrong 1997). The yellow, orange, and red colors of the skin, shell, or exoskeleton of aquatic animals are due to the presence of carotenoids, where they bind to proteins. In nature, carotenoids are the most widespread pigments, and they are found in bacteria, yeasts, mold, all green plants, and many animals. They possess various functions (Shahidi et al. 1998). Animals are incapable of the de novo synthesis of carotenoids and they depend on their diets for the carotenoids requirements. However, animals are capable of modifying dietary plant carotenoids (Bjerkeng et al. 1990). Astaxanthin, canthaxanthin, lutein, zeaxanthin, and tunaxanthin can be considered as the major carotenoids present in aquatic animals (Martínez-Camacho et al. 2001; Maoka 2011). The predominant carotenoid found in crustacean waste is free and esterified astaxanthins (Nègre-Sadargrues et al. 2000). Algae are the primary source of carotenoids present in aquatic animals. There are over 600 carotenoid pigments available, which can be divided into two groups: xanthophylls (which contain oxygen) and carotenes (which are pure hydrocarbons, and contain no oxygen) (Kouchi et al. 2012a, b).

2.5 Chitin, Chitosan, and Glucosamine

Shellfish are aquatic organisms with an exoskeleton. Crustaceans produce considerable amounts of by-products, in which heads account for 30–40 % of the total by-product (Meyers 1986). Shrimp produces 52 % as by-products out of its total body weight (Heu et al. 2003). Chitin is a glucose-based branched polysaccharide, and it

is considered as the second most abundant biopolymer in nature, mainly found in the exoskeleton of crustaceans and insects, and the cell walls of some bacteria and fungi (AbdElhady 2012). Chitosan is the name used for low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2-amino-2-deoxy-β-D-glucose, known as (1 → 4)-2-amino-2-deoxy-D-glucose (Shahidi and Kamil 2001). Glucosamine is obtained by the hydrolysis of chitosan and is considered as one of the most abundant monosaccharides. Under specific conditions, concentrated hydrochloric acid degrades chitosanous materials to form glucosamine and its acetylated form, N-acetylglucosamine (Mojarrad et al. 2007; Abdou et al. 2008).

2.6 Functional Substances and Nutraceuticals

Functional foods are food products that contain added, technologically developed ingredients with specific health benefits (Tahergorabi et al. 2012). The delivery of active substances with pharmaceutical properties through food-based approaches in order to prevent or treat certain diseases can be regarded as nutraceutical applications. Marine foods and by-products can be considered as valuable sources of many healthy food ingredients and biologically active compounds. However, marine functional ingredients have not been fully exploited for food purposes (Kadam and Prabhasankar 2010).

Antioxidant compounds in food products play a major role in the health and wellbeing of humans as important functional ingredients. Antioxidants are used to preserve food products by retarding discoloration and deterioration (development of undesirable off-flavors, odors, and potentially toxic products) as a result of oxidation (Decker et al. 2005; Bougatef et al. 2010). Furthermore, antioxidants can protect the human body against damage by reactive oxygen species (ROS), which attack macromolecules such as membrane lipids, proteins, and DNA, leading to many health problems, such as cancer, diabetes mellitus, coronary heart disease, Alzheimer's disease, neurodegenerative, and inflammatory diseases with severe tissue injuries (Bougatef et al. 2010; Ngo et al. 2011). Therefore, antioxidants in food play an important role in human health. Furthermore, other marine foods, such as brown algae, and by-product-derived antioxidants, such as chitooligosaccharide derivatives, sulfated polysaccharides, phlorotannins, and carotenoids, also possess potential applications in the food industry, due to their ability to protect food products against oxidative degradation, as well as preventing and/or treating free radical-related diseases (Heo et al. 2005; Kang et al. 2005; Kim and Mendis 2006; Alemán et al. 2011; Ngo et al. 2011). The use of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, *t*-butylhydroquinone, and propyl gallate is under strict regulation due to the potential health hazards caused by such compounds. This indicates the importance of identifying new sources of safe and inexpensive antioxidants of natural origin, especially for use in the food industry (Je et al. 2005; Kim and Wijesekara 2010). Fish with low economic value and by-products from the fishing industry, such as the skin and frames of jumbo flying squid (*Dosidicus gigas*), tuna (*Thunnus* spp.) and halibut (*Hypoglossus* spp.) (Alemán et al. 2011), yellowfin sole (*Limanda aspera*) (Jun et al. 2004),

Table 23.1 Fish and aquatic invertebrate-derived functional ingredients and potential health and nutritional benefits

Functional ingredient	Sources	Potential health/nutritional effect
Chitin, chitosan	Crab, shrimp, prawn, squid, lobster, cuttlefish	Prevention of inflammatory disorders
Chitooligosaccharide		Antimicrobial activity
		Reduction of lipid absorption
Omega-3 oils	Salmon, cod liver oil	Prevention of cardiovascular diseases
	Pelagic reduction fisheries	Brain function in children
Polysaccharides	Carrageenan	Anticarcinogenic effect
		Antiviral effect
Carotenoids	Algae and crustaceans	Antioxidants
Astaxanthin		Prevention of neurodegenerative diseases
Fucoxanthin		
Vitamin and minerals	Marine fish, fish bone	Growth and physiology of the body
		Teeth and bones strength and antitumor agent
Shark cartilage	Shark	Anticarcinogenic agent
Bioactive peptides	Fish protein, Alaska pollock backbone	Obesity control and Ca-binding activity
	Atlantic cod stomach	Immunomodulatory activities
Fish protein hydrolysates	Fish protein	Protein supplement
Taurine	Cod, mackerel, and other fish species	Prevention of cardiovascular diseases, Alzheimer's disease, and cystic fibrosis

Source: Kadam and Prabhasankar (2010), Freitas et al. (2012)

Alaska pollock (*Theragra chalcogramma*) (Je et al. 2005), Pacific hake (*Merluccius productus*) (Samaranayaka and Li-Chan 2008), and round scad (*Decapterus maruadsi*) (Thiansilakul et al. 2007), have been utilized in isolating new secondary metabolites and/or antioxidants that have novel activity in biological systems. Other than the antioxidant activity, many other fish and aquatic invertebrate-derived functional ingredients play an important role in improving nutritional condition and human health. For example, recent studies have provided evidence that fish and aquatic invertebrate-derived functional ingredients such as bioactive peptides play a vital role in human health and nutrition (Zhao et al. 2009; Samaranayaka and Li-Chan 2011). Also, fish proteins have been recognized to be beneficial in counteracting metabolic syndrome appearance in both animal models and human studies (Pilon et al. 2011). Table 23.1 demonstrates some of these functional ingredients, their sources, and potential beneficial health effects.

2.7 *Antifreeze Compounds*

Antifreeze proteins, also known as thermal hysteresis proteins, are ice-structuring proteins able to influence the growth of ice crystals and inhibit ice recrystallization. These molecules were first discovered in the blood of fish living in frozen Arctic

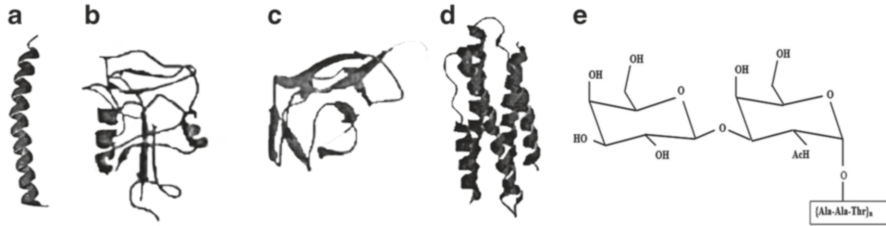

Fig. 23.2 Structural diversity based on the molecular structures of different types of antifreeze proteins: (**a**) type I; (**b**) type II; (**c**) type III; (**d**) type I; (**e**) antifreeze glycoprotein. *Source*: Rasika et al. (2013)

and Antarctic areas (Ferraro et al. 2010). Large amounts of antifreeze proteins have been found in the body fluids of coldwater marine fish, such as cod, because these compounds prevent fish from freezing. antifreeze proteins found in marine sources can be categorized into two types: glycoproteins and non-glycoproteins, based on the presence or absence of carbohydrates (Harding et al. 2003). Based on the structural diversity, non-glycoproteins have been further classified as type I, type II, type III, and type IV antifreeze proteins. Figure 23.2 demonstrates the molecular structures of different antifreeze proteins and non-glycoprotein. Both glycoprotein and non-glycoprotein antifreeze compounds can lower the freezing point of an aqueous solution via a non-colligative mechanism, through binding to ice crystal surfaces and inhibiting further growth of ice crystals (Harding et al. 2003; Evans and Fletcher 2004). Although yeasts and some other types of microorganisms are suitable sources of antifreeze proteins, the most promising is fish by-products (Ferraro et al. 2010). The reason for this could be due to the abundant availability of raw materials and comparatively easy isolation procedures. Antifreeze protein compounds can be isolated not only from the body fluids/blood, but also from certain fish skin tissues, such as snailfish, cunner, and sea raven (Evans and Fletcher 2004). Moreover, antifreeze protein compounds have been extracted from many other marine fish species, such as righteye flounder, longhorn sculpin, shorthorn sculpin, winter flounder, sea raven, Atlantic herring, Atlantic eelpout, northern cods, and northern notothenioids (Fletcher et al. 2001; Harding et al. 2003; Jørgensen et al. 2008).

2.8 Surimi and Surimi-Based Products

Surimi is produced by the heading, gutting, and cleaning of fresh fish, and mechanically separating the edible muscle from the skin and bone, followed by washing, refining, dewatering, and mixing of fish mince with sugar and cryoprotective food ingredients, and, finally, freezing (Sen 2005; Codex Alimentarius 2009). Surimi is also described as moist frozen concentrated myofibrillar protein of fish meat. Fish mince resulting from the separation of skeletal muscle of fish from bones and skin

is very often the starting material for surimi production because access to the Alaska pollock (*Theragra chalcogramma*), white croaker (*Argyrosomus argentatus*), Pacific whiting (*Merluccius productus*), hoki (*Macruronus novaezealandiae*), and blue whiting (*Micromesistius*) sources has been limited in recent years (Chen et al. 1997; Mahawanich et al. 2010). In addition to these traditional marine fish species, the possibility of making surimi using freshwater fish species such as Indian major carps, rohu (*Labeo rohita*), catla (*Catla catla*), and mrigal (*Cirrhinus mrigala*) (Sankar and Ramachandran 2002), grass carp (*Ctenopharyngodon idellus*) (Luo et al. 2006), and common carp (*Cyprinus carpio*) (Jafarpour and Gorczyca 2008) have been reported in the literature. Moreover, fish mince produced from underutilized fish, bycatch species, and by-products such as trimmings, cut-offs, frames, etc. resulting from the seafood processing industries have the potential for surimi production (Venugopal and Shahidi 1995).

Raw surimi is produced by washing out fat and water-soluble compounds from minced fish using water, and mainly contains wet concentrated myofibrillar proteins of fish. Many factors such as lipids, water-soluble proteins, pigments, and trimethylamine oxide in fish muscle have an adverse effect on the quality of the surimi. Hence, excessive washing of fish mince is required in order to remove such compounds and manufacture high-quality surimi (Chen et al. 1997; Bentis et al. 2005). Furthermore, low-temperature setting (suwari) and high temperature degradation are two important aspects that affect the formation of the gel during surimi production (Shimizu et al. 1981). Once it is prepared, raw surimi has enhanced functional properties, such as gel-forming ability, water-holding capacity, fat-binding ability, etc., compared to the fish mince. Nevertheless, surimi is an inexpensive source of protein that can be used as a food item in its raw form (Arason et al. 2009). In addition, surimi can also be used in the preparation of a wide array of products, such as kamaboko (chikuwa, fried surimi, hanpen), seafood analogues, and fabricated meats. Moreover, surimi can be included as a binder in many fish-based products (artificial fish eggs, fish sausages, fish balls, etc.) (Kamal 1994).

Japanese fish cake, or kamaboko, is one of the traditional products produced using surimi gel. In general, kamaboko is produced via a multistep process. In kamaboko production, the first step is the tempering of frozen surimi at a temperature of −4 °C to −8 °C. Then, the surimi gel is mixed with salt and disintegrated using a grinder to induce the dissociation of actomyosin. Ingredients are added to the salted surimi paste, depending on the variety of kamaboko to be produced (Sen 2005). For example, in order to improve the kamaboko's gel strength, which is one of the characteristics associated with high-quality surimi products, functional additives such as egg white could also be added (Techaratanakrai et al. 2011). In addition to that, the setting process is often optimized by incubation of the surimi at temperatures lower than 40 °C in order to enhance the gel strength. Ground product is then shaped using molds. The next step in kamaboko production is heating the molded products at over 80 °C in order to cook them. While cooking, suwari gel is formed, which is then transferred into kamaboko gel (Sen 2005).

2.9 Fish Protein Hydrolysate and Fish Protein Concentrate

The utilization of proteins recovered from by-products as ingredients for food has become a new trend in the food industry. Furthermore, it has become a current challenge to develop technologies to recover usable ingredients for the food industry from the prevailing fish processing industries. Fish protein hydrolysates are breakdown products of the enzymatic conversion of fish proteins into smaller peptides. Generally, protein hydrolysates are small fragments of peptides that contain 2–20 amino acids. These protein hydrolysates are produced by the enzymatic hydrolysis of native fish proteins (Chalamaiah et al. 2012). Many studies have been carried out on seafood by-products, including as a potential raw material for fish protein hydrolysate production. These by-products contain a wide range of underutilized body parts from fish and fisheries industries (Liaset et al. 2000; Martone et al. 2005; Nilsang et al. 2005; Souissi et al. 2007; Cudennec et al. 2008; Ovissipour et al. 2009; Bougatef et al. 2010; Motamedzadegan et al. 2010; Uddin et al. 2010; Benhabiles et al. 2012a; Muzaifa et al. 2012; Tanuja et al. 2012).

Fish protein concentrate is defined as any stable and wholesome product with higher protein and other nutrients content than fresh fish, which is also produced hygienically at low cost (Pariser 1973). Generally, fish protein concentrate can be prepared from any type of fish or fishery waste. It is prepared from fish or fish-by-products by removing the oil, screening or settling out the bones, and drying; hence, the resultant product is higher in protein (85–95 %) and lower in ash content than fish meal. Three types of fish protein concentrates have been identified. Type A is a virtually odorless and tasteless powder, having a maximum total fat content of 0.75 %. Type B has no specific limits as to odor or flavor, but definitely has a fishy flavor and a maximum fat content of 3 %. Type C is normal fish meal produced under satisfactorily hygienic conditions (Windsor 1977). In the preparation of fish protein concentrates, most of the lipids and water are removed from the raw material. Methods for preparing fish protein concentrates can be classified as chemical, biological, and physical. However, chemical methods of the preparation of fish protein concentrate are the most commonly used (Dubrow and Stilling 1971).

2.10 Fermented Fish Products

Fermented fish products are processed using finfish, shellfish, and crustaceans of fresh water or marine origin, with salt to cause fermentation and, thereby, to prevent putrefaction. Different traditional fermented products are produced and used in different parts of the world, mainly in Southeast Asia. As a traditional fermented food product, its recipes vary by region throughout the world, depending upon local consumer preferences and ingredient availability. Fish sauce is the most commonly known fermented fish product among the category. In the recent past, Japan has quadrupled the amount of fish sauce produced in order to make maximum use of fish materials (Françoise 2010). Fish sauce is a water-soluble clear brown liquid

hydrolysate derived after several months of storage of heavily salted fish in closed tanks at tropical temperatures. A great variety of raw materials can be used for sauce production, provided that the content of proteolytic enzymes is sufficient to give efficient tissue solubilization and protein hydrolysis (Gildberg 2001). The annual world production of fish sauce is more than 400 million liters. There are approximately 100 fish sauce producers; however, only 20 producers already hold 80 % of the market share and many of them are from Thailand (Dissaraphong et al. 2006). Fish sauce has been commonly used as a condiment and a seasoning throughout the world, mostly in Southeast Asia. The possibility of using fish, crustacean, and mollusk by-products such as Arctic capelin and Atlantic cod intestines, bonito processing waste, tuna viscera, southern rough shrimp waste, squid head, viscera, skin, and fins for the manufacturing of fish sauce has been demonstrated by many researchers recently (Gildberg 2001; Shih et al. 2003; Kim et al. 2005; Dissaraphong et al. 2006; Xu et al. 2008).

3 Extraction Procedures/Production of Seafood By-products Used in the Food Industry

3.1 Extraction, Purification, and Refining of Fish Oil

The processing of seafood by-products soon after harvesting is vital for maintaining the quality of the fish oil (Wu and Bechtel 2008; Bimbo 2011). This is to avoid degradation of oils and functional compounds by intrinsic enzymes of fish, chemical reactions, and microbial activity. Rai et al. (2010) suggested that fish viscera which are not processed soon after extraction can undergo lipid oxidation; thus, the oil recovered will have to be refined prior to human use. However, Aidos et al. (2003) have shown that oil with the highest content of polyunsaturated fatty acids (PUFAs) was not produced from the freshest herring by-products; instead, the oil with higher PUFAs were recovered from stored by-products compared to the fresh by-products of herring. As suggested by Aidos et al. (2003), the increase of PUFAs in the stored by-products could be due to the release of PUFAs from the cell degradation. Further studies have to be conducted based on the results of Aidos et al. (2003) in order to find out the optimum storage condition for each fish type or fish by-product to obtain the result that they have reported, if the modification during the storage of raw material is to be practiced on a commercial scale.

3.1.1 Methods for the Extraction of Fish Oil

Traditional Method to Extract Oil from Seafood By-products

The fermentation of fish viscera to release oil in adipocytes due to the breakdown of cells and proteins is the basis used in traditional methods of lipid extraction. This process has been widely used as a simple fermentation process in order to extract

the fish oil from fish liver by putrification. Fish liver is put into wooden barrels and left to be putrefied. Usually, these barrels contain seawater to facilitate the putrification process. The oil that leaches out during fermentation is skimmed off (Wetzel 2009). On certain occasions, shark stomachs have been used as pouches to ferment shark liver (Wetzel 2009). However, these traditional methods take a long time to complete the manufacturing process.

Wet Rendering Process to Extract Oil from Seafood By-products

The basis of the wet rendering process is the application of steam to rupture fat-containing cells (adipocytes) and to allow the oil to flow out. The remaining residue following extraction of the oil from fish viscera is usually utilized to process fish meal (FAO Report 1986). As raw materials, by-products such as viscera, bycatch, or fish heads have been used as the starting material in steam cookers to extract oil. Steam ruptures the cells and allows oil to flow out. As steam liquefies, the oil fraction begins to float on the water. Chantachum et al. (2000) have studied the extraction of crude oil from precooked and non-precooked skipjack tuna heads using wet rendering methods. They found that both the heating temperature and heating time affect the separation of oil and the quality of oil from skipjack tuna heads. The optimal conditions as identified by Chantachum et al. (2000) for the rendering of skipjack tuna heads were at 85 °C for 30 min. However, Aidos et al. (2003) found that the temperature of the heat treatment does not influence the quality of the manufactured oil. The main factor that influences the quality of oil according to these researchers is the mono-pump, which they used to pump the ground herring by-products into the heat exchanger. Perhaps, the pump speed to the heat exchanger may have influenced the ultimate temperature of the raw material and the amount of heat passed to the raw material. Therefore, the results of these studies suggest that the time–temperature combination during the heat treatment of raw material influences the ultimate product quality during the wet rendering processing of fish oil. The differences between the results of these two studies could also be due to the variation in composition of by-products and other production parameters, such as the range of temperatures used in testing.

The next step for extracting oil from fish and fish by-products is pressing the rendered material. Pressing separates the liquid fraction (press liquor) from the solid residue. The optimal condition for the pressing of cooked fish viscera as identified by Chantachum et al. (2000) is 140 t/m^2 using a hydraulic press. The solid residue resulting from pressing is sent for the processing of fish meal. Separated liquid has three main fractions, namely, sludge, oil, and water (FAO Report 1986). Fine particles from fish, such as muscle fiber particles, are contained in the sludge, while the water fraction contains water-soluble compounds, such as enzymes extracted from the raw material.

The separation of oil from the press liquor is done using several methods. The cheapest and most basic method is to send the liquid fraction to a tank to allow the two layers (oil and water) to separate under gravity. Another cost-effective method

is using vibrating strainers to separate the oil portion from the water (FAO Report 1986). Both these processes are slow, although cost-effective. These processes are normally practiced in small-scale production, where investing in expensive machinery to separate oil from press liquor is not economically feasible.

Centrifugation is the commonly used commercial operation to separate the oil from press liquor (Bimbo 2011). Centrifugation uses the differences in the densities of the three fractions (sludge, oil, and water) to separate them. First, the sludge is removed from the press liquor. A decanter or desludger uses centrifugal force to remove high-density sludge from the liquor. The liquid fraction after decanting/desludging is called “stickwater” (FAO Report 1986). A disk centrifuge is usually used to separate crude fish oil from water. The difference in density between water and oil results in a rapid phase separation during centrifugation. For both centrifugation processes (desludging and separation of oil), the temperature must be maintained at 90–95 °C to obtain an optimal separation (FAO Report 1986). According to the work done by Aidos et al. (2003), the speed of the decanter is a critical factor to be monitored in maintaining the quality of the final product. The use of centrifugation is rapid and efficient compared to basic gravity systems or vibrating strainers.

Separated crude fish oil is polished to remove impurities in the oil. Hot water is used to extract the impurities into water, and centrifugation is done to remove the water with impurities (Ackman 2005). Further refining is done prior to food application; however, polishing during extraction is essential to prevent quality deterioration of oil during storage prior to further refining.

Hydrolysis Processes to Extract Oil from Seafood By-products

In the hydrolysis processes, enzymes have been used to hydrolyze the fat cells to facilitate the extraction of oil from fish and fish by-products. Hydrolysis is achieved by intrinsic enzymes in fish or added commercial enzymes. The hydrolysis process is done not only to extract oil, but also to produce peptides and amino acid fractions that can be utilized in the further processing of food. Therefore, special care must be taken during the separation of oil from the protein fraction not to harm the functional properties of the peptides, as the functional properties of those peptides are essential in the further processing of the hydrolysate.

Raw materials are pasteurized prior to adding extrinsic enzymes to destroy the intrinsic fish enzymes, as the activity of intrinsic enzymes would result in unintended products which commercial enzymes would not yield. However, this initial heat treatment is associated with the problem of the formation of protein–lipid complexes, which would lead to a reduction in the yield of oil (Šližytė et al. 2005a).

In the process where intrinsic digestive enzymes are used for the hydrolysis of the raw materials, pasteurization is not done. A pH adjustment is done during the production of oil using acids such as formic, propionic, sulfuric, and phosphoric (3–4 %) to prevent the activity of microorganisms, which may result in a reduction

in quality due to the production of hazardous microbial metabolites and hydrolytes (Bimbo 2011). The process of the separation and purification of oil fraction after hydrolysis is similar to that which is used in the wet reduction process.

A common problem associated with the extraction of oil from hydrolyzed fish (in both processes that use intrinsic enzymes and extrinsic enzymes) is the formation of an emulsion (Šližytė et al. 2005a). Hydrolyzed protein fraction have groups ($-NH_3^+$and $-COO^-$) which can bind with water and saturated hydrocarbon groups in protein chains that can attract non-polar groups in fish oil. Thus, the hydrolyzed fish proteins act as emulsifiers to form an emulsion. This emulsion reduces the yield of oil. This problem could be minimized by reducing the amount of water added to the hydrolysate (Daukšas et al. 2005; Šližytė et al. 2005a, b).

The application of proteases to facilitate the extraction of oil from fish by-products has been studied by several researchers (Dumay et al. 2004; Kechaou et al. 2009). The findings from these studies revealed that enzymatic methods could generate greater amounts of oils compared to that of direct use of unfermented fisheries by-products. The rupturing of cells during the hydrolysis process by enzymes may have facilitated the extraction of oil (Kechaou et al. 2009). The process that had been used by Kechaou et al. (2009) to extract oil from fish by-products is a solvent extraction process, which is not commonly practiced in industry to extract fish oil. Recently, Rai et al. (2010) used a centrifugation method to separate fish oil from fermented fish by-products, which is a conventional method of extraction practiced in the industry. However, in their study, Rai et al. (2010) have not compared the fish yield of fish oil resulting from the unfermented processes and the hydrolyzed procedure. Furthermore, more than 80 % of the lipids has been recovered from the fish by-products when both enzymatic and centrifugation methods were used for the extraction process (Kechaou et al. 2009; Rai et al. 2010) It would be interesting to study the application of the enzymatic hydrolysis method combined with wet or dry rendering methods in a pilot plant to find out how the combined procedures may affect the quality and quantity of oil recovered from fish processing by-products.

Dry Rendering Procedure to Extract Oil from Seafood By-products

In the dry rendering process, water is not added to the raw material during cooking. Cooking results in the further removal of water from the raw material. The pressing of cooked raw material is practiced after heat treatment in order to obtain oil from fish processing by-products. Separated oil is further purified to remove water and impurities from the oil.

Dry processing yields a high amount of phospholipids compared to wet rendering (Bimbo 2011). The reason for this is the amphiphilic characteristics of phospholipids, which allows them to bind with water, thus a major fraction of phospholipids is removed with water during wet rendering, which is not removed during dry rendering (Jaczynski 2008). These phospholipids may contribute to rancidity development in fish oil (Hultin 2002). Therefore, the refining of fish oil from dry rendering is essential in order to obtain a quality end product.

Chemical Rendering Procedure to Extract Oil from Seafood By-products

Bimbo (2011) described a chemical process used in Iceland to extract oil from fish liver. Fish liver residue, which resulted after the extraction of oil from conventional methods, can be treated with sodium hydroxide for deproteinization and release of oil (Bimbo 2011).

Supercritical Fluid Extraction Procedure to Extract Oil from Seafood By-products

Supercritical fluid extraction (SFE) is a method for extracting metalloid and metal species from a solid or liquid material by exposing the material to a supercritical fluid solvent containing a chelating agent (Wai and Laintz 1994). The SFE procedure has been applied to extract oil from fish offcuts, which resulted in good quality extraction of fish oil with a lower amount of free fatty acids, as well as less oxidized products (Rubio-Rodríguez et al. 2012). However, due to the high initial cost involved in the SPE process, the method may be suitable for industries in which fish oil has been extracted for nutraceutical or pharmaceutical purposes.

Isoelectric Precipitation/Solubilization Procedure to Extract Oil from Seafood By-products

Precipitation of proteins at the isoelectric point is the basis used in the isoelectric extraction of lipids from seafood by-products (Jaczynski 2008). At the isoelectric point, the total charge on the protein molecule is zero; this reduces the interaction of protein molecules with water and results in a reduction of the water solubility of protein (the interaction of protein molecules with water is arrested by $–NH_3^+$ and $–COO^-$ groups, which forms hydrogen bonds with water). Therefore, this process facilitates the precipitation of solubilized protein.

To extract fish oil from seafood by-products, first, the by-products are minced, and then homogenization is done. The homogenized sample is solubilized by changing the pH of the mixture beyond the isoelectric point. Centrifugation is used to separate the oil fraction from the mixture and the protein fraction is sent for further extraction of peptides and proteins (Chen and Jaczynski 2007a, b; Jaczynski 2008).

Okada and Morrissey (2007) used a different process to extract oil from fish involving the isoelectric precipitation of fish protein. They adjusted the pH of the extraction medium to the isoelectric point of sardine muscle (pH 5.5) using HCl or food-grade organic acids. The mixture was then centrifuged to separate the oil. This method is different to the processes proposed by Chen and Jaczynski (2007a, b) and Jaczynski (2008), in which the oil is extracted after the isoelectric solubilization of protein by changing the pH of the mixture beyond the isoelectric point.

Usually, a significant change in the pH of the fish oil may result in a pH-induced degradation of PUFAs during the isoelectric precipitation/solubilization process.

However, low temperatures and short durations can be adopted to reduce the chance of a pH-induced degradation of lipids (Gehring et al. 2011; Jaczynski 2008). Furthermore, Okada and Morrissey (2007) revealed that the use of calcium prior to the pH adjustment has a beneficial effect in terms of lipid oxidation stability and color of the final fish oil products. Despite the possibility of pH-induced degradation of PUFAs, the isoelectric process has been proven to yield oil with oxidative stability. Perhaps this could be due to the production of lower amounts of impurities in the isoelectric precipitation procedure when compared to the oils rendered using heat treatments. For example, impurities such as hemoglobin from the blood of fish may accelerate the oxidation of lipids (Fu et al. 2009).

3.2 Extraction of Enzymes from Seafood By-products

The extraction of enzymes is done mainly for industrial and research purposes. The process of extraction of enzymes depends upon their intended use. Enzyme extraction for research and analytical purposes are extensively purified, while enzymes for commercial purposes are less purified. The main reasons behind the lower purification on the industrial scale are the high cost involved and the reduction of yield associated with further purification. Commercial extraction of enzymes is dependent on the intended final use. For example, enzymes for animal feed processing are less purified, while enzymes for food application are purified further (Chaplin and Bucke 1990).

The basic process for the extraction of enzymes involves the mashing of tissues to extract enzymes to an aqueous solution, separation of the aqueous layer, and concentration and purification of the solution to obtain the enzyme.

3.2.1 Extraction of Enzymes to Water (Cell Rupture)

Seafood by-products are cleaned and washed prior to enzyme extraction in order to remove unwanted material, such as blood. Size reduction of tissues is an optional unit operation, especially in the case of tissues from large fish species. Viscera from smaller fish such as squids do not need to be size reduced.

Usually, enzymes are located inside the cells. Hence, cells are ruptured either by mechanical or non-mechanical methods to extract enzymes. Rupturing is usually done with added buffers, such as Tris–HCl, sodium phosphate, or sodium acetate, to preserve the enzyme activity (Cardenas-Lopez and Haard 2005; Lu et al. 2008). Mechanical methods include mincing, ultrasonic cell disruption, high-pressure homogenization, bead milling, freeze-pressing, and solid or liquid shearing. Physiochemical parameters such as heat, shear, proteases, pH, and oxidation influence the rate of extraction of seafood enzymes. Non-mechanical methods adopted by researchers include osmotic shocking, freezing followed by thawing, cold shocking, desiccation, enzymatic lysis, and chemical lysis (Chaplin and Bucke 1990).

3.2.2 Removal of Fat from the Mixture

Most fish tissues such as liver of lean fish and muscle of fatty fish store fats and oils. Therefore, fats and oils are solubilized by organic solvents to remove fats (Hatate et al. 2000). Another method available to remove lipids is centrifuging at low temperatures. Most unsaturated lipids tend to solidify at low temperatures compared to water-soluble enzymes, which remain in solution; thus, low temperatures facilitate the removal of fat. Lipids may harm the enzymes by accelerating protein oxidation (Castro-Ceseña et al. 2012).

3.2.3 Isolation and Purification of Enzymes

The purification process can be applied in an appropriate order designed by the respective operator, depending on the buyer's requirements. A combination of methods can also be adopted by the producer, depending on the degree of purity required and the cost involved (Bougatef et al. 2007; Balti et al. 2009). Purification and clarification can be done using microfiltration and using clarifiers. Biomaterials remaining in the solution other than enzymes are removed in the clarification process. Further purification of the solution to extract protein fraction which contain enzymes can be obtained using salts such as ammonium sulfate or organic solvents such as methanol, ethanol, propan-2-ol, and acetone (Bougatef et al. 2007; Souza et al. 2007; Espósito et al. 2009; Ktari et al. 2012). These chemicals precipitate the enzymes from the mixture. The precipitation of enzymes at the isoelectric point is the basis to this method. The use of aqueous two-phase systems, which involves the mixing of one or two hydrocolloids, resulting in separation of the mixture, is also applied to purify enzymes (Schmidt et al. 1994; Venâncio et al. 1996).

To fractionate and isolate the intended enzymes from the protein mixture, chromatographic methods such as ion-exchange, affinity, and gel exclusion have been extensively used in the industry (An and Visessanguan 2000; Bougatef et al. 2007; Souza et al. 2007; Sila et al. 2012). Bougatef et al. (2007) and Ktari et al. (2012) used Sephadex G-100 gel filtration, while Souza et al. (2007) used Sephadex G-75 filtration to purify the enzyme extract. Many other researchers have used ion exchange chromatography linked with gel filtration to purify the enzyme extract (Ktari et al. 2012; Sila et al. 2012). Heat treatment and selective precipitation are other possible methods to retain the desired enzymes. Furthermore, these methods destroy undesirable enzymes. Different enzymes have differing susceptibilities to heat denaturation and precipitation (Chaplin and Bucke 1990; Matsumiya et al. 2003; Bougatef et al. 2007). To remove salts in the purified enzyme mix, dialysis, ultrafiltration, or desalting methods have been widely employed (Ktari et al. 2012).

3.2.4 Concentration and Drying of the Enzyme Mixture

Enzymes are sold in solid form or as a concentrated liquid. Therefore, concentration is carried out using ultrafiltration or diafiltration, whereby water and low molecular weight materials are removed, while the required enzymes are retained.

Lyophilization is also practiced in order to concentrate or dry the extract. Lyophilization is essential in the drying of heat-labile seafood enzymes. However, loss of enzyme activity can be expected, even in the lyophilization process (Castro-Ceseña et al. 2012).

Spray drying can be used as a cheap and rapid method to obtain dry enzymes. For example, Gildberg and Xian-Quan (1994) used spray drying to concentrate fish enzymes extracted from cod viscera with minor losses in the activity of the enzymes. However, the heat-liable enzymes may be destroyed during the heating process. Inert materials such as starch, lactose, carboxymethylcellulose, and other polyelectrolytes are added during spray drying to protect the enzymes (Chaplin and Bucke 1990).

3.3 Extraction of Chitin, Chitosan, and Glucosamine

Shrimp and crab shell wastes have been extensively used for the isolation of chitin among the many different species of crustaceans (Muzzarelli 1977; Johnson and Peniston 1982; Wang and Xing 2007). Shell wastes of crustaceans are mainly composed of 30–40 % protein, 30–50 % calcium carbonate, and 20–30 % chitin. This composition manly differs with different species and seasonal variations (Cho et al. 1998). Other than that, the shell waste of crustaceans contains carotenoids (Sachindra et al. 2007; Khanafari et al. 2008).

Traditionally, the separation of chitin includes deproteinization, demineralization, and decolorization. Deproteinization or removal of protein is usually done by alkali treatment (NaOH), whereas demineralization or the removal of calcium carbonate and calcium phosphate is done by acid treatments (HCl, HNO_3, H_2SO_4, CH_3COOH, and HCOOH) under high temperatures (No and Hur 1998; Percot et al. 2003). Other than the chemical deproteinization, enzymatic and microbial methods are utilized for the deproteinization of shell waste of crustaceans (Synowiecki and Al-Khateeb 2003). Furthermore, microbial demineralization of crustacean shell waste has been reported by using *Pseudomonas aeruginosa* F722 (Oh et al. 2007), lactic acid bacteria (Oh et al. 2008), *Bacillus cereus*, and *Exiguobacterium acetylicum* (Sorokulova et al. 2009).

The deacetylated polymer of acetyl glucosamine obtained through alkaline deacetylation of chitin is known as chitosan. Chitosan is a liner, polycationic polymer of D-glucosamine and N-acetyl glucosamine. The two monosaccharides are linked by β-(1 → 4) glycosidic bonds and the relative amounts of these two monosaccharides varies remarkably, resulting in a varied degree of deacetylation, ranging from 75 % to 95 %. The molecular weight of chitosan can be in the range from 50 to 2,000 kDa (Tharanathan and Kittur 2003). Hence, the term "chitosan" refers to a group of polymers varying in molecular weight by up to several million Daltons (Şenel and McClure 2004).

As the world's leading chitosan manufacturer, each year, Japan manufactures more than 100 billion tons of chitosan and contributes to more than 90 % of the world's chitosan production (Palpandi et al. 2009). Chitosan is commercially

prepared by boiling chitin in concentrated alkali solution for several hours to achieve extensive alkaline deacetylation of the chitin. It is considered that the process of N-deacetylation is not competed; hence, chitosan is a form of partially N-deacetylated derivative of chitin. Chitosan shows poor solubility above pH 6.5, hence its biological activities are confined only to acidic mediums. Consequently, chitosan has been converted into chitooligosaccharides, which dissolve in water and possess a variety of functional properties (Park et al. 2003). Jeon et al. (2000) and Vidanarachchi et al. (2009) have documented comprehensive accounts on the separation of chitin, chitosan, and the preparation of their oligosaccharides. Glucosamine is produced by the hydrolysis of chitin with hydrochloric acid solution (Mojarrad et al. 2007).

3.4 Extraction of Gelatin and Collagen

The main raw material for the production of gelatin in the food industry is skin and bones from bovine and porcine sources (Binsi et al. 2009). However, marine by-products such as fish skin have a significant potential for the production of high-quality gelatin with different melting and gelling temperatures over a much wider range than mammalian gelatins, with sufficiently high gel strength and viscosity (Boran et al. 2010). Regardless of the raw material used, all gelatin manufacturing processes consist of three main stages: pretreatment of the raw material, extraction of the gelatin, and purification and drying. Since the collagen of warm- and cold-blooded animals differ in some physicochemical properties, such as amino acid composition, solubility, and thermal stability, the same procedures used for the isolation of collagen and preparation of gelatin from bovine and porcine connective tissues cannot be directly applied in the case of fish offal (Kołodziejska et al. 2004). Gelatin's properties for a particular application is greatly influenced not only by the raw material (species or tissue) from which it is extracted, but also by the extraction procedure, which may depend on the pH, temperature, and time during both pretreatment and extraction processes, because the extraction process can influence the length of the polypeptide chains and the functional properties of the gelatin (Karim and Bhat 2009; Tabarestani et al. 2010). In general, collagen is extracted with acid treatment (such as sulfuric acid) and solubilized without altering its triple helix structure. It is also possible to convert collagen into soluble gelatin by using an alkali, such as sodium hydroxide. Thermal treatment cleaves hydrogen and covalent bonds that stabilizes the triple helix configuration of collagen and converts its helical conformation into a coiled conformation (gelatin state). Therefore, as a general procedure, hot water treatment is used to solubilize collagen in fish skin and extract as gelatin (Kim and Mendis 2006). Two different types of gelatin, which possess differing characteristics, can be produced from raw materials such as fish skin according to the method in which the collagens are pretreated. Type A gelatin (isoelectric point at pH 6–9) is produced from acid-treated collagen, and type B gelatin (isoelectric point at approximately pH 5) is produced from alkali-treated collagen. Mild acid pretreatment of the raw material (instead of alkali treatments) and mild

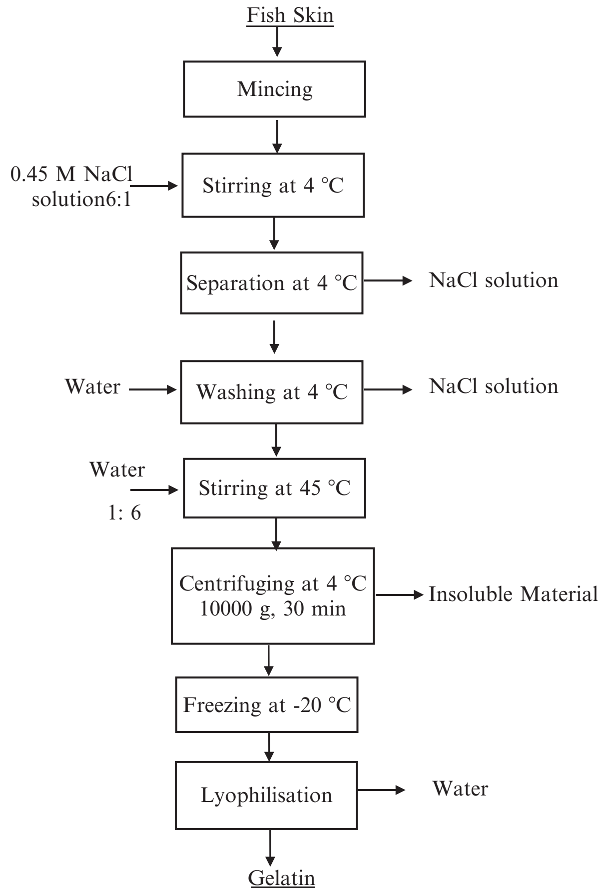

Fig. 23.3 Gelatin extraction procedure from fish offal (Source: Kolodziejska et al. 2004)

temperature conditions during the extraction process could also be used in preparing certain marine-derived gelatins, such as fish gelatin (Karim and Bhat 2009).

The extraction procedure used by Kolodziejska et al. (2004) (Fig. 23.3) recorded gelatin yields of 70–100 % from different kinds of fish offal: heads and backbones of Baltic cod, skin of fresh and cold-smoked salmon, and skin of salted and marinated herrings under different extraction temperatures and extraction durations.

3.5 *Extraction of Carotenoids from Seafood By-products*

Solvents such as methanol, acetone, ethyl acetate, hexane, isopropanol, methyl ethyl ketone, and ethanol can be used in the food industry as organic solvents for the extraction of carotenoids. Decrease in the stability of carotenoids due to oxidation (if antioxidants have not been used) and environmental issues are the major

concerns of organic solvent extraction of carotenoids. The other disadvantage is the low yield of carotenoids resulting from this method (Kouchi et al. 2012a). Generally, the dried wastes of crustaceans are pulverized, enzymatically treated, and extracted with organic solvents. However, Mezzomo et al. (2011) reported that pretreatments such as cooking, drying, and milling could maximize the carotenoids extraction from shrimp residue. Moreover, by separating bound proteins using proteolytic enzymes, the pigments extractability can also be enhanced (Venugopal 2008). The effect of enzyme treatment on carotenoid extraction has been evaluated by many researchers and it was found that trypsin is more effective than pepsin and papain (Chakrabarti 2002; Babu et al. 2008). Also, bacterial fermentation can be effectively used for the deproteination of shell waste from crustaceans (Simpson et al. 1994). Lactic acid fermentation helps in the extraction of carotenoids, by deproteinization without damage to pigments. Lactic acid fermentation can reduce the protein content in crustacean residues by 50 %. Especially, since astaxanthin is highly unstable, lactic acid fermentation can stabilize the astaxanthins molecule and, thereby, increase the astaxanthins production (Hall and Da Silva 1992). Armenta-López et al. (2002) inoculated shrimp waste with *Pediococcus pentosaceus* P-1, *Staphylococcus carnosus* MC-1, and *Lactobacillus* species isolated from shrimp waste, and they found that *Lactobacillus* spp. was more efficient than the other two bacterial spp. with regard to the production of lactic acid and, thereby, reduction of pH in the medium.

Depending on the species of shrimp, the recovery percentage of carotenoids can be varied from shrimp shells. Babu et al. (2008) reported that the recovery percentage of carotenoids from shrimp waste showed a trend of *Penaeus indicus* > *Penaeus monodon* (culture) > *Metapenaeus monoceros* > *Penaeus monodon* (wild). Furthermore, in the same study, the application of heat had a positive effect on the amount of extractable carotenoids. Low extraction yield of carotenoids from in natura samples (uncooked) has been reported compared to cooked lobster carapaces (Babu et al. 2008).

Other than organic solvent extraction, vegetable oil and supercritical CO_2 extraction of carotenoids are also popular (Félix-Valenzuela et al. 2001; Sachindra et al. 2007; Kouchi et al. 2012b). Among sunflower oil, groundnut oil, gingelly oil, mustard oil, soy oil, coconut oil, and rice bran oil, sunflower oil resulted in the highest yield of carotenoids (Sachindra and Mahendrakar 2005). López et al. (2004) studied the effect of online SFE of astaxanthin from crayfish and proposed a clean, swift, and highly selective automated supercritical extraction method for the isolation of carotenoids from crustaceans. This process can reduce the amount of solvent, waste, and handling times. Sánchez-Camargo et al. (2011a) observed that the combination of 60 % (v/v) n-hexane and 40 % (v/v) isopropyl alcohol is more efficient in the extraction of carotenoids formed from spotted shrimp waste compared to acetone, supercritical CO_2, or supercritical CO_2 plus ethanol. Furthermore, Sánchez-Camargo et al. (2011b) reported that the temperature and pressure are very critical when performing supercritical CO_2 extraction for the yield of astaxanthin extraction from Brazilian red spotted shrimp (*Farfantepenaeus paulensis*) waste. At 43 °C and 370 bar, the highest recovery of astaxanthin is seen (39 % recovery).

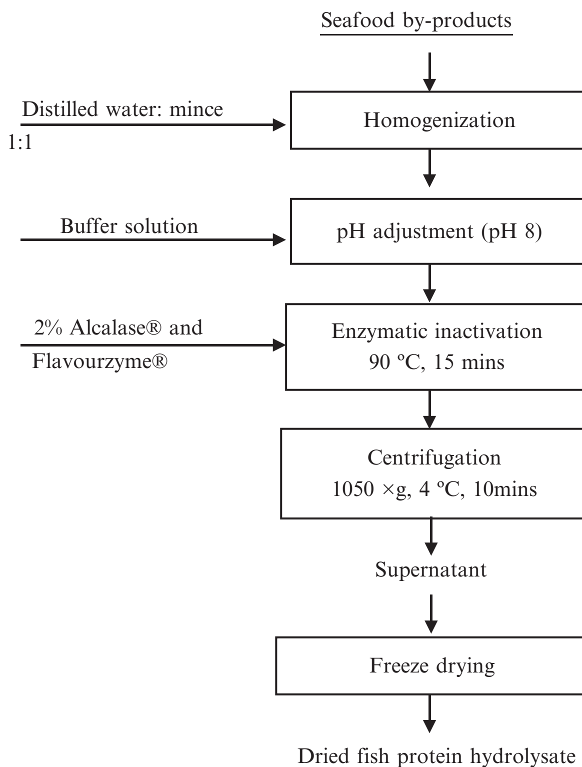

Fig. 23.4 Production of fish protein hydrolysate by enzymatic hydrolysis of fish by-products (Source: Muzaifa et al. (2012))

3.6 Production of Fish Protein Hydrolysate and Concentrate Using Seafood By-products

Protein hydrolysates can be produced by using acids, proteolytic enzymes, and by subcritical water hydrolysis (Grimble 2000; Uddin et al. 2010). However, proteolytic enzyme-assisted fish protein hydrolysate production is the most preferred among all the methods (Grimble 2000). Moreover, it is found that the use of strong chemicals and solvents, and the use of extreme temperatures and pH have adverse effects on nutritional qualities, causing poor functionality in the resulting hydrolysate. Therefore, enzymatic hydrolysis using proteolytic enzymes is preferred for the food industry mainly because it could generate hydrolysate having high functional and nutritional properties (balanced amino acid composition and high digestibility) (Quaglia and Orban 1990; Kristinsson and Rasco 2000; Nesse et al. 2011). Not only the type of enzymes but also the duration of hydrolysis has an impact on the quality of the final product. Amiza et al. (2012) reported that the amino acid content, emulsifying capacity, foaming capacity, and foaming stability of cobia fish frame hydrolysate depend on the extent of hydrolysis, whereas the water-holding capacity, oil-holding capacity, and solubility are not affected. Muzaifa et al. (2012) developed a general procedure for the enzymatic hydrolysis of fish and fish by-products (Fig. 23.4). Liaset et al. (2000), in their novel approach of two-step enzymatic

hydrolysis, found that the degree of hydrolysis can be increased from 20 % to 50–60 % by 2 h of prehydrolysis with Alcalase®, followed by 12 h of hydrolysis using Kojizyme™. A recent study by Benhabiles et al. (2012a) revealed that crude pepsin extract prepared by autolysis of the mucous membranes of sheep stomach can be satisfactorily utilized for the enzymatic hydrolysis of solid fish waste at optimal conditions of pH 1.5–2 and incubation period 6 h. Moreover, crude enzyme extract from sardine viscera has been used in the preparation of sardinelle protein hydrolysates. When using crude enzyme extract from sardine viscera, sardine by-products used for hydrolysate preparation were cooked at 90 °C for 20 min to deactivate the endogenous enzymes as an additional step to the normal enzymatic hydrolysis procedure. In addition, hydrolysis with crude extract from sardine viscera resulted in hydrolysates with the highest antioxidant activity (Bougatef et al. 2010).

On the other hand, enzymatic hydrolysis has its own drawbacks as well. Bitterness of the final product is of the greatest concern to the food industry (Dauksas et al. 2004; Liaset et al. 2000). According to Dauksas et al. (2004), bitterness is caused by the bile, fat, and ash in whole fish and fish viscera, whereas the total amino acids and hydrophobic amino acids have no correlation with bitterness. The recommended solutions to eliminate bitterness in the final hydrolyzed product are the use of commercial enzymes (Imm and Lee 1999), extraction with butanol, and treatment with cholestyramine resin (Dauksas et al. 2004). However, Nilsang et al. (2005) reported that Kojizyme™, which is a commercially available enzyme, enhances some bitter-tasting amino acids contents, such as tryptophan, during the hydrolysis process.

Apart from the enzymatic method of preparing fish protein hydrolysates, subcritical water hydrolysis is recommended for the recovery of amino acids from deoiled material in a shorter reaction time (Uddin et al. 2010).

By-products from the seafood industry can be effectively utilized in the production of fish protein concentrates. When whole fish from bycatch or trash fish are used, they must be washed thoroughly, followed by evisceration. The resulting products can be fed into a mechanical deboner with other by-products received from different seafood processing industries, such as trimmings, trash fish, etc. (Windsor 1977). In the extraction step, isopropanol and fish by-products are mixed at a ratio of 2:1. For the sieving of the ground product after final extraction, a 100-μm filter is recommended. Muraleedharan and Gopakumar (1998) developed a functional protein concentrate by using mechanically separated tuna fish mince. For the production of the powdered form of fish protein concentrate, they washed the mince with water, 2 % (w/v) aqueous solution of sodium bicarbonate, acidified with acetic acid, and spray dried (Fig. 23.4).

3.7 Extraction of Important Functional Substances and Nutraceuticals from Seafood Wastes

Various marine foods and by-products have been successfully utilized in isolating compounds with functional and nutraceutical values, such as antioxidant. Antioxidant assays can be performed in vitro or in vivo to identify the antioxidant

properties from various sources. Commercial enzymes such as pepsin and collagenase and combinations of enzymes have been used to generate hydrolysates from marine by-products. After ultrafiltration using 10-, 5-, 3-, or 1-kDa MWCO membranes, hydrolysates are tested for antioxidant activity using one or several assay methods, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, thiobarbituric acid reactive substance assay (TBARS), metal chelating activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, peroxyl radical scavenging activity, and the peroxide value and carbonyl value method. When antioxidant activity is detected, further purification steps are required before identification of the bioactive peptide sequence. Purification techniques such as ion-exchange chromatography, gel filtration chromatography, and high-performance liquid chromatography (HPLC) can be performed for further purification. When hydrolysates are separated into peptidic fractions, these fractions are further tested for their antioxidant activities, and the most active fractions are sequenced by using mass spectrometry techniques, including matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF), electrospray ionization (ESI), and Edman degradation (Di Bernardini et al. 2011).

Enzymatic hydrolysis can be identified as the most common method for producing bioactive peptides with functional and nutritive values from marine sources, where the precursor proteins are hydrolyzed using endogenous proteolytic enzymes already present in the muscle or viscera of fish or shellfish or/and by adding exogenous enzymes from other sources (Guerard 2007). Ultimately, peptides having the desired characteristics are separated by an ultrafiltration membrane system (Kim and Mendis 2006). Further, it is possible to isolate fish proteins as well as oil which retain the functionality and nutritive value of food products from materials not intended for human consumption, such as bones, scales, and skin (Gehring et al. 2011; Tahergorabi et al. 2012). The recovery of such fish oil and protein compounds has been discussed in Sects. 3.1 and 3.6, respectively.

3.8 Extraction of Antifreeze Compounds From Seafood By-products

Procedures for the extraction of antifreeze compounds from seafood by-products have been developed over the last few decades. Before the recovery process, blood or fish skin can be frozen in liquid nitrogen and stored at −70 °C. The next step is the pulverization process (using a mortar and pestle containing liquid nitrogen prior and homogenized in 0.1 M NH_4HCO_3, pH 8.5). Then, the samples should be homogenized in the same alkaline medium and the supernatant is separated after centrifugation (5,000 rpm for 10 min), followed by lyophilization. The lyophilized supernatant is then dissolved in the same alkaline medium and subjected to preparative chromatography reverse-phase HPLC for purification and separation (under a gradient of 35–65 % acetonitrile as solvent A and 0.1 % trifluoroacetic acid as solvent B, using a specific silica gel column) (Evans and Fletcher 2004).

Alternatively, antifreeze compounds can be isolated using specially designed equipment such as cold finger apparatus followed by chromatographic purification (Kuiper et al. 2003; Ferraro et al. 2010).

3.9 Production of Surimi Using Seafood By-products

The traditional method of surimi production, or "washing" method, is basically carried out by the separation of muscle from bones and skins, washing, refining, addition of cryoprotectants, and freezing.

During surimi production, fresh fish are sorted and dressed by removing various structures and tissues, such as heads, viscera, roe, scales, bones, and peritoneum. Fish muscle is minced and sarcoplasmic proteins are removed from it by leaching in order to increase the myofibrillar protein concentration and, thereby, the gel-forming property. To avoid heat denaturation of myofibrillar proteins, cold water of temperature less than 10 °C is normally used for the leaching procedure. In leaching, the number of washing cycles and the ratio between minced fish and water based on the fish species should be employed in order to optimize the process. Refining is done in order to remove any connective tissue, skin, or other undesirable material. The moisture content of the resulting product is reduced 80–84 % by dewatering. Cryoprotectants are used to stabilize fish protein from freeze denaturation. In general, 4 % sugar along with 4–5 % sorbitol and 0.2–0.3 % polyphosphate are used as cryoprotectants (Sen 2005).

Surimi powder has also been produced and used in different parts of the world for various food applications. Powder has been produced by using different drying methods, such as oven drying, freeze drying, etc. However, to avoid denaturation of proteins during the process, dryoprotectants such as sucrose and polyols can be added (Ramírez et al. 1999; Huda et al. 2001; Chin et al. 2012; Santana et al. 2012).

3.10 Production of Fermented Fish Sauce

The production of fermented fish sauce using marine-derived foods and by-products have been well known procedures for many years. However, several researchers have suggested various novel methods for the production of fish sauce by utilizing seafood by-products as raw material (Klomklao et al. 2006; Xu et al. 2008; Takano et al. 2012). Lopetcharat and Park (2002) reported a procedure for unripened fish sauce production using Pacific whiting and surimi by-products within a shorter period than the traditional method. The results of the study show that the procedure developed gives a product equivalent to the commercial fish sauce available on the market in terms of total nitrogen, soluble solids, and relative gravity. These authors suggest that the appropriate color could be developed by subjecting the unripened product to a ripening process.

Furthermore, two types of fish sauces have been developed using waste from kamaboko processing factories in Japan recently (Takano et al. 2012). Whole fish and wastes removed from kamaboko production were minced and mixed with soy sauce koji (prepared by incubating a mixture of steamed defatted soybean and roasted wheat in a 1:1 ratio with *Aspergillus oryzae*), salt, and water to produce one type of fish sauce, named "waste sauce", whereas minced whole fish and waste were mixed with deep-sea smelt meat, salt, and water to produce the other type of sauce, called "mixed sauce". Fermentation was done for 6 months with stirring by paddles at 2-week intervals, followed by filtering to separate the sauces from the fermented mixture. The study revealed that the kamaboko waste-incorporated fish sauce has less off-flavor, less fishy odor, and higher umami taste, with an agreeable soy sauce-like flavor (Takano et al. 2012).

Squid by-products have also been reported as a potential raw material to produce good quality fish sauce (Xu et al. 2008). In this study, three processing methods have been studied to produce low-salt fish sauce using squid by-products, such as heads, viscera, skin, and fins. According to the results, the authors have reported that the best method for processing squid by-products involves autolysis and mixing with salt, koji, and flavourzyme prior to fermentation. In addition, acceptable sensory qualities have been recorded for mixing with salt, koji, and flavourzyme without autolysis, suggesting that the by-products of squids can be effectively used for the production of fish sauce.

4 Food Applications of Seafood-Derived By-products

4.1 Fish Oil

Beneficial functional and nutritional properties of fish oil have led to its incorporation in food or the modification of food processing raw materials, such as margarine spread, milk, sausages, meat, snacks, and cheese.

4.1.1 Modification of Livestock and Fish Feed to Improve Lipid Profiles of Animal-Derived Food Products

In general, it is accepted that the composition of animal-derived food can be modified through the alteration of feed, up to a certain extent. Livestock and aquatic animal feed can be enriched with fish oil in order to improve the lipid profile of meat, fish, milk, and eggs obtained from these animals (Bimbo 2009; Kouba and Mourot 2011; Tyagi et al. 2011).

Many studies have been conducted to enrich milk with fish oil and to evaluate the effect of the nutritional status of consumers (Lovegrove et al. 1997; Metcalf et al. 2003; Timm-Heinrich et al. 2004; Let et al. 2007; Romeo et al. 2011). Modification of the lipid profile of milk is of greater importance than other animal products

because milk fat is a major ingredient in producing various dairy products, such as cheese, butter, and paneer. Feed and drinking water fortified with fish oil have been successfully used to improve the lipid profile of cow's milk and value-added dairy products (AbuGhazaleh and Holmes 2007; Bobe et al. 2007; Bu et al. 2007; Murphy et al. 2004; Osborne et al. 2008).

Feeding layers with fish oil (rich in essential fatty acids) supplemented feed can be recognized as an easier and appealing method for favorably modification of the lipid profile of chicken eggs (Bovet et al. 2007; Cachaldora et al. 2008; García-Rebollar et al. 2008; Fraeye et al. 2012). Increasing the fish oil content in the feed of layer chicken results in an increase of n-3 fatty acids in egg yolk (García-Rebollar et al. 2008). In their study, García-Rebollar et al. (2008) reported that a 15–17-g/kg increase of marine fish oil in feed resulted in a significant increase in n-3 fatty acids in the egg yolk of laying hens. Furthermore, these modifications in feed have been reported to not having any negative impact on the sensory attributes of chicken eggs. Moreover, improvement in the fatty acid profile of chicken eggs is reflected by the alterations of the lipid profile of humans fed with these modified eggs. For example, Bovet et al. (2007) reported that the consumption of enriched eggs (5 g of fish oil mixed with 100 g of feed) was associated with a significant decrease (16–18 %) in serum triacylglycerols.

According to Hallenstvedt et al. (2010), pigs fed with a mixture of mackerel, sardines, and anchovy oils with proportions in the range 0.0–0.7 % showed a significant impact on the fatty acid composition in pork, based on their gender. Sows demonstrated a higher percentage of monounsaturated fatty acids (C18:1) compared to boars. Furthermore, 0.7 % fish oil in feed resulted in a slight increase in fish oil flavor in pig belly over a long storage period (12 months at −80 °C or 6 months at −20 °C). Bryhni et al. (2002) reported that, when 0.4 % fish oil is used in swine feed, the sensory qualities of meat from the loin are not altered. Accordingly, low levels of fish oil (below 0.7 %) in swine feed can be used to improve the lipid profile of pork without resulting in a negative effect on the sensory qualities of pork. A common problem encountered in animal products following the provision of fish oil-based feed is the alteration of organoleptic properties, mainly the formation of off-flavor (fishy odor) in meat, milk, and eggs. This problem is very common when fish oil has been incorporated beyond certain levels in the feed. Kolanowski et al. (1999) reported that the enrichment of fish oil at high levels (more than 0.1 %, which provides 0.03 % EPA and DHA) in milk strongly decreases its palatability. Therefore, it is advisable to incorporate fish oil with flavored milk products, which can mask unpleasant fishy flavors (Kolanowski et al. 1999; Kolanowski et al. 2007).

4.1.2 Application of Fish Oil in Sausages

Due to the unique food structure (meat emulsion), the incorporation of fish oil in sausages is relatively easy. The production of sausages by replacing vegetable fat and animal fat in sausage emulsion with fish oil has been tested by many researchers (Table 23.2). The findings from many studies suggested that it is possible (in both

Table 23.2 Recent literature on the incorporation of fish oil in sausages

Type of sausage	Form of fish oil used	Level of fish oil in the final product	References
Mortadella (Spanish bologna-type sausage)	Fish oil pre-emulsified with caseinates and water	1–6 %	Cáceres et al. (2008)
Fresh pork sausages	Fish oil (omega-3 18:12)	15 % pork back fat substitution	Valencia et al. (2008)
Dry fermented pork sausages	Deodorized fish oil		Valencia et al. (2006) Muguerza et al. (2004)
Dutch-style fermented-beef sausages	Pure, pre-emulsified, or encapsulated fish oil	15 % and 30 % pork back fat substitution	Josquin et al. (2012)
	Encapsulated fish oil	5 % and 20 % pork back fat substitution	Pelser et al. (2007)

technological and sensory aspects) to use fish oil as a substitute to enrich sausages with essential PUFAs (Metcalf et al. 2003; Valencia et al. 2006, 2008; Muguerza et al. 2004; Cáceres et al. 2008; Josquin et al. 2012).

4.1.3 Application of Fish Oil in the Production of Cheese

Fish oil has been effectively utilized in producing cheese. The highest level of fish oil (8.69 mg/g) retention in cheese curd has been observed when fish oil was added during the salting process of cheddar cheese under high hydrostatic pressure (Bermúdez-Aguirre and Barbosa-Cánovas 2011, 2012). Bermúdez-Aguirre and Barbosa-Cánovas (2012) also reported that non-thermal approaches such as high hydrostatic pressure, pulsed electric fields, and ultrasound can be used to retain more fatty acids from fish oil in queso fresco cheese. Non-thermal methods are advantageous, as the thermal oxidation of essential fatty acids in both fish oil and cheese can be minimized with non-thermal unit operations. Furthermore, for mozzarella cheese, adding microencapsulated fish oil during curdling and the use of high hydrostatic pressure have been identified as the best methods to achieve maximum retention of fish oil (Bermúdez-Aguirre and Barbosa-Cánovas 2012).

Identifying the maximum level of fish oil that can be added without altering the sensory characteristics beyond threshold levels is vital for the successful incorporation of fish oil into cheese. The aroma and flavor of the fish oil-incorporated cheese were less preferred by panelists when compared to that of flaxseed oil-incorporated cheese (Bermúdez-Aguirre and Barbosa-Cánovas 2011). In addition, processed cheese slices containing a high level of non-encapsulated fish oil (10 g fish oil/kg) has been reported to have fishy odor (Ye et al. 2009). Kolanowski et al. (2007) also reported the development of unacceptable fishy off-flavor in fish oil-incorporated processed cheese. The oxidation of fish oil during the processing of cheese may lead to unacceptable flavor development in the final product (Ye et al. 2009). However, off-flavor and off-odor problems in fish oil-incorporated cheese can be avoided by

using moderate levels of fish oil and adding flavor compounds to mask the fishy flavor (Kolanowski and Weißbrodt 2007; Hughes et al. 2012b). Furthermore, many recent studies confirmed that fish oil, when added in suitable amounts, can be used to improve the sensory as well as nutritive value of cheese without having negative effects on consumer preference (Ye et al. 2009; Bermúdez-Aguirre and Barbosa-Cánovas 2011, 2012).

4.1.4 Fortification of Yoghurt with Fish Oil

The fortification of yoghurt with fish oil is possible with careful monitoring and control of the level of fish oil in the product and by adding flavor compounds to mask the fish flavor. Furthermore, the fortification of yoghurt with fish oil is relatively easy, as the food system of yoghurt is composed of fat. The fortification of yoghurt with fish oil was recently tested by several researchers (Higuchi et al. 2008; Estrada et al. 2011; Rognlien et al. 2012; Sanguansri et al. 2013; Tamijidi et al. 2012). Estrada et al. (2011) showed that the fortification of yoghurt with microencapsulated salmon oil before homogenization and pasteurization had no negative effect on the physicochemical properties, such as pH or syneresis, when compared to that of the control sample. However, Tamijidi et al. (2012) demonstrated that the acidity, apparent viscosity, and water-holding capacity were higher and the gel strength and amount of whey separation were lower in the fish oil-fortified yoghurt when compared to that of the control.

4.1.5 Incorporation of Fish Oil into Butter

As a food system made out of water in fat emulsion, butter is a potential food that could be incorporated with fish oil. Porsgaard et al. (2007) used an enzymatic interesterification method to successfully incorporate fish oil into butter. Positive nutritional effects of fish oil-incorporated butter have been observed by Porsgaard et al. (2007) and Overgaard et al. (2008). Porsgaard et al. (2007) reported that the intake of butter product with fish oil resulted in a higher level of n-3 PUFAs in plasma, erythrocytes, and liver of hamsters compared to the control group. Overgaard et al. (2008) reported that the consumption of butter incorporated with fish oil resulted in a lower concentration of triacylglycerols in the plasma levels of humans compared to the control group.

4.1.6 Nutrition Bars and Snacks Enriched with Fish Oil

The nutritional quality of nutrition bars and snacks could be further enhanced when incorporated with fish oil. Hughes et al. (2012a) were able to produce consumer-acceptable fish oil-incorporated nutrition bars having an oxidative stability of over 10 weeks. Pansawat (2007) reported that nutrition bars containing fish powder at a

concentration of 25 g per 100 g of extrusion mixture (dry basis) and fish oil at a concentration of 1.78 g per 100 g of extrusion mixture (dry basis) can be developed without reducing the consumer acceptability in terms of sensory qualities.

4.1.7 Fish Oil in Mayonnaise and Salad Dressings

Mayonnaise and salad dressings are food products often made of oils and form a food system of emulsions of water and oil. The possibility of replacing oil in salad dressings with fish oil has been studied recently. Furthermore, many studies have been focused on the evaluation of oxidative stability and means to enhance the shelf life of fish oil-enriched mayonnaise and salad dressings (Takai et al. 2003; Timm-Heinrich et al. 2004; Sørensen et al. 2010a, b). In general, cod liver oil has been used in these studies and the amount of fish oil used were varied in the range 4–14 % (Sørensen et al. 2010a, b).

4.1.8 Fish Oil as Functional Foods and in Nutraceutical Applications

Lipids (such as omega-3 fatty acids) from fish and fish waste can be used to produce fish oil capsules with potential use in other foods, bakery, and confectionary products (Freitas et al. 2012). The enrichment of bread and other bakery products with fish oil is widely utilized around the world, due to their increased content in eicosapentaenoic acid and docosahexaenoic acid with functional properties (Kadam and Prabhasankar 2010). These functional ingredients can be added at different stages of the food production process (from processing to storage). Foods containing fish and fish by-products-derived oils rich in omega-3 fatty acids are commercially available in the developed countries, such as the United States, Japan, and some European countries (Kadam and Prabhasankar 2010; Freitas, et al. 2012). As explained in Sect. 4.1.1, omega-3-enriched eggs with functional and desirable nutritional properties are also commercially available in many countries (Freitas et al. 2012). White bread enriched with omega-3 fatty acids in the form of gelatin-coated fish oil is available in Denmark under the name of "Omega Bread" (Kadam and Prabhasankar 2010). Functional spaghetti pasta product enriched with refined marine oil (containing long-chain omega-3 fatty acids) and fish gelatin was reported to be an effective means of delivering these functional ingredients to humans (Verardo et al. 2009). Furthermore, spaghetti pasta enriched with long-chain omega-3 fatty acids possessed acceptable sensory characteristics (Iafelice et al. 2008). The incorporation of omega-3 oils in dairy products (such as milk and yoghurt), juices, and nutrition bars contributes to food quality by improving flavor, aroma, color, texture, taste, and, also, nutritive value (carrier of fat-soluble vitamins: A, D, E. K) (Kadam and Prabhasankar 2010). Tahergorabi et al.(2012) demonstrated the feasibility in developing functional food products made out of muscle protein isolate recovered with isoelectric solubilization/precipitation from whole gutted rainbow trout fish (bones in, skin and scales on). These functional food products

were nutritionally enhanced with omega-3 PUFAs, with lower sodium and higher potassium content, while the color and textural properties were good and the gelation properties were improved.

4.2 Fish Enzymes

Fish enzymes derived from marine by-products have a wide range of applications in the food industry.

4.2.1 Tenderization of Meat

Collagen and elastin are proteins that are known to increase the toughness of meat (Ponnampalam et al. 2002; Aoki et al. 2004; Lepetit 2008), and these proteins can be hydrolyzed using photolytic enzymes to result more tender meat products. The beef treated with partially purified shrimp head extracts (*Pandalus borealis*) containing mainly collagenase-like, trypsin-like, and elastase-like proteases has significantly reduced the toughness of meat analyzed by the Warner–Bratzler shear force (Aoki et al. 2004). Similar results were observed by Kim et al. (2005), indicating a higher tenderness in shrimp sauce-treated pork during storage at 4 °C for 5 days compared to that of untreated pork. The temperature employed to store/treat meat can affect the activity of the added enzymes and, thereby, the degree of tenderizing achieved. For example, a study done by Kołodziejska et al. (1992) reported that the toughness of crude squid liver extract-treated, cooked squids mantle decreased by about 65 % (stored at 20 °C) and 40 % (stored at 4 °C) when compared to that of the untreated samples. In contrast to common methods that mainly target the hydrolysis of collagen and elastin, which are connective tissues, the study by Kołodziejska et al. (1992) achieved tenderness of meat mainly by hydrolyzing myosins, which are myofibrillar proteins. Therefore, crude squid liver enzyme extract is not suitable to be applied in tenderizing meat in which the myofibrillar fraction is important, for example, in the production of surimi. Table 23.3 shows some examples for the tenderization of meat by enzymes derived from marine by-products.

4.2.2 Hydrolysis of Collagen and Extraction of Gelatin

Enzymes can be used to pretreat raw materials during the extraction of collagen/gelatin, as they can specifically target and hydrolyze unwanted components in raw materials, such as fat and hemoglobin, facilitating the removal of those compounds (Ofori 1999). For example, pepsin from all tuna species can hydrolyze hemoglobin in fish tissues at optimal conditions of pH 2.0 and 50 °C (Nalinanon et al. 2008a).

During the extraction of collagen, the amount of soluble collagen content is increased when the enzymes are used to hydrolyze the cross-linked regions at the

Table 23.3 Tenderization of meat by enzymes extracted from seafood by-products

Source of enzymes	Meat type used	Amount added	Treatment conditions	References
Shrimp head extracts (*Pandalus borealis*)	Beef	Enzyme solution 10 mL to meat 10 g (concentration of collagenase-like, trypsin-like, and elastase-like proteases extracted from shrimp heads)	1 h at 10 °C	Aoki et al.(2004)
Liver extract of squid (*Illex argentinus*)	Squid mantle	0.02, 0.05, and 0.1 % of liver extract	24 h at 20 or 4 °C	Kołodziejska et al. (1992)
Sauce from shrimp processing by-products (Southern rough shrimp; *Trachypenaeus curvirostris*)	Pork	Dip in sauce containing crude shrimp by-product extract for 3 min	20 ± 2 °C for 3 h, followed by storage at 4 °C for 5 days, wrapped in aluminum foil	Kim et al. (2005)

telopeptide, resulting in an increase in the solubility of collagen in acid (Bama et al. 2010). Collagen has to be hydrolyzed to hot water-soluble gelatin fractions during the extraction of gelatin (refer to the extraction of gelatin and collagen). Thus, increase in the water solubility of collagen results in an increase of the yield of collagen and gelatin extracted (Nalinanon et al. 2007, 2008a; Balti et al. 2011; Bougatef et al. 2012). For example, Nalinanon et al. (2007) reported that the yield of collagen extracted from bigeye snapper (*Priacanthus tayenus*) skin using acetic acid and bigeye snapper pepsin were 5.31 % and 18.74 % (dry basis), respectively. Similar results (almost three-fold increase in the yield of collagen/gelatin) from marine-derived enzymes were observed by Balti et al. (2011). They gained gelatin yields of 2.21 % (using acetic acid for 48 h) and 7.84 % (using crude smooth hound protease, 15 units/g alkaline-treated skin) from cuttlefish skin. However, enzymes may result in a reduction of yield and quality of gelatin/collagen due to the excessive hydrolyzation of proteins (Nalinanon et al. 2008b; Phanturat et al. 2010). This problem can be solved by the use of enzyme inhibitors such as soybean trypsin inhibitor and pepstatin to inhibit the hydrolysis process at the desired levels (Nalinanon et al. 2008b; Phanturat et al. 2010). Examples for the application of enzymes derived from marine by-products used in the extraction of gelatin and collagen are shown in Table 23.4.

4.2.3 Bone Separation and Digestion

Fish enzymes can be used in the process of preparation of fish bone hydrolysate. The removal of remaining muscle parts in fish can be done by the use of fish enzymes to clean the fish bones for further processing. However, the removal of muscle

Table 23.4 Enzymes derived from marine by-products that have been used in gelatin and collagen extraction

Species and organ used in extraction	Compound extracted	Fish enzyme used	References
Bigeye snapper skin (*Priacanthus tayenus*)	Gelatin	Bigeye snapper pepsin	Nalinanon et al. (2008b)
Threadfin bream skin (*Nemipterus* spp.)	Collagen	Pepsin from the stomach of albacore tuna, skipjack tuna, and tongol	Nalinanon et al. (2008a)
Bigeye snapper skin (*Priacanthus tayenus*)	Collagen	Bigeye snapper pepsin	Nalinanon et al. (2007)
Smooth hound skin (*Mustelus mustelus*)	Gelatin	Smooth hound crude acid protease extract	Bougatef et al. (2012)
Cuttlefish skin (*Sepia officinalis*)	Gelatin	Smooth hound crude acid protease extract	Balti et al. (2011)

proteins by fish enzymes may not be effective compared to commercial or microbial enzymes. This could be due to the enzyme inhibitors present in the sarcoplasmic fraction of cod muscle (Gildberg et al. 2002). The separation of fish bones using mackerel intestine crude enzyme has also been reported by Kim et al. (2003). These researchers were able to obtain approximately 90 % bone recovery from hoki (*Johnius belengerii*) after 6 h of extraction using crude enzymes of mackerel at optimum conditions of pH 9.0 and 40 °C. In this experiment, mackerel intestine crude enzyme yielded a higher rate of recovery compared to other commercial enzymes, such as subtilisin, trypsin, α-, and chymotrypsin (Kim et al. 2003). The cleaned and separated fish bones using fish enzymes can further be hydrolyzed using fish enzymes to produce hydrolyzed fractions with functional properties. For example, Jung et al. (2005) reported that heterogeneous enzyme extracted from the intestines of a carnivorous fish, bluefin tuna (*Thunnus thynnus*), could degrade the hoki (*Johnius belengerii*) bone. They suggest that fish bone oligophosphopeptide prepared by the enzymatic degradation of bones could be utilized as a nutraceutical with a potential calcium-binding activity, which inhibits the formation of insoluble calcium phosphate, thus increasing the bioavailability of calcium extracted from fish bone (Jung et al. 2005).

4.2.4 Rennet Substitute in Cheese Making

Rennet is the most commonly used enzyme for the coagulation of milk in cheese making. However, various studies have been employed to evaluate the applicability of new enzyme types, such as fish proteases, seed extracts, and fungal proteases to replace rennet (Talib et al. 2009; Merheb-Din et al. 2012). Some examples of marine by-product-derived enzymes and optimum processing conditions used in cheese production are shown in Table 23.5.

Table 23.5 Enzymes extracted from seafood by-products with potential application in the production of cheese

Source	Type of enzyme(s) in the extract	Type of cheese	Optimum conditions	References
Hepatopancreas of the crustaceans (*Munida*)	Isotrypsin-like and isochymotrypsin-like enzymes, aminopeptidases, and carboxypeptidases A and B	Not given	pH 7.5 40–45 °C	Rossano et al. (2011a, b)
Crustaceans (*Munida*)	Aspartic and cysteine proteases	Not given	pH 6.5–7.5 55–60 °C	D'Ambrosio et al. (2003)
Clam viscera	Thiol protease such as cathepsin B-like protease	Cheddar cheese	Not given	Chen and Zall (1986)

Enzymes from different marine by-products can result in cheese with different sensory qualities due to variation in the enzymes in the extract, which determines the type of peptides hydrolyzed and the types of peptide fractions produced (Visser et al. 1983; Kelly et al. 1996; Singh et al. 2004). For example, isotrypsin-like and isochymotrypsin-like enzymes, aminopeptidases, and carboxypeptidases A and B extracted from the hepatopancreas of the crustaceans *Munida* have been found to degrade the chymosin-derived β-casein fragment f193–209, one of the peptides associated with bitterness in cheese, thus resulting in the reduction of bitterness (Rossano et al. 2011a). Furthermore, cathepsin B-like protease from clam viscera was reported to be more proteolytic and produced softer curd compared to calf rennet (Chen and Zall 1986). However, the sensory results of Colby cheese made out of tuna protease showed lower preference in terms of flavor, texture, and overall acceptability compared to that of cheese made using rennet (Tavares et al. 1997).

The use of enzymes derived from marine by-products in cheese making has several advantages over commercial enzymes. The cost of enzymes is lower when they are extracted from fish by-products. The ability of fish enzymes to function at low temperature is useful in cheese ripening, which is usually done at mild temperatures. The ability of fish enzymes to hydrolyze undesirable compounds such as derived β-casein fragment f193–209 is also an advantage (Rossano et al. 2011a).

4.2.5 Production of Food-Grade Detergents

Enzymes are a good alternative to increase the cleaning power of a detergent (especially those used in the food industry) with minimum health and environmental hazards. However, not all enzymes can be used in detergents. They have to be compatible with the pH range of the detergent, which is usually alkaline. Therefore, alkaline-stable enzymes are used in detergents. Table 23.6 shows enzymes extracted from marine-derived by-products with potential application in detergents due to their alkaline stability.

Table 23.6 Enzymes extracted from seafood by-products with potential application in detergents

Enzyme	Source	Optimal pH and stable temperatures	References
Trypsin	Zebra blenny (*Salaria basilisca*) viscera	pH 9.5 and 60 °C	Ktari et al. (2012)
Proteases	Sea cucumber (*Stichopus japonicus*) digestive tract	pH 8 and pH 13.5 Temperature not detected	Fu et al. (2005)
Trypsin	Striped sea bream (*Lithognathus mormyrus*) viscera	pH 10 and 50 °C	El Hadj Ali et al. (2009)
Trypsin	Cuttlefish (*Sepia officinalis*) hepatopancreas	pH 8 and 70 °C	Balti et al. (2009)
Proteases	*Colossoma macropomum* viscera	pH 10–12 and 60 °C	Espósito et al. (2009)
Trypsin	Goby (*Zosterisessor ophiocephalus*) viscera	pH 9 and 60 °C	Nasri et al. (2012)

Table 23.7 Use of enzymes from seafood by-products in extracting carotenoproteins

Source of carotenoproteins	Type and origin of the enzyme	Conditions used	References
Black tiger shrimp shells	Bluefish trypsin	1.2 trypsin units/g shrimp shells, kept for 1 h at 25 °C	Klomklao et al.(2005)
Shrimp shells (*Parapenaeus longirostris*)	Trypsin barbel (*Barbus callensis*)	1.0 U barbel trypsin/g shrimp shells, kept for 1 h at 30 °C	Sila et al.(2012)
Shrimp wastes	Atlantic cod trypsin	25 mg cod trypsin containing 0.5 N EDTA at 4 °C	Cano-Lopez et al.(1987)

4.2.6 Extraction of Carotenoproteins from Seafood By-product-Derived Enzymes

Carotenoproteins are proteins bound with carotene, which can be found in the shells of crustaceans (Cianci et al. 2002; Pilbrow et al. 2012). Enzymes extracted from seafood by-products can be used to hydrolyze the shells and extract these carotenoproteins. Cano-Lopez et al. (1987) compared the yield of astaxanthin from shrimp wastes digested by Atlantic cod trypsin and bovine trypsin. Accordingly, cod trypsin has resulted in more astaxanthin (64 %) compared to bovine trypsin (49 %). However, semi-purified cod trypsin was not as effective as pure trypsin in facilitating the recovery of carotenoproteins from shrimp waste. Studies conducted to extract carotenoproteins using enzymes extracted from seafood by-products are shown in Table 23.7.

Carotenoids can be effectively used as natural colorants and antioxidants (Ötles and Çagindi 2008; Qian et al. 2012). According to epidemiological studies, high intake of foods rich in carotenoids is associated with decreased risk for some

cancers, cardiovascular disease, age-related macular degeneration, and cataracts (Gerster 1993; von Lintig 2010). Preventing oxidative damage, quenching singlet oxygen, altering transcriptional activity, and serving as precursors for vitamin A have been identified as physiological mechanisms which render all these health benefits of carotenoids (Qian et al. 2012). In a review, Agarwal et al. (2012) described in detail the effects of carotenoids, with special emphasis on cardiovascular diseases.

4.3 Utilization of Chitin, Chitosan, and Their Oligosaccharides in the Food Industry

Chitin, chitosan, and their oligosaccharides can be considered as natural materials with antioxidant, antibacterial, and antifungal properties. Also, due to their biocompatibility, biodegradability, non-toxicity, and bioadhesion nature, they have attracted the attention of many industries other than the food industry, such as pharmaceutical, cosmetics, medical, wastewater treatment, paper finishing, photographic paper, and in agriculture (Vidanarachchi et al. 2009; Alishahi and Aïder 2012).

4.3.1 Antioxidant Activity of Chitin, Chitosan, and Chitooligosaccharides

Substances that delay or avert the oxidation of substrates and present in food at minute concentrations compared to the oxidizable substrate are considered as antioxidants. Thereby, the degradation of food quality and nutritive value of food can be controlled (Halliwell et al. 1995; Shahidi 2000). Antioxidants can be categorized into two groups, as primary and secondary antioxidants. Primary antioxidants are compounds with phenolic groups which react at the initial stages of oxidation by behaving as H donors. The metal-chelating molecules are the second group which exert their effect on the catalysis of oxidative reactions (Agulló et al. 2003). It has been found that chitin and chitosan can behave as secondary antioxidants. Kamil et al. (2002) observed effective inhibition of lipid oxidation of cooked comminuted herring (*Clupea harengus*) with different molecular weight chitosans. These authors suggested that the observed antioxidant activity of chitosan is due to their metal chelating activity, where chitosan behaves as a secondary antioxidant. The abundance of amine groups in chitosan molecules has resulted in its ability to chelate iron (Peng et al. 1998; Winterowd and Sanford 1995).

Park et al. (2004) evaluated the free radical scavenging activity of deacetylated heterochitosans against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, alkyl radicals, hydroxyl radicals, and superoxide radicals using an electron spin resonance spectrometer, and found that the scavenging activity of deacetylated heterochitosans increased with increasing alkyl radicals. Furthermore, the same authors also observed that chitosan with 90 % deacetylation has the highest radical scavenging activity. Chitobiose and chitotriose obtained by partial hydrolysis of chitosan have

been evaluated for their antioxidant activity (Chen et al. 2003). Chitobiose and chitotriose have shown higher hydroxyl radical scavenging activity than that of the three reference compounds aminoguanidine, pyridoxamine, and trolox (Chen et al. 2003). The same authors also found that exerting an antioxidant activity presence of more than two amino groups in chitooligosaccharides is important. Huang et al. (2006) concluded that the ability of H donation by chitosan and its derivatives is important for the scavenging of DPPH and carbon-centered radicals. In addition, the Fe^{2+} binding activity helps in the H donation activity of chitosan and it derivatives during the latter part of the reaction. The antioxidant activity of carboxymethyl chitosan has been evaluated recently and it has been found that the DPPH radicals and superoxide radicals scavenging activities of carboxymethyl chitosan are higher than that of chitosan (Zhao et al. 2011). Furthermore, the Fe^{2+} chelation ability of carboxymethyl chitosan reaches 100 % at a concentration of 1.2 mg/mL, and, at the same concentration, chitosan showed only 22 % chelation activity. Thus, the use of carboxymethylation of chitosan could be a promising way to maximize the functional properties of chitosan in applications of the food and nutraceuticals industries (Zhao et al. 2011). Furthermore, Xing et al. (2005) evaluated the effect of different molecular weights of chitosan and sulfated chitosan derivatives on superoxide, hydroxyl radicals scavenging, and reducing power and ferrous ion chelating activities. It has been observed that the chitosan with low molecular weight has shown strong scavenging activity on superoxide and hydroxyl radicals. Among similar molecular weight chitosan and sulfated chitosan molecules, sulfated chitosan has shown a marked increase of scavenging activities compared to chitosan. In the same study, Xing et al. (2005) showed that both chitosan and sulfated chitosan possess considerable reducing power and different ion binding activities, depending on the molecular weights.

Researchers have attempted to produce new derivatives of chitosan by introducing phenolic groups into the chitosan molecule. Thereby, the chitosan molecule can be exploited as both primary and secondary antioxidants (Agulló et al. 2003). Substituting groups may have an effect on the antioxidant activity of chitosan and its derivatives by affecting its charge properties. Fras-Zemljič et al. (2011) introduced two chemically similar phenolics, namely fisetin and quercetin, to chitosan-based viscous fiber and they observed a significant increase of the antioxidant activity of such fibers. Ngo et al. (2011) synthesized a novel derivative of chitooligosaccharides (COS) by covalently linking gallic acid and COS (gallate-COS) via carbodiimide to discover the antioxidant activity at the cellular level. It was found that COS and gallate-COS are non-toxic and could inhibit oxidative damage to lipids, proteins, and DNA in RAW264.7 cells. Furthermore, gallate-COS suppressed the ROS, increased the expression of the level of intracellular antioxidant enzymes (superoxide dismutase and Glutathione), and induced the activation of the nuclear transcription factor in oxidative stress-induced RAW264.7 cells. Based on these findings, the authors concluded that gallate-COS can be used effectively as a natural antioxidant in the functional food and pharmaceutical industries. Recently, Torres et al. (2012) attached quercetin and rutin quinones chemically to low molecular weight chitosan (LMWC) and, subsequently, antioxidant properties have been evaluated with DPPH

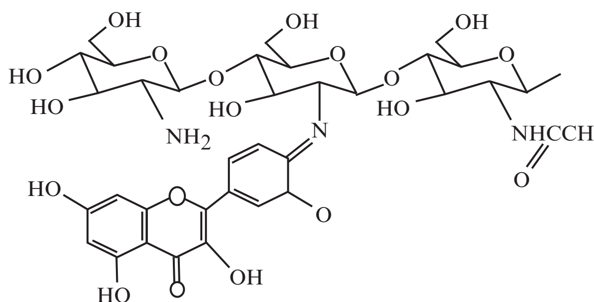

Fig. 23.5 Molecular structure of a modified chitosan (Source: Torres et al. (2012))

and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) methods, and found that modified chitosan (Fig. 23.5) is superior compared to chitosan in regards to antioxidant activities. Furthermore, Cho et al. (2011) showed that chitosan gallate could effectively suppress lipid peroxidation in RAW264.7 macrophage cells and subdue the generation of intracellular ROS. Interestingly, the same researchers observed that chitosan gallate is capable of protecting DNA from damage caused by hydroxyl radicals. Moreover, chitosan gallate has increased the upregulation of protein expression of some of the antioxidant enzymes, such as superoxide dismutase-1 and glutathione reductase, under H_2O_2-mediated oxidative stress in RAW264.7 macrophage cells (Cho et al. 2011). Therefore, as a derivative of chitosan, chitosan gallate could be a natural substance that can be exploited in the food and nutraceutical industries as a potent antioxidant.

4.3.2 Antibacterial Activity of Chitin, Chitosan, and Chitooligosaccharides

Kong et al. (2010) carried out a detailed review of the antibacterial effects of chitosan, which included an in-depth analysis of the microbial factors, intrinsic factors, physical state, environmental factors, mode of antibacterial effect of chitosan, and antimicrobial application of chitosan and its derivatives in the food, medical, and textile industries. Vinsova and Vavrikova (2011) reviewed the antimicrobial activity of chitosan derivatives. Hence, the current chapter mainly emphasizes the recent antibacterial advances of chitosan and its derivatives in the food sector.

Interestingly, chitosan shows a wide spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, and, more importantly, low toxicity levels toward mammalian cells (Kong et al. 2010). However, chitosan has illustrated a stronger antibacterial activity against Gram-negative bacteria than Gram-positive bacteria (No et al. 2002; Chung et al. 2004). In contrast to that, some authors have found that Gram-positive bacteria are more susceptible than Gram-negative bacteria for chitosan (Zhong et al. 2008). Gram-negative bacteria are inhibited by chitosan through the interaction of polycationic amino groups with anionic components of the bacterial surface and, thereby, changing its permeability (Tao et al. 2011).

Another mode of action proposed to explain the antibacterial activity of chitosan is its ability to form a membrane around the bacteria, which prevents the absorption of nutrient by bacterial cells (Liu et al. 2004). The difference in zeta potential between the surface of LMWC particles and the bacteria has an effect on the antioxidant activity of chitosan. A study which evaluated the effect of LMWCs on three strains of *Staphylococcus aureus* and two strains of *Escherichia coli* showed differential sensitivities to LMWCs (Chen et al. 2012). This study revealed that the cationic charge of LMWCs is primarily responsible for its antibacterial effect against three strains of *S. aureus* (Chen et al. 2012). The antimicrobial activities of both commercial chitosan and chitosans extracted from shrimp (*Metapenaeus stebbingi*) shell wastes have been tested in vitro by using the disk diffusion method with standard microorganisms of *Pseudomonas putida*, *Pseudomonas fluorescens*, *Vibrio parahaemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Enterecoccus faecalis*, and *Aeromonas caviae*. The results showed that chitosan has different antibacterial effects, depending on the chitosan concentration, solvent material, and bacteria type. For example, an antibacterial effect has been observed on all types of bacteria except *E. coli* in the range of 0.10 % and 0.50 % chitosans (both commercial and extracted) concentrations. These results suggest the potent use of chitosan in food preservation as a natural antibacterial agent (Küçükgülmez et al. 2012). Chitin, chitosan, and its oligomers N-acetyl chitooligosaccharides and chitooligosaccharides have been tested for their antibacterial effect against Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300, *Bacillus subtilis* and *Bacillus cereus*, and seven Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium*, *Vibrio cholerae*, *Shigella dysenteriae*, *Prevotella melaninogenica*, and *Bacteroides fragilis*, and two anaerobic bacteria, namely *P. melaninogenica* and *B. fragilis* (Benhabiles et al. 2012b). Chitin has shown a bacteriostatic effect on Gram-negative bacteria, *E. coli* ATCC 25922, *V. cholerae*, *S. dysenteriae*, and *B. fragilis*. A bacteriostatic effect of chitosan has been observed on all the bacteria tested, except *S. typhimurium*. Nevertheless, chitosan oligomers exhibited a bactericidal effect on all the bacteria tested. Therefore, the possibilities of using chitooligomers have potent advantages due to their high antibacterial effect and ready solubility in water (Benhabiles et al. 2012b).

A recent study revealed the possibility of the use of chitosan in food packaging. The effect of arginine-functionalized chitosan has been evaluated against *E. coli* O157 in chicken drip (liquid accumulated inside package) stored at 4 °C and 40 °C. It has been found that arginine-functionalized chitosan effectively reduces the *E. coli* O157 in a dose-dependent manner (Lahmer et al. 2012). In addition, it suppressed the growth of spoilage bacteria and off-odor produced by bacteria. Thus, the results suggest the possible use of arginine-functionalized chitosan in fresh chicken meat packaging to overcome the risk of food poisoning and to extend the shelf life of chicken meat (Lahmer et al. 2012). Another study has focused on the effect of chitosan for the extension of shelf life of filleted tilapia. Tilapia fillets inoculated with bacteria (*Pseudomonas*, *Aeromonas*, and *Staphylococcus*) isolated from tilapia spoiled fish muscle were treated with 5.0 g/L chitosan. The results revealed a wide

spectrum of antibacterial effects of chitosan and it extended the shelf life of tilapia fillets to up to 12 days at 4 °C, whereas untreated tilapia showed a shelf life of 6 days at the same storage conditions (Cao et al. 2012).

4.3.3 Antifungal Activity of Chitin, Chitosan, and Chitooligosaccharides

Agricultural products can be contaminated with toxigenic fungi and the occurrence of mycotoxins causes serious economic losses and harmful effects to humans and animals. The antifungal activity of chitosan has been evaluated both in vitro and in vivo in several studies, although chitosan's activity against fungi has been shown to be less efficient compared to its activity against bacteria (Tsai et al. 2000).

Chitosan exerts its fungistatic activity against *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Monilinia fructicola*, *Penicillium digitatum*, *Rhizopus stolonifer*, and *Aspergillus niger*, etc., causing damage to the microbial cell membrane and altering the morphology of fungi (Cota-Arriola et al. 2011). Chitosan prepared from shrimp waste and commercial chitosan have been evaluated against *Aspergillus niger*, and both types of chitosans inhibited the growth of fungus by 47 % and 56 % compared to the control, respectively (Martínez-Camacho et al. 2001). In order to extend the shelf life of food items, coating them with chitosan films has been evaluated extensively. Chitosan added with 3 % lemon essential oil has shown significant antifungal activity during the cold storage of strawberries inoculated with a spore suspension of *Botrytis cinerea* (Perdones et al. 2012). The coating of strawberries with pure chitosan reduced the growth of bacterium compared to the control. The addition of lemon essential oil has significantly improved the antifungal activity of chitosan. However, coated strawberries with chitosan containing lemon essential oil significantly reduced the typical strawberry aroma and flavor compared to the chitosan-coated and control samples. This was due to the masking effect exerted by the lemon essential oil (Perdones et al. 2012). The antifungal effect of chitosan with or without essential oils of bergamot, thyme, and tea tree oil was evaluated with oranges inoculated with *Penicillium italicum* CECT 2294 (105 spores/mL) (Cháfer et al. 2012). The findings revealed that the chitosan coating containing tea tree oil showed a 50 % reduction of infection of fruit with the inoculated fungus compared to the control. However, chitosan alone has not shown any antifungal activity against *P. italicum* (Cháfer et al. 2012). This demonstrates the importance of the combination of plant-derived oils with chitosan in order to enhance its effectiveness in terms of antifungal activity. In general, the coating of food products extends the shelf life by reducing respiration and moisture loss. The enhanced antimicrobial effect of chitosan films added with essential oils could be due to the antimicrobial effect of essential oils.

Zahid et al. (2012) evaluated the antifungal activity of chitosan and chitosan-loaded nanoemulsions on anthracnose caused by *Colletotrichum* spp. on different tropical fruits. Chitosan at a concentration of 1–5 % inhibited the radial mycelial growth, conidial germination, sporulation, and dry weight of mycelia for *Colletotrichum musae* and *Colletotrichum gloeosporioides*. For banana, chitosan

at 1.0 % concentration with a droplet size of 200 nm, and for papaya and dragon fruits, a droplet size of 600 nm delayed the anthracnose occurrences and maintained the quality of fruits for up to 28 days during cold storage (Zahid et al. 2012). This could be due to the direct contact of chitosan with the fungal cell wall, causing chitosan to penetrate the fungal cell and invoke configurational changes of the DNA, which ultimately inhibits the synthesis of m-RNA and proteins (Hadwiger and Loschke 1981). Furthermore, the chelating activity of chitosan towards metals, spore elements, and essential elements retard the microbial growth (Cuero et al. 1991; Al-Hetar et al. 2010).

4.3.4 Other Functional and Nutraceutical Properties of Chitosan and Its Derivatives

Chitosan and its derivatives also possess other valuable functional properties, such as hypocholesterolemic, immunostimulating, antitumor and anticancer effects, accelerating calcium and iron absorption, anti-inflammatory, and angiotensin-I-converting enzyme, etc. Furthermore, chitin, chitosans, and chitooligosaccharide derivatives may have potential functional applications in the food industry as gelling agents, emulsifying agents, food preservatives, and dietary fiber (Freitas et al. 2012). Commercial functional products such as chitosan capsules are available in Korea, Japan, China, and Norway. Edible chitosan films have also been prepared for food storage due to its antimicrobial activity (Xia et al. 2011). Food products including soybean paste, potato chips, and noodles with added chitosan are available as cholesterol-lowering functional foods (Borderías et al. 2005). Xia et al. (2011) performed a detailed review of the biological activities of chitosan and chitooligosaccharides. Furthermore, another comprehensive review describes the hypocholesterolemic, immunomodulating, blood hemostasis, anticancer, and antidiabetes effect of chitosan and its derivatives (Zhang et al. 2010). Moreover, Vidanarachchi et al. (2009) also discussed in detail the applications of chitin, chitosan, and their chitooligosaccharides in the food industry.

4.4 Food Applications of Gelatins Extracted from Fish and Aquatic Invertebrates

Gelatin is considered a highly digestible dietary food, ideal as a complement in certain types of diet. Furthermore, gelatin has found wide applications not only in the food but also in the materials, pharmaceuticals, photographic, and cosmetics industries over many years due to its unique chemical and physical properties (Yang et al. 2007; Binsi et al. 2009; Tabarestani et al. 2010). There is an increasing demand from the food industry for alternative collagen/gelatin sources, such as fish and aquatic invertebrates-derived gelatin compared to traditional pork or beef

Table 23.8 Examples of fish and aquatic invertebrate-derived by-products used in extracting collagen/gelatin

Aquatic species	By-product(s)	References
Giant red sea cucumber (*Parastichopus californicus*)	Skin and connective tissues	Liu et al. (2009)
Deep-sea redfish (*Sebastes mentella*)	Skin, scales, and bones	Wang et al. (2008)
Lizardfish (*Saurida* spp.)	Scales	Wangtueai and Noomhorm (2009)
Sin croaker (*Johnius dussumieri*)	Skin	Cheow et al. (2007)
Shortfin scad (*Decapterus macrosoma*)	Skin	Cheow et al. (2007)
Alaska pink salmon (*Oncorhynchus gorbuscha*)	Skin	Chiou et al. (2006)
Giant squid (*Dosidicus gigas*)	Skin	Uriarte-Montoya et al. (2011)
Bigeye snapper (*Priacanthus tayenus*)	Skin	Rattaya et al. (2009)
Cuttlefish (*Sepia pharaonis*)	Skin	Aewsiri et al. (2009)
Skipjack tuna (*Katsuwonus pelamis*)	Fins	Aewsiri et al. (2008)

gelatin due to having fewer religious and sociocultural barriers (Islam, Hinduism, and Judaism) and health concerns related to diseases such as mad cow disease. This could potentially provide a greater economic return for fishermen and fish processors, since by-products from fish and aquatic invertebrates-based industries such as skin, bones, and scales of teleosts, selachians, and mollusks can be considered as valuable alternative sources for collagen/gelatin. This also helps in reducing waste and pollution caused by the marine industries (Kim and Mendis 2006; Bae et al. 2009; Boran et al. 2010; Zhu et al. 2010). Some examples for marine fish and aquatic invertebrates-derived by-products used in extracting collagen/gelatin are illustrated in Table 23.8. Although gelatin from warmwater fish species possesses physical properties more similar to those of mammalian gelatin, coldwater fish gelatin (which represents the majority of the industrial fish gelatin) has low gelation and melting temperatures and low gel modules (Bae et al. 2009). Hence, the commercial interest to date in gelatin extracted from coldwater fish species has been constrained to a considerable extent. However, gelatins from warmwater fish species have physical properties more similar to those of mammalian gelatins. The differences in the physical properties of mammalian and coldwater fish gelatins are due to the lower content of amino acids proline and hydroxyproline in coldwater fish gelatin (Bae et al. 2009). Fishy odor and certain rheological properties of fish-derived gelatin may also contribute to its limited applications in the food industry (Cheow et al. 2007; Gómez-Guillén et al. 2007). However, fish and aquatic invertebrate-derived collagen/gelatin has been utilized or has great potential in various applications in the food industry, including confectionary, dairy, and meat products. Other than providing specific product properties in these foods, such as chewiness, gelling, and mouth feel, another advantage of food-grade fish gelatin is lower or no risk to fish-allergic subjects (Hansen et al. 2004).

4.4.1 Edible Gelatin Films/Coatings from Fish and Aquatic Invertebrate By-products

There is an increasing demand for edible and biodegradable films with potential in extending the shelf life and improving the quality of food products during storage. Generally, fish gelatin possesses excellent film-forming properties (Karim and Bhat 2009). However, the quality of a food-grade gelatin depends to a large extent on its rheological properties (mainly gel strength), absence of color and flavor, and easy dissolution (Gómez-Guillén et al. 2007). These properties of marine gelatin can be significantly varied according to the type of raw materials used in isolating marine derived-gelatin (Gómez-Guillén et al. 2002). The quality of fish gelatin films can be improved by various approaches, including the use of enzymes, addition of salts and ultraviolet irradiation (Bhat and Karim 2009; Karim and Bhat 2009), blending of fish gelatin with other biopolymers, such as chitosan and pectin, and the addition of plasticizers, such as glycerol and sucrose (Bae et al. 2009). Plant extracts have also been reported to alter the physical properties of fish gelatin films beneficially. The addition of murta (*Ugni molinae* Turcz) leaves extracts into tuna fish (*Thunnus thynnus*) gelatin-based edible films led to transparent films with increased protection against UV light as well as the antioxidant capacity of the extract, demonstrating their wide potential applications in the food packaging industry (Gómez-Guillén et al. 2007). Seaweed (*Turbinaria ornata*) extract has been effectively utilized as the natural protein cross-linkers, in order to modify the properties (through modification of the polymer network by the cross-linking of the polymer chains) of film-forming gelatin from bigeye snapper skin (Rattaya et al. 2009). Moreover, edible films with antimicrobial properties have also been produced from coldwater fish skin gelatin with added lysozyme, a food-safe antimicrobial enzyme (Bower et al. 2006; Gómez-Guillén et al. 2009).

Edible films have been successfully manufactured from various fish and aquatic invertebrate by-products, such as surimi processing waste (Al-Hassan and Norziah 2012), skin of cod, haddock, and pollock fish species (Krishna et al. 2012), cuttlefish skin (Hoque et al. 2010), brownstripe red snapper skin, bigeye snapper skin (Jongjareonrak et al. 2006), and blue shark skin (Limpisophon et al. 2009), with acceptable physical and mechanical properties. Potential applications of these edible films in the food industry may include the transport of gases (O_2 and CO_2), water vapour, and flavors for fruits and vegetables, confectioneries, frozen foods, and meat products such as sausages (Jongjareonrak et al. 2006; Bae et al. 2009). Although the inherent fishy odor of fish and aquatic invertebrate-derived gelatin may limit its application as edible films and coatings in certain foods, gelatin derived from such by-products can be recognized as an ideal source in the manufacture of edible films and coatings for seafood. A recent study revealed that the edible films based on fish skin gelatin incorporated with chitosan and/or clove essential oil have improved the shelf life of raw sliced salmon (Gómez-Estaca et al. 2009). Moreover, fish gelatin melts more slowly than pork gelatin in the mouth (Choi and Regenstein 2000), and this feature may likely positively influence the sensory properties of the foods produced with fish gelatin edible films.

4.4.2 Use of Gelatin Derived from Fish and Aquatic Invertebrates for Microencapsulation

The microencapsulation of food ingredients is often achieved with biopolymers of various sources, such as natural gums (gum Arabic, alginates, etc.), proteins (whey protein, gelatin, etc.), maltodextrins with different dextrose equivalences, waxes, and their blends. Gelatin possesses an encapsulating ability, which is useful in the spray drying of food matrixes as well (Gharsallaoui et al. 2007). Furthermore, the low gelation temperature of fish gelatin can provide advantages in the microencapsulation process, since the process can be carried out at lower temperatures (Karim and Bhat 2009). The incorporation of coldwater fish skin gelatin with gum Arabic (acacia gum) has been reported to provide many useful potential applications of fish gelatin in microcapsule formation (Yang et al. 2012). Fish gelatin has been utilized in the microencapsulation of vitamins, colorants, and other pharmaceutical additives, such as azoxanthine. It is also possible to microencapsulate food flavors such as vegetable oil, lemon oil, garlic flavor, apple flavor, or black pepper with warmwater fish gelatin (Karim and Bhat 2009). Furthermore, soft gel capsules produced from fish gelatin has been widely used as nutrition supplements (Karim and Bhat 2009). However, applications of fish and aquatic invertebrate-derived gelatin in the microencapsulation food matrices have not yet been well developed.

4.4.3 Gelatin Derived from Fish and Aquatic Invertebrates as an Emulsifier and a Gelling Agent

The amphoteric characteristic of gelatin as well as its hydrophobic areas on the peptide chain make it a strong emulsifying and forming agent (Galazka et al. 1999; Cheng et al. 2008). Unique melt-in-the-mouth properties and variety in texture and gel melting behavior of various gelatins offer opportunities for developing gelatin-based desserts (Zhou and Regenstein 2007; Gómez-Guillén et al. 2011). Desserts made from fish-derived gelatin from Alaskan pollock skin (by increasing the gelatin concentrations or by using gelatin mixtures) were found to be more similar to desserts made from high-bloom pork skin gelatin (Zhou and Regenstein 2007). Furthermore, the same researchers also found that the lower melting temperature in gel desserts made from the Alaskan pollock skin gelatins accelerated flavor release in the gel desserts (Zhou and Regenstein 2007). The flavored fish skin gelatin desserts were reported to possess less undesirable off-flavor and off-odor and more desirable release of flavor and aroma than the same product made with an equal bloom value, but higher melting point, pork gelatin (Choi and Regenstein 2000). The gelatin produced from the skin of fish living in cold waters does not gel at room temperature and its gelling temperature is below 8–10 °C. This feature limits the use of such gelatin as a gelling component in food production. However, enzyme treatments such as transglutaminase can improve such disadvantages of fish skin gelatin. For example, megrim and Baltic cod skin gelatin demonstrated better rheological properties after transglutaminase treatments (Kołodziejska et al. 2004; Gómez-Guillén et al. 2011).

Furthermore, reducing drip loss and impairing juiciness, which aid in improving the sensory and physicochemical properties of frozen fish or meat products when thawed or cooked, also seem possible with the use of fish-derived solubilized collagen and gelatin (Gómez-Guillén et al. 2011). Allam et al. (1997) reported that gelatin extracted from the wastes of Aswan bolti fish (*Tilapia nilotica*) could be used to replace between 50 % and 100 % of commercial gelatin in strawberry jam, as well as 10 % of wheat flour in baby food formula, without affecting the quality of the products.

Fish gelatin may also aid in preventing unattractive clumping in dairy foods such as yoghurts (Karim and Bhat 2009). A gelling agent is one of the essential ingredients for the production of a low-fat spread. Fish gelatin and pectin were reported to significantly influence the rheological properties, such as firmness, elasticity, compressibility, adhesiveness, and melting ability of low-fat spreads (Cheng et al. 2008). The gel-forming capability of threadfin bream (*Nemipterus japonicus*) mince was substantially increased by adding gelatin from the skin of bigeye snapper (Binsi et al. 2009). Furthermore, a recent study by Taherian et al. (2011) demonstrated the ability of whey protein isolates and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. Hence, such properties of combined macromolecules would have potential practical applications for the design of industrial dispersions to deliver functional ingredients into beverages.

4.5 Applications of Fish Protein Hydrolysates and Concentrates in the Food Industry

Fish protein hydrolysates can be recommended as an alternative for other expensive animal-derived protein sources currently being used in the processed food industry (Liceaga-Gesualdo and Li-Chan 1999). Furthermore, there is a good potential of fish protein hydrolysate as a component of food formulation for human consumption. For example, fish protein hydrolysate from Bluewing searobin (*Prionotus punctatus*) has been effectively utilized in food formulations for human consumption (Santos et al. 2011). Fish protein hydrolysates possess important functional properties such as emulsifying, foaming, and whipping abilities, which facilitate their applications in the food industry (Souissi et al. 2007; Amiza et al. 2012). Further, proteins, bioactive peptides, and amino acids from fish and fish wastes can be utilized as stabilizing and thickness agents, protein replacements, and gelling agents (Freitas et al. 2012).

The supplementation of fish protein hydrolysates in the human diet has positive impacts on the IgA concentration level in human serum as well s saliva. Boutin et al. (2012) reported that the supplementation of fish protein hydrolysates for 4 weeks may influence the serum IgA concentration in men aged between 18 and 60 years, non-smokers, and with a body mass index (BMI) between 20 and 30 kg/m^2. Moreover, fish protein hydrolysates may have a role as a cardioprotective nutrient. For example, Wergedahl et al. (2004) observed that fish protein hydrolysate has the ability to reduce the plasma total cholesterol and acyl-CoA:cholesterol

acyltransferase activity in the liver of Zucker rats while increasing the proportion of high-density lipoprotein. Fish protein hydrolysate has shown an increasing effect on the red blood cells count, white blood cells count, and hemoglobin content of anemic mice in a feeding trial, indicating the antianemic biological activities of fish protein hydrolysate (Dong et al. 2005). The same authors further indicate that the antianemic activity of fish protein hydrolysates is due to the amino acid composition, including essential amino acids and the biological active peptides present. Fish protein hydrolysate made from salmon fish protein has demonstrated safe use for malnourished children as an alternative nutritional source without altering their immune status (Nesse et al. 2011).

As fish protein concentrate is a low-cost animal protein of high quality, it can be used as a supplementary protein to increase the nutritive value of nutritionally low-valued foods (Córdova Murueta et al. 2007). Fish is a good source of lysine; hence, good quality fish protein concentrate can be used as a supplement for rice and other cereal-based products, which are low in lysine (Shamay et al. 2005). In addition, fish protein concentrate can also be effectively used for the preparation of protein-rich diets. Hussain et al. (2007) prepared infant foods for humans using fish protein concentrate (up to 10 %) with better net protein utilization (NPU), biological value, protein efficiency ratio (PER), and true digestibility (TD) than the weaning food prepared with no fish protein concentrate.

Different types of biscuits have developed by incorporating fish protein concentrate made from a freshwater whole fish (*Tilapia nilotica*) (El-Bedawey et al. 1986) and their by-products (Ibrahim 2009). The results of these studies illustrate the possibility of incorporating fish protein concentrate of marine origin into food products such as biscuits. It has been shown that *Tilapia nilotica* fish protein concentrate can be incorporated up to a level of 6 % in biscuits (El-Bedawey et al. 1986). Furthermore, *Tilapia nilotica* by-products have been incorporated up to 5 % in biscuits without having any adverse effects on their appearance or other sensory attributes (Ibrahim 2009).

In the Republic of Sierra Leone, Bonga fish meal has been used as a fish protein concentrate in bread baking to address the problem of inadequate intake of proteins. The product with 10 % added fish protein concentrate has been accepted by a sensory panel for its appearance and aroma (Olapade and Karim 2011). The findings showed the possibility of incorporating fish protein concentrate for cereal-based products and its potential for serving as a low cost, good quality protein source to overcome protein malnutrition in less developed countries.

4.6 Functional Foods and Nutraceuticals from Seafood By-products

Potential food applications of functional ingredients from fish and aquatic invertebrates have recently been reviewed by Freitas et al. (2012). Some functional applications and nutraceutical values of seafood by-products have also been briefly discussed in Sects. 4.1, 4.3, 4.4, 4.6, and 4.9.

4.7 Use of Antifreeze Compounds in the Food Industry

Antifreeze proteins have found a vast array of applications in frozen food technology and low-fat content food manufacturing (Ferraro et al. 2010). The unique functional properties of antifreeze proteins include lowering the freezing point without affecting the non-colligative properties, inhibition of recrystallization at very low concentrations (<0.1 μg/mL), and very high non-colligative activity compared to colligatively active substances. These properties of antifreeze proteins have received the attention of food scientists to be incorporated into various processed food products (Feeney and Yeh 1998). However, antifreeze proteins from natural sources may have limited applications in the food industry due to the high cost, because synthesized antifreeze protein analogues and mass-produced transgenic antifreeze proteins can be a cheaper source.

Two applications of antifreeze proteins in the food industry arise as being particularly important in terms of frozen foods: ice cream manufacturing and frozen meat technology. Antifreeze proteins may improve the quality of frozen foods, hence allowing for the maintenance of their natural texture, reduction of cellular damage, and loss of nutrients, all of which contribute to preserve their nutritional value (Li and Sun 2002; Ferraro et al. 2010). Antifreeze proteins have a great potential to avoid or minimize certain defects (such as dripping and loss of nutrients during thawing due to the recrystallization of intracellularly produced large ice crystals) in the frozen meat products due to the inhibition of recrystallization of water. There are some evidences on the formation of smaller ice crystals when antifreeze proteins derived from Antarctic cod and winter flounder were used as soaking agents (at 0.1 mg/mL up to 1 mg/mL) for bovine and ovine meat frozen at −20 °C (Payne et al. 1994; Ferraro et al. 2010). Preslaughter administration of antifreeze glycoproteins (from Antarctic cod) to lambs reduced the damages that occurred in meat during frozen storage (Payne and Young 1995).

Antifreeze proteins have been used in ice cream manufacturing to reduce the cost of production by some ice cream manufacturers (Feeney and Yeh 1998; Ferraro, et al. 2010), due to its ability in preventing ice crystals formation. Natural antifreeze proteins purified from coldwater ocean pout have been used as a preservative in ice cream (Goodsell 2009). Low fat, low caloric ice cream produced by incorporating a genetically modified ice-structuring protein compound isolated from antifreeze proteins of ocean pout (*Macrozoarces americanus*) is available in the USA, Australia, and New Zealand. These types of ice cream products have high consumer demand not only due to their low fat and low caloric value, but also because of their pleasing texture (Sigman-Grant et al. 2003; Ferraro et al. 2010).

Fish and aquatic invertebrate-derived antifreeze proteins also possess great potential in surimi production as a preservative of the gel-forming ability of fish muscle, which is one of the most important quality determinants of surimi products. Furthermore, the introduction of antifreeze proteins with its ability to inhibit recrystallization may be a better and more cost-effective solution for freezing-induced defects in the cryopreservation of fruits and vegetables (Rasika et al. 2013).

4.8 Applications of Fish Mince in the Food Industry

Fish muscle protein has a unique ability to set when macerated with salt and incubated below 40 °C. This phenomenon is known as "setting" or "suwari" in the Japanese language and has been used for the manufacturing of various ready-to-eat products utilizing fish mince (Binsi and Shamasundar 2012). Minced fish has been used in a number of products as it has several beneficial functional properties, such as succulence, juiciness, cohesion–adhesion, desired texture, emulsifying and fat-absorbing capacity, absence of bitterness, toughness, granularity, chewiness, heat-setting capacity, gel-forming ability, and resilience (Sen 2005). Minced muscle blocks, surimi, breaded patties, cutlet, creamy fish bites, fish balls, sausages, fish fingers, fish wafers, fish soup powder, fish noodles, fish crackers, protein concentrate tablets, dehydrated salt mince, etc. have been developed with the above-mentioned functional properties of fish mince (Kamal 1994; Sen 2005).

Sen (2005) reported that commercially available processed fish products could also be developed by using minced fish such as canned specialty products and various preparations such as crisps, seafood chowders, gefilte fish, and molded fish salads. In addition, Saritha and Patterson (2012) developed two innovative ready-to-fry crackers using powdered *Penaeus japonicus* and rice which demonstrated an extended shelf life (180 days) and improved nutritional value due to the presence of long-chain omega-3 fatty acids. Neiva et al. (2011) reported that minced fish of different low-value species can be used in the production of two types of fish crackers, namely the traditional keropok cracker and a low-fat fish cracker. The findings of Filho et al. (2010) showed that minced fish obtained from Nile tilapia waste could be incorporated up to 60 % (w/w) for the manufacturing of fish-based sausage with high digestibility and acceptable sensory properties. Recently, a fried cutlet was developed by Reddy et al. (2012) using minced meat prepared from reef cod with acceptable sensory and microbial qualities. These findings showed the potential of utilizing fish mince for the preparation of battered and breaded products.

4.9 Applications of Surimi in the Food Industry

Interest on surimi and surimi-based products has risen rapidly throughout the food industry, especially because surimi-based products are becoming popular as crab analogues. A cold extrusion and molding process can be used to produce imitation seafood products such as shrimp analogues and crab leg analogues (Sen 2005). Alaska pollock surimi has been used in Japan for the commercialized production of an extruded crab analog with a desirable texture by mixing egg white and 1 % starch (Cheftel et al. 1992).

Venugopal and Shahidi (1995) reported that surimi has ideal functionality for sausage manufacturing. In line with their suggestion, Murphy et al. (2004) showed that levels of 25 % surimi in combination with 6.3 % fat and 28.5 % water or 22 % fat and 12.6 % water may be used in the manufacture of pork sausages without

adversely affecting its flavor, color, and overall acceptability. Furthermore, Ramírez et al. (1999) also suggested that surimi can be used as a functional ingredient in sausage manufacturing due to its ability for stabilizing emulsions. Moreover, meat-flavored surimi-based sausage (containing no meat) has been produced by First Alaska Surimi, Inc., USA, which has been named “Seattle sausage”. The Seattle sausage has the appearance and taste of a meat frankfurter. The same company has also manufactured a surimi-based lobster analog called “lobster roll”, which is similar to chunk-style imitation crab but made with lobster flavoring and packed in a sausage casing (Alaska Fisheries Development Foundation Report 1990). In addition, a recent study has also shown that adding 1.0 % of fish surimi had no adverse effect on the sensory properties of Gouda cheese (Kim et al. 2012).

Apart from the applications of surimi in its original form, dried surimi incorporated into noodles has been produced in Malaysia. Wet surimi is oven dried at 60 °C to reduce the moisture content to less than 10 % prior to incorporation into noodles. Researchers have found that 5 % of dried surimi is the most suitable level of surimi to be incorporated into noodles (Chin et al. 2012). Surimi in dried form is useful as a raw material for the preparation of seafood products, as it has its own advantages in industrial applications, such as easy handling, low distribution cost, physical convenience for addition to dry mixtures, etc. On the other hand, it is rich with several functional properties, such as gelation, water-holding capacity, emulsifying ability, and foaming properties. Furthermore, surimi powder prepared from threadfin bream has been reported to have higher functional properties, such as solubility, gelation, water-holding capacity, color characteristics, emulsification, and foaming properties compared to lizardfish and purple-spotted bigeye-based surimi, in which the food applications require further studies. However, with all these functional properties, powdered surimi is readily applicable for gel-based products and fish snacks like food items (Huda et al. 2001; Santana et al. 2012).

5 Conclusions

Seafood by-products are bycatch or discarded body parts of commercial fish such as skin, gills, heads, fins, shells, blood, and viscera generated during seafood processing. Although these by-products are composed of the same constituents as edible seafood, undesirable organoleptic properties and certain hazardous compounds associated with these by-products may limit their applications in the food industry. However, various techniques can be applied to extract the useful compounds from seafood by-products and to eliminate the hazardous compounds. These extracted compounds, such as enzymes, fish oil, chitosan, collagen/gelatin, colorants, and various other functional compounds, can be applied in food processing as ingredients or be fortified with food products to enhance the nutritional value. Potential health claims of these compounds together with less expensive extraction methods of some compounds further promote their excessive use in the food industry. Furthermore, the current trend of using natural compounds in food manufacturing

has a positive influence towards the use of these compounds in the food industry. However, unpleasant fishy odors and flavors, certain complex and expensive extraction procedures associated with some compounds, and undesirable changes in the physicochemical properties and oxidative non-stability during the processing and storage of food products may limit the successful application of seafood by-products in the food industry. More research is needed in order to explore the ways and means to address these limitations of seafood by-products.

References

AbdElhady MM (2012) Preparation and characterization of chitosan/zinc oxide nanoparticles for imparting antimicrobial and UV protection to cotton fabric. Int J Carbohydr Chem (in press)

Abdou ES, Nagy KS, Elsabee MZ (2008) Extraction and characterization of chitin and chitosan from local sources. Bioresour Technol 99(5):1359–1367

AbuGhazaleh AA, Holmes LD (2007) Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J Dairy Sci 90(6):2897–2904

Ackman RG (2005) Chapter 11: fish oils. In: Shahidi F (ed) Bailey's industrial oil and fat products. Wiley, New York, pp 279–317

Aewsiri T, Benjakul S, Visessanguan W, Tanaka M (2008) Chemical compositions and functional properties of gelatin from pre-cooked tuna fin. Int J Food Sci Technol 43(4):685–693

Aewsiri T, Benjakul S, Visessanguan W (2009) Functional properties of gelatin from cuttlefish (*Sepia pharaonis*) skin as affected by bleaching using hydrogen peroxide. Food Chem 115(1): 243–249

Agarwal M, Parameswari RP, Vasanthi HR, Das DK (2012) Dynamic action of carotenoids in cardioprotection and maintenance of cardiac health. Molecules 17:4755–4769

Agulló E, Rodríguez MS, Ramos V, Albertengo L (2003) Present and future role of chitin and chitosan in food. Macromol Biosci 3:521–530

Aidos I, Padt A, Boom RM, Luten JB (2003) Quality of crude fish oil extracted from herring byproducts of varying states of freshness. J Food Sci 68(2):458–465

Alaska Fisheries Development Foundation Report (1990) Seattle sausage: development and market evaluation of a surimi product, final report. First Alaska Surimi, Inc., Seattle, Washington, DC

Alemán A, Giménez B, Montero P, Gómez-Guillén MC (2011) Antioxidant activity of several marine skin gelatins. LWT-Food Sci Technol 44(2):407–413

Al-Hassan AA, Norziah MH (2012) Starch–gelatin edible films: water vapor permeability and mechanical properties as affected by plasticizers. Food Hydrocolloid 26(1):108–117

Al-Hetar MY, Zainal Abidin MA, Sariah M, Wong MY (2010) Antifungal activity of chitosan against *Fusarium oxysporum* f. sp. *cubense*. J Appl Polym Sci 120:2434–2439

Alishahi A, Aïder M (2012) Applications of chitosan in the seafood industry and aquaculture: a review. Food Bioprocess Technol 5:817–830

Allam MH, Mustafa MM, Dessouki TM, Atia AM, Hussein SA (1997) Food utilizations of gelatin extracted from Bolti fish wastes. Ann Agric Sci Moshtohor 35(3):1439–1453

Amiza MA, Kong YL, Faazaz AL (2012) Effects of degree of hydrolysis on physicochemical properties of Cobia (*Rachycentron canadum*) frame hydrolysate. Int Food Res J 19(1): 199–206

An H, Visessanguan W (2000) Recovery of enzymes from seafood processing wastes. In: Haard NF, Simpson BK (eds) Seafood enzymes: utilization and influence on postharvest seafood quality. CRC Press, Boca Raton, pp 641–664

Aoki H, Ahsan MN, Matsuo K, Hagiwara T, Watabe S (2004) Partial purification of proteases that are generated by processing of the Northern shrimp *Pandalus borealis* and which can tenderize beef. Int J Food Sci Technol 39(5):471–480

Arason S, Karlsdottir M, Valsdottir T, Slizyte R, Rustad T, Falch E, Eysturskard J, Jakobsen G (2009) Maximum resource utilisation—value added fish by-products. Nordic Innovation Centre, Stensberggata, Oslo

Armenta-López R, Guerrero IL, Huerta S (2002) Astaxanthin extraction from shrimp waste by lactic fermentation and enzymatic hydrolysis of the carotenoprotein complex. J Food Sci 67(3):1002–1006

Armstrong GA (1997) Genetics of eubacterial carotenoid biosynthesis: a colorful tale. Ann Rev Microbiol 51(1):629–659

Ásgeirsson B, Bjarnason JB (1993) Properties of elastase from Atlantic cod a cold-adapted proteinase. Biochim Biophys Acta (BBA)-Protein Struct Mol Enzymol 1164(1):91–100

Babu CM, Chakrabarti R, Sambasivarao KRS (2008) Enzymatic isolation of carotenoid–protein complex from shrimp head waste and its use as a source of carotenoids. LWT-Food Sci Technol 41:227–235

Bae HJ, Darby DO, Kimmel RM, Park HJ, Whiteside WS (2009) Effects of transglutaminase-induced cross-linking on properties of fish gelatin–nanoclay composite film. Food Chem 114(1):180–189

Bailey AJ, Paul RG, Knott L (1998) Mechanisms of maturation and ageing of collagen. Mech Ageing Dev 106(1–2):1–56

Balti R, Barkia A, Bougatef A, Ktari N, Nasri MA (2009) A heat-stable trypsin from the hepatopancreas of the cuttlefish (*Sepia officinalis*): purification and characterisation. Food Chem 113(1):146–154

Balti R, Jridi M, Sila A, Souissi N, Nedjar-Arroume N, Guillochon D, Nasri M (2011) Extraction and functional properties of gelatin from the skin of cuttlefish (*Sepia officinalis*) using smooth hound crude acid protease-aided process. Food Hydrocolloid 25(5):943–950

Bama P, Vijayalakshimi M, Jayasimman R, Kalaichelvan PT, Deccaraman M, Sankaranarayanan S (2010) Extraction of collagen from cat fish (*Tachysurus maculatus*) by pepsin digestion and preparation and characterization of collagen chitosan sheet. Int J Pharm Pharm Sci 2(4):133–137

Benhabiles MS, Abdi N, Drouiche N, Lounici H, Pauss A, Goosen MFA, Mameri N (2012a) Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. Mat Sci Eng C 32:922–928

Benhabiles MS, Salah R, Lounici H, Drouiche N, Goosen MFA, Mameri N (2012b) Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. Food Hydrocolloid 29:48–56

Bentis CA, Zotos A, Petridis D (2005) Production of fish-protein products (surimi) from small pelagic fish (*Sardinops pilchardusts*), underutilized by the industry. J Food Eng 68:303–308

Bermúdez-Aguirre D, Barbosa-Cánovas GV (2011) Quality of selected cheeses fortified with vegetable and animal sources of omega-3. LWT-Food Sci Technol 44(7):1577–1584

Bermúdez-Aguirre D, Barbosa-Cánovas GV (2012) Fortification of queso fresco, cheddar and mozzarella cheese using selected sources of omega-3 and some nonthermal approaches. Food Chem 133(3):787–797

Bhat R, Karim AA (2009) Ultraviolet irradiation improves gel strength of fish gelatin. Food Chem 113(4):1160–1164

Bimbo AP (2009) Alaska seafood by-products: potential products, markets and competing products. Alaska Fisheries Development Foundation, Anchorage, Alaska

Bimbo AP (2011) The production and processing of marine oils. Updated 19th May 2011. Available online at: http://lipidlibrary.aocs.org/processing/marine/index.htm. Accessed 22 Sept 2012

Binsi PK, Shamasundar BA (2012) Purification and characterisation of transglutaminase from four fish species: effect of added transglutaminase on the viscoelastic behaviour of fish mince. Food Chem 132:1922–1929

Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK (2009) Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel-forming ability of fish mince. Food Hydrocolloid 23(1):132–145

Bjerkeng BT, Storebakken T, Liaaen JS (1990) Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. Aquaculture 91(1–2):153–162

Bobe G, Zimmerman S, Hammond EG, Freeman AE, Porter PA, Luhman CM, Beitz DC (2007) Butter composition and texture from cows with different milk fatty acid compositions fed fish oil or roasted soybeans. J Dairy Sci 90(6):2596–2603

Boran G, Lawless HT, Regenstein JM (2010) Effects of extraction conditions on the sensory and instrumental characteristics of fish gelatin gels. J Food Sci 75:S469–S476

Borderías AJ, Sánchez-Alonso I, Pérez-Mateos M (2005) New applications of fibres in foods: addition to fishery products. Trends Food Sci Technol 16(10):458–465

Bougatef A, Souissi N, Fakhfakh N, Ellouz-Triki Y, Nasri M (2007) Purification and characterization of trypsin from the viscera of sardine (*Sardina pilchardus*). Food Chem 102(1):343–350

Bougatef A, Nedjar-Arroume N, Manni L, Ravallec R, Barkia A, Guillochon D, Nasri M (2010) Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinella aurita*) by-products proteins. Food Chem 118(3):559–565

Bougatef A, Balti R, Sila A, Nasri R, Graiaa G, Nasri M (2012) Recovery and physicochemical properties of smooth hound (*Mustelus mustelus*) skin gelatin. LWT-Food Sci Technol 48(2):248–254

Boutin Y, Paradis M-E, Couture P, Lamarche B (2012) Immunological effects of fish protein supplementation on healthy adults. J Nat Prod 5:37–44

Bovet P, Faeh D, Madeleine G, Viswanathan B, Paccaud F (2007) Decrease in blood triglycerides associated with the consumption of eggs of hens fed with food supplemented with fish oil. Nutr Metab Cardiovasc Dis 17(4):280–287

Bower CK, Avena-Bustillos RJ, Olsen CW, McHugh TH, Bechtel PJ (2006) Characterization of fish-skin gelatin gels and films containing the antimicrobial enzyme lysozyme. J Food Sci 71(5):M141–M145

Bryhni EA, Kjos NP, Ofstad R, Hunt M (2002) Polyunsaturated fat and fish oil in diets for growing-finishing pigs: effects on fatty acid composition and meat, fat, and sausage quality. Meat Sci 62(1):1–8

Bu DP, Wang JQ, Dhiman TR, Liu SJ (2007) Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. J Dairy Sci 90(2):998–1007

Cáceres E, García ML, Selgas MD (2008) Effect of pre-emulsified fish oil – as source of PUFA n-3 – on microstructure and sensory properties of mortadella, a Spanish bologna-type sausage. Meat Sci 80(2):183–193

Cachaldora P, García-Rebollar P, Alvarez C, De Blas JC, Méndez J (2008) Effect of type and level of basal fat and level of fish oil supplementation on yolk fat composition and n-3 fatty acids deposition efficiency in laying hens. Anim Feed Sci Technol 141(1–2):104–114

Cano-Lopez A, Simpson BK, Haard NF (1987) Extraction of carotenoprotein from shrimp process wastes with the aid of trypsin from Atlantic cod. J Food Sci 52:503–504

Cao R, Liu Q, Yin BZ, Wu B (2012) Chitosan extends the shelf-life of filleted tilapia (*Oreochromis niloticus*) during refrigerated storage. J Ocean Univ of China 11(3):408–412

Cardenas-Lopez JL, Haard NF (2005) Cysteine proteinase activity in jumbo squid (*Dosidicus gigas*) hepatopancreas extracts. J Food Biochem 29(2):171–186

Castro-Ceseña AB, Sánchez-Saavedra MP, Márquez-Rocha FJ (2012) Characterisation and partial purification of proteolytic enzymes from sardine by-products to obtain concentrated hydrolysates. Food Chem 135(2):583–589

Cháfer M, Sánchez-González L, Gonález-Martínez C, Chiralt A (2012) Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. J Food Sci 77(8):E182–E187

Chakrabarti R (2002) Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. Food Biotechnol 16(1):81–90

Chalamaiah M, Kumar BD, Hemalatha R, Jyothirmayi T (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chem 135:3020–3038

Chantachum S, Benjakul S, Sriwirat N (2000) Separation and quality of fish oil from precooked and non-precooked tuna heads. Food Chem 69(3):289–294

Chaplin M, Bucke C (1990) Enzyme technology. Cambridge University Press, Cambridge, UK

Cheftel JC, Kitagawa M, Queguiner C (1992) New protein texturization processes by extrusion cooking at high moisture levels. Food Rev Int 8(2):235–275

Chen Y-C, Jaczynski J (2007a) Protein recovery from rainbow trout (*Oncorhynchus mykiss*) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. J Agric Food Chem 55:9079–9088

Chen Y-C, Jaczynski J (2007b) Gelation of protein recovered from whole Antarctic krill (*Euphausia superba*) by isoelectric solubilization/precipitation as affected by functional additives. J Agric Food Chem 55:1814–1822

Chen HC, Zall RR (1986) Evaluation of thiol activated proteases from clam viscera as a rennet substitute for cheese-making. J Food Sci 51(3):815–820

Chen H-H, Chiu E-M, Huang J-R (1997) Color and gel-forming properties of horse mackerel (*Trachurus japonicus*) as related to washing conditions. J Food Sci 62(5):985–991

Chen A-S, Taguchi T, Sakai K, Kikuchi K, Wang M-W, Miwa I (2003) Antioxidant activities of chitobiose and chitotriose. Biol Pharm Bull 9:1326–1330

Chen L-C, Chiang W-D, Chen W-C, Chen H-H, Huang Y-W, Chen W-J, Lin S-B (2012) Influence of alanine uptake on *Staphylococcus aureus* surface charge and its susceptibility to two cationic antibacterial agents, nisin and low molecular weight chitosan. Food Chem 135:2397–2403

Cheng LH, Lim BL, Chow KH, Chong SM, Chang YC (2008) Using fish gelatin and pectin to make a low-fat spread. Food Hydrocolloid 22(8):1637–1640

Cheow CS, Norizah MS, Kyaw ZY, Howell NK (2007) Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). Food Chem 101(1):386–391

Chin CK, Huda N, Yang TA (2012) Incorporation of surimi powder in wet yellow noodles and its effects on the physicochemical and sensory properties. Int Food Res J 19(2):701–707

Chiou B-S, Avena-Bustillos RJ, Shey J, Yee E, Bechtel PJ, Imam SH, Glenn GM, Orts WJ (2006) Rheological and mechanical properties of cross-linked fish gelatins. Polymer 47(18): 6379–6386

Cho YI, No HK, Meyers SP (1998) Physicochemical characteristics and functional properties of various commercial chitin and chitosan products. J Agric Food Chem 46:3839–3843

Cho Y-S, Kim S-K, Je J-Y (2011) Chitosan gallate as potential antioxidant biomaterial. Bioorg Med Chem Lett 21:3070–3073

Choi S-S, Regenstein JM (2000) Physicochemical and sensory characteristics of fish gelatin. J Food Sci 65(2):194–199

Chung Y-C, Su Y-P, Chen C-C, Jia G, Wang H-L, Wu JCG, Lin J-G (2004) Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacol Sin 25:932–936

Cianci M, Rizkallah PJ, Olczak A, Raftery J, Chayen NE, Zagalsky PF, Helliwell JR (2002) The molecular basis of the coloration mechanism in lobster shell: beta-crustacyanin at 3.2-A resolution. Proc Natl Acad Sci USA 99(15):9795–9800

Codex Alimentarius (2009) Code of practice for fish and fishery products, 1st edn. Food & Agriculture Organization of the United Nations and World Health Organization, Rome

Córdova Murueta JH, Toro MAN, Carreño FG (2007) Concentrates of fish protein from bycatch species produced by various drying processes. Food Chem 100(2):705–711

Cota-Arriola O, Cortez-Rocha MO, Rosas-Burgos EC, Burgos-Hernández A, López-Franco YL, Plascencia-Jatomea M (2011) Antifungal effect of chitosan on the growth of *Aspergillus parasiticus* and production of aflatoxin B1. Polym Int 60:937–944

Cudennec B, Ravallec-Plé R, Courois E, Fouchereau-Peron M (2008) Peptides from fish and crustacean by-products hydrolysates stimulate cholecystokinin release in STC-1 cells. Food Chem 111:970–975

Cuero RG, Duffus E, Osuji G, Petit R (1991) Aflatoxin control in preharvest maize: effects of chitosan and two microbial agents. J Agric Sci 117:165–169

D'Ambrosio A, Rossano R, Ungaro N, Riccio P (2003) Proteolytic and milk clotting activities in extracts obtained from the crustaceans *Munida*. J Mol Catal B Enzym 22(3–4):145–150

Danielewicz MA, Anderson LA, Franz AK (2011) Triacylglycerol profiling of marine microalgae by mass spectrometry. J Lipid Res 52(11):2101–2108

Dauksas E, Slizyte R, Rustad T, Storro I (2004) Bitterness in fish protein hydrolysates and methods for removal. J Aquat Food Prod Technol 13(2):101–114

Daukšas E, Falch E, Šližytė R, Rustad T (2005) Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochem 40(8):2659–2670

Decker EA, Warner K, Richards MP, Shahidi F (2005) Measuring antioxidant effectiveness in food. J Agric Food Chem 53(10):4303–4310

Di Bernardini R, Harnedy P, Bolton D, Kerry J, O'Neill E, Mullen AM, Hayes M (2011) Antioxidant and antimicrobial peptidic hydrolysates from muscle protein sources and by-products. Food Chem 124(4):1296–1307

Dissaraphong S, Benjakul S, Visessanguan W, Kishimura H (2006) The influence of storage conditions of tuna viscera before fermentation on the chemical, physical and microbiological changes in fish sauce during fermentation. Bioresour Technol 97:2032–2040

Dong Y-L, Sheng G-Y, Fu J-M, Wen K-W (2005) Chemical characterization and anti-anaemia activity of fish protein hydrolysate from *Saurida elongata*. J Sci Food Agric 85:2033–2039

Dubrow DJ, Stillings BR (1971) Chemical and nutritional characteristics of fish protein concentrate processed from heated whole red hake, *Urophycis chuss*. Fish Bull 69(1):141–144

Dumay J, Barthomeuf C, Bergé JP (2004) How enzymes may be helpful for upgrading fish by-products: enhancement of fat extraction. J Aqua Food Prod Technol 13(2):69–84

El Hadj Ali N, Hmidet N, Bougatef A, Nasri R, Nasri M (2009) A laundry detergent-stable alkaline trypsin from striped seabream (*Lithognathus mormyrus*) viscera: purification and characterization. J Agric Food Chem 57(22):10943–10950

El-Bedawey AEI-F, Zein GN, El-Sherbiney AM, Dawoud FMA (1986) Effect of supplementation with fish protein concentrate on protein and amino acid content of salt biscuit. Mol Nutr Food Res 30(1):19–24

Espósito TS, Amaral IPG, Buarque DS, Oliveira GB, Carvalho LB, Bezerra RS (2009) Fish processing waste as a source of alkaline proteases for laundry detergent. Food Chem 112(1): 125–130

Estrada JD, Boeneke C, Bechtel P, Sathivel S (2011) Developing a strawberry yogurt fortified with marine fish oil. J Dairy Sci 94(12):5760–5769

Evans RP, Fletcher GL (2004) Isolation and purification of antifreeze proteins from skin tissues of snailfish, cunner and sea raven. Biochim Biophys Acta (BBA)-Protein Proteom 1700(2): 209–217

Falch E, Rustad T, Aursand M (2006) By-products from gadiform species as raw material for production of marine lipids as ingredients in food or feed. Process Biochem 41(3):666–674

FAO (1986) The production of fish meal and oil. FAO fisheries technical paper 142. Fishery Industries Division, Food and Agriculture Organization of the United Nations, Rome

FAO (2012) The state of world fisheries and aquaculture. Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome

Feeney RE, Yeh Y (1998) Antifreeze proteins: current status and possible food uses. Trends Food Sci Technol 9:102–106

Félix-Valenzuela L, Higuera-Ciapara I, Goycoolea-Valencia F, Argüelles-Monal W (2001) Supercritical CO_2/ethanol extraction of astaxanthin from blue crab (*Callinectes sapidus*) shell waste. J Food Process Eng 24:101–112

Ferraro V, Cruz IB, Jorge RF, Malcata FX, Pintado ME, Castro PML (2010) Valorisation of natural extracts from marine source focused on marine by-products: a review. Food Res Int 43(9):2221–2233

Filho PRCO, Netto FM, Ramos KK, Trindade MA, Viegas EMM (2010) Elaboration of sausage using minced fish of Nile tilapia filleting waste. Braz Arch Biol Technol 53(6):1383–1391
Fletcher GL, Hew CL, Davies PL (2001) Antifreeze proteins of teleost fishes. Annu Rev Physiol 63:359–390
Fraeye I, Bruneel C, Lemahieu C, Buyse J, Muylaert K, Foubert I (2012) Dietary enrichment of eggs with omega-3 fatty acids: a review. Food Res Int 48(2):961–969
Françoise L (2010) Occurrence and role of lactic acid bacteria in seafood products. Food Microbiol 27(6):698–709
Fras-Zemljič L, Kokol V, Čakara D (2011) Antimicrobial and antioxidant properties of chitosan-based viscose fibres enzymatically functionalized with flavonoids. Text Res J 81(15):1532–1540
Freitas AC, Rodrigues D, Rocha-Santos TAP, Gomes AMP, Duarte AC (2012) Marine biotechnology advances towards applications in new functional foods. Biotechnol Adv 30(6):1506–1515
Fu X-Y, Xue C-H, Miao B-C, Li Z-J, Gao X, Yang W-G (2002) Characterization of proteases from the digestive tract of sea cucumber (*Stichopus japonicus*): high alkaline protease activity. Aquaculture 246(1–4):321–329
Fu X et al. (2009) Kinetics of lipid oxidation and off-odor formation in silver carp mince: the effect of lipoxygenase and hemoglobin. Food Res Int 42(1):85–90
Galazka VB, Dickinson E, Ledward DA (1999) Emulsifying behaviour of 11S globulin *Vicia faba* in mixtures with sulphated polysaccharides: comparison of thermal and high-pressure treatments. Food Hydrocolloid 13(5):425–435
García-Rebollar P, Cachaldora P, Alvarez C, De Blas C, Méndez J (2008) Effect of the combined supplementation of diets with increasing levels of fish and linseed oils on yolk fat composition and sensorial quality of eggs in laying hens. Anim Feed Sci Technol 140(3–4):337–348
Gehring CK, Gigliotti JC, Moritz JS, Tou JC, Jaczynski J (2011) Functional and nutritional characteristics of proteins and lipids recovered by isoelectric processing of fish by-products and low-value fish: a review. Food Chem 124(2):422–431
Gerster H (1993) Anticarcinogenic effect of common carotenoids. Int J Vitam Nutr Res 63(2): 93–121
Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R (2007) Applications of spray-drying in microencapsulation of food ingredients: an overview. Food Res Int 40(9):1107–1121
Gildberg A (2001) Utilisation of male Arctic capelin and Atlantic cod intestines for fish sauce production–evaluation of fermentation conditions. Bioresour Technol 76:119–123
Gildberg A (2004) Enzymes and bioactive peptides from fish waste related to fish silage, fish feed and fish sauce production. J Aquat Food Prod Technol 13(2):3–11
Gildberg A, Xian-Quan S (1994) Recovery of tryptic enzymes from fish sauce. Process Biochem 29(2):151–155
Gildberg A, Arnesen JA, Carlehög M (2002) Utilisation of cod backbone by biochemical fractionation. Process Biochem 38(4):475–480
Gómez-Estaca J, López de Lacey A, Gómez-Guillén MC, López-Caballero ME, Montero P (2009) Antimicrobial activity of composite edible films based on fish gelatin and chitosan incorporated with clove essential oil. J Aquat Food Prod Technol 18(1–2):46–52
Gómez-Guillén MC, Turnay J, Fernández-Díaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloid 16(1):25–34
Gómez-Guillén MC, Ihl M, Bifani V, Silva A, Montero P (2007) Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae* Turcz). Food Hydrocolloid 21(7):1133–1143
Gómez-Guillén MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez B, Montero P (2009) Fish gelatin: a renewable material for developing active biodegradable films. Trends Food Sci Technol 20(1):3–16
Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocolloid 25(8):1813–1827

Goodsell DS (2009) Molecule of the month: antifreeze proteins. Worldwide Protein Data Bank, New Jersey, USA

Grimble GK (2000) Mechanisms of peptide and amino acid transport and their regulation. In: Furst P, Young V (eds) Proteins, peptides and amino acids in enteral nutrition, vol 3. Karger and Nestec, Basel, pp 63–88

Guerard F (2007) Enzymatic methods for marine by-products recovery. In: Shahidi F (ed) Maximizing the value of marine by-products. Woodhead Press, Cambridge, UK, pp 107–143

Hadwiger LA, Loschke DC (1981) Molecular communication in host–parasite interactions: hexosamine polymers (chitosan) as regulator compounds in race-specific and other interactions. Phytopathology 71:756–762

Hall GM, Da Silva S (1992) Lactic acid fermentation of shrimp (*Penaeus monodon*) waste for chitin recovery. In: Brine CJ, Sandford PA, Zikakis JP (eds) Advances in chitin and chitosan. Elsevier, London, pp 633–638

Hallenstvedt E, Kjos NP, Rehnberg AC, Øverland M, Thomassen M (2010) Fish oil in feeds for entire male and female pigs: changes in muscle fatty acid composition and stability of sensory quality. Meat Sci 85(1):182–190

Halliwell B, Aeschbach R, Löliger J, Aruoma OI (1995) The characterization of antioxidants. Food Chem Toxicol 33:601–617

Hansen TK, Poulsen LK, Stahl Skov P, Hefle SL, Hlywka JJ, Taylor SL, Bindslev-Jensen U, Bindslev-Jensen C (2004) A randomized, double-blinded, placebo-controlled oral challenge study to evaluate the allergenicity of commercial, food-grade fish gelatin. Food Chem Toxicol 42(12):2037–2044

Harding MM, Anderberg PI, Haymet ADJ (2003) 'Antifreeze' glycoproteins from polar fish. Eur J Biochem 270(7):1381–1392

Hatate H, Tanaka R, Suzuki N, Hama Y (2000) Comparison of protease activity in liver among several species of squid and cuttlefish. Fish Sci 66(1):182–183

Heo S-J, Park E-J, Lee K-W, Jeon Y-J (2005) Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 96(14):1613–1623

Heu MS, Kim JS, Shahidi F (2003) Components and nutritional quality of shrimp processing by-products. Food Chem 82(2):235–242

Higuchi T, Shirai N, Suzuki H, Kawashima M, Tamura Y (2008) Effects of yogurt supplemented with fish oil on plasma lipid and glucose concentrations, and liver lipid contents in mice. Int J Vitam Nutr Res 78(3):129–138

Hoque MS, Benjakul S, Prodpran T (2010) Effect of heat treatment of film-forming solution on the properties of film from cuttlefish (*Sepia pharaonis*) skin gelatin. J Food Eng 96(1):66–73

Huang R, Rajapakse N, Kim S-K (2006) Structural factors affecting radical scavenging activity of chitooligosaccharides (COS) and its derivatives. Carbohydr Polym 63:122–129

Huda N, Abdullah A, Babji AS (2001) Functional properties of surimi powder from three Malaysian marine fish. Int J Food Sci Technol 36:401–406

Hughes BH, Muzzy HM, Laliberte LC, Grenier HS, Perkins LB, Skonberg DI (2012a) Oxidative stability and consumer acceptance of fish oil fortified nutrition bars. J Food Sci 77(9):S329–S334

Hughes BH, Perkins LB, Calder BL, Skonberg DI (2012b) Fish oil fortification of soft goat cheese. J Food Sci 77(2):S128–S133

Hultin HO (2002) Recent advances in surimi technology. In: Fingerman M, Nagabhushanam R (eds) Recent advances in marine biotechnology. Science Publisher, New Hampshire, pp 241–251

Huss HH (1988) Fresh fish–quality and quality changes. A training manual prepared for the FAO/DANIDA training programme on fish technology and quality control. Food and Agriculture Organization of the United Nations, Danish International Development Agency, Rome

Hussain I, Akhtar N, Hussain S (2007) Evaluation of weaning food "khitchri" incorporated with different levels of fish protein concentrate. J Anim Plant Sci 17(1–2):33–35

Iafelice G, Caboni MF, Cubadda R, Di Criscio T, Trivisonno MC, Marconi E (2008) Development of functional spaghetti enriched with long chain omega-3 fatty acids. Cereal Chem J 85(2): 146–151

Ibrahim SM (2009) Evaluation of production and quality of salt-biscuits supplemented with fish protein concentrate. World J Dairy Food Sci 4(1):28–31

Imm JY, Lee CM (1999) Production of seafood flavor from red hake (*Urophycis chuss*) by enzymatic hydrolysis. J Agric Food Chem 47:2360–2366

Jaczynski J (2008) Protein and lipid recovery from food processing by-products using isoelectric solubilization/precipitation. In: Papadopoulos KN (ed) Food chemistry research developments. Nova Science Publishers, Hauppauge, pp 167–198

Jafarpour A, Gorczyca EM (2008) Alternative techniques for producing a quality surimi and kamaboko from common carp (*Cyprinus carpio*). J Food Sci 73(9):E415–E424

Je J-Y, Park P-J, Kim S-K (2005) Antioxidant activity of a peptide isolated from Alaska Pollack (*Theragra chalcogramma*) frame protein hydrolysate. Food Res Int 38(1):45–50

Jeon Y-J, Shahidi F, Kim S-K (2000) Preparation of chitin and chitosan oligomers and their applications in physiological functional foods. Food Rev Int 16(2):159–176

Johnson EL, Peniston QP (1982) Utilization of shellfish waste for chitin and chitosan production. In: Martin RE, Flick GJ, Hebard CE, Ward DR (eds) Chemistry and biochemistry of marine food products. AVI Publishing, Westport, pp 415–428

Jongjareonrak A, Benjakul S, Visessanguan W, Prodpran T, Tanaka M (2006) Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloid 20(4):492–501

Jørgensen SKK, Keskin S, Kitsios D, Commerou PW, Nilsson B, Vincents B (2008) Antifreeze proteins: the applications of antifreeze proteins in the food industries. Ph.D. thesis, Roskilde University, Denmark

Josquin NM, Linssen JPH, Houben JH (2012) Quality characteristics of Dutch-style fermented sausages manufactured with partial replacement of pork back-fat with pure, pre-emulsified or encapsulated fish oil. Meat Sci 90(1):81–86

Jun S-Y, Park P-J, Jung W-K, Kim S-K (2004) Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. Eur Food Res Technol 219(1):20–26

Jung W-K, Park P-J, Byun H-G, Moon S-H, Kim S-K (2005) Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. Food Chem 91(2):333–340

Kadam SU, Prabhasankar P (2010) Marine foods as functional ingredients in bakery and pasta products. Food Res Int 43(8):1975–1980

Kamal MD (1994) Status and prospects of value addition of by-catch and their marketing. In: Proceedings of the workshop on substainable development of marine fisheries resources in Bangladesh. FAO Corporate Document Repository, Fisheries and Aquaculture Department, Food and Agriculture Organization, Rome

Kamil JYVA, Jeon Y-J, Shahidi F (2002) Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (*Clupea harengus*). Food Chem 79:69–77

Kang KA, Lee KH, Chae S, Koh YS, Yoo B-S, Kim JH, Ham YM, Baik JS, Lee NH, Hyun JW (2005) Triphlorethol-A from *Ecklonia cava* protects V79-4 lung fibroblast against hydrogen peroxide induced cell damage. Free Radic Res 39(8):883–892

Karim AA, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloid 23(3):563–576

Kechaou ES, Dumay J, Donnay-Moreno C, Jaouen P, Gouygou J-P, Bergé J-P, Amar RB (2009) Enzymatic hydrolysis of cuttlefish (*Sepia officinalis*) and sardine (*Sardina pilchardus*) viscera using commercial proteases: effects on lipid distribution and amino acid composition. J Biosci Bioeng 107(2):158–164

Kelly M, Fox PF, McSweeney PLH (1996) Influence of salting-moisture on proteolysis in Cheddar-type cheese. Milchwissenschaft 51:498–501

Khanafari A, Mari R, Sanatei SH (2008) Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. Iran J Environ Health Sci Eng 5(1):1–24

Kim S-K, Mendis E (2006) Bioactive compounds from marine processing byproducts–a review. Food Res Int 39(4):383–393

Kim S-K, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2(1):1–9

Kim S-K, Park P-J, Byun H-G, Je J-Y, Moon S-H, Kim S-H (2003) Recovery of fish bone from hoki (*Johnius belengeri*) frame using a proteolytic enzyme isolated from mackerel intestine. J Food Biochem 27(3):255–266

Kim J-S, Shahidi F, Heu M-S (2005) Tenderization of meat by salt-fermented sauce from shrimp processing by-products. Food Chem 93(2):243–249

Kim K-H, Choi H-Y, Chun S-S, Bae I-H (2012) Quality properties of Gouda cheese added with fish surimi. J Anim Sci Technol 54(1):23–28

Klomklao S, Benjakul S, Visessanguan W, Simpson BK, Kishimura H (2005) Partitioning and recovery of proteinase from tuna spleen by aqueous two-phase systems. Process Biochem 40(9):3061–3067

Klomklao S, Benjakul S, Visessanguan W, Kishimura H, Simpson BK (2006) Effects of the addition of spleen of skipjack tuna (*Katsuwonus pelamis*) on the liquefaction and characteristics of fish sauce made from sardine (*Sardinella gibbosa*). Food Chem 98:440–452

Kolanowski W, Jaworska D, Laufenberg G, Weißbrodt J (2007) Evaluation of sensory quality of instant foods fortified with omega-3 PUFA by addition of fish oil powder. Eur Food Res Technol 225(5–6):715–721

Kolanowski W, Weißbrodt J (2007) Sensory quality of dairy products fortified with fish oil. Int Dairy J 17(10):1248–1253

Kolanowski W, Swiderski F, Berger S (1999) Possibilities of fish oil application for food products enrichment with omega-3 PUFA. Int J Food Sci Nutr 50:39–49

Kolodziejska I, Pacana J, Sikorski ZE (1992) Effect of squid liver extract on proteins and on the texture of cooked squid mantle. J Food Biochem 16(3):141–150

Kołodziejska I, Kaczorowski K, Piotrowska B, Sadowska M (2004) Modification of the properties of gelatin from skins of Baltic cod (*Gadus morhua*) with transglutaminase. Food Chem 86(2):203–209

Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol 144:51–63

Kouba M, Mourot J (2011) A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie 93(1):13–17

Kouchi HH, Moosavi-Nasab M, Shabanpour B (2012a) Extraction of carotenoids from crustacean waste using organic solvents. The 1st International and The 4th National Congress on Recycling of Organic Waste in Agriculture, Isfahan, 26–27 Apr 2012

Kouchi HH, Shabanpour B, Moosavi-Nasa M (2012b) Extraction of carotenoids from crustacean waste with vegetable oils. The 1st International and The 4th National Congress on Recycling of Organic Waste in Agriculture, Isfahan, 26–27 Apr 2012

Krishna M, Nindo CI, Min SC (2012) Development of fish gelatin edible films using extrusion and compression molding. J Food Eng 108(2):337–344

Kristinsson HG, Rasco BA (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Crit Rev Food Sci Nutr 40(1):43–81

Ktari N, Khaled HB, Nasri R, Jellouli K, Ghorbel S, Nasri M (2012) Trypsin from zebra blenny (*Salaria basilisca*) viscera: purification, characterisation and potential application as a detergent additive. Food Chem 130(3):467–474

Küçükgülmez A, Gülnaz O, Celik M, Yanar Y, Kadak AE, Gerçek G (2012) Antimicrobial activity of the chitosan extracted from *Metapenaeus stebbingi* shell wastes. J Polym Environ 20: 431–437

Kuiper MJ, Lankin C, Gauthier SY, Walker VK, Davies PL (2003) Purification of antifreeze proteins by adsorption to ice. Biochem Biophys Res Commun 300(3):645–648

Lahmer RA, Williams AP, Townsend S, Baker S, Jones DL (2012) Antibacterial action of chitosan-arginine against *Escherichia coli* O157 in chicken juice. Food Control 26:206–211

Lepetit J (2008) Collagen contribution to meat toughness: theoretical aspects. Meat Sci 80(4): 960–967

Let MB, Jacobsen C, Sørensen A-D, Meyer AS (2007) Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: lipid oxidation. J Agric Food Chem 55(5): 1773–1780

Li B, Sun D-W (2002) Novel methods for rapid freezing and thawing of foods–a review. J Food Eng 54(3):175–182

Liaset B, Lied E, Espe M (2000) Enzymatic hydrolysis of by-products from the fish-filleting industry; chemical characterization and nutritional evaluation. J Sci Food Agric 80:581–589

Liceaga-Gesualdo AM, Li-Chan ECY (1999) Functional properties of fish protein hydrolysate from herring (*Clupea harengus*). J Food Sci 64(6):1000–1004

Limpisophon K, Tanaka M, Weng W, Abe S, Osako K (2009) Characterization of gelatin films prepared from under-utilized blue shark (*Prionace glauca*) skin. Food Hydrocolloid 23(7):1993–2000

Liu H, Du YM, Wang XH, Sun LP (2004) Chitosan kills bacteria through cell membrane damage. Int J Food Microbiol 95(2):147–155

Liu Z, Oliveira ACM, Su Y-C (2009) Purification and characterization of pepsin-solubilized collagen from skin and connective tissue of giant red sea cucumber (*Parastichopus californicus*). J Agric Food Chem 58(2):1270–1274

Lopetcharat K, Park JW (2002) Characteristics of fish sauce made from Pacific whiting and surimi by-products during fermentation stage. J Food Sci 67(2):511–516

López M, Arce L, Garrido J, Rıos A, Valcárcel M (2004) Selective extraction of astaxanthin from crustaceans by use of supercritical carbon dioxide. Talanta 64:726–731

Lovegrove JA, Brooks CN, Murphy MC, Gould BJ, Williams CM (1997) Use of manufactured foods enriched with fish oils as a means of increasing long-chain n-3 polyunsaturated fatty acid intake. Br J Nutr 78:223–236

Lu B-J, Zhou L-G, Cai Q-F, Hara K, Maeda A, Su W-J, Cao M-J (2008) Purification and characterisation of trypsins from the pyloric caeca of mandarin fish (*Siniperca chuatsi*). Food Chem 110(2):352–360

Luo YK, Shen HX, Pan DD (2006) Gel-forming ability of surimi from grass carp (*Ctenopharyngodon idellus*): influence of heat treatment and soy protein isolate. J Sci Food Agric 86:687–693

Mahawanich T, Lekhavichitr J, Duangmal K (2010) Gel properties of red tilapia surimi: effects of setting condition, fish freshness and frozen storage. Int J Food Sci Technol 45:1777–1786

Maoka T (2011) Review: carotenoids in marine animals. Mar Drugs 9:278–293

Martínez-Camacho AP, Cortez-Rocha MO, Ezquerra-Brauer JM, Graciano-Verdugo AZ, Matsuno T (2001) Aquatic animal carotenoids. Fish Sci 67(5):771–783

Martone CB, Borla OP, Sánchez JJ (2005) Fishery by-product as a nutrient source for bacteria and archaea growth media. Bioresour Technol 96(3):383–387

Matsumiya M, Miyauchi K, Mochizuki A (2003) Purification and some properties of a chitinase isozyme from the liver of Japanese common squid *Todarodes pacificus*. Fish Sci 69(2):427–429

Merheb-Dini C, Garcia GAC, Penna ALB, Gomes E, da Silva R (2012) Use of a new milk-clotting protease from *Thermomucor indicae-seudaticae* N31 as coagulant and changes during ripening of Prato cheese. Food Chem 130(4):859–865

Metcalf RG, James MJ, Mantzioris E, Cleland LG (2003) A practical approach to increasing intakes of n-3 polyunsaturated fatty acids: use of novel foods enriched with n-3 fats. Eur J Clin Nutr 57:1605–1612

Meyers SP (1986) Utilization of shrimp processing waste. Infofish Mark Digest 4:18–19

Mezzomo N, Maestri B, dos Santos RL, Maraschin M, Ferreira SRS (2011) Pink shrimp (*Penaus brasiliensis* and *Penaus paulensis*) residue: influence of extraction method on carotenoid concentration. Talanta 85:1383–1391

Mojarrad JS, Nemati M, Valizadeh H, Ansarin M, Bourbour S (2007) Preparation of glucosamine from exoskeleton of shrimp and predicting production yield by response surface methodology. J Agric Food Chem 55(6):2246–2250

Motamedzadegan A, Davarniam B, Asadi G, Abedian A, Ovissipour M (2010) Optimization of enzymatic hydrolysis of yellowfin tuna *Thunnus albacares* viscera using Neutrase. Int Aquat Res 2:173–181

Muguerza E, Ansorena D, Astiasarán I (2004) Functional dry fermented sausages manufactured with high levels ofn-3 fatty acids: nutritional benefits and evaluation of oxidation. J Sci Food Agric 84(9):1061–1068

Muraleedharan V, Gopakumar K (1998) Preparation and properties of functional protein concentrate from tuna (*Euthynnus affinis*). In: Fish utilization in Asia and the Pacific. Proceedings of the APFIC symposium, Beijing, 24–26 Sept 1998

Murphy SC, Gilroy D, Kerry JF, Buckley DJ, Kerry JP (2004) Evaluation of surimi, fat and water content in a low/no added pork sausage formulation using response surface methodology. Meat Sci 66:689–701

Muzaifa M, Safriani N, Zakaria F (2012) Production of protein hydrolysates from fish by-product prepared by enzymatic hydrolysis. Int J Bioflux Soc 5:36–39

Muzzarelli RAA (1977) Chitin. Pergamon Press, Oxford

Nalinanon S, Benjakul S, Visessanguan W, Kishimura H (2007) Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). Food Chem 104(2):593–601

Nalinanon S, Benjakul S, Visessanguan W, Kishimura H (2008a) Tuna pepsin: characteristics and its use for collagen extraction from the skin of threadfin bream (*Nemipterus* spp.). J Food Sci 73(5):C413–C419

Nalinanon S, Benjakul S, Visessanguan W, Kishimura H (2008b) Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. Food Hydrocolloid 22(4):615–622

Nasri R, Sila A, Ktari N, Lassoued I, Bougatef A, Karra-Chaâbouni M, Nasri M (2012) Calcium dependent, alkaline detergent-stable trypsin from the viscera of Goby (*Zosterisessor ophiocephalus*): purification and characterization. Proc Biochem 47(12):1957–1964

Neiva CRP, Machado TM, Tomita RY, Furlan EF, Neto MJL, Bastos DHM (2011) Fish crackers development from minced fish & starch: an innovative approach to a traditional product. Ciência e Tecnologia de Alimentos 31(4):973–979

Nesse KO, Nagalakshmi AP, Marimuthu P, Singh M (2011) Efficacy of a fish protein hydrolysate in malnourished children. Indian J Clin Biochem 26(4):360–365

Nègre-Sadargues G, Castillo R, Segonzac M (2000) Carotenoid pigments and trophic behaviour of deep-sea shrimps (Crustacea, decapoda, alvinocarididae) from a hydrothermal area of the mid-atlantic ridge. Comp Biochem Physiol Mol Integr Physiol 127(3):293–300

Ngo D-H, Wijesekara I, Vo T-S, Van Ta Q, Kim S-K (2011) Marine food-derived functional ingredients as potential antioxidants in the food industry: an overview. Food Res Int 44(2):523–529

Nilsang S, Lertsiri S, Suphantharika M, Assavanig A (2005) Optimization of enzymatic hydrolysis of fish soluble concentrate by commercial proteases. J Food Eng 70(4):571–578

No HK, Hur EY (1998) Control of foam formation by antifoam during demineralization of crustacean shell in preparation of chitin. J Agric Food Chem 46:3844–3846

No HK, Park NY, Lee SH, Meyers SP (2002) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol 74:65–72

Ofori RA (1999) Preparation of gelatin from fish skin by an enzyme aided process. M.Sc. thesis. Department of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, Montreal

Oh K-T, Kim Y-J, Nguyen VN, Jung W-J, Park R-D (2007) Demineralization of crab shell waste by *Pseudomonas aeruginosa* F722. Process Biochem 42:1069–1074

Oh KT, Kim YJ, Nguyen NV, Jung WJ, Park RD (2008) Effect of crab shell size on bio-demineralization with lactic acid-producing bacterium, *Lactobacillus paracasei* subsp. *tolerans* KCTC-3074. Biotechnol Bioprocess Eng 13:566–570

Okada T, Morrissey MT (2007) Recovery and characterization of sardine oil extracted by pH adjustment. J Agric Food Chem 55(5):1808–1813

Olapade OJ, Karim MA (2011) Utilization of bonga (*Ethmalosa fimbriata* Bodwich) fish concentrate in bread baking in Njala-Mokonde community, Sierra Leone. J Res Forest Wildl Environ 3(2):119–125

Osborne VR, Radhakrishnan S, Odongo NE, Hill AR, McBride BW (2008) Effects of supplementing fish oil in the drinking water of dairy cows on production performance and milk fatty acid composition. J Anim Sci 86(3):720–729

Ötles S, Çagindi Ö (2008) Carotenoids as natural colorants. In: Socaciu C (ed) Food colorants: chemical and functional properties. CRC Press, Boca Raton, pp 51–70
Overgaard J, Porsgaard T, Guo Z, Lauritzen L, Mu H (2008) Postprandial lipid responses of butter blend containing fish oil in a single-meal study in humans. Mol Nutr Food Res 52(10):1140–1146
Ovissipour M, Safari R, Motamedzadegan A, Rasco B, Pourgholam R, Mohagheghi E, Molla AE (2009) Use of hydrolysates from yellowfin tuna *Thunnus albacares* fisheries by-product as a nitrogen source for bacteria growth media. Int Aquat Res 1:73–77
Palpandi C, Shanmugan V, Shanmugan A (2009) Extraction of chitin and chitosan from shell and operculum of mangrove gastropod *Nerita* (Dostia) *crepidularia* Lamarck. Int J Med Med Sci 1:198–205
Pansawat N (2007) Development of an extruded snack supplemented with fish protein and n-3 fatty acids. Doctor of philosophy thesis, Kasetsart University, Thailand
Pariser ER (1973) Proteins of aquatic origin as foods for human consumption. In: Chichester CO (ed) Advances in food research, vol 20. Academic Press, New York, pp 189–216
Park P-J, Je J-J, Kim S-K (2003) Free radical scavenging activity of chitooligosaccharides by electron spin resonance spectrometry. J Agric Food Chem 51:4624–4627
Park P-J, Je J-Y, Kim S-K (2004) Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. Carbohydr Polym 55(1):17–22
Payne SR, Sandford D, Harris A, Young OA (1994) The effects of antifreeze proteins on chilled and frozen meat. Meat Sci 37:429–438
Payne SR, Young OA (1995) Effect of pre-slaughter administration of antifreeze proteins on frozen meat quality. Meat Sci 41:147–155
Pelser WM, Linssen JPH, Legger A, Houben JH (2007) Lipid oxidation in n-3 fatty acid enriched Dutch style fermented sausages. Meat Sci 75(1):1–11
Peng C, Wang Y, Tang Y (1998) Synthesis of crosslinked chitosan-crown ethers and evaluation of these products as adsorbents for metal ions. J Appl Polym Sci 70:501–506
Percot A, Viton C, Domard A (2003) Optimization of chitin extraction from shrimp shells. Biomacromolecules 4(1):12–18
Perdones A, Sánchez-González L, Chiralt A, Vargas M (2012) Effect of chitosan–lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest Biol Technol 70:32–41
Phanturat P, Benjakul S, Visessanguan W, Roytrakul S (2010) Use of pyloric caeca extract from bigeye snapper (*Priacanthus macracanthus*) for the production of gelatin hydrolysate with antioxidative activity. LWT-Food Sci Technol 43(1):86–97
Pilbrow J, Garama D, Carne A (2012) Carotenoid-binding proteins; accessories to carotenoid function. Acta Biochim Pol 59(1):163–165
Pilon G, Ruzzin J, Rioux L-E, Lavigne C, White PJ, Frøyland L, Jacques H, Bryl P, Beaulieu L, Marette A (2011) Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats. Metabolism 60(8):1122–1130
Ponnampalam EN, Sinclair AJ, Hosking BJ, Egan AR (2002) Effects of dietary lipid type on muscle fatty acid composition, carcass leanness, and meat toughness in lambs. J Anim Sci 80(3):628–636
Porsgaard T, Overgaard J, Krogh AL, Jensen MB, Guo Z, Mu H (2007) Butter blend containing fish oil improves the level of n-3 fatty acids in biological tissues of hamster. J Agric Food Chem 55(18):7615–7619
Qian C, Decker EA, Xiao H, McClements DJ (2012) Inhibition of β-carotene degradation in oil-in-water nanoemulsions: influence of oil-soluble and water-soluble antioxidants. Food Chem 135:1036–1043
Quaglia GB, Orban E (1990) Influence of enzymatic hydrolysis on structure and emulsifying properties of sardine (*Sardina pilchardus*) protein hydrolysate. J Food Sci 55:1571–1573
Rai AK, Swapna HC, Bhaskar N, Halami PM, Sachindra NM (2010) Effect of fermentation ensilaging on recovery of oil from fresh water fish viscera. Enzyme Microb Technol 46(1):9–13
Ramírez JA, Díaz-Sobac R, Morales OG, Vázquez M (1999) Evaluation of freeze-dried surimi from tilapia and fat sleeper as emulsifiers. Ciência Tecnol Aliment 2(4):210–214

Rasika DMD, Ranadheera CS, Vidanarachchi JK (2013) Applications of marine derived peptides and proteins in the food industry. In: Kim S-K (ed) Marine proteins and peptides: biological activities and applications. Wiley-Blackwell, Oxford, pp 545–587

Rattaya S, Benjakul S, Prodpran T (2009) Properties of fish skin gelatin film incorporated with seaweed extract. J Food Eng 95(1):151–157

Reddy MA, Elavarasan K, Reddy DA, Bhandary MH (2012) Suitability of reef cod (*Epinephelus diacanthus*) minced meat for preparation of ready to serve product. Adv Appl Sci Res 3(3):1513–1517

Robert SS, Petrie JR, Zhou X-R, Mansour MP, Blackburn SI, Green AG, Singh SP, Nichols PD (2009) Isolation and characterisation of a delta5-fatty acid elongase from the marine microalga *Pavlova salina*. Marine Biotechnol 11(3):410–418

Rognlien M, Duncan SE, O'Keefe SF, Eigel WN (2012) Consumer perception and sensory effect of oxidation in savory-flavored yogurt enriched with n-3 lipids. J Dairy Sci 95(4):1690–1698

Romeo J, Wärnberg J, García-Mármol E, Rodríguez-Rodríguez M, Diaz LE, Gómez-Martínez S, Cueto B, López-Huertas E, Cepero M, Boza JJ, Fonollá J, Marcos A (2011) Daily consumption of milk enriched with fish oil, oleic acid, minerals and vitamins reduces cell adhesion molecules in healthy children. Nutr Metab Cardiovasc Dis 21(2):113–120

Rossano R, Larocca M, Lamaina A, Viggiani S, Riccio P (2011a) The hepatopancreas enzymes of the crustaceans *Munida* and their potential application in cheese biotechnology. LWT-Food Sci Technol 44(1):173–180

Rossano R, Larocca M, Riccio P (2011b) Digestive enzymes of the crustaceans Munida and their application in cheese manufacturing: a review. Mar Drugs 9(7):1220–1231

Rubio-Rodríguez N, de Diego SM, Beltrán S, Jaime I, Sanz MT, Rovira J (2012) Supercritical fluid extraction of fish oil from fish by-products: a comparison with other extraction methods. J Food Eng 109(2):238–248

Sachindra NM, Mahendrakar NS (2005) Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. Bioresour Technol 96:1195–1200

Sachindra NM, Bhaskar N, Siddegowda GS, Sathisha AD, Suresh PV (2007) Recovery of carotenoids from ensilaged shrimp waste. Bioresour Technol 98(8):1642–1646

Samaranayaka AGP, Li-Chan ECY (2008) Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (*Merluccius productus*). Food Chem 107(2):768–776

Samaranayaka AGP, Li-Chan ECY (2011) Food-derived peptidic antioxidants: a review of their production, assessment, and potential applications. J. Funct Foods 3:229–254

Sánchez-Camargo AP, Meireles MÂA, Lopes BLF, Cabral FA (2011a) Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). J Food Eng 102:87–93

Sánchez-Camargo AP, Martinez-Correa HA, Paviani LC, Cabral FA (2011b) Supercritical CO_2 extraction of lipids and astaxanthin from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). J Supercrit Fluid 56:164–173

Sanguansri L, Shen Z, Weerakkody R, Barnes M, Lockett T, Augustin MA (2013) Omega-3 fatty acids in ileal effluent after consuming different foods containing microencapsulated fish oil powder–an ileostomy study. Food Funct 4(1):74–82

Sankar TV, Ramachandran A (2002) Rheological characteristics of suwari and kamaboko gels made of surimi from Indian major carps. J Sci Food Agric 82:1021–1027

Santana P, Huda N, Yang TA (2012) Technology for production of surimi powder and potential of applications. Int Food Res J 19(4):1313–1323

Santos SD, Martins VG, Salas-Mellado M, Prentice C (2011) Evaluation of functional properties in protein hydrolysates from bluewing searobin (*Prionotus punctatus*) obtained with different microbial enzymes. Food Bioprocess Technol 4:1399–1406

Saritha K, Patterson J (2012) Processing of innovative ready to fry crackers from *Penaeus japonicas*. World J Dairy Food Sci 7(1):66–73

Schmidt AS, Ventom AM, Asenjo JA (1994) Partitioning and purification of α-amylase in aqueous two-phase systems. Enzyme Microb Technol 16(2):131–142

Sen DP (2005) Advances in fish processing technology. Allied Publishers, New Delhi

Şenel S, McClure SJ (2004) Potential applications of chitosan in veterinary medicine. Adv Drug Deliv Rev 56(10):1467–1480

Shahidi F (2000) Antioxidants in food and food antioxidants. Mol Nutr Food Res 44(3):158–163

Shahidi F, Kamil JYVA (2001) Enzymes from fish and aquatic invertebrates and their application in the food industry. Trends Food Sci Technol 12(12):435–464

Shahidi F, Metusalach G, Brown JA (1998) Carotenoid pigments in seafoods and aquaculture. Crit Rev Food Sci Nutr 38(1):1–67

Shamay A, Werner D, Moallem U, Barash H, Bruckental I (2005) Effect of nursing management and skeletal size at weaning on puberty, skeletal growth rate, and milk production during first lactation of dairy heifers. J Dairy Sci 88:1460–1469

Shih I-L, Chen L-G, Yu T-S, Chang W-T, Wang S-L (2003) Microbial reclamation of fish processing wastes for the production of fish sauce. Enzyme Microb Technol 33:154–162

Shimizu Y, Machida R, Takenami S (1981) Species variation in the gel-forming characteristics of fish meat paste. Nippon Suisan Gakk 47:95–104

Sigman-Grant M, Warland R, Hsieh G (2003) Selected lower-fat foods positively impact nutrient quality in diets of free-living Americans. J Am Diet Assoc 103(5):570–576

Sila A, Nasri R, Jridi M, Balti R, Nasri M, Bougatef A (2012) Characterisation of trypsin purified from the viscera of Tunisian barbel (*Barbus callensis*) and its application for recovery of carotenoproteins from shrimp wastes. Food Chem 132(3):1287–1295

Simpson BK, Gagne N, Simpson MV (1994) Bioprocessing of chitin and chitosan. In: Martin AM (ed) Fisheries processing. Chapman and Hall, London, pp 155–175

Singh TK, Young ND, Drake MA, Cadwallader KR (2004) Production of a bitter peptide from casein and its sensory characterization. In: 2004 Institute of food technology annual meeting, Las Vegas, 12–16 Jul 2004

Šližytė R, Daukšas E, Falch E, Storrø I, Rustad T (2005a) Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochem 40(3–4):1415–1424

Šližytė R, Rustad T, Storrø I (2005b) Enzymatic hydrolysis of cod (*Gadus morhua*) by-products: optimization of yield and properties of lipid and protein fractions. Process Biochem 40(12): 3680–3692

Sørensen A-DM, Nielsen NS, Jacobsen C (2010a) Oxidative stability of fish oil-enriched mayonnaise-based salads. Eur J Lipid Sci Technol 112(4):476–487

Sørensen A-DM, Nielsen NS, Hyldig G, Jacobsen C (2010b) Influence of emulsifier type on lipid oxidation in fish oil-enriched light mayonnaise. Eur J Lipid Sci Technol 112(9):1012–1023

Sorokulova I, Krumnow A, Globa L, Vodyanoy V (2009) Efficient decomposition of shrimp shell waste using *Bacillus cereus* and *Exiguobacterium acetylicum*. J Ind Microbiol Biotechnol 36:1123–1126

Souissi N, Bougatef A, Triki-Ellouz Y, Nasri M (2007) Biochemical and functional properties of Sardinella (*Sardinella aurita*) by-product hydrolysates. Food Technol Biotechnol 45(2):187–194

Souza AAG, Amaral IPG, Santo ARE, Carvalho LB, Bezerra RS (2007) Trypsin-like enzyme from intestine and pyloric caeca of spotted goatfish (*Pseudupeneus maculatus*). Food Chem 100(4):1429–1434

Swatschek D, Schatton W, Kellermann J, Müller WEG, Kreuter J (2002) Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. Eur J Pharm Biopharm 53(1):107–113

Synowiecki J, Al-Khateeb NA (2003) Production, properties, and some new applications of chitin and its derivatives. Crit Rev Food Sci Nutr 43(2):145–171

Tabarestani HS, Maghsoudlou Y, Motamedzadegan A, Sadeghi Mahoonak AR (2010) Optimization of physico-chemical properties of gelatin extracted from fish skin of rainbow trout (*Onchorhynchus mykiss*). Bioresour Technol 101(15):6207–6214

Tabarsa M, Rezaei M, Ramezanpour Z, Waaland JR (2012) Chemical compositions of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. J Sci Food Agric 92(12):2500–2506

Tahergorabi R, Beamer SK, Matak KE, Jaczynski J (2012) Functional food products made from fish protein isolate recovered with isoelectric solubilization/precipitation. LWT-Food Sci Technol 48(1):89–95

Taherian AR, Britten M, Sabik H, Fustier P (2011) Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. Food Hydrocolloid 25(5):868–878

Takai J-i, Endo Y, Okuzaki M, Fujimoto K (2003) Autoxidation of fish oil in mayonnaise-like O/W type emulsion. Food Sci Technol Res 9(4):383–386

Takano T, Shozen K, Satomi M, Taira W, Abe H, Funatsu Y (2012) Quality of fish sauce products from recycled by-products from fish gel and Kamaboko processing. J Food Qual 35:217–227

Talib MA, Abubakar MM, Jideani IA, Hassan A (2009) Use of Jiben seeds extract to manufacture soft white cheese. Am J Appl Sci 6(4):551–554

Tamijidi F, Nasirpour A, Shahedi M (2012) Physicochemical and sensory properties of yogurt enriched with microencapsulated fish oil. Food Sci Technol Int 18(4):381–390

Tanuja S, Viji P, Zynudheen AA, Joshy C (2012) Composition, functional properties and antioxidative activity of hydrolysates prepared from the frame meat of Striped catfish (*Pangasianodon hypophthalmus*). Egypt J Biol 14:27–35

Tao Y, Qian L-H, Xie J (2011) Effect of chitosan on membrane permeability and cell morphology of *Pseudomonas aeruginosa* and *Staphyloccocus aureus*. Carbohydr Polym 86:969–974

Tavares JFP, Baptista JAB, Marcone MF (1997) Milk-coagulating enzymes of tuna fish waste as a rennet substitute. Int J Food Sci Nutr 48(3):169–176

Techaratanakrai B, Nishida M, Igarashi Y, Watanabe M, Okazaki E, Osako K (2011) Effect of setting conditions on mechanical properties of acid-induced Kamaboko gel from squid *Todarodes pacificus* mantle muscle meat. Fish Sci 77:439–446

Tharanathan RN, Kittur FS (2003) Chitin—the undisputed biomolecule of great potential. Crit Rev Food Sci Nutr 43:61–87

Thiansilakul Y, Benjakul S, Shahidi F (2007) Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem 103(4):1385–1394

Timm-Heinrich M, Xu X, Nielsen NS, Jacobsen C (2004) Oxidative stability of mayonnaise and milk drink produced with structured lipids based on fish oil and caprylic acid. Eur Food Res Technol 219(1):32–41

Torres E, Marín V, Aburto J, Beltrán HI, Shirai K, Villanueva S, Sandoval G (2012) Enzymatic modification of chitosan with quercetin and its application as antioxidant edible films. Appl Biochem Microbiol 48(2):151–158

Tsai C-J, Pan SB (2012) Identification of sulfoglycolipid bioactivities and characteristic fatty acids of marine macroalgae. J Agric Food Chem 60(34):8404–8410

Tsai G-J, Wu Z-Y, Su W-H (2000) Antibacterial activity of a chitooligosaccharide mixture prepared by cellulase digestion of shrimp chitosan and its application to milk preservation. J Food Prot 63:747–752

Tyagi A, Tyagi AK, Singh RRB, Hossain SA (2011) Designing milk and meat products with enhanced conjugated linoleic acid content: a review. Indian J Anim Nutr 28(1):1–17

Uddin M, Ahn H-M, Kishimura H, Chun B-S (2010) Production of valued materials from squid viscera by subcritical water hydrolysis. J Environ Biol 31(5):675–679

Uriarte-Montoya MH, Santacruz-Ortega H, Cinco-Moroyoqui FJ, Rouzaud-Sández O, Plascencia-Jatomea M, Ezquerra-Brauer JM (2011) Giant squid skin gelatin: chemical composition and biophysical characterization. Food Res Int 44(10):3243–3249

Valencia I, Ansorena D, Astiasarán I (2006) Nutritional and sensory properties of dry fermented sausages enriched with n-3 PUFAs. Meat Sci 72(4):727–733

Valencia I, O'Grady MN, Ansorena D, Astiasarán I, Kerry JP (2008) Enhancement of the nutritional status and quality of fresh pork sausages following the addition of linseed oil, fish oil and natural antioxidants. Meat Sci 80(4):1046–1054

Vecchi S, Coppes Z (1996) Marine fish digestive proteases—relevance to food industry and the South-West Atlantic region—a review. J Food Biochem 20(1):193–214

Venâncio A, Almeida C, Teixeira J (1996) Enzyme purification with aqueous two-phase systems: comparison between systems composed of pure polymers and systems composed of crude polymers. J Chromatogr B Biomed Sci Appl 680(1):131–136
Venugopal V (2008) Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC Press, Boca Raton
Venugopal V, Shahidi F (1995) Value-added products from underutilized fish species. Crit Rev Food Sci Nutr 35(5):431–453
Verardo V, Ferioli F, Riciputi Y, Iafelice G, Marconi E, Caboni MF (2009) Evaluation of lipid oxidation in spaghetti pasta enriched with long chain n-3 polyunsaturated fatty acids under different storage conditions. Food Chem 114(2):472–477
Vidanarachchi JK, Kurukulasuriya MS, Kim S-K (2009) Chitin, chitosan, and their oligosaccharides in food industry. In: Kim S-K (ed) Chitin, chitosan, oligosaccharides and their derivatives. CRC Press, Boca Raton, pp 543–560
Vinsova J, Vavrikova E (2011) Chitosan derivatives with antimicrobial, antitumour and antioxidant activities-a review. Curr Pharm Des 17(32):3596–3607
Visser S, Slangen KJ, Hup G, Stadhouders J (1983) Bitter flavour in cheese, 3: comparative gel-chromatographic analysis of hydrophobic peptide fractions from twelve Gouda-type cheeses and identification of bitter peptides isolated from a cheese made with *Streptococcus cremoris* strain HP. Neth Milk Dairy J 37(3):181–192
von Lintig J (2010) Colors with functions: elucidating the biochemical and molecular basis of carotenoid metabolism. Annu Rev Nutr 30:35–56
Wai CM, Laintz KE (1994) Supercritical fluid extraction. US Patent 5,356,538
Wang X, Xing B (2007) Importance of structural makeup of biopolymers for organic contaminant sorption. Environ Sci Technol 41:3559–3565
Wang L, An X, Yang F, Xin Z, Zhao L, Hu Q (2008) Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*). Food Chem 108(2):616–623
Wangtueai S, Noomhorm A (2009) Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT-Food Sci Technol 42(4):825–834
Wergedahl H, Liaset B, Gudbrandsen OA, Lied E, Espe M, Muna Z, Mørk S, Berge RK (2004) Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. J Nutr 134(6):1320–1327
Wetzel D (2009) Update on cod liver oil manufacture. Available online at: http://www.westonaprice.org/cod-liver-oil/update-on-cod-liver-oil-manufacture. Accessed 22 Sep 2012
Windsor ML (1977) Fish protein concentrates. Issue 39, Torry Research Station, Food and Agriculture Organization, Rome
Winterowd JG, Sanford PA (1995) Chitin and chitosan. In: Stephen AM (ed) Food polysaccharides and their applications. Marcel Decker, New York, pp 441–462
Wu TH, Bechtel PJ (2008) Salmon by-product storage and oil extraction. Food Chem 111(4):868–871
Xia W, Liu P, Zhang J, Chen J (2011) Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloid 25(2):170–179
Xing R, Liu S, Guo Z, Yu H, Wang P, Li C, Li Z, Li P (2005) Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. Bioorg Med Chem 13(5):1573–1577
Xu W, Yu G, Xue C, Xue Y, Ren Y (2008) Biochemical changes associated with fast fermentation of squid processing by-products for low salt fish sauce. Food Chem 107:1597–1604
Yang H, Wang Y, Jiang M, Oh J-H, Herring J, Zhou P (2007) 2-Step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J Food Sci 72(4):C188–C195
Yang Y, Anvari M, Pan C-H, Chung D (2012) Characterisation of interactions between fish gelatin and gum arabic in aqueous solutions. Food Chem 135(2):555–561
Ye A, Cui J, Taneja A, Zhu X, Singh H (2009) Evaluation of processed cheese fortified with fish oil emulsion. Food Res Int 42(8):1093–1098

Zahid N, Ali A, Manickam S, Siddiqui Y, Maqbool M (2012) Potential of chitosan-loaded nano-emulsions to control different *Colletotrichum* spp. and maintain quality of tropical fruits during cold storage. J Appl Microbiol 113(4):925–939

Zhang J, Xia W, Liu P, Cheng Q, Tahi T, Gu W, Li B (2010) Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs 8:1962–1987

Zhao Y, Li B, Dong S, Liu Z, Zhao X, Wang J, Zeng M (2009) A novel ACE inhibitory peptide isolated from *Acaudina molpadioidea* hydrolysate. Peptides 30(6):1028–1033

Zhao D, Huang J, Hu S, Mao J, Mei L (2011) Biochemical activities of N,O-carboxymethyl chitosan from squid cartilage. Carbohydr Polym 85:832–837

Zhong ZM, Xing RG, Liu S, Wang L, Cai SB, Li PC (2008) Synthesis of acyl thiourea derivatives of chitosan and their antimicrobial activities in vitro. Carbohydr Res 343:566–570

Zhou P, Regenstein JM (2007) Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. J Food Sci 72(4):C196–C201

Zhu C-F, Li G-Z, Peng H-B, Zhang F, Chen Y, Li Y (2010) Effect of marine collagen peptides on markers of metabolic nuclear receptors in type 2 diabetic patients with/without hypertension. Biomed Environ Sci 23(2):113–120

Chapter 24
Antidiabetic and Obesity Effects of Materials from Seafood By-products

Fatih Karadeniz, Mustafa Zafer Karagozlu, and Se-Kwon Kim

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1 Introduction

Obesity and increased body weight are recent crucial health risks of the modern world. In general terms, obesity might be defined as a part of complex complications identified as obesity-related metabolic syndrome, which is directly related to low HDL cholesterol, hypertension, hyperglycemia, and insulin resistance (Eckel et al. 2005; Ford et al. 2002). In this manner, the high morbidity and mortality rates of obesity, diabetes, and related complications urge the need for novel, natural treatment or prevention methods (Ivorra et al. 1989).

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Seafood waste has enormous potential that has not yet been fully exposed for utilization and adding value. Around the world, millions of tons of waste are dumped every year. Food processing industries leave around 21 % of the total seafood catch as waste, which includes a large amount of biomass of organism waste (e.g., skin, bones, and shells). Unfortunately, most of this mass is discarded. Several industries, researchers, and centers are now focusing on increasing the use of this waste and developing preventive and functional nutrition as well as bioactive substances. Normally, most of these wastes possess relatively low economic value. However, with the help of recent studies, numerous bioactive agents can be obtained from the remaining fish muscle proteins, collagen, gelatin, fish bone, fish oil, crustacean shell, internal organs, and shellfish. These bioactive substances are reported to possess various bioactivities, ranging from acting against oxidative stress, tumor cell proliferation, and aging to preventing metabolic diseases such as cardiovascular complications, diabetes, and obesity. In the light of recent results and the potential of waste utilization, more value will be obtained from what is today considered a waste, and novel bioactive compounds will pave the way for drug development and opportunities for the seafood industry.

2 Marine Oil

One of the main products of seafood waste is marine oils. These oils are extracted from waste fish muscle, internal organs, shellfish remains, etc. using several techniques, varying from steam stripping to solvent extraction in order to release lipids, which are considered to be unstable and require low-temperature methods. Marine oils are mainly formed by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are commonly classified as omega-3 fatty acids of polyunsaturated fatty acid (PUFA). These PUFAs are widely found in most marine animals, especially in coldwater fish species with a higher unsaturated fat content. Interestingly, scientific evidence has expressed that the beneficial effects of seafood consumption are mostly credited to its PUFAs (Kromhaut et al. 1985; Marckmann and Grønbaek 1999; Menotti et al. 1999; Mori et al. 1999). Especially, fish oil is shown to reduce the risk of coronary heart disease, decreases mild hypertension, prevents certain cardiac arrhythmia, and lowers the risk of diabetes, as well as preventing diabetes-related complications. However, due to depleting marine fisheries sources, fishing for obtaining fish oils is not preferred and is harmful for marine ecology. Therefore, the utilization of seafood processing by-products by the isolation of fish oil would be of great benefit to functional food development.

As a dietary component, PUFAs have also been shown to confer some cardiac benefits in patients with type 2 diabetes (Djousse et al. 2011; Jenkins et al. 2008) and overweight individuals. However, there are either limited or inconsistent data on the possible effects of these PUFAs on glucose or insulin pathways. On the other hand, some studies strongly suggest that omega-3 fatty acids might play a beneficiary role for overweight people with hypertension who are on a diet to lose weight. Bao et al. (1998) studied this effect and showed that consuming fat-rich seafood

daily improved glucose and insulin metabolism in individuals on a weight-loss diet. In the same study, people on the same diet who did not consume any seafood had no improvement in their glucose and insulin metabolism. Even though both groups lost the same amount of body weight, the seafood-consuming individuals had better values for their blood pressure and glucose level reductions. Hence, diabetic patients who consume oil-rich seafood on a regular basis can have improved health benefits on their glucose metabolism and blood pressure while losing body weight.

It has been known that diets with high fat content other than seafood lead to insulin resistance. In an earlier study, Storlien et al. showed that replacing safflower oil with omega-3 fatty acids prevented the high-fat diet rats developing insulin resistance. Liver and skeletal muscle, which play crucial roles in the glucose metabolism, were affected most by oil replacement. These results might be significant for the development of agents to prevent and treat insulin resistance in patients with diabetes.

In addition to direct effects on diabetic complications, marine oils rich in omega-3 fatty acids aid type 2 diabetic patients to have improved quality of life. In a study by Kesavulu et al. (2002), 34 non-insulin-dependent patients were tested for the activity of omega-3 fatty acids to elevate the antidiabetic drugs' effects. Following a month of antidiabetic drug only treatment, patients were supplied with 1,080 mg EPA and 720 mg DHA, along with antidiabetic drugs for 2 months. The comparison clearly showed that supplementation with marine fatty acids has a notable effect on lowering serum triglycerides (2.07 +/− 0.94 mmol/l before fatty acid therapy and 1.54 +/− 0.49 mmol/l after fatty acid therapy) and increasing HDL cholesterol levels (0.93 +/− 0.099 mmol/l before fatty acid therapy and 1.04 +/− 0.098 mmol/l after fatty acids therapy). Similar beneficiary effects were observed on lipid peroxidation and antioxidant enzymes, which, altogether, might suggest that EPA and DHA supplementation decrease the rate of diabetic complications caused by vascular deterioration.

3 Marine Peptides

As well as lipids, discarded seafood waste also contains proteins from bones, cut-offs, organs, and various muscle-including parts. For a well-balanced amino acid consumption, these proteins are of high nutritious value. In this context, marine proteins can be recovered by enzymatic hydrolysis processes from several seafood processing by-products. In addition to its nutritional value, scientists added more value to marine proteins by showing that enzymatic hydrolysates of seafood by-products possess functional properties. Mainly, researches have focused on the possibility of obtaining biologically active peptides which can act as agents against several diseases and syndromes. Supported by the evidence, bioactive peptides are supposed to be responsible for the health-promoting effects of marine proteins. As the molecular weight and chemical properties of peptide are crucial for its bioactivity, separating peptides from marine by-products is of the same importance. Therefore, it is a common method to use enzymatic hydrolysis for digesting

by-product proteins in order to obtain bioactive peptides. Peptides isolated from various seafood hydrolysates have shown numerous biological activities, such as antihypertensive, antioxidative, antithrombotic, and immunomodulatory activities. Each day, studies add more value to the utilization of seafood waste for bioactive peptide isolation.

In several studies, numerous peptides and protein hydrolysates, such as protamine, wheat proteins, soybean proteins, bovine hemoglobin hydrolysates, and rice proteins, have been shown to have anti-obesity activities. In the case of marine, especially fish, proteins, studies reported cholesterol-lowering, antihypertensive, antioxidative, as well as very few anti-obesity effects compared to terrestrial sources. Recent improvements and trends focus more on marine-based peptides and hydrolysates by using different methods and adding various value to the seafood waste to be utilized as a functional nutrition source.

Improving glucose metabolism is a significant way to prevent and inhibit type 2 diabetes. In this context, a number of fish protein hydrolysates have been shown to possess glucose uptake-stimulating activity, which adds notable potential to be active in hyperglycemia management. A previous study showed two suggested pathways for the effect; either affecting the receptors for glucose uptake or improving insulin sensitivity in target cells. Similarly, Zhu et al. (2010) reported that wild marine fish collagen-derived peptides increased insulin sensitivity and, hence, glucose metabolism in type 2 diabetes patients. The oral administration of these peptides are closely associated with the serum level changes of three diabetes-related hormones, leptin, resistin, and adiponectin. In other studies, some fish hydrolysates were reported to promote higher serum levels of bradykinin, along with improved glucose transport and insulin sensitivity (Henriksen and Jacob 2003; Henriksen et al. 1998). Their efficiency on glucose and insulin metabolism is linked to their ACE inhibition. Similarly, ACE inhibitory peptides in sardine protein hydrolysates are shown to suppress rising blood glucose levels with improved glucose metabolism (Otani et al. 2009).

4 Chitin and Chitosan

Crustacean shells and shellfish wastes make up another important category of by-products released from processing seafood. Chitin is one of the major structural components of these by-products and, up to now, it has been studied in detail for its various activities, both biologically and physiologically. Crustacean shells are the main sources of chitin isolation on a large scale from by-products, as well as being a potential source for chitosan and other oligomers, which are hydrolyzed derivatives of chitin (Hai et al. 2003; Jeon and Kim 2000). Chitin-based molecules are reported to possess several bioactivities, varying from anticancer, antitumor, antibacterial, and antiviral to skin protection and fat-lowering activities.

Chitosan-based products are known to have many biological activities, such as antitumor, antiHIV, antifungal, antibiotic, and preventing against oxidative stress

(Artan et al. 2010; Cho et al. 2011; Kendra and Hadwiger 1984; Kim and Rajapakse 2005; Kim et al. 2008; Nishimura et al. 1998; Xiao et al. 2000; Xie et al. 2001). Activities can be grouped into two categories according to the use of chitin-based products. These products are often used as indirect helping agents to enhance the effectiveness of other active compounds through chemical modification or non-chemical linkage against diabetes and obesity. On the other hand, the main role of chitin-based products is known as therapeutic nutraceutical agents, acting directly against diabetes and obesity. In both cases, derivatives of these natural products express a high and significant potential in the light of searching for bioactive pharmaceuticals against obesity and obesity-related diabetes.

4.1 Supportive Applications

The preferred route of drug administration for patients is mostly the oral route on chronic therapy of diseases and complications. However, the delivery of many therapeutic peptides and proteins through the digestion system is still an unsolved problem, basically because of the size, hydrophilicity, and unstable conditions of these molecules. Thus, several chitosan derivatives have been developed over the years with improved properties for enhanced applicability (Fernández-Urrusuno et al. 1999; Jiang et al. 2007; Prabaharan 2008; Thanou et al. 2001). Therefore, recent studies have focused on carrier products for the administration of insulin efficiently in pre- or postdiabetic patients and, lately, one of these products is chitosan derivatives. It has been reported by Portero et al. (2007) that chitosan sponges are quite successful in the buccal administration of insulin. Moreover, up-to-date studies presumed that chitosan-derived particles are entirely usable for insulin administration by the oral route, with their high protective effect and harmless structure (Hari et al. 1998; Krauland et al. 2004, 2006). The results of some related studies have suggested that the observed drug delivery activity of chitosan is highly promising in the case of insulin. For example, studies showed that chitosan–insulin nanoparticles have a strong affinity to rat intestinal epithelium after 3 h following oral administration (Ma et al. 2005). This suggests that chitosan as a cofactor for drug delivery makes insulin absorption safe and rapid. Carboxymethyl–hexanoyl chitosan is an amphiphilic chitosan derivative with important swelling ability and water solubility under natural conditions, and studies have shown that these hydrogels can be used for encapsulating poorly water-soluble drugs for effective drug delivery (Liu and Lin 2010), which opens up the path for efficient insulin delivery by chitosan derivatives. Furthermore, Mao et al. (2005) showed that PEG–trimethyl chitosan complexes are efficiently coupled with insulin and easily taken up by Caco-2 cells.

Besides drug delivery activity for insulin, studies have shown that chitosan complexes can be efficiently used for gene delivery in gene therapy (Köping-Höggård et al. 2001). Therefore, it can be easily adduced that chitosan complex derivatives are potent gene delivery targets for highly prevalent diseases, such as diabetes. Furthermore, it has been reported that these chitosan complexes possess relatively

higher uptake and transfection efficiency compared to that of other polysaccharide complexes used for both drug and gene delivery (Huang et al. 2004). Several researches were conducted in order to prove chitosan as a non-toxic alternative to other cationic polymers, and the results demonstrated a prominent potential for further studies of chitosan-based gene delivery systems (Sato et al. 2001). All these results suggest that chitosan and chitosan-based derivatives are the main focus in the search for a harmless agent for drug and gene delivery, which is extremely crucial for the improvement of a diabetic patient's life standards.

Moreover, studies on streptozotocin (STZ)-induced diabetic rats discovered that chitosan-based sponges are highly effective at healing diabetic wounds, in addition to the treatment of diabetic patients. Wang et al. (2008) suggest that application of the chitosan–collagen complex is an ideal wound-healing cover to enhance the recovery and healing of wounds, such as diabetic skin wounds, which demonstrates the great potential of chitosan and its derivatives to be used clinically for diabetic patients.

To conclude, chitosan-based polymers show great potential for the therapeutic treatment of diabetes, with their highly efficient drug and gene delivery properties, as well as effectiveness on diabetic wound healing.

4.2 Antidiabetic Applications

Overweight and obesity, two common health-threatening conditions, are considered to result in diabetes worldwide, although there are nowhere near enough treatment options (Leong and Wilding 1999). Therefore, studies of chitosan are focused on its fat-lowering and fat-preventing activities. Several researchers have demonstrated that chitosan tends to bond with the ingested dietary fat and carry it out in the stool, while preventing their absorption through the gut (Kanauchi et al. 1995). Relevant researches about the fat-lowering activity of chitosan have also shown that chitosan is capable of absorbing fat up to five times its own weight. In respect of these results, there are several studies showing that chitosan derivatives lower the level of LDL while increasing the HDL levels. Studies of chitosan and its fat-lowering activity suggest that it and its derivatives are highly effective hypocholesterolemic agents with the ability to decrease the blood cholesterol level by as much as 50 % (Jameela et al. 1995; Maezaki et al. 1993). Moreover, diabetic patient-based studies clearly showed that the daily administration of chitosan could reduce the blood cholesterol levels by 6 %, with an increased level of HDL (Maezaki et al. 1993). In addition to chitosan, chitosan oligosaccharides (COS), an oligomerized derivative of chitosan, show high activity in regulating blood cholesterol levels. Especially, previous studies reported that COS are capable of regulating cholesterol levels even in the liver. COS prevent the development of fatty liver caused by the action of hepatotropic poisons. Despite the fact that few studies have been carried out for the action mechanism of COS in regulating the serum cholesterol level, several investigations suggested a possible mechanism of COS lowering the LDL levels. As Remuñán-López

et al. (1998) suggested, the ionic structure of COS binds bile salts and acid, which inhibit lipid digestion through micelle formation. However, Tanaka et al. (1997) suggest a different mechanism of chitosan and COS, where lipids and fatty acids are directly bonded by chitosan.

In addition to the fat-lowering mechanisms of chitosan and its derivatives, studies have also proven that chitosan administration can lead to an increase in the insulin sensitivity of animal models (Neyrinck et al. 2009). It has been shown that 3 months of administration of chitosan significantly increased insulin sensitivity in obese patients and expressed a highly notable decrease in body weight and triglyceride levels (Hernández-González et al. 2010).

On the other hand, glucosamine and its derivatives are reported to be highly effective at inhibiting adipogenesis in vitro. Recent studies showed that phosphorylated derivatives of glucosamine inhibited the adipogenesis of 3T3-L1 cells, as well as fat accumulation (Kim et al. 2010; Kong et al. 2010). Several researches suggested that acetylated chitin treatment causes adipocytes to break down fats and lower their triglyceride accumulation by as much as half compared to control cells (Kong et al. 2011). Kong et al. (2009) demonstrated clearly that sulfated derivatives of glucosamine inhibited the proliferation and adipogenesis mechanism through AMPK pathways in 3T3-L1 cells. Glucosamine, acetylated-, sulfated-, and phosphorylated-glucosamine derivatives are reported as successful adipogenic inhibitors with intense potential to prevent weight gain by adipogenesis in patients who are at risk for diabetes. Also, it has been reported that chitosan oligosaccharides inhibit fat accumulation and adipogenesis in the 3T3-L1 cell line (Cho et al. 2008). In addition, studies have shown that treatment with glucosamines reduced the triglyceride content of adipocytes and enhanced glycerol secretion as a lipid-lowering effect. Most of these studies have expressed the better activity of chitosan-based compounds, such as chitosan oligosaccharides and glucosamines, after derivation by adding a charged side chain via phosphorylation and sulfation. Therefore, it can be suggested that the cationic power of glucosamine and chitosan oligosaccharides plays the main role in its anti-obesity effect. Further, a selective synthesis of phosphorylated or sulfated derivatives of chitosan and glucosamine will open up the path to a better understanding behind the structure–mechanism relation. However, up-to-date researchers have formed strong proofs that chitosan shows its anti-obesity effect through the PPAR-γ pathway of adipogenic differentiation, resulting in fewer adipocytes and lipid accumulation (Cho et al. 2008). Collectively, chitosan and its derivatives, such as glucosamines and chitosan oligosaccharides, successfully inhibit the differentiation of cells into adipocytes, as well as enhancing adipocytes to hydrolyze the triglycerides, which show a significant effect against lipid accumulation of the body (Langin 2006). This effect of chitosan and its derivatives demonstrates an important impact against obesity in the progression of diabetes. Hence, it shows a great amount of potential to be used as pharmaceutical agents.

Furthermore, chitosan and its oligosaccharides act as antidiabetic agents for the treatment of diabetes in the manner of protecting pancreatic β-cells. In type 2 diabetes, although patients can retain healthy pancreatic β-cells for many years after the disease onset, chronic exposure to high glucose will impair β-cell function in later stages.

Impaired β-cell functionality leads to cellular damage in type 2 diabetic patients (Ihara et al. 1999). Therefore, the protection of β-cells is of great importance for elevated insulin secretion as a part of diabetes treatment. Recent studies reported that chitosan oligosaccharides were a protective agent for pancreatic β-cells against high glucose-dependent cell deterioration (Karadeniz et al. 2011). It is suggested that, at the same time, COS could effectively accelerate the proliferation of pancreatic islet cells with elevated insulin secretion in the aid of lowering blood glucose levels. Liu et al. (2007) reported that COS treatment could improve the general situation and diabetic symptoms of rats, decrease the blood glucose levels, and normalize the impaired insulin sensitivity. Moreover, COS were reported as a preventive agent in non-obese diabetic mice from developing type 1 diabetes, which might be related to several bioactivities of COS (Cao et al. 2004). These results supported the hypothesis that COS can prevent pancreatic β-cells of diabetic patients and normalize the crucial insulin secretion. The mechanism behind this protection is studied and suggested as being related to the immunopotentiation and antioxidation activity of COS.

Renal failure is one of the most common diseases caused by diabetes mellitus. The metal cross-linked complex of chitosan, chitosan-iron (III), has recently been reported to be highly active in reducing phosphorus serum levels to treat chronic renal failure (Schöninger et al. 2010). This relatively new derivative of chitosan is significantly capable of adsorbing serum phosphorus in alloxan diabetes-induced rats with symptoms of renal failure progression.

Moreover, recent studies indicate that diabetics may be at higher risk for blood coagulation than non-diabetics. This life-threatening condition is urged to be treated for diabetic patients. Therefore, the sulfated derivative of chitosan has been shown to possess anticoagulant potency (Vongchan et al. 2002). Furthermore, studies have reported that sulfated chitosan does not show antiplatelet activity, unlike heparin, which is an effective anticoagulant agent. Collectively, the results proved that sulfated chitosan is a more efficient agent than heparin, although the latter has been used for a long time for blood coagulation treatment (Bourin and Lindahl 1993).

In addition to COS, chitosan has also been reported to prevent the development and symptoms of non-insulin-dependent diabetes in rats as well as complications of STZ inducement (Kondo et al. 2000). Briefly, reports have suggested that chitosan products protect pancreatic cells and the insulin secretion mechanism in diabetic conditions. Furthermore, these compounds can decrease the progression and complication rate of diabetes onset in animal models, demonstrating a great potential for chitosan products to be used as a nutraceutical for the treatment of diabetes.

5 Conclusion

The reported biological activities and natural occurrence of seafood by-products-derived molecules, agents, and hydrolysates and interest in marine seafood processing wastes has been increased notably in the past several years. The lack of research and utilization urges further detailed studies for both the isolation and obtaining of

bioactive agents from by-products and screening the action mechanism of obtained agents from seafood wastes. In addition to their various biological activities, such as antioxidative, antihypertensive, anticancer, and similar health-promoting effects, promising antidiabetes and anti-obesity effects of these agents have been well-studied and reported recently. Evidently, several by-products-derived compounds such as oils, fatty acids, protein hydrolysates, peptides, chitin, and oligomers are active against obese and diabetic conditions on both preventive and treatment measures. Hence, by adding potential to seafood processing by-products, they deserve to be in the spotlight as being an important source for preventive nutritional and functional foods in order to maintain and promote healthy living for diabetic and obese individuals.

References

Artan M, Karadeniz F, Karagozlu MZ, Kim M-M, Kim S-K (2010) Anti-HIV-1 activity of low molecular weight sulfated chitooligosaccharides. Carbohyd Res 345(5):656–662

Bao DQ, Mori TA, Burke V, Puddey IB, Beilin LJ (1998) Effects of dietary fish and weight reduction on ambulatory blood pressure in overweight hypertensives. Hypertension 32(4):710–717

Bourin M-C, Lindahl U (1993) Glycosaminoglycans and the regulation of blood coagulation. Biochem J 289(Pt 2):313

Cao, Zhaohui, Bangliang Li, Xinhui Qiao (2004) The effect of chitooligosaccharides on preventing the on set of diabetes in nod mice. J Med Theory Pract 12:25–31

Cho EJ, Kim SW, Hwang HJ, Hwang HS, Yun JW (2008) Chitosan oligosaccharides inhibit adipogenesis in 3t3-l1 adipocytes. J Microbiol Biotechnol 18(1):80–87

Cho Y-S, Lee S-H, Kim S-K, Ahn C-B, Je J-Y (2011) Aminoethyl-chitosan inhibits LPS-induced inflammatory mediators, iNOS and COX-2 expression in RAW264. 7 mouse macrophages. Process Biochem 46(2):465–470

Djoussé L, Biggs ML, Lemaitre RN, King IB, Song X, Ix JH, Mukamal KJ, Siscovick DS, Mozaffarian D (2011) Plasma omega-3 fatty acids and incident diabetes in older adults. Am J Clin Nutr 94(2):527–533

Eckel RH, Grundy SM, Zimmet PZ (2005) The metabolic syndrome. Lancet 365:1415–1428

Fernández-Urrusuno R, Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ (1999) Enhancement of nasal absorption of insulin using chitosan nanoparticles. Pharmaceut Res 16(10):1576–1581

Ford ES, Giles WH, Dietz WH (2002) Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287:356–359

Hai L, Diep TB, Nagasawa N, Yoshii F, Kume T (2003) Radiation depolymerization of chitosan to prepare oligomers. Nucl Instrum Methods Phys Res Sect B 208:466–470

Hari PR, Chandy T, Sharma CP (1998) Chitosan/calcium–alginate beads for oral delivery of insulin. J Appl Polym Sci 59(11):1795–1801

Henriksen EJ, Jacob S, Fogt DL, Dietze GJ (1998) Effect of chronic bradykinin administration on insulin action in an animal model of insulin resistance. J Physiol 275(1):40–45

Hernández-González SO, González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA (2010) Chitosan improves insulin sensitivity as determined by the euglycemic-hyperinsulinemic clamp technique in obese subjects. Nutr Res 30(6):392–395

Huang M, Khor E, Lim L-Y (2004) Uptake and cytotoxicity of chitosan molecules and nanoparticles: effects of molecular weight and degree of deacetylation. Pharmaceut Res 21(2):344–353

Ihara Y, Toyokuni S, Uchida K et al (1999) Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. Diabetes 48(4):927–932

Ivorra MD, Paya M, Villar A (1989) A review of natural products and plants as potential antidiabetic drugs. J Ethnopharmacol 27(3):243–275
Jameela SR, Misra A, Jayakrishnan A (1995) Cross-linked chitosan microspheres as carriers for prolonged delivery of macromolecular drugs. J Biomater Sci Polym Ed 6(7):621–632
Jenkins DJ, Hu FB, Tapsell LC, Josse AR, Kendall CW (2008) Possible benefit of nuts in type 2 diabetes. J Nutr 138(9):1752–1756
Jeon Y-J, Kim S-K (2000) Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. Carbohydr Polym 41(2):133–141
Jiang L, Qian F, He X et al (2007) Novel chitosan derivative nanoparticles enhance the immunogenicity of a DNA vaccine encoding hepatitis b virus core antigen in mice. J Gene Med 9(4):253–264
Kanauchi O, Deuchi K, Imasato Y, Shizukuishi M, Kobayashi E (1995) Mechanism for the inhibition of fat digestion by chitosan and for the synergistic effect of ascorbate. Biosci Biotechnol Biochem 59(5):786–790
Karadeniz F, Karagozlu MZ, Pyun S-Y, Kim S-K (2011) Sulfation of chitosan oligomers enhances their anti-adipogenic effect in 3t3-l1 adipocytes. Carbohydr Polym 86(2):666–671
Kendra DF, Hadwiger LA (1984) Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin formation in *Pisum sativum*. Exp Mycol 8(3):276–281
Kesavulu MM, Kameswararao B, Apparao Ch, Kumar EG, Harinarayan CV (2002) Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients. Diabetes Metab 28(1):20–26
Kim S-K, Rajapakse N (2005) Enzymatic production and biological activities of chitosan oligosaccharides (COS): a review. Carbohydr Polym 62(4):357–368
Kim J-H, Kim Y-S, Park K et al (2008) Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. J Control Release 127(1):41–49
Kim J, Kong C-S, Pyun SY, Kim S-K (2010) Phosphorylated glucosamine inhibits the inflammatory response in LPS-stimulated PMA-differentiated THP-1 cells. Carbohyd Res 345(13):1851–1855
Kromhaut D, Bosschieter EB, de Lezenne Coulandar C (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 312(19):1205–1209
Kondo Y, Nakatani A, Hayashi K, Ito M (2000) Low molecular weight chitosan prevents the progression of low dose streptozotocin-induced slowly progressive diabetes mellitus in mice. Biol Pharm Bull 23(12):1458–1464
Kong C-S, Kim J, Kim S-K (2009) Anti-obesity effect of sulfated glucosamine by AMPK signal pathway in 3T3-L1 adipocytes. Food Chem Toxicol 47(10):2401–2406
Kong C-S, Kim J, Eom TK, Kim S-K (2010) Phosphorylated glucosamine inhibits adipogenesis in 3T3-L1 adipocytes. J Nutr Biochem 21(5):438–443
Kong C-S, Kim J, Bak S-S, Byun H-G, Kim S-K (2011) Anti-obesity effect of carboxymethyl chitin by AMPK and aquaporin-7 pathways in 3T3-L1 adipocytes. J Nutr Biochem 22(3):276–281
Köping-Höggård M, Tubulekas I, Guan H et al (2001) Chitosan as a nonviral gene delivery system. Structure–property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. Gene Ther 8:1108–1121
Krauland AH, Guggi D, Bernkop-Schnürch A (2004) Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats. J Control Release 95(3):547–555
Krauland AH, Guggi D, Bernkop-Schnürch A (2006) Thiolated chitosan microparticles: a vehicle for nasal peptide drug delivery. Int J Pharm 307(2):270–277
Langin D (2006) Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacol Res 53(6):482–491
Leong KS, Wilding JP (1999) Obesity and diabetes. Best Pract Res Clin Endocrinol Metab 13(2):221–237
Liu T-Y, Lin Y-L (2010) Novel pH-sensitive chitosan-based hydrogel for encapsulating poorly water-soluble drugs. Acta Biomater 6(4):1423–1429

Liu B, Liu W-S, Han B-Q, Sun Y-Y (2007) Antidiabetic effects of chitooligosaccharides on pancreatic islet cells in streptozotocin-induced diabetic rats. World J Gastroenterol 13(5):725

Ma Z, Lim TM, Lim L-Y (2005) Pharmacological activity of peroral chitosan–insulin nanoparticles in diabetic rats. Int J Pharmaceut 293(1):271–280

Maezaki Y, Tsuji K, Nakagawa Y et al (1993) Hypocholesterolemic effect of chitosan in adult males. Biosci Biotechnol Biochem 57(9):1439–1444

Mao S, Germershaus O, Fischer D, Linn T, Schnepf R, Kissel T (2005) Uptake and transport of PEG-graft-trimethyl-chitosan copolymer–insulin nanocomplexes by epithelial cells. Pharmaceut Res 22(12):2058–2068

Marckmann P, Grønbaek M (1999) Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. Eur J Clin Nutr 53(8):585-590

Menotti A, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A (1999) Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. Eur J Epidemiol 15(6):507–515

Mori TA, Bao DQ, Burke V, Puddey IB, Watts GF, Beilin LJ (1999) Lipids and cardiovascular risk. Am J Clin Nutr 70(5):817–825

Neyrinck AM, Bindels LB, De Backer F, Pachikian BD, Cani PD, Delzenne NM (2009) Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid-lowering action. Int Immunopharmacol 9(6):767–773

Nishimura S-I, Kai H, Shinada K et al (1998) Regioselective syntheses of sulfated polysaccharides: specific anti-HIV-1 activity of novel chitin sulfates. Carbohydr Res 306(3):427–433

Otani L, Ninomiya T, Murakami M, Osajima K, Kato H, Murakami T (2009) Sardine peptide with angiotensin I-converting enzyme inhibitory activity improves glucose tolerance in stroke-prone spontaneously hypertensive rats. Biosci Biotechnol Biochem 73(10):2203–2209

Portero A, Teijeiro-Osorio D, Alonso MJ, Remuñán-López C (2007) Development of chitosan sponges for buccal administration of insulin. Carbohydr Polym 68(4):617–625

Prabaharan M (2008) Review paper: chitosan derivatives as promising materials for controlled drug delivery. J Biomater Appl 23(1):5–36

Remuñán-López C, Portero A, Vila-Jato JL, Alonso MJ (1998) Design and evaluation of chitosan/ethylcellulose mucoadhesive bilayered devices for buccal drug delivery. J Control Rel 55(2):143–152

Sato T, Ishii T, Okahata Y (2001) In vitro gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. Biomaterials 22(15):2075–2080

Schöninger LMR, Dall'Oglio RC, Sandri S, Rodrigues CA, Bürger C (2010) Chitosan iron(III) reduces phosphorus levels in alloxan diabetes-induced rats with signs of renal failure development. Basic Clin Pharmacol Toxicol 106(6):467–471

Tanaka Y, Tanioka S-i, Tanaka M et al (1997) Effects of chitin and chitosan particles on BALB/c mice by oral and parenteral administration. Biomaterials 18(8):591–595

Thanou M, Verhoef JC, Junginger HE (2001) Oral drug absorption enhancement by chitosan and its derivatives. Adv Drug Deliv Rev 52(2):117–126

Vongchan P, Sajomsang W, Subyen D, Kongtawelert P (2002) Anticoagulant activity of a sulfated chitosan. Carbohyd Res 337(13):1239–1242

Wang W, Lin S, Xiao Y et al (2008) Acceleration of diabetic wound healing with chitosan-crosslinked collagen sponge containing recombinant human acidic fibroblast growth factor in healing-impaired STZ diabetic rats. Life Sci 82(3):190–204

Xiao FL, Guan YL, Yang DZ, Li Z, Yao KD (2000) Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci 79(7):1324–1335

Xie W, Xu P, Liu Q (2001) Antioxidant activity of water-soluble chitosan derivatives. Bioorg Med Chem Lett 11(13):1699–1701

Zhu CF, Li GZ, Peng HB, Zhang F, Chen Y, Li Y (2010) Effect of marine collagen peptides on markers of metabolic nuclear receptors in type 2 diabetic patients with/without hypertension. Biomed Environ Sci 23(2):113–120

Chapter 25
Bioluminescence Isolated from Seafood By-products and Its Applications

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1 Introduction

Bioluminescence is simply the production of light without heat in living organisms by chemical reactions. It has been found in a wide range of major groups of organisms from bacteria to fish, and it has been found in marine as well as terrestrial organisms. Although the frequency of luminescence production in marine organisms is related to depth, due to the many kinds of usage in marine organisms, bioluminescence enables organisms to see at all depths or regions (Herring 1987). On the other hand, due to the absence of light, most luminescence-producing organisms live in the deep sea and it is essential in fresh waters. Mostly, luminescence is produced by the organisms themselves, but occasionally, it occurs as bacterial symbiosis, which is sometimes very hard to distinguish. For example, in the past, many organisms such as the sponges, bryozoans, and *Cyclosalpa* species have all been reported as luminous but now, many of the pharmacologically interesting compounds isolated from sponges have turned out to be bacterial in origin (Schmidt et al. 2000; Taylor et al. 2007).

In the past decade, the application of bioluminescence, especially bioluminescent imaging, has become indispensable for the non-invasive monitoring of biologic phenomena in vivo. Understanding the background of the mechanism of luminescence at the chemical and biological levels has led us to improve its applications in many ways.

2 Chemistry of Bioluminescence

Simply, bioluminescence is energy released in the form of light by chemical reactions. Mostly, these chemical reactions are oxidation of light-emitted luciferin molecules or conformational changes of photoproteins by the binding of ions or cofactors such as Mg^{+2} or Ca^{+2}. Since luciferin is a light-emitting molecule, bioluminescence is frequently confused with fluorescence. However, these are two different reactions. In fluorescence, photons are absorbed by molecules, and this absorption causes energy elevation. Consequently, fluorescence is powered by the input of high-energy radiation (Williams 2001). On the other hand, bioluminescence is created by chemical energy. An enzyme called luciferase oxidases the luciferin into the high-energy form called oxyluciferin. Then, the oxyluciferin releases energy to produce visible light. This production of light does not produce any heat (Webster et al. 1991). Luciferins are fluorescent molecules, but the chemical reactions of fluorescence and luminescence are different. In fluorescence, the original molecule is restored following the fluorescent emission; in bioluminescence, the expended oxyluciferin is distinct from the original luciferin and must be replaced, either by biochemical synthesis or from the organism's diet. There are several luciferins which have been isolated from different marine organisms.

Table 25.1 Some marine-derived bioluminescent proteins

Protein	Organism	Mw (kDa)	Required components
Renilla luciferase	*Renilla reniformis* (sea pansy)	36	Coelenterazine[a]; O_2
Gaussia luciferase	*Gaussia princes* (marine copepod)	20	Coelenterazine[a]; O_2
Metridia luciferase	*Metridia longa* (marine copepod)	24	Coelenterazine[a]; O_2
Vargula luciferase	*Vargula hilgendorfii* (marine ostracod)	62	Coelenterazine[a]; O_2
Cypridina luciferase	*Cypridina noctiluca* (marine ostracod)	61	Coelenterazine[a]; O_2

2.1 Photoproteins from the Sea

The most common photoproteins are aequorin from the jellyfish *Aequorea victoria* and obelin from the hydrozoa *Obelia longissima.* They are both coelenterazine-based bioluminescence proteins with behavior quite different from that of luciferases. Indeed, aequorin and obelin can be considered as high-energy enzyme–substrate complexes that are more stable than their dissociated components and can release excess energy in a flash light emission triggered by Ca^{2+} ions.

According to the presence of oxygen and coelenterazine, the reconstitution of photoproteins is a relatively slow process. Photoproteins are important bioanalytical tools, just like luciferase. They can be used in a wide range of applications, from the labeling of biospecific probes to the measurement of intracellular Ca^{2+} levels.

2.2 Luciferins from the Sea

The ostracods, which have been examined since the 1950s, were the first crystallized and well understood luciferins from marine sources (Tsuji 1955; Shinomura et al. 1957). This luciferin is found mainly in *Cypridina*, *Vargula*, which is a cypridinid ostracod, and the midshipman fish (Warner and Case 1980). Other well-characterized luciferins are coelenterazine (Shimomura and Johnson 1972, 1975) and the *Cypridina* luciferin (Kishi et al. 1966; Hori et al. 1977; Inouye 2007). Besides, the dinoflagellate luciferin is a tetrapyrrole, similar to chlorophyll and differing mainly in the metal ions present (Dunlap et al. 1981; Nakamura et al. 1989; Takeuchi et al. 2005). On the other hand, there are several luciferases which have been isolated from marine organisms (Table 25.1.)

3 Use of Luminescence by Marine Organisms

Marine organisms produce their own luminescence or use the luminescence produced by microorganisms for many different functions. Most of the time, bioluminescence serves multiple roles for a single organism. These functions can be grouped under three main headings: defense, offense, and communication.

3.1 Defense

Most organisms use bioluminescence as a defense mechanism instead of offence by blinding predators with a sudden flash in the dark sea (Vallin et al. 2006; Jones and Nishiguchi 2004) or confusing them by preparing a glowing fluid, which allows them to escape (Haddock and Case 1999). The most common method is to sacrifice a body part to distract predator. This detached body part can continue to glow, which gives the prey more time to escape from the predator (Robison 1992; Herring and Widder 2004; Mensinger and Case 1992). On the other hand, in terrestrial areas, the usage of bright colors is a defense mechanism because bright colors advertise toxicity. The same defense mechanism occurs in marine organisms as bioluminescence usage. Some marine organisms, such as scale worms, jellyfish, and brittle stars use, this mechanism to warn predators (Grober 1988; Bassot and Nicolas 1995; Herring 2007).

3.2 Offense

Light glowing also serves as an attracter of prey in marine organisms. This is most prominent in fish. For example, some fishes store the luminescence produced by bacteria to attract prey, such as anglerfishes and dragonfishes (Pietsch 2009; Kenaley and Hartel 2005). On the other hand, dragonfishes use the light glowing under their eyes for visual support in the dark blue sea. Also, some squid and octopuses use the same mechanism for hunting. For example, *Chiroteuthis* is a squid that has special light organs (Voss 1967; Robison et al. 2003) and the octopus *Stauroteuthis* may use its luminous suckers to attract planktonic prey in a similar manner (Johnsen 2005). Confusion by glowing light can also be used as an offense mechanism instead of a defense mechanism. The squid *Taningia*, which flashes its tentacles while attacking bait (Kubodera et al. 2007), supports this hypothesis. In addition, the fish species myctophid uses luminescent heads for same reason. One more extraordinary example for predators that use bioluminescence is megamouth sharks. They use luminescence in a direct way, even though they are not known to be bioluminescent (Herring 2000). The white pigmented band along the upper jaw may reflect luminescence or downwelling light in order to draw plankton closer (Takahashi 2001).

3.3 Communication

Bioluminescence is known to be used for communication, especially for mating systems among marine organisms (Herring and Cope 2005). For example, Caribbean ostracods, which are small crustaceans, use luminescence for mating (Morin and Cohen 2010; Rivers and Morin 2008). Luminescent-based sexual dimorphism can be used by the other organisms, such as the pelagic octopods *Japetella* and *Eledonella*, and the ponyfishes, which produce synchronized group displays (Woodland et al. 2002; Ikejima et al. 2008).

4 Applications of Bioluminescence

In 1986, Ow and colleagues declared that they had successfully transferred the luciferase gene from firefly to tobacco plant. Since then, bioluminescence has been accepted as a promising bioanalytical tool due to its high detectability. Bioluminescence is now a well-established detection principle in many biotechnological fields, especially imaging, including reporter gene technology, gene probe assays, and immunoassays.

4.1 Gene Expression Assay

Bioluminescent reporters have been used to study gene expression. They are used as *Cis*-transcriptional reporter systems and allow the detection of gene expression and gene regulation at the transcriptional level. Their detection is performed by either generating point mutations or deletions in a promoter region of a gene of interest or using different transcription-factor-binding sites linked to a minimal promoter to drive the expression of a luciferase. For example, mRNA stability is imaged by fusing a luciferase reporter to the full-length 3′ untranslated region (UTR) of a gene of interest (Subramaniam 2008). RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression. It is a promising strategy against many diseases. RNAi-mediated silencing of luciferase expression is a useful strategy for testing the delivery and targeting efficiency of small interfering RNAs (siRNAs) on specific tissues. The ability of nanoparticles to deliver siRNAs to the liver was tested using luciferase-targeted siRNAs. Knockdown of luciferase gene expression was efficiently measured in the liver using bioluminescence imaging (Issad 2002). On the other hand, in vivo gene expression patterns have been studied in light-producing transgenic animals that are used as models of human disease, in drug development and discovery, and for target validation (Katz 2006). As an example, a transgenic mouse model has been developed to monitor the in vivo transcriptional regulation of the gene encoding human cytochrome P450 3A4 (CYP3A4), which plays an important role in drug metabolism and contributes to many adverse drug interactions (Lorenz et al. 1991).

4.2 Protein Interaction Assay

Most of the transcriptional activator proteins consist of two main domains, the DNA-binding domain and the activating domain. The most commonly used transcriptional activator system is based on a fusion between the DNA-binding domain of the yeast activator GAL4 and the activator herpes simplex virus protein VP16 (Xia and Rao 2009). Interactions between two different proteins can be determined by using GAL4–VP16 activation. To study the interaction of two proteins of

interest, X and Y, two vectors encoding fusions between GAL4–X and VP16–Y are coexpressed with a third vector encoding the reporter gene under the control of GAL4-responsive elements. On interaction of the X–Y proteins, the GAL4–X–Y–VP16 complex binds to the GAL4-responsive elements, and the transcriptional activator VP16 will then drive the expression of the luciferase reporter (Chen 2010). Using this system, Pichler et al. recently generated transgenic mice in which Fluc is cloned under the control of the GAL4 promoter (Pichler et al. 2008).

4.3 Imaging of Cells

Visible light from bioluminescence reactions is partially transmitted through animal tissues, so that low-light imaging can be performed in whole-animal models. Bioluminescence whole-body cellular and molecular imaging has been proven to be a sensitive, quantitative, non-invasive, and real-time method for monitoring biological processes in living animals, and is expected to have a profound impact in the fields of biotechnology, molecular medicine, gene therapy, and drug discovery and development.

Disease progression and the efficacy of therapeutic agents have been assessed by injecting mice with a bioluminescence marker, such as pathogenic microorganisms engineered to emit light, and then imaging the light transmitted through the layers of the animal's tissues (Francis et al. 2000). In this way, colonization of the gastrointestinal tract of live mice by bioluminescent recombinant *Salmonella enteritidis* and the effects of diet components could be followed (Brovko et al. 2003).

Besides the imaging of cells, bioluminescence imaging is a valuable technique against cancer disease. Tumor growth and metastases, as well as drug efficacy, have been monitored in living animals by injecting mice with bioluminescent recombinant tumor cells and imaging the produced light (Soling and Rainov 2003). Alternatively, primary tumors and unknown metastases can be revealed in vivo by using engineered light-emitting cells as probes for tumor location (Yu et al. 2003).

4.4 Immunoassay

Bioluminescence has been extensively used for the ultrasensitive detection of labels in immunoassays. Bioluminescent immunoassays have been developed using photoproteins (Wu et al. 2007) or luciferase-coding DNA fragments as labels (Inouye et al. 2011). Various biofunctional bioluminescent molecules have been prepared and proposed for the development of immunoassays; for example, a thermostable luciferase–biotin complex obtained by fusing the bioluminescence protein with a biotin acceptor peptide (Nakatsu et al. 2006).

5 Conclusion

The importance of bioluminescence for marine animals can be presumed by its widespread distribution throughout the ocean, from the surface to the deep sea, and from the poles to the tropics. Recent developments on understanding bioluminescence have improved their importance in scientific research. In recent years, new concepts for exploiting bioluminescence in the development of bioanalytical tools have been published and they strongly suggest that the fascinating world of bioluminescence still deserves continuous promising investigation. Especially, bioluminescent imaging provides valuable insight into biological processes in interacting cells and small-animal models. Furthermore, it helps in expediting drug identification and development, and the subsequent functional assessment of new treatment regimens in animal models before translation into the clinic. The future of analytical bioluminescence is extremely bright, since only a few bioluminescent systems have been identified and even fewer have been used for bioanalytical applications.

References

Bassot JM, Nicolas MT (1995) Bioluminescence in scale-worm photosomes: the photoprotein polynoidin is specific for the detection of superoxide radicals. Histochem Cell Biol 104:199–210

Branchini BR, Southworth TL, Khattak NF, Michelini E, Roda A (2005) Red- and green-emitting firefly luciferase mutants for bioluminescent reporter applications. Anal Biochem 345:140–148

Brovko LY, Vandenende C, Chu B, Ng K-Y, Brooks A, Griffiths MW (2003) In vivo assessment of effect of fermented milk diet on course of infection in mice with bioluminescent *Salmonella*. J Food Prot 66:2160–2163

Chen IY (2010) Indirect imaging of cardiac-specific transgene expression using a bidirectional two-step transcriptional amplification strategy. Gene Ther 17:827–838

Dunlap PV, Kita-Tsukamoto K (2006) Luminous bacteria. Prokaryotes 2:863–892

Francis KP, Joh D, Bellinger-Kawahara C, Hawkinson MJ, Purchio TF, Contag PR (2000) Monitoring bioluminescent *Staphylococcus aureus* infections in living mice using a novel *lux*ABCDE construct. Infect Immun 68:3594–3600

Grober MS (1988) Brittle-star bioluminescence functions as an aposematic signal to deter crustacean predators. Anim Behav 36:493–501

Haddock SHD, Case JF (1999) Bioluminescence spectra of shallow and deep-sea gelatinous zooplankton: ctenophores, medusae and siphonophores. Mar Biol 133:571–582

Herring PJ (1987) Systematic distribution of bioluminescence in living organisms. J Biolum Chemilum 1:147–163

Herring PJ (2000) Species abundance, sexual encounter and bioluminescent signalling in the deep sea. Philos Trans R Soc Lond B Biol Sci 355:1273–1276

Herring PJ (2007) Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea. J Mar Biol Assoc UK 87:829–842

Herring PJ, Cope C (2005) Red bioluminescence in fishes: on the suborbital photophores of *Malacosteus*, *Pachystomias* and *Aristostomias*. Mar Biol 148:383–394

Herring PJ, Widder EA (2004) Bioluminescence of deep-sea coronate medusae (Cnidaria: Scyphozoa). Mar Biol 146:39–51

Hori K, Charbonneau H, Hart RC, Cormier MJ (1977) Structure of native *Renilla reinformis* luciferin. Proc Natl Acad Sci U S A 74:4285–4287

Ikejima K, Wada M, Kita-Tsukamoto K, Yamamoto T, Azuma N (2008) Synchronized development of gonad and bioluminescent light organ in a highly sexually dimorphic leiognathid fish, *Photoplagios rivulatus*. Mar Biol 153:1009–1014

Inouye S (2007) Expression, purification and characterization of calcium-triggered luciferin-binding protein of *Renilla reniformis*. Protein Expr Purif 52:66–73

Inouye S, Sato J-I, Sasaki S, Sahara Y (2011) Streptavidin-aequorin fusion protein for bioluminescent immunoassay. Biosci Biotechnol Biochem 75:568–571

Issad T (2002) A homogenous assay to monitor the activity of the insulin receptor using Bioluminescence Resonance Energy Transfer. Biochem Pharmacol 64:813–817

Johnsen S (2005) The red and the black: bioluminescence and the color of animals in the deep sea. Integr Comp Biol 45:234–246

Jones BW, Nishiguchi MK (2004) Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). Mar Biol 144:1151–1155

Katz UF (2006) KM3NeT: towards a km^3 Mediterranean neutrino telescope. Nucl Instrum Meth Phys Res A 567:457–661

Kenaley CP, Hartel KE (2005) A revision of Atlantic species of *Photostomias* (Teleostei: Stomiidae: Malacosteinae), with a description of a new species. Ichthyol Res 52:251–263

Kishi Y, Goto T, Hirata Y, Shimomura O, Johnson FH (1966) *Cypridina* bioluminescence. I: structure of *Cypridina luciferin*. Tetrahedron Lett 7:3427–3436

Kubodera T, Koyama Y, Mori K (2007) Observations of wild hunting behaviour and bioluminescence of a large deep-sea, eight-armed squid, *Taningia danae*. Proc R Soc B Biol Sci 274:1029–1034

Lorenz WW, McCann RO, Longiaru M, Cormier MJ (1991) Isolation and expression of a cDNA encoding *Renilla reniformis* luciferase. Proc Natl Acad Sci U S A 88:4438–4442

Mensinger AF, Case JF (1992) Dinoflagellate luminescence increases susceptibility of zooplankton to teleost predation. Mar Biol 112:207–210

Morin JG, Cohen AC (2010) It's all about sex: bioluminescent courtship displays, morphological variation and sexual selection in two new genera of Caribbean ostracodes. J Crustacean Biol 30:56–67

Nakamura H, Kishi Y, Shimomura O, Morse D, Hastings JW (1989) Structure of dinoflagellate luciferin and its enzymic and nonenzymic air-oxidation products. J Am Chem Soc 111: 7607–7611

Nakatsu T, Ichiyama S, Hiratake J, Saldanha A, Kobashi N, Sakata K, Kato H (2006) Structural basis for the spectral difference in luciferase bioluminescence. Nature 440:372–376

Ow DW (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234:856–859

Pichler A, Prior JL, Luker GD, Worms DP (2008) Generation of a highly inducible Gal4→ Fluc universal reporter mouse for in vivo bioluminescence imaging. Proc Natl Acad Sci U S A 105: 15932–15937

Pietsch TW (2009) Oceanic anglerfishes: extraordinary diversity in the deep sea. University of California Press, Berkeley, 576pp

Rivers TJ, Morin JG (2008) Complex sexual courtship displays by luminescent male marine ostracods. J Exp Biol 211:2252–2262

Robison BH (1992) Bioluminescence in the benthopelagic holothurian *Enypniastes eximia*. J Mar Biol Assoc UK 72:463–472

Robison BH, Reisenbichler KR, Hunt JC, Haddock SHD (2003) Light production by the arm tips of the deep-sea cephalopod *Vampyroteuthis infernalis*. Biol Bull 205:102–109

Schmidt EW, Obraztsova AY, Davidson SK, Faulkner DJ, Haygood MG (2000) Identification of the antifungal peptide-containing symbiont of the marine sponge *Theonella swinhoei* as a novel delta-proteobacterium, "*Candidatus* Entotheonella palauensis". Mar Biol 136:969–977

Shimomura O, Johnson FH (1972) Structure of the light-emitting moiety of aequorin. Biochemistry 11:1602–1608

Shimomura O, Johnson FH (1975) Chemical nature of bioluminescence systems in coelenterates. Proc Natl Acad Sci U S A 72:1546–1549

Shimomura O, Goto T, Hirata Y (1957) Crystalline *Cypridina* luciferin. Bull Chem Soc Jpn 30:929–933

Soling A, Rainov NG (2003) Bioluminescence imaging in vivo–application to cancer research. Expert Opin Biol Ther 3:1163–1172

Subramaniam D (2008) Translation inhibition during cell cycle arrest and apoptosis: Mcl-1 is a novel target for RNA binding protein CUGBP2. Am J Physiol Gastrointest Liver Physiol 294:G1025–G1032

Takahashi T (2001) White band on upper jaw of megamouth shark, *Megachasma pelagios*, and its presumed function (Lamniformes: Mecachasmidae). Bull Fish Sci 52:125–129

Takeuchi A, Nakamura M, Suzuki C, Ryufuku M (2005) Isolation and structure determination of the luciferin of the dinoflagellate, *Noctiluca scintillans*. Nippon Kagakkai Koen Yokoshu 85:1461

Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347

Tsuji FI (1955) The absorption spectrum of reduced and oxidized *Cypridina luciferin*, isolated by a new method. Arch Biochem Biophys 59:452–464

Vallin A, Jakobsson S, Lind J, Wiklund C (2006) Crypsis versus intimidation—anti-predation defence in three closely related butterflies. Behav Ecol Sociobiol 59:455–459

Voss GL (1967) Squids: jet-powered torpedoes of the deep. Natl Geogr Mag 131:386–411

Warner JA, Case JF (1980) The zoogeography and dietary induction of bioluminescence in the midshipman fish, *Porichthys notatus*. Biol Bull 159:231–246

Webster MS, Roos CE, Roberts A, Okada A, Ohashi Y (1991) Mechanical stimulation of bioluminescence in the deep Pacific Ocean. Deep Sea Res A 38:201–217

Williams GC (2001) First record of a bioluminescent soft coral: description of a disjunct population of *Eleutherobia grayi* (Thomson and Dean, 1921) from the Solomon Islands, with a review of bioluminescence in the *Octocorallia*. Proc Calif Acad Sci 52:209–225

Woodland DJ, Cabanban AS, Taylor VM, Taylor RJ (2002) A synchronized rhythmic flashing light display by schooling *Leiognathus splendens* (Leiognathidae: Perciformes). Mar Fresh Res 53:159–162

Wu C, Kawasaki K, Ogawa Y, Yoshida Y, Ohgiya S, Ohmiya Y (2007) Preparation of biotinylated *Cypridina* luciferase and its use in bioluminescent enzyme immunoassay. Anal Chem 79:1634–1638

Xia Z, Rao J (2009) Biosensing and imaging based on bioluminescence resonance energy transfer. Curr Opin Biotechnol 20:37–44

Yu AY, Timiryasova T, Zhang Q (2003) Optical imaging: bacteria, viruses, and mammalian cells encoding light-emitting proteins reveal the locations of primary tumors and metastases in animals. Anal Bioanal Chem 377:964–972

Chapter 26
Preparation of Useful Components from Marine Algal Processing By-products and Their Applications

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1 Introduction

The ocean is an untapped potential of bioresources containing functional and health-promoting effects for mankind. Among the marine bioresources, marine algae have gained a promising position in the ocean, and that enables it to be sustained as natural sources for future prospects. Marine algae exhibit substantial varieties among

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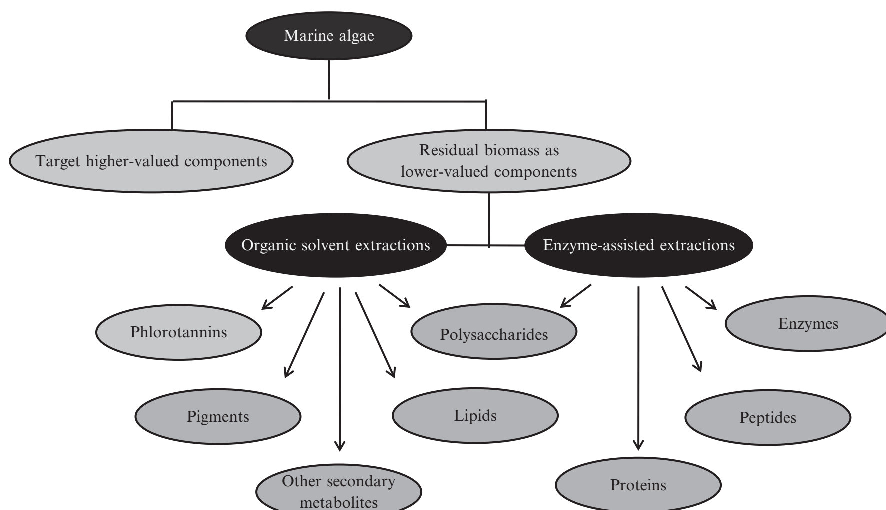

Fig. 26.1 Flow chart for the possible functional ingredients that can be obtained from marine algae processing by-products

the marine species and comprise a broad spectrum of functional ingredients, including proteins, polysaccharides, polyunsaturated fatty acids (PUFAs), minerals, vitamins, and pigments (Becker 2007; Burtin 2003; Gouveia et al. 2009; Aneiros and Garateix 2004). Marine algae can be divided into two major groups, seaweed and microalgae. Furthermore, seaweeds are broadly classified as profound marine organisms which contain three groups based on the pigmentation: brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae), and green seaweed (Chlorophyceae). In contrast to seaweeds, marine microalgae are classified into three major groups: blue-green algae, dinoflagellates, and diatoms (El Gamal 2010).

Marine algae have been utilized in many parts of the world for a long time, especially in Asia-Pacific and Southeast Asian countries, and are traditionally consumed as a part of the diet because they are rich sources of nutrients. On the other hand, western countries have used them for thickening and gelling agents, including alginate, carrageenan, and agar for industrial applications (Kong et al. 2009; Shibata et al. 2008). However, nowadays, marine algae bioresources have become a more sophisticated field of research combining the profound biological activities with active components having different structural integrities for functional applications such as pharmaceuticals, cosmeceuticals, and nutraceuticals (Gómez-Ordóñez et al. 2010; Gupta and Abu-Ghannam 2011). Hence, the harvesting of marine algae from marine systems for possible functional applications has increased tremendously in the past several decades. Based on only the activity or valuable components that are isolated and characterized from the complete marine biomass, the remainder of the residue biomasses will be removed or thrown away as waste products. However, in contrast to the significant output, the wastes and by-products also account for a high percentage of the biomasses and are discarded without being used as economically valued materials (Fig. 26.1).

Therefore, in this chapter, we demonstrate the possible applications of marine algal processing by-products using appropriate extraction methodologies for the isolation of bioactive components. Furthermore, functionalities are discussed with respect to the pharmaceutical and nutraceutical properties which can be employed on marine algae processing by-products in order to review the sustainability of marine bioresources and their perspectives.

2 Different By-products with Marine Algal Processing

The term "by-products" entails the lower valued residual biomasses removed from the processing industries and which require further processing in order to become useful (Rustad et al. 2011). In fact, there is a significant output concerning the marine algal processing by-products identified as valuable therapeutics and industries materials. Especially, marine algae-based applications such as foods, cosmetics, biofuels, and seaweed natural biopolymers are suggested to be the major industries, and they are able to enhance value of the by-products. Due to the scarcity of natural marine algal bioresources and increasing expectations on the health benefits with respect to the marine commodities, the recent trend has been directed towards the marine processing by-products. Indeed, it has huge unexploited potential for value-adding materials compared to the natural biomass. However, the discovery of functional components and their activities from marine algae waste has been poorly discussed. Therefore, major components, including polyphenols, polysaccharides, pigments, proteins, and peptides, can be obtained by either organic solvents or enzyme-assisted extractions from marine algal by-products. Hence, the rest of the chapter will review the applicable extraction techniques and strategies which are undertaken for harnessing the value-added components.

3 Components from Organic Solvent Extractions of Marine Algal By-products

Marine algae provide a substantial opportunity for research and can be attributed to comprehensive studies as well as providing economical benefits for mankind. In fact, in terms of the macroalgae, the annual turnover is estimated to be approximately US$ 7 billion over the whole world. However, this has been contributed only by eight countries and limited to six seaweed genera (Lorbeer et al. 2013). As well as this, a large amount of marine algae has been utilized to screen the bioactive compounds in the field of medicine and related pharmacological aspects by many researchers (Smit 2014). Hence, the pharmacological effects of marine algae have been well documented and described in detail, especially in the region of Asia-Pacific countries, including Korea, Japan, China, Australia, and Malaysia (Lorbeer et al. 2013). However, algae by-products and value-adding materials for therapeutic applications were rarely discussed.

3.1 Polyphenols/Phlorotannins

Over the years, organic solvent extractions have been significantly developed to isolate and characterize secondary metabolites. Among the marine macroalgae, brown seaweeds are well known to accumulate a variety of phloroglucinol-based polyphenols, so-called phlorotannins (Singh and Bharate 2006). These molecules are derived from many species of brown algae, including *Ecklonia cava*, *Ecklonia stolonifera*, *Ecklonia kurome*, *Eisenia bicyclis*, *Ishige okamurae*, *Sargassum thunbergii*, *Hizikia fusiformis*, *Undaria pinnatifida*, and *Laminaria japonica* (Li et al. 2011). Moreover, these seaweeds have become the most popular edible algae in the Asia-Pacific region. Therefore, it is believed that algae processing industries have focused on commercial applications where the by-products can be used, because the available bioactive phlorotannins have been reported to show promising health benefits, including antioxidant activity (Shibata et al. 2008; Kang et al. 2013), ACE inhibitory activity (Jung et al. 2006), anticancer activity (Ryu et al. 2009), antidiabetic activity (Lee and Jeon 2013), etc. Hence, these bio-functionalities have a significant influence on the value-added materials which can be extracted from marine algae processing by-products.

Recently, Wijesinghe et al. (2012c) worked on the *E. cava* processing by-products which are fermented by yeast, *Candida utilis*, to determine the antioxidant effect. According to their studies, *E. cava* fermented by-products showed profound activities, related to the available and retained polyphenolic compounds. Furthermore, the same research group has determined the anti-inflammatory activity of the fermented *E. cava* by-products against lipopolysaccharide-stimulated raw macrophages (Wijesinghe et al. 2013). According to their determinations, *E. cava* extracts were shown to retain their potential activity, even after polyphenol extractions were made for cosmetic industrial applications. In fact, an appropriate fermentation process can be identified as the breakdown process of complex substrates into a mixture of simple compounds without causing oxidation (De Lucas et al. 2007). Hence, marine algal processing by-products can be targeted as important pharmacological tools concerning the value-added screening of bioactive polyphenols.

3.2 Polysaccharides

Marine algae-derived polysaccharides is a recent emergent field of research that is rendering a vast array of solutions for modern applications. The industry based on the hydrocolloids is known as phycocolloids and has shown a global value of over US$ 1 billion in 2009 (Bixler and Porse 2011). With the existing commercial trend for polysaccharides from marine algae, there several few kinds of significant materials available, such agar, carrageenan, and sulfated galactans from red algae, as well as alginate from brown algae (Lorbeer et al. 2013). In addition, laminarins from

brown algae and ulvans from green algae are also an interesting form of polysaccharides (Read et al. 1996; Yaich et al. 2013). On the other hand, fucose-rich sulfated polysaccharides and fucoidans have gained much attention in various industries recently (Wijesinghe and Jeon 2012b). These phycocolloids are extensively used in modern applications, which are listed in Table 26.1.

Table 26.1 Possible phycocolloids isolated from marine algae processing by-products and their applications

Phycocolloids	Marine algae	Applications	Properties	References
Agar	**Rhodophyceae:**	Gelling agents in foods	Colloid formed with water	Selby and Whistler (1993)
	Gracilaria chilensis	Thickeners and emulsifiers	Resistant to high temperatures	
	Gelidium spp.	Solid culture media	Holds large amounts of soluble solids	
	Pterocladia spp.	Gels for separation and molecular biology research	Strong brittle gel formation	
Alginates	**Phaeophyceae:**	Thickeners and emulsifiers	Viscous solution and forming gels with water	Cardozo et al. (2007)
	Laminaria spp.	Paper and textile sizing	Water absorption ability, even when cold	
	Durvillaea potatorum	Fire- and water-proofing of fabrics	Chelating metal ions	
	Ascophyllum	Additives to dehydrated products	Non-melting chemical gel formation	
	Ecklonia cava	Thickening agent for food, beverages, and cosmetics	Stabilizers for beverages	
	Ecklonia radiata *Eisenia* spp. *Sargassum* spp.	Pharmaceuticals as slow release for drugs		
Carrageenans	**Rhodophyceae:**	Stabilizers for cosmetics and toothpaste	High-quality thermal gel formation	McHugh (2003), Smit (2004)
	Chondrus spp.	Gelatinous for lubricants	Protein reactivity	
	Gigartina spp. *Eucheuma* spp. *Furcellaria* spp.	Antiviral agents in pharmaceutical products	Stabilizers and emulsifiers	

(continued)

Table 26.1 (continued)

Phycocolloids	Marine algae	Applications	Properties	References
Fucoidan	*Undaria pinnatifida*	Pharmacological applications and anticoagulant activity	L-fucose monomers and sulfate esters	Li et al. (2008), Athukorala et al. (2006), Wijesinghe and Jeon (2012b)
	Scytosiphon lomentaria	Anti-inflammation		
	Macrocystis pyrifera	Antitumor		
	Durvillaea antarctica	Antiviral		
	Ecklonia cava	Functional foods		
		Cosmeceuticals		
Ulvan	*Ulva lactuca*	Pharmacological applications	High alcohol precipitates	Yaich et al. (2013), Qi et al. (2012)
	Ulva rigida	Antiviral	High uronic acid contents	
		Anticoagulants	High ash contents	
		Antihyperlipidemic		
Laminarins	**Phaeophyceae:**	Pharmacological applications	β-1 → 3 linked D-glucans residue	Lépagnol-Descamps et al. (1993), Choi et al. (2011)
	Haliotis tuberculata	Antioxidant activity		
	Eisenia bicyclis			

The phycocolloid-extracting industries generate a large amount of waste and by-products which are rich with the cell-wall polymers. For example, in the alginate processing industry, brown seaweeds are stabilized by formaldehyde for a while and washed several times using acidic water. Then laminarins and fucans can be removed. However, the resulting product of alginic acid can be further converted into the soluble form of sodium alginate by using sodium carbonate (Fleury and Lahaye 1993). Moreover, extraction methods for polysaccharides can be employed due to the presence of interfering substances of algae. Basically, polysaccharides can be used to extract water- or ethanol-based organic solvents. Despite this, enzyme-assisted extractions have also been employed as an alternative method, which enhanced the efficacy of the extraction process on the industrial scale (Wijesinghe and Jeon 2012b). However, further process development and value-enhancing efficacy is needed in order to sustain the utilization of resources.

3.3 Pigments

Among the important marine algae sources of functional ingredients, marine algae natural pigments are described as some of the most attractive materials (Pangestuti and Kim 2011). Most of the algae processing industries have not been concerned with the pigments that can be removed. However, the potential uses of natural

pigments of been explored, which can be confirmed with the higher amount of bioactivities. Moreover, their efficacy depends upon the structural differences and therapeutic value, which seem to be valuable aspects in advances in biotechnology and pharmacology.

Marine algae pigments are found in three classes: chlorophylls, carotenoids, and phycobiliproteins. Among them, chlorophylls are the most diverse form of pigment found in almost all algae, including seaweeds and microalgae. The natural abundance of this pigment means that its use has great diversity in food, cosmetics, and industrial applications (Frankmolle and Fenical 1994). However, the extraction of chlorophylls has not been studied in detail. In fact, for this purpose, supercritical fluid extraction is facilitated in order to speed up the process and is an alternative method of obtaining chlorophyll extractions with the highest purity (Macías-Sánchez et al. 2007). Among the bioactive potentials of natural pigments, carotenoids take the highest place among marine algae pigments. It is suggested that it consists of unsaturated hydrocarbons in the carotenes and xanthophylls. Moreover, it contained functional groups with oxygen molecules, which also supported their bioactivity. Fucoxanthin is the most abundant pigment from the carotenoid pigments family, and it is found in the brown seaweeds and diatoms (Matsuno 2001). In a recent research, Kim et al. (2013) showed the inhibitory effect of tumor growth in vitro and in vivo by fucoxanthin isolated from brown seaweed, *Ishige okamurae*. This seaweed has been commonly consumed by Koreans and used for folk medicine for a long time. Furthermore, its identified health benefits, including hepatoprotective (Das et al. 2008) and antioxidant activity, were reported by fucoxanthin isolated from *Undaria pinnatifida* (Liu et al. 2011).

Other natural pigments that can be isolated from marine algae processing by-products are phycobiliproteins. There are several major classes of this pigment: phycocyanins, allophycocyanins, and phycoerythrins (Pangestuti and Kim 2011). Among them, C-phycocyanin, a type of water-soluble protein pigment from *Spirulina platensis*, has shown an inhibitory effect against CCl_4-induced lipid peroxidation in the rat liver in vivo. Moreover, this has been tested on the growth and multiplication of the human chronic myeloid leukemia cell line (K562) (Subhashini et al. 2004). In another study, the isolated C-phycocyanin from *S. platensis* treated on the human hepatocarcinoma cell line (HepG2) revealed a downregulation of the expression of multidrug resistance protein-1 (MDR-1). In addition, it was noted that the ROS and cyclooxygenase-2 (COX-2)-mediated pathways are involved through NF-κB and AP-1 (Nishanth et al. 2010). These findings ensure that the marine algae pigments have pharmacological potential and further studies need to be undertaken on its extraction from marine algal processing by-products.

4 Components from the Enzyme-Assisted Extraction of Marine Algal By-products

Bioactive peptides are found naturally in foods as independent components that enable differentiation into active forms by hydrolyzation of their parent proteins (Samarakoon and Jeon 2012). Interestingly, the harnessing of peptides from

Table 26.2 Possible proteolytic enzymes and the optimized conditions used for the isolation of proteins and peptides from marine algae processing by-products

		Optimum conditions	
Proteolytic enzyme	Enzyme origin	Temperature (°C)	pH
Alcalase	*Bacillus licheniformis*	50	7
α-Chymotrypsin	Bovine pancreas	37	8
Flavourzyme	*Aspergillus oryzae*	50	7
Kojizyme	Selected *Bacillus* and *Aspergillus* strains	40	6
Neutrase	*Bacillus amyloliquefaciens*	50	8
Papain	*Carica papaya*	37	6
Pancreatin		45	7.5
Pepsin	Porcine gastric mucosa	50	2
Protamex	*Bacillus* spp.	40	6
Protease S	*Bacillus stearothermophilus*	90	7
Trypsin	Bovine, porcine, or human pancreas	37	5

precursor proteins can be attempted using proteolytic hydrolyzation in vitro, fermentation, and autolysis (Kim and Wijesekara 2010). Hence, the identified rich content of marine microalgae proteins can be described as alternative food sources for the extraction of bioactive proteins and peptides (Samarakoon et al. 2013). Components of proteins containing sequences that comprise 3–20 amino acids residues exert physiological health-promoting effects in the body. Moreover, the identified amino acid sequences and their activity-based entities yield certain benefits, including antioxidative (Sheih et al. 2009b), ACE inhibitory activity (Sheih et al. 2009a), anticancer (Sheih et al. 2010), immune-modulating (Kralovec et al. 2007), and hepatoprotective (Kang et al. 2012) activities by in vitro and in vivo assays (Morris et al. 2007). These reports confirm that marine algae processing waste protein by-products have potential for further applicable utilization. In addition, marine algae processing industrial by-products are targeted as alternative sources for the exploration of enzymes, lectins, and taurines. Despite these facts, in the search for new bioactive proteins and peptides from the marine microalgae, the extraction procedures have to be accomplished using the latest biotechnological tools.

4.1 Proteins and Peptides

Enzymes are catalysts in biological systems and play an important role in food processing and commercial applications. Appropriate physicochemical conditions such as pH and temperatures are needed in order to produce the desired proteolytic enzymes (Table 26.2). Most of the precursor proteins in the marine microalgae are associated with the complex form of proteins. However, the selection of appropriate hydrolytic enzymes can be employed to obtain the desired output. Moreover,

the maximum yield of enzymatic hydrolysates can be recovered using appropriate digestive conditions in order to release bioactive peptides (Wijesinghe and Jeon 2012a). In that case, the incubation time is a crucial factor in the hydrolysis process. Especially, bioactivity-guided evidence must be noteworthy so as to adjust the incubation time in order to obtain the highest activity (Ko et al. 2013). Hence, discovering the best conditions for microalgae protein extractions has become an important practice in recent reports. In fact, blue-green algae possesses the highest content of proteins (6–71 % of the dry weight) and has become a targeted source for the isolation of biologically active proteins and peptides (Samarakoon et al. 2013). A number of studies have shown that protein by-products from *Chlorella vulgaris* were the subjected species from marine microalgae and were digested by different proteases. However, pepsin enzymatic hydrolyzation was achieved in order to derive bioactive proteins and peptides in many instances (Sheih et al. 2009a; Sheih et al. 2010). In contrast to microalgae, the protein content of the seaweeds is low. Besides, brown algae has shown a lower protein content (3–15 % of the dry weight) compared to the green and red seaweeds (10–47 % of the dry weight) (Fleurence 1999). It is believed that the available high phenolic content in the brown and green seaweeds might have a low level of phenol, leading to a high content of proteins. Despite that, the availability of proteins in the sources should be determined, with particular interest for the method of separation, isolation, and characterization.

4.2 Polysaccharides and Oligosaccharides

Marine algae, especially seaweeds, contain various cell-wall-bound compounds. Among them, polysaccharides are described as the major constituents. The basic and classical extraction methods, such as organic solvents or aqueous solvents, are unable to release these components and the extraction yields vary from 8 % to 30 % (Robic et al. 2009). Moreover, the presence of various polysaccharide compounds reduces the extraction efficacy and purity of the extractions with the complex form of counterparts during the use of classical extraction techniques. Importantly, cell-wall-associated polysaccharides are composed of large and complex forms of biopolymers, including cellulose, lignin, pectin, and hemicelluloses (Doi and Kosugi 2004). Hence, high-yielding marine algae processing by-products can be considered as valuable output in regards to containing heterogeneous complexes from proteins and polysaccharides. Therefore, it is impossible separate them by the usual procedures and carbohydrase-based enzyme-assisted extractions will be the focus in the future (Wijesinghe and Jeon 2012a). In fact, appropriate enzymes and their optimum conditions used to degrade the cell-wall polysaccharides were very likely to interfere with access to the inner materials, due to the fundamental steps leading to the release of bioactive components. Furthermore, treating with specific enzymes can improve the extraction efficacy and may have the potential to release oligosaccharides and monosaccharides as well.

5 Industrial Applications of Extracts from Marine Algal By-products

Seaweed processing by-products have gained considerable evidence suggesting that they can be applied for industrial applications with new trends. They are based on micronutrients and auxins like hormones which are found in some seaweed by-products and are known to stimulate the growth of plants through several potential mechanisms. A leading seaweed by-product manufacturer, AlgeFiber™, ProNova Biopolymers, Inc., Norway, has been processing materials for soil amendments (Jeng and Vigerust 1985). This was done by removing the alginic acid from the raw seaweed by extraction using sodium hydroxide and sodium bicarbonate, leaving the fiber by-products containing sodium-enriched nutrients. Moreover, Craigie (2011) has reported that both micro- and macroalgae-soluble constituents are applicable to improving plant and animal nutrition and productivity. They are mainly manufactured from the brown seaweeds, including *Ascophyllum nodosum*, *Laminaria* spp., *Ecklonia maxima*, *Sargassum* spp., and *Durvillaea* spp., as well as their by-products (Gandhiyappan and Perumal 2001). These ideas have been confirmed in a recent report detailing the production of compost from marine waste, allowing conditioning of the soil and enriching its agricultural properties (Illera-Vives et al. 2013). On the other hand, the aquaculture industries have experimented with seaweed water-soluble natural products to eliminate fish pathogens. The red alga *Asparagopsis taxiformis* can be used to release the halogenated metabolites into the surrounding environments and inhibiting the growth of pathogens by seaweed soluble by-products (Mata et al. 2013).

Moreover, there is great interest in microalgae as sources for renewable energy and biofuels (Borowitzka and Moheimani 2013). In addition, recent trends and the development of economically viable strategies are important in order to understand the future industries (Pignolet et al. 2013). This recently published report focused on a novel process for adding value to algae residue. In this case, the currently processed oleaginous microalgae is grown and harvested for lipid production, leaving a lipid-free algae residue. The process is described with conversion of the carbohydrate fraction into glucose prior to lipid extraction. Furthermore, this bioconversion step was found to increase the free water content by 60 % and it was also found that, when the bioconversion was carried out prior to the extraction step, it improved the solvent extractability of lipids from the algae (Trzcinski et al. 2012).

In order to maximize the efficiency of marine algae processing by-products, the downstream processing system needs to be understood, as well as purification and quality control strategies. In addition, the latest improvements in biotechnology have led to the understanding of the different energy requirements, and extraction steps may furnish useful industrial applications. In the case of the increasing trend of harvesting natural products from marine algae, it its use in large-scale industries will increase on a daily basis. In fact, the interesting aspect lies on the future potential of by-products more than what it is today. Therefore, establishing appropriate methodologies in order to manage the scope of harvesting functional ingredients from marine algae is required in the future.

6 Conclusion

In this chapter, we have reviewed the possible applications of the marine algae processing by-products from lower valued residual biomass to increase their efficacy with respect to biotechnological and pharmacological aspects. In the development of marine algae commercialization practices, understanding innovative technology used for the handling and processing of algae low-profile residues will provide opportunities to improve the capabilities and qualities of products. Moreover, this will support reductions in environmental and ecological impacts, and facilitates the management of bioresources in a fruitful manner.

References

Aneiros A, Garateix A (2004) Bioactive peptides from marine sources: pharmacological properties and isolation procedures. J Chromatogr B 803:41–53

Athukorala Y, Jung W-K, Vasanthan T, Jeon Y-J (2006) An anticoagulative polysaccharide from an enzymatic hydrolysate of *Ecklonia cava*. Carbohydr Polym 66:184–191

Becker EW (2007) Micro-algae as a source of protein. Biotechnol Adv 25:207–210

Bixler HJ, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. J Appl Phycol 23:321–335

Borowitzka MA, Moheimani NR (2013) Sustainable biofuels from algae. Mitig Adapt Strat Glob Chang 18:13–25

Burtin P (2003) Nutritional value of seaweeds. Elec J Environ Agr Food Chem 2:498–503

Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E (2007) Metabolites from algae with economical impact. Comp Biochem Physiol C 146:60–78

Choi J-I, Kim H-J, Lee J-W (2011) Structural feature and antioxidant activity of low molecular weight laminarin degraded by gamma irradiation. Food Chem 129:520–523

Craigie JS (2011) Seaweed extract stimuli in plant science and agriculture. J Appl Phycol 23: 371–393

Das SK, Hashimoto T, Kanazawa K (2008) Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. Biochim Biophys Acta (BBA) 1780:743–749

De Lucas A, Rodriguez L, Villaseñor J, Fernández FJ (2007) Fermentation of agro-food wastewaters by activated sludge. Water Res 41:1635–1644

Doi RH, Kosugi A (2004) Cellulosomes: plant-cell-wall-degrading enzyme complexes. Nat Rev Microbiol 2:541–551

El Gamal AA (2010) Biological importance of marine algae. Saudi Pharm J 18:1–25

Fleurence J (1999) Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends Food Sci Technol 10:25–28

Fleury N, Lahaye M (1993) Studies on by-products from the industrial extraction of alginate. J Appl Phycol 5:605–614

Frankmolle WP, Fenical W (1994) Isolation of the chlorophyll-related pigment isochlorin-e4 from the tropical red alga *Dasya pedicellata*. Phytochemistry 36:361–364

Gandhiyappan K, Perumal P (2001) Growth promoting effect of seaweed liquid fertilizer (*Enteromorpha intestinalis*) on the sesame crop plant (*Sesamum indicum L.*). Seaweed Res Util 23:23–25

Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P (2010) Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Res Int 43: 2289–2294

Gouveia L, Batista AP, Sousa I, Raymundo A, Bandarra NM (2009) Microalgae in novel food products. In: Hagen KN (ed) Algae: nutrition, pollution control and energy sources. Nova Science Publishers, New York, pp 265–300

Gupta S, Abu-Ghannam N (2011) Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci Technol 22:315–326

Illera-Vives M, Labandeira SS, López-Mosquera ME (2013) Production of compost from marine waste: evaluation of the product for use in ecological agriculture. J Appl Phycol 25:1395–1403. doi:10.1007/s10811-013-9997-3

Jeng AS, Vigerust E (1985) Investigations on the effects of the kelp-meal (Protatek BF40) on some physical properties of the soil. Rapport 1, Institute for Jordkultur, Agricultural University of Norway

Jung HA, Hyun SK, Kim HR, Choi JS (2006) Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. Fisheries Sci 72:1292–1299

Kang K-H, Qian Z-J, Ryu BM, Kim D, Kim S-K (2012) Protective effects of protein hydrolysate from marine microalgae *Navicula incerta* on ethanol-induced toxicity in HepG2/CYP2E1 cells. Food Chem 132:677–685

Kang M-C, Cha SH, Wijesinghe WAJP, Kang S-M, Lee S-H, Kim E-A, Song CB, Jeon Y-J (2013) Protective effect of marine algae phlorotannins against AAPH-induced oxidative stress in zebrafish embryo. Food Chem 138:950–955

Kim S-K, Wijesekara I (2010) Development and biological activities of marine-derived bioactive peptides: a review. J Funct Foods 2:1–9

Kim K-N, Ahn G, Heo S-J et al (2013) Inhibition of tumor growth in vitro and in vivo by fucoxanthin against melanoma B16F10 cells. Environ Toxicol Pharmacol 35:39–46

Ko J-Y, Lee J-H, Samarakoon K, Kim J-S, Jeon Y-J (2013) Purification and determination of two novel antioxidant peptides from flounder fish (*Paralichthys olivaceus*) using digestive proteases. Food Chem Toxicol 52:113–120

Kong C-S, Kim J-A, Yoon N-Y, Kim S-K (2009) Induction of apoptosis by phloroglucinol derivative from *Ecklonia cava* in MCF-7 human breast cancer cells. Food Chem Toxicol 47:1653–1658

Kralovec JA, Metera KL, Kumar JR, Watson LV, Girouard GS, Guan Y et al (2007) Immunostimulatory principles from *Chlorella pyrenoidosa*—part 1: isolation and biological assessment in vitro. Phytomedicine 14:57–64

Lee S-H, Jeon Y-J (2013) Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. Fitoterapia 86:129–136

Lépagnol-Descamps V, Richards C, Lahaye M et al (1993) Purification and determination of the action pattern of *Haliotis tuberculata* laminarinase. Carbohydr Res 310:283–289

Li B, Lu F, Wei XJ, Zhao RX (2008) Fucoidan: structure and bioactivity. Molecules 13:1671–1695

Li Y-X, Wijesekara I, Li Y, Kim S-K (2011) Phlorotannins as bioactive agents from brown algae. Process Biochem 46:2219–2224

Liu C-L, Liang A-L, Hu M-L (2011) Protective effects of fucoxanthin against ferric nitrilotriacetate-induced oxidative stress in murine hepatic BNL CL.2 cells. Toxicol In Vitro 25:1314–1319

Lorbeer AJ, Tham R, Zhang W (2013) Potential products from the highly diverse and endemic macroalgae of Southern Australia and pathways for their sustainable production. J Appl Phycol 25:717–732. doi:10.1007/s10811-013-0003-x

Macías-Sánchez MD, Mantell C, Rodríguez M, Martínez de la Ossa E, Lubián LM, Montero O (2007) Supercritical fluid extraction of carotenoids and chlorophyll *a* from *Synechococcus* sp. J Supercrit Fluid 39:323–329

Mata L, Wright E, Owens L, Paul N, De Nys R (2013) Water-soluble natural products from seaweed have limited potential in controlling bacterial pathogens in fish aquaculture. J Appl Phycol 25:1963–1973. doi:10.1007/s10811-013-0043-2

Matsuno T (2001) Aquatic animal carotenoids. Fisheries Sci 67:771–783

McHugh DJ (2003) A guide to the seaweed industry. Food and Agriculture Organization of the United Nations, Rome

Morris HJ, Carrillo O, Almarales A, Bermúdez RC, Lebeque Y, Fontaine R et al (2007) Immunostimulant activity of an enzymatic protein hydrolysate from green microalga *Chlorella vulgaris* on undernourished mice. Enzyme Microb Technol 40:456–460

Nishanth RP, Ramakrishna BS, Jyotsna RG, Roy KR, Reddy GV, Reddy PK et al (2010) C-Phycocyanin inhibits MDR1 through reactive oxygen species and cyclooxygenase-2. Eur J Pharmacol 649:74–83

Pangestuti R, Kim S-K (2011) Biological activities and health benefit effects of natural pigments derived from marine algae. J Funct Foods 3:255–266

Pignolet O, Jubeau S, Vaca-Garcia C, Michaud P (2013) Highly valuable microalgae: biochemical and topological aspects. J Ind Microbiol Biotechnol 40:781–796. doi:10.1007/s10295-013-1281-7

Qi H, Liu X, Zhang J, Duan Y, Wang X, Zhang Q (2012) Synthesis and antihyperlipidemic activity of acetylated derivative of ulvan from *Ulva pertusa*. Int J Biol Macromol 50:270–272

Read SM, Currie G, Bacic A (1996) Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectrometry. Carbohydr Res 281:187–201

Robic A, Rondeau-Mouro C, Sassi J-F, Lerat Y, Lahaye M (2009) Structure and interactions of ulvan in the cell wall of the marine green algae *Ulva rotundata* (Ulvales, Chlorophyceae). Carbohydr Polym 77:206–216

Rustad T, Storrø I, Slizyte R (2011) Possibilities for the utilisation of marine by-products. Int J Food Sci Technol 46:2001–2014

Ryu BM, Li Y, Qian Z-J, Kim M-M, Kim S-K (2009) Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: implication for chronic articular disease. Chem Biol Interact 179:192–201

Samarakoon K, Jeon Y-J (2012) Bio-functionalities of proteins derived from marine algae—a review. Food Res Int 48:948–960

Samarakoon KW, Kwon O-N, Ko J-Y, Lee J-H, Kang M-C, Kim D, Lee JB, Lee J-S, Jeon Y-J (2013) Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (*Nannochloropsis oculata*) protein hydrolysate. J Appl Phycol 25:1595–1606. doi:10.1007/s10811-013-9994-6

Selby HH, Whistler RL (1993) Agar. In: Whistler RL, BeMiller JN (eds) Industrial gums: polysaccharides and their derivatives, 3rd edn. Academic Press, San Diego, pp 87–103

Sheih I-C, Fang TJ, Wu T-K (2009a) Isolation and characterisation of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste. Food Chem 115:279–284

Sheih I-C, Wu T-K, Fang TJ (2009b) Antioxidant properties of a new antioxidative peptide from algae protein waste hydrolysate in different oxidation systems. Bioresour Technol 100:3419–3425

Sheih I-C, Fang TJ, Wu T-K, Lin P-H (2010) Anticancer and antioxidant activities of the peptide fraction from algae protein waste. J Agric Food Chem 58:1202–1207

Shibata T, Ishimaru K, Kawaguchi S, Yoshikawa H, Hama Y (2008) Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. J Appl Phycol 20:705–711

Singh IP, Bharate SB (2006) Phloroglucinol compounds of natural origin. Nat Prod Rep 23:558–591

Smit AJ (2004) Medicinal and pharmaceutical uses of seaweed natural products: a review. J Appl Phycol 16:245–262

Subhashini J, Mahipal SVK, Reddy MC, Reddy MM, Rachamallu A, Reddanna P (2004) Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562. Biochem Pharmacol 68:453–462

Trzcinski AP, Hernandez E, Webb C (2012) A novel process for enhancing oil production in algae biorefineries through bioconversion of solid by-products. Bioresour Technol 116:295–301

Wijesinghe WAJP, Jeon Y-J (2012a) Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. Fitoterapia 83:6–12

Wijesinghe WAJP, Jeon Y-J (2012b) Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: a review. Carbohydr Polym 88:13–20

Wijesinghe WAJP, Lee W-W, Kim Y-M, Kim Y-T, Kim S-K, Jeon B-T, Kim J-S, Heu M-S, Jung W-K, Ahn G, Lee K-W, Jeon Y-J (2012) Value-added fermentation of *Ecklonia cava* processing by-product and its antioxidant effect. J Appl Phycol 24:201–209

Wijesinghe WAJP, Ahn G, Lee W-W, Kang M-C, Kim E-A, Jeon Y-J (2013) Anti-inflammatory activity of phlorotannin-rich fermented *Ecklonia cava* processing by-product extract in lipopolysaccharide-stimulated RAW 264.7 macrophages. J Appl Phycol 25:1207–1213. doi:10.1007/s10811-012-9939-5

Yaich H, Garna H, Besbes S, Paquot M, Bleker C, Attia H (2013) Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca*. Food Hydrocoll 31:375–382

Chapter 27
Functional Properties of Ascidians By-products: Nutritional and Medicinal Value

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1 Physiological Viewpoint of Ascidians

Ascidians, also known as sea squirts, are common marine animals which are sessile and attached to docks, rocks, or similarly suitable surfaces. They are the largest and most diverse class of the subphylum *Tunicata*. Because of these close ties, many scientists have shown interest in their genetic relationship to other invertebrate and vertebrate animals, biology, and applied biochemistry. As with many invertebrate groups, they typically have a motile larva that undergoes a metamorphosis into a sessile adult. Their larvae look like small tadpoles or very simplified small fishes,

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having a notochord and are free swimming, flanked by muscle bands and a dorsal hollow neural tube with a very simple body plan. Also, ascidians have openings with mucus-covered gill slits in the front part of their gut. Additionally, most mature ascidians possess a simple nervous system in spite of the complicated larval nervous system, consisting of a main dorsal hollow nerve cord situated on the notochord with small nerves leaving it. As their name implies, ascidians have cellulose as their main feature, which is a tunic or outer supportive/protective layer that almost completely surrounds the body, and secretes a specialized type of secretory epidermis (Millar 1971; Kozloff 1990; Ruppert et al. 2003). In others animal, however, the fibers are found to be deposited within the skin.

There are 2,300 species of ascidians, which consist of three main types: solitary, social ascidians that form clumped distributions by attaching at their bases, and compound ascidians with many small individuals. The ascidians live fastened to the substrate by their tunic and ciliary mucous suspension-feeding animals. Unlike other marine animals, ascidians extend inside the tunic without molting, which is probably due to their ability to resorb and redeposit tunic materials at the mantle–tunic interface. Some of the ascidians have channels in the tunic that are continuous with internal blood spaces, which allow for the secretion and redeposition of tunic material, as well as for the secretion of defensive materials into the tunic.

Various ascidians are commonly used as food, with their peculiar flavor originating from unsaturated alcohol called cynthiaol, ever since ancient times in Asia, particularly in Japan and Korea (i.e., *Halocynthia roretzi*, *Styela clava*) and also in some Western countries. There exists an aquaculture industry where it is cultivated on dangling cords made of palm fronds. In 1994, over 42,800 tons were produced, but since then, there have been problems with mass mortality events and only 4,500 tons were produced in 2004. Increased cultivation of ascidians leads to the generation of a large amount of biomass from the remainder of the catch, but there is no special utilization processing except for the intake, thus, they cause many problems related to environmental or resource utilization (Kelleher 2005). Consequently, much research has been concerned with the utilization of those by-products of ascidians that have potential as bioactive materials which have high value.

2 Utilization of Seafood Processing By-products

There is great potential in the marine bioprocess industry to convert and utilize the marine food by-products as valuable functional materials. There are number of examples on the utilization of by-products; isolation of bioactive compounds and rediscovery from the remaining fish muscle proteins, collagen, gelatin, fish oil, fish bone, internal organs, shellfish, and crustacean shells through suitable bioprocesses (Je et al. 2005; Jeon and Kim 2002; Kim et al. 2001). Followed by the application of technologies on these by-products, functional bioactive compounds have been found and investigated for nutraceutical and medicinal applications. The development of technologies on the reuse of by-products can result in high profitability and

also gives an important purpose to the rediscovery of bioactive compounds derived from by-products on resource utilization. Also, these bioactive compounds could be extracted through bioconversion technologies varying from simple to complex, and may include the preparation and isolation of bioactive peptides, oligosaccharides, fatty acids, enzymes, water-soluble minerals, and biopolymers to obtain nutraceutical potentials that are of benefit to human health promotion. Therefore, the development of technologies in search of novel bioactive compounds from by-products will yield more value from what is today considered a waste and represents unique challenges and opportunities for the seafood industry.

3 Nutritional and Medicinal Values of Ascidians Processing By-products

Ascidian is cultured on a large scale, thus bringing down the economic value of ascidians, in spite of the improving ascidians industry. As a result, these phenomenon lead to a considerable increase in the amount of discarded ascidians as processing leftovers, and that includes tunic. According this, questions regarding the utilization of the remainder of the tunic from ascidians after processing are proposed, possibly leading to serious problems. Much research has been carried out on the subject, which has found great potential in ascidians tunic to convert it into more valuable products that have nutritional and medicinal properties. These innovative efforts of incorporating the ascidians tunic in useful developments and applications came into the mainstream to realize their potential benefits.

3.1 Nutritional Value of Ascidians Tunic

Nutrition substances such as carbohydrates, proteins, lipids, and minerals in food and their biological studies are important in order to understand the possibility to be able to extend the properties of their nutrition. The ascidians from the *Halocynthia* genus are mostly useful only in terms of their contents and not their shells. However, a great number of researches on these by-products have investigated the nutritional values by comparison with the raw contents of ascidians. As a result, the decolorized tunic of the ascidian *Halocynthia roretzi* has similar components to raw *H. roretzi*, with crude protein comprising about 40 % and the total carbohydrate being almost 46 %, respectively, in the proximate composition (Lee et al. 1998, Table 27.1). Lee et al (1998) analyzed the amino acid profile of the extraction method among the decolorized tunic with acetone treatment, hydrolysis with neutrase, and autoclaving (Table 27.2). Their major amino acids contained 34.4 % of the total amino acid, that is, histidine, glutamic acid, and aspartic acid, and exhibited 31.7 % essential amino acid. Moreover, Lee et al. suggested that large quantities of Ca in *H. roretzi* tunic are similar to the raw contents of *H. roretzi* (Lee et al. 1998, Table 27.3).

Table 27.1 Proximate composition of raw *H. roretzi* and removed pigment from *H. roretzi* tunic (Lee et al. 1998)

	Dried in raw form[a]	Treated with acetone[b]
Moisture	8.5 ± 0.3	7.4 ± 0.2
Ash	4.1 ± 1.1	7.2 ± 0.5
Protein	40.3 ± 2.4	39.1 ± 1.8
Lipid	1.2 ± 0.1	0.3 ± 0.1
Carbohydrate	46.7 ± 1.6	46 ± 1.3

[a]Dried and ground ascidians tunic
[b]Pigments were removed from ascidians tunic

Table 27.2 Amino acid profiles of *H. roretzi* with different extraction methods (Lee et al. 1998; Jo et al. 2010)

		Ascidians tunic		
Amino acid	Raw *H. roretzi* (Unit: μmol/g)	Treated with acetone[a]	Neutrase[b]	Autoclaving[c]
Tau	–	0.5	0.2	0.0
Asp	3,192.99 ± 85.4	10.4	10.1	11.8
Thr	1,834.36 ± 46.3	4.7	5.1	5.3
Ser	2,066.61 ± 42.5	4.3	3.0	3.8
Glu	3,702.29 ± 88.2	11.0	9.1	12.2
Gly	3,656.93 ± 694.7	5.4	4.6	4.5
Ala	2,412.82 ± 107.7	4.5	2.6	3.8
Cys	200.91 ± 17.3	2.8	4.8	2.7
Val	1,731.96 ± 50.1	4.5	3.8	5.9
Met	624.48 ± 27.9	2.2	1.5	1.6
Ile	1,448.94 ± 46.3	4.2	2.6	3.8
Leu	2,105.15 ± 71.3	5.2	2.4	4.4
Tyr	751.10 ± 42.8	4.0	2.9	3.4
Phe	1,130.97 ± 34.5	4.6	2.8	3.7
Lys	2,202.08 ± 80.2	6.2	8.1	4.7
NH_3	4,376.55 ± 268.7	1.9	0.2	0.2
His	806.01 ± 89.7	13.0	29.2	20.4
Arg	1,542.91 ± 91.2	6.1	4.0	3.9
Pro	2,581.17 ± 65.4	4.5	3.1	3.8

[a]Pigments were removed from ascidians tunic
[b]Neutrase digestibles from ascidians tunic
[c]Water solubles prepared at 125 °C for 6 h (100 g of sample in 1.5 L of water)

Table 27.3 Mineral content of ascidians tunic (Lee et al. 1998)

	Ascidians tunic	
Mineral	Dried in raw form[a]	Treated with acetone[b]
Ca	512.8	563.6
Na	193.5	580.4
Cu	1.0	1.2
Mg	482.1	375.8
Mn	9.8	6.8
Fe	21.6	26.9
Zn	4.1	4.0
P	33.6	28.8
K	487.6	342.2

[a]Dried and ground ascidians tunic
[b]Pigments were removed from the ascidians tunic

Table 27.4 Proximate composition of *H. roretzi* and *H. aurantium* tunic using autoclave treatment (Kang and Choi 2004)

ä	Ascidians tunic[a]	
	H. roretzi	*H. aurantium*
Yield	5.4 ± 0.8	3.8 ± 0.3
Moisture	3.1 ± 0.1	4.2 ± 0.1
Ash	30.1 ± 1.1	27.9 ± 0.9
Protein	18.4 ± 0.8	19.7 ± 0.7
Lipid	0.5 ± 0.1	0.5 ± 0.1
Carbohydrate	48.0 ± 0.7	47.7 ± 0.9

[a]Dried and ground ascidians tunic

Table 27.5 Proximate composition of *Styela clava* tunic (Ahn et al. 2003)

	Styela clava tunic
Moisture	74.0 ± 0.1
Ash	0.8 ± 0.2
Protein	8.1 ± 0.1
Lipid	0.4 ± 0.1
Carbohydrate[a]	16.7 ± 0.2

[a]100 – (moisture + lipid + ash + protein)

These genetic variations do not make any difference to the nutritional values of *H. roretzi* tunic and *H. aurantium* tunic (Table 27.4) (Kang and Choi 2004). In addition, another ascidians tunic from *Styela clava* has the proximate composition as presented in Table 27.5 (Ahn et al. 2003).

3.2 Medicinal Value of Ascidians Tunic

Ascidians tunic was revealed to have biological compounds such as glycosaminoglycan, chondroitin sulfate, carotenoid, and pigments. Since Lönnberg (1931) first identified several kinds of specific xanthophyll and carotene in tunicate, Lederer (1938) also purified the α- and β-carotenes, astaxanthin, astaxanthin esters, cynthiaxanthin, and lycopene . Those efforts enabled the separation of astacene and cynthiaxanthin in *H. roretzi* tunic (Nishibori 1958). Tsuchiya and Suzuki (1960) reported that cynthiaxanthin and astaxanthin were the major carotenoids in *H. roretzi* tunic, and alloxanthin (cynthiaxanthin, pectenoxanthin) was identified by Campbell et al. (1967). These findings are very useful in helping to identify the properties of positive compounds from ascidians tunic associated with human health.

3.2.1 Antioxidants

The production of oxygen radicals in cellular systems is part of the normal metabolism, as oxygen is the ultimate electron acceptor in the electron flow system. However, if produced in excess, electron flow generates uncoupled radical species

Table 27.6 Biological activity of ascidians tunic

Bioactivity	Activity (%)	Species of ascidians tunic	Reference
Antioxidant			
DPPH radical assay	42.90 ± 0.74	*H. roretzi*	Jo et al. (2010)
	56.11 ± 3.64	*H. aurantium*	Jo et al. (2010)
	37.39 (1.5 mg)	*S. plicata*	Kim et al. (2005)
	29.9 %	*S. clava* (1 mg/ml)	Nacional et al. (2011)
ABTS radical assay	3.24 ± 0.92	*H. roretzi*	Jo et al. (2010)
	30.08 ± 0.11	*H. aurantium*	Jo et al. (2010)
	1.06 mM	*S. clava*	Park et al. (2010)
Total phenol (ppm, 10 mg/mL)	300.21 ± 3.82	*H. roretzi*	Jo et al. (2010)
	176.04 ± 0.72	*H. aurantium*	Jo et al. (2010)
TRAP activity	0.36 mM	*S. clava*	Park et al. (2010)
ABTS radical assay	1.06 mM	*S. clava*	Park et al. (2010)
Anti-inflammation			
VCAM-1/COX-2 inhibition		*H. roretzi*	Xu et al. (2008a)
VCAM-1/iNOS inhibition		*S. clava*	Xu et al. (2008b)
Anticancer			
Toxicity activity	29.0 μg/ml (ED_{50})	*S. clava* (50 μg/mL)	Park et al. (2010)
Antiproliferative	12.0 %	*S. clava* (500 μg/mL)	Seo et al. (2006)
Antihypertension			
Anticoagulants	20 %	*H. roretzi*	Hong et al. (2002)
ACE inhibition activity	34 %	*H. roretzi*	Hong et al. (2002)
Antibacterial	–	*H. roretzi*	Tsukamoto et al. (1994)

such as hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), and superoxide anions (O_2^-), which lead to an increase of reactive oxygen species (ROS), which significantly contribute to cell damage in aerobic cells. Specifically, oxidative free radicals leads to the oxidation of significant cellular biomolecules such as DNA, membrane protein, and membrane lipids, which are implicated in the etiology of a wide variety of diseases, including cutaneous fibrosis, chronic inflammation, and atherosclerosis, as well as in aging (Halliwell and Gutteridge 1999; Liu and Connally 1998). Therefore, antioxidant-rich sources and supplements are under the spotlight to offer extra protection. Although many chemical substances have been proved for their antioxidant activity, there has been a continuous search for new antioxidants from natural sources because of the poor bioavailability, cumulative effects inside the body, and adverse side effects of synthetic substances (Jorge et al. 2011). Many researches among ascidians tunics showed their strong antioxidant effects and they have similar or higher defense activity as the raw contents of ascidians (Table 27.6, Jo et al. 2010; Kim et al. 2005; Nacional et al. 2011; Park et al. 2010). From those researches, many kinds of compounds have been derived from ascidians tunic, such as cynthiaxanthin, and halocynthiaxanthin has been suggested as one of the best antioxidants (Table 27.7).

Table 27.7 Secondary metabolites of ascidians tunic

Compounds	Bioactivity	Species	Reference
Cynthiaxanthin	Antioxidant		Ha et al. (2000)
Halocynthiaxanthin	Antioxidant		Ha et al. (2000)
β-cryptoxanthin	Antioxidant		Zulueta et al. (2007)
Sulfate–polysaccharide	Anticancer		Okutani and Morikawa (1978), Okutani (1982)
α- and β-carotenes	DNA protection		Astley et al. (2004)
Lycopene	DNA protection		Torbergsen and Collins (2000)
Astaxanthin	DNA protection		Santocono et al. (2006)
Zeaxanthin	DNA protection		Santocono et al. (2006)
Lutein	DNA protection		Santocono et al. (2006)
2,6-Dimethylheptyl sulfate (4Z,7E)-4,7decadienyl sulfate (32,62)-3,6,9-Decatrienyl sulfate	Antibacterial and antifungal activity	*H. roretzi* *H. aurantium*	Tsukamoto et al. (1994)
Alloxanthin			Choi et al. (1994)
Halocynthiaxanthin			Choi et al. (1994)
Diatoxanthin			Choi et al. (1994)
Diadinochrome			Choi et al. (1994)
Mytiloxanthin			Choi et al. (1994)
Astaxanthin			Choi et al. (1994)
Sulfate–polysaccharide		*H. roretzi*	Okutani and Morikawa (1978), Okutani (1982)
Chondroitin sulfate	Anti-inflammatory activity		Xu et al. (2008a, b)
Halocynthiaxanthin	Inflammation		Ha et al. (2000)
Chondroitin sulfate	Cell proliferation activity		Kim et al. (2004)
	Collagen synthesis, recovery ability after UV damage		Kim et al. (2004)
Sulfate–polysaccharide	Anticoagulant	*H. roretzi*	Li and Lian (1988), Hong et al. (2002)
Sulfate–glycosaminoglycan	Anticoagulant		Gandra et al. (2000)
Dermatan sulfate		*Ascidia nigra*	Pavão et al. (1995); Merton and Thomas (1987), Maggi et al. (1987), Thomas et al. (1989)

3.2.2 Anti-inflammatory Compounds

Inflammation is a defense response in a wide variety of physiological and pathological processes caused by stress, injury, and infection. This process contributes to impairment on the immune system when persistent for a long period of time, as activated macrophages produce toxic factors (Medzhitov 2008). Nitric oxide

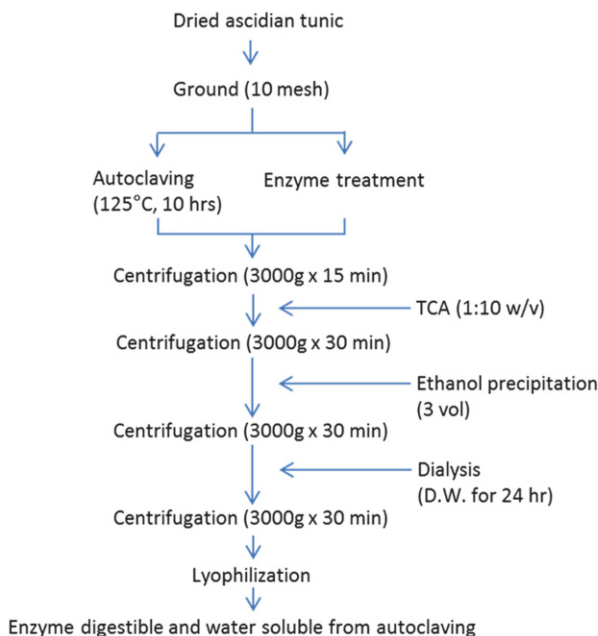

Fig. 27.1 Preparation of enzyme digestibles and water solubles from ascidians tunic (Lee et al. 1998)

(NO)/cyclooxygenase (COX) and nuclear factor-κB (NF-κB) are well-known inflammatory pathways which induce the production of inflammatory mediators such as prostaglandins, thromboxanes, leukotrienes, and cytokines. Thus, those kinds of inflammatory mediators or cytokines are studied in order to search for sources having anti-inflammatory activity, along with effects on the set of extracellular stimuli-dependent signal transduction cascades. Taken together, there has been much research in the field of searching for natural anti-inflammatory sources that are safer for consumption than synthetic compounds, as well as having a high potent biological activity (Yamamoto and Maruyama 1985; Okai et al. 1994). Therefore, some studies have proposed effective extraction methods for producing anti-inflammatories from ascidians tunic (Fig. 27.1, Lee et al. 1998; Okutani and Morikawa 1978; Okutani 1982).

Chondroitin sulfate is a member of the glycosaminoglycans (GAGs), which are naturally present in the extracellular matrix of articular cartilage. The GAGs are a group of structurally related polysaccharides known to have various activities found in the carbohydrate moieties of proteoglycans and sometimes as free polysaccharides (Mathews 1975). GAGs and sulfated GAGs may be components of connective tissue or cell surface carbohydrates involved in the cell's interaction with and response to its surroundings, together with participation in immune and inflammatory responses (Ruoslahti 1989; Okutani and Shigeta 1993).

From the ascidian *S. clava* tunic, the derived chondroitin sulfates are potent anti-inflammatory metabolites which inhibit the inflammatory factor, vascular cell

adhesion molecule-1 (VCAM-1), and iNOS, though by Akt/NF-κB in murine epidermal JB6 P+cells (transformation-sensitive subclone of JB6 cells, Xu et al. 2008a). Xu et al (2008b) also investigated the effect of chondroitin sulfate extracted from ascidians tunic on TPA-induced inflammation in mouse skin. In the inflammation response in mouse skin in vivo, chondroitin sulfate significantly inhibited TPA-induced NF-κB activation and subsequent inflammation cytokines, VCAM-1 and COX-2, were suppressed by IKKβ and Akt/PKB signaling; thus, they suggested that the chondroitin sulfate extracted from ascidians tunic was an effective anti-inflammation agent. Many kinds of biological compounds from ascidians tunic such as halocynthiaxanthin have been shown to have anti-inflammatory activity (Ha et al. 2000). These researches supported the observation that biological compounds isolated from ascidians tunic with special characteristics have significant effects on the human immune system.

3.2.3 Anticancer Activity

Apoptosis, or programmed cell death, is an active physiological process that occurs during embryonic development and tissue remodeling, and is a natural process for removing unneeded or damaged cells with potentially harmful mutations. However, the deregulation of apoptosis can disrupt the delicate balance between cell proliferation and cell death, and can lead to diseases such as cancer (Danial and Korsmeyer 2004). Most cancer cells block apoptosis via antiapoptotic signaling pathways in order to survive, despite undergoing genetic and morphologic transformations. Therefore, drugs promoting apoptosis may be effective against many cancers and should become an important strategy to counteract cancer (Fesik 2005). Some marine animals secrete toxic substances to protect themselves from their foe, and these substances in their natural forms have been studied as an effective anticancer source. Metabolites derived from ascidian *H. roretzi* tunic, sulfate polysaccharides, and pigments such as carotene, astaxanthin, and zeaxanthin have exhibited anticancer activity (Okutani and Morikawa 1978; Okutani 1982; Astley et al. 2004; Torbergsen and Collins 2000; Santocono et al. 2006). The acetone extract of *S. clava* also showed a significant anticancer effect on human colon cancer (HT-29) cells (Seo et al. 2006). Especially, among the acetone extractions of *S. clava* at different parts, tunic has shown to inhibit the growth of HT-29 cells (12.0 %) at the highest concentration of extracts (500 μg/ml) compared to whole or flesh (26.9 % and 30.6 %, respectively).

3.2.4 Antihypertensive and Anticoagulant Compounds

The GAGs consist of dermatan sulfate, chondroitin sulfate, and heparan sulfate. The glycosaminoglycan heparin is a crucial therapeutic agent used in the treatment of thrombosis and hypertension (Kakkar and Hedges 1989), and dermatan sulfate has been shown to have anticoagulant activity, although it has a lower

potency than heparin (Merton and Thomas 1987; Maggi et al. 1987; Thomas et al. 1989). Pavão et al (1995) compared the dermatan sulfate-like glycosaminoglycan from the body of the ascidian *Ascidia nigra* with mammalian dermatan sulfate and investigated the relationships in their structure and anticoagulant activity. From the results, they suggested that the unique glycosaminoglycan may help to determine the structural requirement for the anticoagulant activity of dermatan sulfate and, specifically, for binding to heparin cofactor II. Besides, sulfated polysaccharide from *H. roretzi* showed both anticoagulant and ACE inhibitory activity, displaying 34 % ACE inhibitory activity on the DEAE cellulose ion exchange active fraction (Hong et al. 2002).

3.2.5 Ingredients for Cosmaceuticals

Sulfate chondroitin in GAGs derived from ascidians tunic has a high content of SO_4^- or COO^- in chains that act to attract moisture, which might help to protect the skin against stress. Therefore, sulfate chondroitin, which has an anti-aging effect, is used as a cosmetic ingredient. Kim et al. tested the biological activities and safety with in vivo and in vitro experiments of sulfate chondroitin derived from ascidians *S. clava* and *H. roretzi* by examining the cell toxicity, collagen synthesis, cell growth, and recovery ability after UV irradiation (Kim et al. 2004). The results showed that sulfate chondroitin extracted from ascidians tunic can be considered as suitable cosmetic materials.

3.2.6 Other Biologically Active Compounds Derived from Ascidians Tunic

The effect of ascidians for their biologically active metabolites on human health has become one of the most active fields of studies among marine animals, due to their unusual structure with significant bioactivities (Faulkner 2002; Blunt et al. 2010). The chemistry of ascidians is dominated by the presence of nitrogenous metabolites, which could be divided into two structural type-based groups, peptides and polycyclic aromatic alkaloids (Menna et al. 2011). Pyridoacridine alkaloids isolated from ascidians are typically *tetra*- or *penta*-cyclic aromatic alkaloids based on the pyrido[*k,l*]acridine skeleton, usually possessing a functionalized alkylamine side chain; they are cytotoxic and some of them possess potent antiviral, antifungal, antibacterial, antitumor, and antiparasitic activity (Marshall and Barrows 2004). Shishijimicins which are perhaps the most complex carboline-based, alkaloids-derived ascidians, and *Didemnum proliferum* are reported as antibiotics which are also potent antitumor agents (Oku et al. 2003). There are other kinds of carboline-based alkaloids; bengacarboline exhibited the cytotoxicity towards a 26-cell line human tumor panel in vitro and inhibited the catalytic activity of TOPO II (Foderaro et al. 1997). Moreover, alkaloids isolated from *Eudistoma* ascidians have been shown to be potent regulators of cellular growth and differentiation, and affect

cAMP-mediated processes (He and Faulkner 1991; Rudi et al. 1988a, b; Searle and Molinski 1994; Viracaoundin et al. 2001).

Indole-based alkaloids, fascaplysin and its derivatives, exhibit a broad range of bioactivities, including antibacterial, antifungal, antiviral, HIV-1-RT, p56 tyrosine kinase, antimalarial, cytotoxicity against numerous cancer cell lines, and specific inhibition of CDK4 and DNA intercalation, demonstrating huge potential for therapeutic assays (Zhidkov et al. 2007). Tyrosine-derived alkaloids from ascidians and botryllamides (*Botryllus* species) exhibit activity as selective inhibitors of ABCG2, a human ATP-binding cassette (ABC) transporter gene usually associated with multidrug resistance in cancer (Henrich et al. 2009; Takada et al. 2010). Furthermore, lamellarin α 20-sulfate exhibited potent in vitro inhibition against HIV-1 integrase (Reddy et al. 1999). These biological components of ascidians have already received significant attention and several intriguing aspects of their chemistry are still being reported.

4 Concluding Remarks

An intensive research effort has been ongoing around the world to utilize seafood processing by-products, owing to the presence of functional materials. Among them is marine ascidians tunic, a processing waste identified as a potential source of nutritional medicinal substances. Previous research on ascidians tunics was mainly focused on their rich nutritional value; however, recent studies have revealed the potential of this ascidians by-product to be a source of biologically active compounds commonly bearing antioxidant, anti-inflammation, anticancer, antihypertension, and anticoagulant properties.

The discovery of nutritional and medicinal metabolites from ascidians tunic opens up novel avenues to the utilization and processing of this waste material, and, thereby, reduce the impact on pollution to the environment. With these interesting findings, ascidians tunic, a processing by-product, has great potential to be developed and bioconverted into value-added ingredients in the food, medicine, and cosmetics industries.

References

Ahn SH, Jung SH, Kang SJ, Jeong TS, Choi BD (2003) Extraction of glycosaminoglycans from *Styela clava* tunic. Kor J Biotechnol Bioeng 18(3):180–185

Astley SB, Elliott RM, Archer DB, Southon S (2004) Evidence that dietary supplementation with carotenoids and carotenoid-rich foods modulates the DNA damage: repair balance in human lymphocytes. Brit J Nutr 91:63–72

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2010) Marine natural products. Nat Prod Rep 27:165–237, and earlier reports in the series

Campbell SA, Matlams AK, Waight ES, Weedon BCL (1967) Pectenoxanthin, cynthiaxanthin and a new acetylenic carotenoid, pectenolone. Chen Commun 18:941–942

Choi BD, Kang SJ, Choi YJ, Youm MG, Lee KH (1994) Utilization of ascidian (*Halocynthia roretzi*) tunic. 3. Carotenoid compositions of ascidian tunic. Bull Kor Fish Soc 27(4):344–350

Danial NN, Korsmeyer SJ (2004) Cell death: critical control points. Cell 116(2):205–219

Faulkner DJ (2002) Marine natural products: metabolites of marine invertebrates. Nat Prod Rep 1:551–598, and earlier reports in the series

Fesik SW (2005) Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 5(11):876–885

Foderaro TA, Barrows LR, Lassota P, Ireland CM (1997) Bengacarboline, a new β-carboline from a marine ascidian *Didemnum* sp. J Org Chem 62:6064–6065

Gandra M, Cavalcante MCM, Pavão MSG (2000) Anticoagulant sulfated glycosaminoglycans in the tissues of the primitive chordate *Styela plicata* (*Tunicata*). Glycobiology 10(12): 1333–1340

Ha B-S, Baek S-H, Kim S-Y (2000) Carotenoids components of tunicate, shellfish and its inhibitory effects on mutagenicity and growth of tumor cell. J Kor Soc Food Sci Nutr 29:922–934

Halliwell B, Gutteridge JMC (1999) Free radicals, other reactive species and disease. In: Halliwell B, Gutteridge JMC (eds) Free radicals in biology and medicine, 3rd edn. Clarendon Press, Oxford, pp 617–783

He HY, Faulkner DJ (1991) Eudistones A and B: two novel octacyclic alkaloids from a Seychelles tunicate. *Eudistoma* sp. J Org Chem 56:5369–5371

Henrich CJ, Robey RW, Takada K, Bokesch HR, Bates SE, Shukla S, Ambudkar SV, McMahon JB, Gustafson KR (2009) Botryllamides: natural product inhibitors of ABCG2. Chem Biol 4:637–647

Hong BI, Jung BC, Son BY, Jung WJ, Ruck JH, Choi BD, Lee KH (2002) Utilization of pigments and tunic components of ascidian as an improved feed aids for aquaculture. J Kor Fish Soc 35(6):671–675

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

Jeon Y-J, Kim S-K (2002) Antitumor activity of chitosan oligosaccharides produced in ultrafiltration membrane reactor system. J Microbiol Biotechnol 12:503–507

Jo J-E, Kim K-H, Yoon M-H, Kim N-Y, Lee C, Yook H-S (2010) Quality characteristics and antioxidant activity research of *Halocynthia roretzi* and *Halocynthia aurantium*. J Kor Soc Food Sci Nutr 39(10):1481–1486

Jorge ATS, Arroteia KF, Lago JC, Sá-Rocha VMD, Gesztesi J, Moreira PL (2011) A new potent natural antioxidant mixture provides global protection against oxidative skin cell damage. Int J Cosmet Sci 33:113–119

Kakkar VV, Hedges AR (1989) In: Lane DA, Lindahl U (eds) Heparin. Edward Arnold, London, pp 455–473

Kang S-J, Choi B-D (2004) Extraction and application of bioactive compounds from ascidian tunic. J Agric Life Sci 38(4):103–111

Kelleher K (2005) Discards in the world's marine fisheries. An update. FAO Fisheries technical paper 470. FAO, Rome, 131pp

Kim S-K, Kim Y-T, Byun H-G, Nam K-S, Joo D-S, Shahidi F (2001) Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. J Agric Food Chem 49:1984–1989

Kim YR, Shin GW, Jung TS (2004) In vitro examination of chondroitin sulfates extracted Midduck (*Styela clava*) and Munggae Tunics (*Halocynthia roretzi*) as a cosmetic material. J Kor Soc Food Sci Nutr 33(4):646–652

Kim JJ, Kim SJ, Kim SH, Park HR, Lee SC (2005) Antioxidant and anticancer activities of extracts from *Styela plicata*. J Kor Soc Food Sci Nutr 34(7):937–941

Kozloff EN (1990) Invertebrates. Saunders College Publishing, Philadelphia, p 866

Leaderer E (1938) Recherches sur les carotenoids des invertebres (Research on carotenoid of invertebrate). Bull Soc Chim Bio 20:567–610

Lee K-H, Hong B-I, Choi B-D, Kang S-J, Ruck J-H, Jung B-C (1998) Utilization of pigments and tunic components of ascidian as an improved feed aids for aquaculture : effective extraction

methods of crude polysaccharides in ascidian (*Halocynthia roretzi*) tunic. J Kor Fish Soc 31(3):423–428

Li JZ, Lian EC (1988) Aggregation of human platelets by acidic mucopolysaccharide extracted from *Stichopus japonicus* Selenka. Thromb Haemost 59(3):435–439

Liu B, Connolly MK (1998) The pathogenesis of cutaneous fibrosis. Semin Cutan Med Surg 17:3–11

Lönnberg E (1931) Untersuchungen ueber das vorkommen carotenoid stoffe bei marine vertebrate (Study on carotenoid originated from marine vertebra). Ark Zool 22:1–49

Maggi A, Abbadini M, Pagella PG, Borowska A, Pangrazzi J, Donati MB (1987) Antithrombotic properties of dermatan sulphate in a rat venous thrombosis model. Haemostasis 17:329–335

Marshall KM, Barrows LR (2004) Biological activities of pyridoacridines. Nat Prod Rep 21:731–751

Mathews MB (1975) Polyanionic proteoglycans. In: Kleinzeller A, Springer GF, Wittmann HG (eds) Connective tissue: macromolecular structure and evolution. Springer, Berlin, pp 93–125

Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454:428–435

Menna M, Fattorusso E, Imperatore C (2011) Alkaloids from marine ascidians. Molecules 16:8694–8732

Merton RE, Thomas DP (1987) Experimental studies on the relative efficacy of dermatan sulphate and heparin as antithrombotic agents. Thromb Haemost 58:839–842

Millar RH (1971) The biology of ascidians. Adv Mar Biol 9:1–100

Nacional LM, Kang S-J, Choi B-D (2011) Antioxidative activity of carotenoids in Mideodeok *Styela clava*. Fish Aquat Sci 14(4):243–249

Nishibori K (1958) Studies on the pigments of marine animals-VI. Carotenoids of some tunicates. Publ Seto Mar Biol Lab Kyoto Univ Jpn 7:181–192

Okai Y, Higashi-Okai K, Nakamura S-i, Yano Y, Otani S (1994) Suppressive effects of the extracts of Japanese edible seaweeds on mutagen-induced *umu* C gene expression in *Salmonella typhimurium* (Ta 1535/pSK 1002) and tumor promotor-dependent ornithine decarboxylase induction in BALB/c 3T3 fibroblast cells. Cancer Lett 87(1):25–32

Oku N, Matsunaga S, Fusetani N (2003) Shishijimicins AC, novel enediyne antitumor antibiotics from the ascidian *Didemnum proliferum*. J Am Chem Soc 125:2044–2045

Okutani K (1982) Further investigation of the antitumor activity of the squid internal shell. Bull Japan Soc Sci Fish 48:421–424

Okutani K, Morikawa N (1978) Purification and characterization of the polysaccharide obtained from squid internal shell. Bull Jpn Soc Sci Fish 44:749–753

Okutani K, Shigeta S (1993) Inhibitory effect of sulfated derivatives of a marine bacterium polysaccharide on replication of human immune-deficiency virus in vitro. Nippon Suisan Gakk 59:1433–1448

Park J-H, Seo B-Y, Lee S-C, Park E (2010) Effects of ethanol extracts from stalked sea squirt (*Styela clava*) on antioxidant potential, oxidative DNA damage and DNA repair. Food Sci Biotechnol 19(4):1035–1040

Pavão MSG, Mourão PAS, Mulloy B, Tollefsen DM (1995) A unique dermatan sulfate-like glycosaminoglycan from ascidian: its structure and the effect of its unusual sulfation pattern on anticoagulant activity. J Biol Chem 270:31027–31036

Reddy MVR, Rao MR, Rhodes D, Hansen MST, Rubins K, Bushman FD, Venkateswarlu Y, Faulkner DJ (1999) Lamellarin α 20-sulfate, an inhibitor of HIV-1 integrase active against HIV-1 virus in cell culture. J Med Chem 42:1901–1907

Ruoslahti E (1989) Proteoglycans in cell regulation. J Biol Chem 264:13369–13372

Rudi A, Benayahu Y, Goldberg I, Kashmana Y (1988a) Alkaloid metabolites of the marine tunicate *Eudistoma* sp.: Segoline A, isosegoline A and nor-segoline. Tetrahedron Lett 29:3861–3862

Rudi A, Benayahu Y, Goldberg I, Kashman Y (1988b) Eilatin, a novel alkaloid from the marine tunicate *Eudistoma* sp. Tetrahedron Lett 29:6655–6656

Ruppert EE, Fox RS, Barnes RD (2003) Invertebrate zoology: a functional evolutionary approach, 7th edn. Brooks/Cole-Thomson Learning, Belmont, xvii (963):11–126

Santocono M, Zurria M, Berrettini M, Fedeli D, Falcioni G (2006) Influence of astaxanthin, zeaxanthin and lutein on DNA damage and repair in UVA-irradiated cells. J Photochem Photobiol B 85:205–215

Searle PA, Molinski TF (1994) Five new alkaloids from the tropical ascidian, *Lissoclinum* sp. lissoclinotoxin A is chiral. J Org Chem 59:6600–6605

Seo B-Y, Jung E-S, Kim J-Y, Park H-R, Lee S-C, Park E (2006) Effect of acetone extract from *Styela clava* on oxidative DNA damage and anticancer activity. J Kor Soc Appl Biol Chem 49:227–232

Takada K, Imamura N, Gustafson KR, Henrich CJ (2010) Synthesis and structure–activity relationship of botryllamides that block the ABCG2 multidrug transporter. Bioorg Med Chem Lett 20:1330–1333

Thomas DP, Merton RE, Barrowcliffe TW (1989) Relative efficacy of heparin and related glycosaminoglycans as antithrombotic drugs. Ann N Y Acad Sci 556:313–322

Torbergsen AC, Collins AR (2000) Recovery of human lymphocytes from oxidative DNA damage; the apparent enhancement of DNA repair by carotenoids is probably simply an antioxidant effect. Eur J Nutr 39:80–85

Tsuchiya Y, Suzuki Y (1960) Biochemical studies of the ascidian, *Cynthia roretzi* v. Drasche IV. Carotenoids in test. Tohoku J Agr Res 10:397–407

Tsukamoto S, Kato H, Hirota H, Fusetani N (1994) Antibacterial and antifungal sulfated alkane and alkenes from the hepatopancreas of the ascidian *Halocynthia roretzi*. J Nat Prod 57(11):1606–1609

Viracaoundin I, Faure R, Gaydou EM, Aknin M (2001) A new alkaloid from the purple Indian Ocean tunicate *Eudistoma bituminis*. Tetrahedron Lett 42:2669–2671

Xu C-X, Jin H, Chung Y-S, Shin J-Y, Lee K-H, Beck GR Jr, Palmos GN, Choi B-D, Cho M-H (2008a) Chondroitin sulfate extracted from ascidian tunic inhibits phorbol ester-induced expression of Inflammatory factors VCAM-1 and COX-2 by blocking NF-kappaB activation in mouse skin. J Agric Food Chem 56(20):9667–9675

Xu C-X, Jin H, Chung Y-S, Shin J-Y, Woo M-A, Lee K-H, Palmos GN, Choi B-D, Cho M-H (2008b) Chondroitin sulfate extracted from the *Styela clava* tunic suppresses TNF-alpha-induced expression of inflammatory factors, VCAM-1 and iNOS by blocking Akt/NF-kappaB signal in JB6 cells. Cancer Lett 264(1):93–100

Yamamoto I, Maruyama H (1985) Effect of dietary seaweed preparations on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. Cancer Lett 26(3):241–251

Zhidkov ME, Baranova OV, Balaneva NN, Fedorov SN, Radchenko OS, Dubovitskii SV (2007) The first syntheses of 3-bromofascaplysin, 10-bromofascaplysin and 3,10-dibromofascaplysin—marine alkaloids from *Fascaplysinopsis reticulata* and *Didemnum* sp. by application of a simple and effective approach to the pyrido[1,2-*a*:3,4-*b*′]diindole system. Tetrahedron Lett 48:7998–8000

Zulueta A, Esteve MJ, Frasquet I, Frígola A (2007) Vitamin C, vitamin A, phenolic compounds and total antioxidant capacity of new fruit juice and skim milk mixture beverages marketed in Spain. Food Chem 103:1365–1374

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