Marine Biotechnology (2018) 20:118–130
https://doi.org/10.1007/s10126-018-9799-3

REVIEW ARTICLE

Peptides from Fish By-product Protein Hydrolysates and Its Functional Properties: an Overview

Juan Zamora-Sillero[1,2] · Adem Gharsallaoui[3] · Carlos Prentice[1,2]

Received: 19 November 2017 / Accepted: 4 December 2017 / Published online: 13 March 2018

Abstract

The inadequate management of fish processing waste or by-products is one of the major problems that fish industry has to face nowadays. The mismanagement of this raw material leads to economic loss and environmental problems. The demand for the use of these by-products has led to the development of several processes in order to recover biomolecules from fish by-products. An efficient way to add value to fish waste protein is protein hydrolysis. Protein hydrolysates improve the functional properties and allow the release of peptides of different sizes with several bioactivities such as antioxidant, antimicrobial, antihypertensive, anti-inflammatory, or antihyperglycemic among others. This paper reviews different methods for the production of protein hydrolysates as well as current research about several fish by-products protein hydrolysates bioactive properties, aiming the dual objective: adding value to these underutilized by-products and minimizing their negative impact on the environment.

Keywords Fish by-product · Hydrolysis methods · Protein hydrolysates · Bioactive peptides

Introduction

Many countries worldwide have the fish industry as a pillar of their economy with an annual production of approximately140 million tons, of which about 80% is destined for human consumption (Benhabiles et al. 2012). It has been estimated that 1 billon people depend directly or indirectly on the trade and fish production (Oosterveer 2008). Nevertheless, fish trade presents underutilized fish by-products which include head, skin, trimmings, fins, frames, viscera, and roe that account for more than 60% of total biomass. Fish processing by-products are fish material left over from the primary processing of fish manufacturing process (He et al. 2013). In most cases, these fish by-products are discarded without intention of recovery (Halim et al. 2016). Moreover, discards of fish by-products have a great ecological impact and also considerably affect the economic viability of the fishing and aquaculture sector. The European Commission is carrying out modifications in the common fishery policy in order to the complete elimination of discards. Technical solutions are required to use fish by-products as raw materials for the production of added-value compounds (Morales-Medina et al. 2016).

Fish processing by-products are a great source of high quality compounds that may be used for human consumption. These by-products can be a great source of value added products such as proteins, amino acids, collagen, gelatin, oils, and enzymes (Ghaly et al. 2013). Moreover, up to 10–20% (*w*/*w*) of total fish protein can be found in fish by-products. In addition, the crude protein content of fish by-products varies from 8 to 35% (Sila and Bougatef 2016). The essential amino acids and bioactive peptides found in fish proteins have great potential for their use in the production of drugs and functional foods (Sila and Bougatef 2016). In order to recovery protein and peptides from fish by-products several methods such as acid or alkaline hydrolysis, autolysis and enzymatic hydrolysis have been developed. Non-hydrolyzed fish proteins do not possess these properties due to the poor accessibility to the

✉ Carlos Prentice
dqmprent@furg.br

[1] FURG, Universidade Federal do Rio Grande, Escola de Química e Alimentos, Laboratório de Tecnologia de Alimentos, Avenida Itália km 8 Campus Carreiros, Cx. P. 474, 96.203-900 Rio Grande, RS, Brazil

[2] FURG, Universidade Federal do Rio Grande, Estação Marinha de Aquicultura, Rua do Hotel 2 Querência, Cx. P. 474, Rio Grande, RS 96210-030, Brazil

[3] Univ Lyon, Université Lyon 1-ISARA Lyon, BioDyMIA (Bioingénierie et Dynamique Microbienne aux Interfaces Alimentaires), EA n°3733, IUT Lyon 1, Technopole Alimentec—Rue Henri de Boissieu, 01000 Bourg-en-Bresse, France

functional peptide sequence (Ghaly et al. 2013; Kim and Wijesekara 2010).

Enzymatic hydrolysis is a process carried out under moderate conditions of pH and temperature. Furthermore, it is a specific process with an easy control of the degree of hydrolysis as well as allows retaining the nutritional value of the source protein. In this process, several proteolytic enzymes are commonly used to hydrolyze the proteins and converting them into high added-value products with functional, biological, and nutritional properties (Kristinsson and Rasco 2000; Shahidi et al. 1995). Protein hydrolysates are proteins broken into peptides that contain between 2 and 20 amino acids. In this process, the source not only maintains a high content of essential amino acids but also generate other activities with potential use as food additives (García-Moreno et al. 2014; Pasupuleti and Braun 2010) such as antioxidant, antihypertensive, antithrombotic, immunomodulatory, antimicrobial, among others (Kim and Wijesekara 2010). Several proteolytic enzymes are commonly used to hydrolyze proteins by-products which include Alcalase, papain, pepsin, trypsin, alpha-chymotrypsin, pancreatin, Flavourzyme, Pronase, Neutrase, Protamex, bromelain, cryotin F, protease N, protease A, Orientase, thermolysin, and Validase (Hsu 2010; Je et al. 2007; Ngo et al. 2010; Raghavan and Kristinsson 2008). In order to obtain bioactive peptides with functional properties, it is essential to control the hydrolysis time as well as to establish ideal values of pH and temperature to optimize enzyme activity.

The molecular weights and sizes of the peptides have a significant impact on their bioactive properties. Therefore, the purification and characterization of the peptides resulting from protein hydrolysis is a common practice nowadays in order to study the properties of the hydrolyzed product (Halim et al. 2016). Due to the molecular complexity of protein hydrolysates, it is difficult to use similar methods to those applied in the purification of other organic compounds such as crystallization. The high performance liquid chromatography (HPLC) is widely used for the separation, purification and identification of bioactive peptides. Moreover, reversed phase chromatography allows rapid separation and detection of peptide fractions while normal phase chromatography is used for the separation of hydrophilic peptides. Furthermore, the ion exchange chromatography can separate peptides based on their charge; while the gel filtration chromatography (in aqueous systems) and gel permeation chromatography (in non-aqueous systems) allows a separation based on the molecular weight. However, industrial production of purified peptides is hampered by low production yields. On the other hand, the industry has in consideration the purification of peptides for having lower production costs when compared with chemical synthesis of peptides (Agyei and Danquah 2011).

Recently, several authors have purified fish by-product hydrolysates and have reported sequences of peptides with different bioactivities such as antioxidant (Ahn et al. 2014; Cai et al. 2015; Chi et al. 2015a, b), antihypertensive activity (Intarasirisawat et al. 2013), antibacterial activity (Ennaas et al. 2015), cholecystokinin release activity (Cudennec et al. 2008), and antiproliferative (Picot et al. 2006). Therefore, fish processing by-products can be used as a source for producing nutraceuticals and food additives for functional foods for human consumption. The present paper provides an overview about specific characteristics, production, and purification as well as current and future trends of fish and shellfish by-product protein hydrolysates and bioactive peptides. Recent research of bioactive functionalities will be also briefly discussed.

Fish Protein Hydrolysate Production Methods

Today, the production of protein hydrolysates is massive worldwide. The most widely used methods for the production of protein hydrolysates in industrial practices are chemical and biological methods. Chemical methods involve acid and alkaline hydrolysis. Since these are methods relatively inexpensive and easy to operate, they have been the preferred practices to produce protein hydrolysates at industrial scale. However, chemical hydrolysis is difficult to control due to its harsh reaction and unspecific peptide bonds cleaving, giving a heterogeneous yield of peptides and reduces the nutritional quality of products (Celus et al. 2007). On the other hand, biochemical methods include autolysis and enzymatic hydrolysis. Autolysis process involves the action of endogenous proteolytic enzymes (endo- and exo-proteases) on the animal proteins. The main limitation of the production of bioactive peptides or added value products by autolytic hydrolysis is the reduced functionality and the difficulty to obtaining a homogenous hydrolysate. However, some authors have also produced protein hydrolysates via thermal hydrolysis by retorting the raw material in an autoclave at 121 °C (Wang et al. 2013) or by bacterial fermentation (Jemil et al. 2014). Figure 1 summarizes hydrolysis production methods.

Enzymatic Hydrolysis

The hydrolysis of proteins by exogenous enzymes or enzymatic hydrolysis allows a better control of the hydrolysis process and the resulting product. Therefore, enzymatic hydrolysis is considered as the most effective way to obtain protein hydrolysates with bioactive properties (Clemente 2000; Shahidi et al. 1995). Any hydrolysis process involves at least five independent variables: (i) protein substrate concentration, (ii) enzyme-substrate ratio (E/S), (iii) pH, (iv) temperature, and (v) time (Adler-Nissen 1984). Generally, there is an optimum combination of both pH and temperature, where the enzyme shows the highest activity. Physicochemical

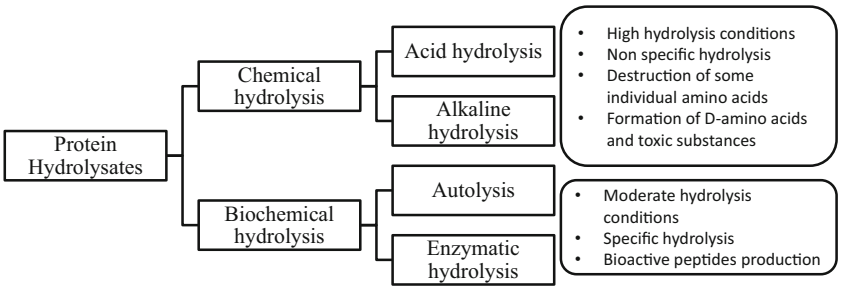

Fig. 1 Production methods of protein hydrolysates

conditions of the hydrolysis reaction such as temperature, pH and enzyme/substrate ratio must be adjusted to optimize the activity of the proteolytic enzyme (Kim and Wijesekara 2010; Santos et al. 2011). At the beginning of hydrolysis process, the mixture is often heated to about 85–95 °C for 5–20 min in order to terminate the endogenous enzyme activity respectively. Some Industrial food-grade proteinases derived from microorganisms have been used to produce bioactive peptides by enzymatic hydrolysis such as Alcalase®, Flavourzyme®, and Protamex®, as well as enzymes from plants such as papain or bromelain and animal sources such as pepsin and trypsin (Samaranayaka and Li-Chan 2011).

Protease specificity affects the size, the amount, the composition of free amino acid and peptides and their amino acid sequence which influences the bioactivity of the obtained hydrolysate (Sarmadi and Ismail 2010). The degree of hydrolysis (DH) is a fundamental parameter for the characterization and the production and of protein hydrolysates. It is defined as the percentage of broken peptide bonds in relation to the original protein. The degree of hydrolysis achieved in the hydrolysis is determined by the conditions used in the process such as substrate concentration, enzyme/substrate ratio, incubation time as well as the physicochemical conditions such as pH and temperature. Moreover, another factor that will determine the degree of hydrolysis is the nature of the enzyme, characterized by its specific activity and type of activity. Thus, the nature of the enzyme used will not only influence the degree of hydrolysis but also in the type of peptides produced (Wang et al. 2013). The higher the DH, more number of peptides would be produced in the solution that will result in an increase of protein solubility and the possibility to recover the protein to be used as a food additive (Sheriff et al. 2014).

When the desired DH is attained, it is necessary to terminate the enzymatic reaction by heating the slurry to 85–95 °C for 5–20 min or by acidifying the hydrolysate mixture to an extreme acidic pH value to inactivate the enzyme activity, this step is often used for the preparation of antimicrobial peptides. In industry, the production process of protein hydrolysates can be coupled to membrane technology, reducing the cost associated with enzyme inactivation at the end of the hydrolysis process (Guerard 2007). Subsequently, it is necessary to separate the different fractions (sludge of solids and non-soluble proteins at the bottom, aqueous layer at the middle and lipid phase at the top) by centrifugation. The oil phase over the aqueous phase is removed and the soluble fraction collected (Kristinsson and Rasco 2000). Commonly, after this step, the hydrolysates are dried by spray- or freeze-drying and stored until further analysis or application.

Bioactive Properties of Fish By-products' Protein Hydrolysates

Bioactive or biologically active peptides have been defined as "food derived components (naturally occurring or enzymatically generated) that, in addition to their nutritional value exert a physiological effect in the body" (Vermeirssen et al. 2004). Moreover, protein hydrolysates and peptides from waste processing fish by-products can promote human health and may help in the prevention of chronic diseases (Kim and Wijesekara 2010). In order to promote the biological activity of peptides, which are inactive in the structure of the native protein, they must be liberated by proteolysis (digestion in vivo) or hydrolysis (in vitro by enzymes). Thus, the resulting peptides may possess biological activities such as antioxidant, antimicrobial, antihypertensive, anti-inflammatory, and antidiabetic potential activity against cancer, among others. In this section, we will provide a review of studies on hydrolyzed fish products having bioactive properties.

Antioxidant Activity

The paradox of the oxygen is the fact that oxygen is essential for energy production in most of living organisms but at the same time, reactive oxygen species (ROS) are continuously generated in cellular metabolism. High ROS concentrations can be extremely deleterious to cell constituents (Amado et al. 2009). Oxidative damage is caused by the depletion of antioxidants in the body due to the formation of ROS by physiological processes or by exogenous molecules (Shackelford et al. 2000; Valavanidis et al. 2006). Organisms protect themselves from such harmful effects with a complex antioxidant defense system that include a number of enzymatic and non-enzymatic defenses (Monserrat et al. 2008). In this regard, the oxidative stress starts when the formation of ROS exceeds the antioxidant defenses capacity (Amado et al. 2009;

Jones 2006). In fact, the oxidative stress is related to a number of deleterious processes, such as protein damage, lipid peroxidation, enzyme inactivation and DNA breakage. These processes favor the occurrence of various diseases or pathologies such as the formation of tumors or cancer, heart disease, rheumatoid arthritis and aging (Sohal 2002; Klaunig and Kamendulis 2004).

Lipid oxidation mediated by free radicals, oxidative stress, and antioxidants has been widely discussed in many research areas (Sila and Bougatef 2016). Although synthetic antioxidants as butylated hydroxy-anisole (BHA) and butylated hydroxytoluene (BHT) show stronger antioxidant activities than natural antioxidant, as α-tocopherol, ascorbic acid, are used in foods to prevent deterioration. However, the use of these synthetic compounds has begun to be restricted due to their potential health hazards and toxicity (Centenaro et al. 2014; Sabeena Farvin et al. 2014). Protein hydrolysates and peptides from fish by-products have shown antioxidant activities and they can be considered as potential substitutes of synthetic antioxidants to reduce oxidative processes as well as ingredients for producing functional foods (Chi et al. 2015a; Frankel and Meyer 2000; Wiriyaphan et al. 2012).

Some authors reported that the hydrophobic amino acids as alanine, phenylalanine, isoleucine, leucine, valine and glycine and proline, methionine, tyrosine, histidine, lysine and cysteine may improve the efficiency of antioxidant peptides. These amino acids can act as proton donors or electron and/or as lipid radicals scavengers (Je et al. 2007; Samaranayaka and Li-Chan 2011; Sarmadi and Ismail 2010). In the same way, it has been demonstrated that acidic amino acids such as glutamic acid and aspartic acid as well as basic amino acids such as arginine, lysine and histidine present antioxidant capacity as chelator of metal ions due to carboxyl and amino groups in the side chains (Sarmadi and Ismail 2010; Udenigwe and Aluko 2012). Also, amino acids with aromatic residues can act as proton donors to radicals with electron deficiency (Sarmadi and Ismail 2010).

Several recent works have proved the antioxidant activity of fish by-product protein hydrolysates (Table 1). Lassoued et al. (2015) hydrolyzed thornback ray (*Raja clavata*) skin gelatin with four different proteases to obtain peptides with antioxidant activity (proteolytic proteases from *Bacillus subtilis* A26, *Raja clavata* crude alkaline protease extract, Alcalase and Neutrase). Results showed that the highest antioxidant activity was obtained with protein hydrolysates generated by bacillus A26 proteases. Further, the authors purified the pentapeptide Ala-Val-Gly-Ala-Thr which showed the highest antioxidant activity using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical-scavenging assay. The octapeptide Phe-Leu-Asn-Glu-Phe-Leu-His-Val isolated from salmon by-product protein hydrolysate exhibited DPPH and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity and strong ferric reducing activity (Ahn et al. 2014). In a recent study Chi et al. (2015b) hydrolyzed skin from Bluefin leatherjacket (*Navodon septentrionalis*) processing by-product with several proteolytic enzymes. The hydrolysate produced with the enzyme Alcalase showed the highest antioxidant activity against DPPH$^{\cdot}$, HO$^{\cdot}$, and $O2^{\cdot -}$ radicals. In the same study, the antioxidant peptides Gly-Ser-Gly-Gly-Leu, Gly-Pro-Gly-Gly-Phe-Ile, and Phe-Ile-Gly-Pro were purified. The authors assumed that the isolated peptides could exert the antioxidant activities due to the hydrophobic nature and/or the aromatic residues of some amino acids contained in the peptidic chains. Recently, Pan et al. (2016) isolated three bioactive hexapeptides (Phe-Ile-Met-Gly-Pro-Tyr, Gly-Pro-Ala-Gly-Asp-Tyr and Ile-Val-Ala-Gly-Pro-Gln) from *Raja porosa* cartilage that demonstrated good scavenging activities against DPPH$^{\cdot}$, HO$^{\cdot}$, $O_2^{\cdot -}$ and $ABTS^{\cdot +}$ due to their small molecular structure and the presence of hydrophobic amino acid residues within the peptide sequences..

Antimicrobial Activity

Currently, the research focusing on the characterization and isolation of antimicrobial peptides from fish processing by-products is lower when compared to research on antioxidant peptides (Di Bernardini et al. 2012). Antimicrobial peptides are chains of amino acids with a molecular weight below 10 kDa that usually contain less than 50 amino acids of which nearly a half are hydrophobic (Najafian and Babji 2012). Moreover, through modification of the net charge or the hydrophobicity ratio, the antibacterial activity of cationic peptides can be modulated (Sila et al. 2014). The interaction of these peptides with the bacterial membrane could form pores or block the membrane ion gradients leading to the destruction of the cell constituents. Moreover, several peptides could also generate bacterial depletion without membrane lysis probably by modifying the cellular metabolism (Wald et al. 2016). However, the mechanism through the peptides exert antibacterial activity is not yet completely understood.

Almost all antimicrobial peptides from fish possess antibacterial activities against numerous Gram-negative and Gram-positive strains. These antimicrobial peptides are potential candidates for new antibiotic development in the pharmaceutical field as well as antimicrobial agents for the food industry. In this manner, these antimicrobial peptides may be used as antibacterial, antiviral, antifungal, immunomodulatory, and antitumor agents (Kim and Wijesekara 2010; Rajanbabu and Chen 2011).

Table 2 shows recently published works that reported fish by-product protein hydrolysates with antimicrobial activity. Ennaas et al. (2015) hydrolyzed mackerel by-products using Protamex, Neutrase, papain, and Flavourzyme as proteolytic enzymes. These hydrolysates showed antibacterial activity against Gram-positive (*L. innocua*) and Gram-negative

Table 1 Antioxidant activity of fish by-product protein hydrolysates (works published since 2012)

Fish	Source	Enzyme	Purified Sequences	Activity	Reference
Thornback ray (*Raja clavata*)	Skin gelatin	*Bacillus subtilis* A26, *R. clavata* crude alkaline protease extract, Alcalase 2,4 L ®, Neutrase 0,5 L®	AVGAT	DPPH radical-scavenging, reducing power, preventing β-carotene bleaching, total antioxidant capacity, Inhibition of supercoiled plasmid DNA scission inducedby hydroxyl radicals	Lassoued et al. (2015)
Salmon (Scientific name not specified)	Pectoral Fin	Alcalase, Flavourzyme, Neutrase, pepsin, Protamex, trypsin	FLNEFLHV	DPPH and ABTS radical-scavenging activity, Ferric reducing power, protection of plasmid pBR322 DNA against hydroxyl radical-induced damage, Protection effect on hydrogen peroxide-induced hepatic damage in Chang liver cells	Ahn et al. (2014)
Bluefin leatherjacket (*Navodon septentrionalis*)	Skin	Trypsin, Flavourzyme, Neutrase, papain, Alcalase, pepsin,	GSGGL GPGGFI, FIGP	DPPH, superoxide and hydroxyl radical-scavenging activity, lipid peroxidation inhibition	Chi et al. (2015a)
Bluefin leatherjacket (*Navodon septentrionalis*)	Heads	Papain	WEGPK, GPP, GVPLT	DPPH, hydroxyl, superoxide and ABTS radical-scavenging activity, lipid peroxidation inhibition	Chi et al. (2015b)
Skate (*Raja porosa*)	Cartilage	Trypsin and Alcalase	FIMGPY), GPAGDY, IVAGPQ	DPPH, hydroxyl, superoxide and ABTS radical-scavenging activity, lipid peroxidation inhibition	Pan et al. (2016)
Giant catfish (*Pangasianodon gigas*)	Skin gelatin	Visceral alkaline-proteases from Giant catfish, commercial trypsin, Izyme AL®	–	ABTS radical-scavenging, Ferric reducing antioxidant power (FRAP) and metal (ferrous) chelating ability	Ketnawa et al. (2016)
Blue shark (*Prionace glauca*)	Skin gelatin	Protamex	Fractions with highest antioxidant capacity: EGR, GPR, GY, GF and four amino acids of R, L,Y and F.	DPPH and Hydroxyl radical-scavenging activity	Weng et al. (2014)
Salmon (Scientific name not specified)	Pectoral Fin	Alcalase, Flavourzyme, Neutrase, Protamex, pepsin, trypsin	Fraction with molecular weight between 1000 and 2000 Da	DPPH and hydrogen peroxyde scavenging activity, inhibition of intracellular reactive oxygen species generation and lipid peroxidation, enhanced thelevel of glutathione in Chang liver cells	Ahn et al. (2012)
Smooth hound (*Mustellus mustellus*)	Viscera	*M. mustellus* crude alkaline protease extract, Neutrase®, Esperase®, Purafect®, and combinations between endogenous and commercial proteases	–	DPPH scavenging activity, FRAP, iron chelating activity, β-carotene bleaching prevention, lipid peroxidation inhibition, DNA breakage protection	Abdelhedi et al. (2016)
Amur sturgeon (*Acipenser schrenckii*)	Skin gelatin	Alcalase, Flavourzyme	–	Lipid peroxidation inhibition, protein oxidation prevention, loss in sulfhydryl content prevention	Nikoo et al. (2015)
Tuna (Scientific name not specified)	Dark muscle	Alcalase	–	DPPH, superoxide and hydroxyl radical-scavenging activity, reducing power, ferrous ion chelating activity, Inhibition of linoleic acid autoxidation	Saidi et al. (2014)

Table 1 (continued)

Fish	Source	Enzyme	Purified Sequences	Activity	Reference
Grass carp (*Ctenopharyngodon idella*)	Skin	Alcalase	PYSFK, GFGPEL, VGGRP	DPPH, hydroxyl, and ABTS radical-scavenging activity, lipid peroxidation inhibition	Cai et al. (2015)
Indian mackerel (*Rastrelliger kanagurta*)	Backbones	Pepsin, papain	–	DPPH and Hydroxyl radical-scavenging activity, reducing power, lipid peroxidation inhibition	Sheriff et al. (2014)
Asian seabass (*Lates calcarifer*)	Skin	Protease from hepatopancreas of Pacific white shrimp, Alcalase	–	DPPH and ABTS radical-scavenging activity, Ferric reducing antioxidant power, metal (ferrous) chelating activity, inhibition of lipid peroxidation	Senphan and Benjakul (2014)
Threadfin bream (*Nemipterus spp.*)	Surimi by-products	Protease from *Virgibacillus sp.* SK33, Alcalase, pepsin, tripsin	–	ABTS radical-scavenging, Ferric reducing antioxidant power, β-carotene bleaching prevention, Cytoprotective activity	Wiriyaphan et al. (2012)
Unicorn leatherjacket (*Aluterus monoceros*)	Skin gelatin	Partially purified glycyl endopeptidase (Autolysis)	–	Protection effect against H2O2-induced DNA damage, on induction of antioxidant enzyme activities,	Karnjanapratum et al. (2016)
Unicorn leatherjacket (*Aluterus monoceros*)	Skin gelatin	protease from *Bacillus amyloliquefaciens* H11, Alcalase	–	ABTS radical-scavenging activity, Ferric-reducing antioxidant power, metal (ferrous) chelating activity, inhibition of lipid peroxidation	Sai-Ut et al. (2015)
Cod (*Gadus morhua*)	Backbones	Protease P "Amano" 6	–	Oxygen radical absorbance capacity, metal chelating ability, intracellular antioxidant activity in HepG2 cell	Halldorsdottir et al. (2014)
Tilapia (*Oreochromis niloticus*)	Frame	Flavourzyme 1000 L	–	DPPH radical-scavenging activity, Metal chelating activity, inhibition of lipid peroxidation	Chuesiang and Sanguandeekul (2015)
Smooth hound (*Mustelus mustelus*)	Heads and viscera	*M. mustellus* gastric protease extract, *M. mustellus* intestine protease extract, porcine pancreatine	–	DPPH radical-scavenging activity, Ferric reducing power, β-carotene bleaching prevention	Sayari et al. (2016)
Whitemouth croaker (*Micropogonias furnieri*)	Carcasses	Flavourzyme 1000 L	–	Lipid peroxidation inhibition	Zavareze et al. (2014)
Asian seabass (*Lates calcarifer*)	Skin gelatin	Alcalase	–	Protection against H2O2-induced DNA damage	Sae-leaw et al. (2016)
Tilapia (*Oreochromis niloticus*)	Skin gelatin	Bromelain, papain, trypsin,Flavourzyme, Alcalase, Neutrase	–	ABTS radical-scavenging activity, Ferric-reducing antioxidant power, metal (ferrous) chelating activity, inhibition of lipid peroxidation	Choonpicharn et al. (2015)
Skipjack (*Katsuwana pelamis*)	Roe	Alcalase	DWMKGQ, MLVFAV, MCYPAST, FVSACSVAG, LADGVAAPA, YVNDAATLLPR, DLDLRKDLYAN	ABTS, hydrogen peroxide and singlet oxygen scavenging-activity, metal chelating activity	Intarasirisawat et al. (2013)
Skate (*Raja porosa*)	Skin gelatin	Alcalase, flavourzyme, Neutrase, Protamex	MVGSAPGVL, LGPLGHQ	Intracellular radical-scavenging effects, enhanced expression of antioxidative enzymes (superoxide dismutase and glutathione)	Ngo et al. (2014)

Table 1 (continued)

Fish	Source	Enzyme	Purified Sequences	Activity	Reference
Pacific cod (*Gadus macrocephalus*)	Skin gelatin		LLMLDNDLPP	Hydroxyl radical-scavenging activity, protective effects on cell membrane lipid peroxidation, protective effects on cell membrane protein oxidation, protective effects on cellular DNA oxidation, intracellular radical-scavenging activity, enhanced expression of antioxidative enzymes (superoxide dismutase, glutathione and catalase)	Himaya et al. (2012)
Horse mackerel (*Magalaspis cordyla*)	Skin	Pepsin + trypsin + α-chymotrypsin	NHRYDR	DPPH and hydroxyl radical-scavenging activity, ferric-reducing antioxidant power, metal (ferrous) chelating activity, inhibition of lipid peroxidation	Sampath Kumar et al. (2012)
Croaker (*Otolithes ruber*)	Skin	Pepsin + trypsin + α-chymotrypsin	GNRGFACRHA	DPPH and hydroxyl radical-scavenging activity, ferric-reducing antioxidant power, metal (ferrous) chelating activity, inhibition of lipid peroxidation	Sampath Kumar et al. (2012)

(*E. coli*) strains. The highest antibacterial activity of these hydrolysates was showed when they were fractionated with acetone, which suggests the hydrophobic nature of these bioactive peptides. In the other hand, Trout pepsin was used to produce antibacterial trout by-products protein hydrolysates against food contaminants and fish farming pathogens (Wald et al. 2016). Hydrolysates with hydrolysis degree of 30% showed the highest activity against *Flavobacterium psychrophilum* and *Salmoninarum renibacterium*. Moreover, the amino acids lysine, leucine, alanine, arginine, glycine, aspartic acid, and glutamic acid were the most abundant in this hydrolysate. Furthermore, head, frames, and viscera from tilapia were submitted to enzymatic hydrolysis using Protamex enzyme by Robert et al. (2015). These hydrolysates possessed well-balanced amino acid profile and showed antimicrobial activity against *Edwardsiella tarda* and *Bacillus megaterium*.

Antihypertensive Activity

As reported by World Health Organization (WHO 2010), about 30% of the deaths in the world are owed to cardiovascular diseases and it is estimated that by 2020 stroke and heart disease will be the major cause of death worldwide. Antihypertensive peptides act in the reduction of arterial blood pressure by inhibiting the action of Angiotensin-I converting enzyme (ACE). This enzyme is able to catalyze the conversion of angiotensin I to the active vasoconstrictor angiotensin II as well as to inactivate a vasodilator (bradykinin), thus resulting in an increase in blood pressure (Lee and Hur 2017). Therefore, inhibition of ACE has become the main target in the treatment of hypertension (Himaya et al. 2012). Although several synthetic ACE inhibitors such as enalapril, alacepril, or lisinopril have an effective result against hypertension, it is reported that they have side effects including inflammatory response, dry cough, taste disturbance, skin eruptions or angioneurotic oedema (Intarasirisawat et al. 2013). Hence, food-derived ACE inhibitory peptides are being considered as an alternative.

The relationship between structure and activity of food-derived ACE inhibitory peptides has not been fully established. However, ACE inhibitory peptides generally contain zinc-binding ligands, a hydrogen-bond donor and carboxyl terminal group (Andrews et al. 1985). Also, ACE activity could be inactivated by the presence of hydrophobic amino acids at the C-terminal tail by the alteration of the catalytic site of ACE (Kang et al. 2003). Peptides containing branched-chain aliphatic amino acids at N-terminal end are also suggested to have strong activity as ACE inhibitors (Wijesekara and Kim 2010).

Recently, several studies have highlighted ACE inhibitor activity of fish by-products protein hydrolysates. Intarasirisawat et al. (2013) hydrolyzed skipjack

Table 2 Antimicrobial activity of fish by-product protein hydrolysates (works published since 2012)

Fish	Source	Enzyme	Purified Sequences	Microbial strains	Reference
Atlantic mackerel (*Scomber scombrus*)	Viscera, digestive gland, stomach gonads, heart, intestines, liver and spleen	Protamex	SIFIQRFTT, RKSGDPLGR, AKPGDGAGSGPR, GLPGPLGPAGPK	Gram-positive (*Listeria innocua*), Gram-negative (*Escherichia coli*)	Ennaas et al. (2015)
Rainbow trout (*Oncorhynchus mykiss*)	Viscera	Trout pepsin, porc pepsin	–	Gram-positive (*R. salmoninarum, W. minor, W. paramesentoides, Micrococcus luteus, B. cereus, Ent. faecalis*), Gram-negative (*A. media, A. salmonicida, F. araucananum, F. psychrophilum, C. freundii, E. Coli, Pro. Mirabilis, P. flureszens*)	Wald et al. (2016)
Tilapia (*Oreochromis niloticus*)	Heads, frames and viscera	Protamex	–	Gram-positive (*Bacillus megaterium*), Gram-negative (*Yersinia ruckeri, Edwardsiella tarda*)	Robert et al. (2015)
Anchovy (*Engraulis japonicus*)	Cooking wastewater	Protamex	GLSRLFTALK	Gram-positive (*Staphylococcus aureus, Bacillus subtilis, S. pneumoniae*), Gram-negative (*E. coli, S. dysenteriae, P. aeruginosa, Salmonella typhimurium*)	Tang et al. (2015)
Smooth hound (*Mustellus mustellus*)	Viscera	*M. mustellus* crude alkaline protease extract, Neutrase®, Esperase®, Purafect®, and combinations between endogenous and commercial proteases	–	Gram-positive (*Staphylococcus aureus, Micrococcus luteus, Bacillus cereus*), Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella enterica, Salmonella typhi, Enterobacter sp.*)	Abdelhedi et al. (2016)

(*Katsuwonus pelamis*) roe with Alcalase to 5% DH. They purified the hydrolysate by ultrafiltration, cation exchange column chromatography, and reverse phase high performance liquid chromatography (RP-HPLC). The hexapeptide Met-Leu-Val-Phe-Ala-Val showed the highest ACE inhibitory activity. Although the ACE inhibitory capacity of this hexapeptide was weaker than the commercial synthetic ACE inhibitors, the authors assumed that it could be potentially used as a functional food ingredient against hypertension symptom. In other study, ACE inhibitory capacity of protein hydrolysates from salmon pectoral fin was evaluated (Ahn et al. 2012b). Salmon by-product proteins were hydrolyzed using Alcalase, Flavourzyme, Neutrase, pepsin, Protamex, and trypsin. The hydrolysates produced with the enzyme Alcalase showed the highest ACE inhibitory activity. The authors identified three peptides (Val-Trp-Asp-Pro-Pro-Lys-Phe-Asp, Phe-Glu-Asp-Tyr-Val-Pro-Leu-Ser-Cys-Phe and Phe-Asn-Val-Pro-Leu-Tyr-Glu). They supposed that the presence of Phe, Leu, and Tyr residues at the C-terminal play an important role in their ACE inhibition activity. Salmon skin is a normally discarded by-product in the Atlantic salmon industry. Gu et al. (2011) hydrolyzed Atlantic salmon skin protein using Alcalase and Papain in a two-step hydrolysis process. Two dipeptides, Ala-Pro and Val-Arg, were isolated from Atlantic salmon skin protein hydrolysates. These peptides were found to be the major contributors to the ACE inhibitory capacity peptides in the protein hydrolysate. Gu et al. (2011) suggested that salmon skin collagen peptides might be useful as functional foods and antihypertensive agents. Several other recent studies that have produced fish by-product protein hydrolysates with ACE inhibitory activity are listed in Table 3.

Other Bioactivities

Some researchers have demonstrated that fish by-product protein hydrolysates possess other bioactivities with promissory applications in the pharmaceutical field or as food additives in functional foods. Although, some works have been published reporting different bioactivities from fish by-product protein hydrolysates to those commented in this section, the authors have preferred to include those that are currently being studied widely. At this point, even though fish by-product protein hydrolysates have been reported to show antitumor or antiproliferative activities, the research in this field remains limited compared to vegetative peptides (Suarez-Jimenez et al. 2012). Hsu et al. (2011) produced and identified two antiproliferative peptides active against human breast cancer cell line MCF-7 from tuna dark muscle by using papain and Protease XXIII. The isolated amino acid sequences for both peptides were Leu-Pro-His-Val-Leu-Thr-Pro-Glu-Ala-Gly-Ala-Thr and Pro-Thr-Ala-Glu-Gly-Gly-Val-Tyr-Met-Val-Thr, respectively. The authors concluded that tuna dark muscle by-product would be a good source to produce antiproliferative peptides. Moreover, proteins from tuna cooking juice, a by-product produced during the processing of canned tuna hydrolysates by using protease XXIII, have shown antiproliferative activity (Hung et al. 2014). Tuna cooking juice hydrolysates showed antiproliferative activities up to 25% against MCF-7 cells without affecting normal breast epithelial cells. Two peptides were identified as Lys-Pro-Glu-Gly-Met-Asp-Pro-Pro-Leu-Ser-Glu-Pro-Glu-Asp-Arg-Arg-Asp-Gly-Ala-Ala-Gly-Pro-Lys and Lys-Leu-Pro-Pro-Leu-Leu-Leu-Ala-Lys-Leu-Leu-Met-Ser-Gly-Lys-Leu-Leu-Ala-Glu-Pro-Cys-Thr-Gly-Arg.

Table 3 Antihypertensive activity of fish by-product protein hydrolysates (works published since 2012)

Fish	Source	Enzyme	Purified Sequences	Reference
Skipjack (*Katsuwana pelamis*)	Roe	Alcalase	DWMKGQ, MLVFAV, MCYPAST, FVSACSVAG, LADGVAAPA, YVNDAATLLPR, DLDLRKDLYAN	Intarasirisawat et al. (2013)
Salmon (Scientific name not specified)	Pectoral fin	Alcalase, Flavourzyme, Neutrase, pepsin, Protamex, trypsin	VWDPPKFD, FEDYVPLSCF, FNVPLYE	Ahn et al. (2012b)
Skate (*Okamejei kenojei*)	Skin gelatin	Alcalase, flavourzyme, Neutrase, Protamex	MVGSAPGVL, LGPLGHQ	Ngo et al. (2014)
Pacific cod (*Gadus macrocephalus*)	Skin gelatin	Pepsin + trypsin + α-chymotrypsin	LLMLDNDLPP	Himaya et al. (2012)
Smooth hound (*Mustelus mustelus*)	Heads and viscera	*M. mustellus* gastric protease extract, *M. mustellus* intestine protease extract, porcine pancreatine	–	Sayari et al. (2016)
Tilapia (*Oreochromis niloticus*)	Skin gelatin	Bromelain, papain, trypsin, Flavourzyme, Alcalase, Neutrase	–	Choonpicharn et al. (2015)
Tilapia (*Oreochromis niloticus*)	Frame	Flavourzyme 1000 L	–	Chuesiang and Sanguandeekul (2015)

Table 4 Fish by-product protein hydrolysates with antiproliferative, antidiabetic, anti-inflamatory or immunomodulatory activity

Fish	Source	Enzyme	Purificated Sequences	Bioactivity	Reference
Tuna (*Thunnus tonggol*)	Dark muscle	Papain, Protease XXIII	LPHVLTPEAGAT, PTAEGGVYMVT	Antiproliferative	Hsu et al. (2011)
Tuna (*Thunnus tonggol*)	Cooking juice	Protease XXIII	KPEGMDPPLSEPEDRRDGAAGPK, KLPPLLLAKLLMSGKLLAEPCTGR	Antiproliferative	Hung et al. (2014)
Atlantic salmon (*Salmo salar*)	Frames	Pepsin + trypsin + chymotrypsin	–	Antidiabetic	Roblet et al. (2016)
Tilapia (*Oreochromis niloticus*)	Skin	Flavourzyme 1000 L	IPGDPGPPGPPGP, LPGERGRPGAPGP, GPKGDRGLPGPPGRDGM	Antidiabetic	Wang et al. (2015)
Atlantic salmon (*Salmo salar*)	Skin gelatin	Alcalase, bromelain, Flavourzyme	GPAE, GPGA	Antidiabetic	Li-Chan et al. (2012)
Salmon (Scientific name not specified)	Pectoral fin	Alcalase, Flavourzyme, Neutrase, Protamex, pepsin, trypsin	Fraction with molecular weight between 1000 and 2000 Da	Anti-inflamatory	Ahn et al. (2012a)
Unicorn leatherjacket (*Aluterus monoceros*)	Skin gelatin	Partially purified glycyl endopeptidase (Autolysis)	–	Immunomodulatory and antiproliferative	Karnjanapratum et al. (2016)
Asian seabass (*Lates calcarifer*)	Skin gelatin	Alcalase	–	Immunomodulatory and antiproliferative	Sae-leaw et al. (2016)

Although the anti-proliferation activity demonstrates a correlation with antioxidant activities, it seems that there is no correlation between the peptides molecular weight and their antiproliferative activity and more works should be carried out to clarify this concern (Hsu et al. 2011; Hung et al. 2014; Lee et al. 2003).

Fish by-products protein hydrolysates have also demonstrated potential activities against several diseases or health disorders that concern world population nowadays. Indeed, salmon frame and tilapia skin gelatin protein hydrolysates have shown potential applications as antihyperglycaemic agent as potent as other antidiabetic drugs (Roblet et al. 2016; Wang et al. 2015). In their work, Wang et al. (2015) stated that the gelatin hydrolysates of warm-water fish skin had more potential for the production of antidiabetic drugs as precursors of DPP-IV inhibitors than those of cold-water fish. Li-Chan et al. (2012) identified two tetrapeptides (Gly-Pro-Ala-Glu, Gly-Pro-Gly-Ala) obtained from Atlantic salmon skin gelatin hydrolyzed with Flavourzyme with high DPP-IV inhibitory activity and could be used for the treatment or prevention of type 2 diabetes.

In an inflammatory process, activated macrophages of the immune system secrete nitric oxide in the inflammation sites to repair tissue and to remove the cause of the inflammation (Ahn et al. 2012a; Guastadisegni et al. 2002). However, various inflammatory diseases are related to the overproduction of nitric oxide. In this way, salmon by-products were hydrolyzed by Ahn et al. (2012a), and the obtained hydrolysates showed an anti-inflammatory activity by inhibiting nitric oxide production and proinflammatory cytokines.

Collagen and gelatin are excellent functional ingredients for the cosmetics industry for the manufacture of anti-aging and anti-wrinkle products due to their excellent moisturizing property. Traditionally, these compounds were obtained from terrestrial animals but owing to certain animal diseases and ethnic and religious barriers, collagen and gelatin from fish are becoming more important as a preference of the cosmetic industry for the preparation of functional cosmeceuticals (Kim 2014).

Table 4 shows some recent works of fish by-products protein hydrolysates presenting antiproliferative, antidiabetic, anti-inflammatory, or immunomodulatory activity.

Conclusion

In this work, we briefly reviewed some important aspects about the production of fish by-products protein hydrolysates, as well as some recent works testing their bioactive properties. Fish waste is presented as an important source of proteins, peptides and amino acids with high potential to develop novel nutraceuticals that may replace or minimize the potential deleterious effects of synthetic drugs. Additionally, the use of this

technology would serve the dual purpose of developing a high added value product from a cheap and abundant raw material and minimizing the polluting potential of fish waste. The knowledge about the recovery of fish by-products and their potential bioactivities has hugely increased during the past decades. Nevertheless, more studies are needed to lead a better understanding of the mechanism through which fish by-product protein hydrolysates exert their biological activities. Moreover, further in vivo studies must be carried out to answer the questions of their absorption in the gastrointestinal track and bioavailability, in order to develop food additives like nutraceuticals for human functional foods and natural drugs against diverse diseases.

Funding Information This work was supported by the Brazilian Council of Research (CNPq) and the Coordination for the Improvement of High Education Personnel (CAPES) from the Ministry of Education of Brazil.

References

Abdelhedi O, Jridi M, Jemil I, Mora L, Toldrá F, Aristoy M, Boualga A, Nasri M, Nasri R (2016) Combined biocatalytic conversion of smooth hound viscera: protein hydrolysates elaboration and assessment of their antioxidant, anti-ACE and antibacterial activities. Food Res Int 86:9–23

Adler-Nissen J (1984) Control of the proteolytic reaction and of the level of bitterness in protein hydrolysis processes. J Chem Technol Biotechnol 34:215–222

Agyei D, Danquah MK (2011) Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. Biotechnol Adv 29:272–277

Ahn CB, Je JY, Cho YS (2012a) Antioxidant and anti-inflammatory peptide fraction from salmon byproduct protein hydrolysates by peptic hydrolysis. Food Res Int 49:92–98

Ahn CB, Jeon YJ, Kim YT, Je JY (2012b) Angiotensin I converting enzyme (ACE) inhibitory peptides from salmon byproduct protein hydrolysate by Alcalase hydrolysis. Process Biochem 47:2240–2245

Ahn CB, Kim JG, Je JY (2014) Purification and antioxidant properties of octapeptide from salmon byproduct protein hydrolysate by gastrointestinal digestion. Food Chem 147:78–83

Amado LL, Garcia ML, Ramos PB, Freitas RF, Zafalon B, Ferreira JL, Yunes JS, Monserrat JM (2009) A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci Total Environ 407:2115–2123

Andrews PR, Carson JM, Caselli A, Spark MJ, Woods R (1985) Conformational analysis and active site modeling of angiotensin converting enzyme inhibitors. J Med Chem 28:393–399

Benhabiles MS, Abdi N, Drouiche N, Lounici H, Pauss A, Goosend MFA, Mameri N (2012) Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit. Mater Sci Eng 32:922–928

Cai L, Wu X, Zhang Y, Li X, Ma S, Li J (2015) Purification and characterization of three antioxidant peptides from protein hydrolysate of grass carp (*Ctenopharyngodon idella*) skin. J Funct Foods 16:234–242

Celus I, Brijs K, Delcour JA (2007) Enzymatic hydrolysis of brewers' spent grain proteins and technofunctional properties of the resulting hydrolysates. J Agric Food Chem 55:8703–8710

Centenaro GS, Salas-Mellado MM, Pires C, Batista I, Nunes ML, Prentice C (2014) Fractionation of protein hydrolysates of fish and chicken using membrane ultrafiltration: investigation of antioxidant activity. Appl Biochem Biotech 172:2877–2893

Chi CF, Wang B, Hu FY, Wang YM, Zhang B, Deng SG, Wu CW (2015a) Purification and identification of three novel antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) skin. Food Res Int 73:124–129

Chi CF, Wang B, Wang YM, Zhang B, Deng SG (2015b) Isolation and characterization of three antioxidant peptides from protein hydrolysate of bluefin leatherjacket (*Navodon septentrionalis*) heads. J Funct Foods 12:1–10

Choonpicharn S, Jaturasitha S, Rakariyatham N, Suree N, Niamsup H (2015) Antioxidant and antihypertensive activity of gelatin hydrolysate from Nile tilapia skin. J Food Sci Technol 52:3134–3139

Chuesiang P, Sanguandeekul R (2015) Protein hydrolysate from tilapia frame: antioxidant and angiotensin I converting enzyme inhibitor properties. Int J Food Sci Technol 50:1436–1444

Clemente A (2000) Enzymatic protein hydrolysates in human nutrition. Trends Food Sci Technol 11:254–262

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

Di Bernardini R, Mullen AM, Bolton D, Kerry J, O'Neill E, Hayes M (2012) Assessment of the angiotensin-I-converting enzyme (ACE-I) inhibitory and antioxidant activities of hydrolysates of bovine brisket sarcoplasmic proteins produced by papain and characterisation of associated bioactive peptidic fractions. Meat Sci 90:226–235

Ennaas N, Hammami R, Beaulieu L, Fliss I (2015) Purification and characterization of four antibacterial peptides from protamex hydrolysate of Atlantic mackerel (*Scomber scombrus*) by-products. Biochem Bioph Res Co 462:195–200

Frankel EN, Meyer AS (2000) The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J Sci Food Agric 80:1925–1941

García-Moreno PJ, Batista I, Pires C, Bandarra NM, Espejo-Carpio FJ, Guadix A, Guadix EM (2014) Antioxidant activity of protein hydrolysates obtained from discarded Mediterranean fish species. Food Res Int 65:469–476

Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D (2013) Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. J Microb Biochem Technol 5:107–129

Gu RZ, Li CY, Liu WY, Yi WX, Cai MY (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

Guastadisegni C, Nicolini A, Balduzzi M, Ajmone-Cat MA, Minghetti L (2002) Modulation of pge2 and tnfα by nitric oxide in resting and lps-activated raw 264.7 cells. Cytokine 19:175–180

Guerard F (2007) In: Shahidi F (ed) Maximising the value of marine by-products. Cambridge, Woodhead Publishing Ltd

Halim NRA, Yusof HM, Sarbon NM (2016) Functional and bioactive properties of fish protein hydolysates and peptides: a comprehensive review. Trends Food Sci Technol 51:24–33

Halldorsdottir SM, Sveinsdottir H, Gudmundsdottir A, Thorkelsson G, Kristinsson HG (2014) High quality fish protein hydrolysates prepared from by-product material with *Fucus vesiculosus* extract. J Funct Foods 9:10–17

He S, Franco C, Zhang W (2013) Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Res Int 50:289–297

Himaya SWA, Ngo DH, Ryu B, Kim SK (2012) 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

Hsu KC (2010) Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product. Food Chem 122:42–48

Hsu KC, Li-Chan ECY, Jao CL (2011) Antiproliferative activity of peptides prepared from enzymatic hydrolysates of tuna dark muscle on human breast cancer cell line MCF-7. Food Chem 126:617–622

Hung CC, Yang YH, Kuo PF, Hsu KC (2014) Protein hydrolysates from tuna cooking juice inhibit cell growth and induce apoptosis of human breast cancer cell line MCF-7. J Funct Foods 11:563–570

Intarasirisawat R, Benjakul S, Wu J, Visessanguan W (2013) Isolation of antioxidative and ACE inhibitory peptides from protein hydrolysate of skipjack (*Katsuwana pelamis*) roe. J Funct Foods 5:1854–1862

Je JY, Qian ZJ, Byun HG, Kim SK (2007) Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process Biochem 42:840–846

Jemil I, Jridi M, Nasri R, Ktari N, Salem RBS, Mehiri M, Hajji M, Nasri M (2014) Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis* A26. Process Biochem 49:963–972

Jones DP (2006) Disruption of mitochondrial redox circuitry in oxidative stress. Chem Biol Interact 163:38–53

Kang DG, Oh H, Chung HT, Lee HS (2003) Inhibition of angiotensin converting enzyme by lithospermic acid B isolated from radix *Salviae miltiorrhiza* Bunge. Phytother Res 17:917–920

Karnjanapratum S, O'Callaghan YC, Benjakul S, O'Brien N (2016) Antioxidant, immunomodulatory and antiproliferative effects of gelatin hydrolysate from unicorn leatherjacket skin. J Sci Food Agric 96:3220–3226

Ketnawa S, Martínez-Alvarez O, Benjakul S, Rawdkuen S (2016) Gelatin hydrolysates from farmed Giant catfish skin using alkaline proteases and its antioxidative function of simulated gastro-intestinal digestion. Food Chem 192:34–42

Kim SK (2014) Marine cosmeceuticals. J Cosmet Dermatol 13:56–67

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

Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol 44:239–267

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

Lassoued I, Mora L, Nasri R, Jridi M, Toldrá F, Aristoy M, Barkia A, Nasri M (2015) Characterization and comparative assessment of antioxidant and ACE inhibitory activities of thornback ray gelatin hydrolysates. J Funct Foods 13:225–238

Lee SY, Hur SJ (2017) Antihypertensive peptides from animal products, marine organisms, and plants. Food Chem 228:506–517

Lee SH, Lee MY, Kang HM, Han DC, Son KH, Yang DC, Sung ND, Lee CW, Kim HM, Kwon BM (2003) Anti-tumor activity of the farnesyl-protein transferase inhibitors arteminolides, isolated from Artemisa. Bioorg Med Chem 11:4545–4549

Li-Chan ECY, Hunag SL, Jao CL, Ho KP, Hsu KC (2012) Peptides derived from atlantic salmon skin gelatin as dipeptidyl-peptidase IV inhibitors. J Agric Food Chem 60:973–978

Monserrat JM, Lima JV, Ferreira JLR, Acosta D, Garcia ML, Ramos PB, Moraes TB, Dos Santos LC, Amado LL (2008) Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). Comp Biochem Physiol C 148:287–292

Morales-Medina R, Tamm F, Guadix AM, Guadix EM, Drusch S (2016) Functional and antioxidant properties of hydrolysates of sardine (*S. pilchardus*) and horse mackerel (*T. mediterraneus*) for the microencapsulation of fish oil by spray-drying. Food Chem 194:1208–1216

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

Ngo DH, Qian ZJ, Ryu B, Park JW, Kim S (2010) In vitro antioxidant activity of a peptide isolated from Nile tilapia (*Oreochromis niloticus*) scale gelatin in free radical-mediated oxidative systems. J Funct Foods 2:107–117

Ngo DH, Ryu B, Kim SK (2014) Active peptides from skate (*Okamejei kenojei*) skin gelatin diminish angiotensin-I converting enzyme activity and intracellular free radical-mediated oxidation. Food Chem 143:246–255

Nikoo M, Benjakul S, Xu X (2015) Antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysate in unwashed fish mince. Food Chem 181:295–303

Oosterveer P (2008) Governing global fish provisioning: ownership and management of marine resources. Ocean Coast Manag 51:797–805

Pan X, Zhao YQ, Hu FY, Wang B (2016) Preparation and identification of antioxidant peptides from protein hydrolysate of skate (*Raja porosa*) cartilage. J Funct Foods 25:220–230

Pasupuleti VK, Braun S (2010) In: Pasupuleti VK, Demain AL (eds) Protein hydrolysates in biotechnology. Springer Dordrecht Heidelberg, New York

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

Raghavan S, Kristinsson HG (2008) Antioxidative efficacy of alkali-treated tilapia protein hydrolysates: a comparative study of five enzymes. J Agric Food Chem 56:1434–1441

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

Robert M, Zatylny-Gaudin C, Fournier V, Corre E, Le Corguillé G, Bernay B, Henry J (2015) Molecular characterization of peptide fractions of a tilapia (*Oreochromis niloticus*) by-product hydrolysate and in vitro evaluation of antibacterial activity. Process Biochem 50: 487–492

Roblet C, Akhtar MJ, Mikhaylin S, Pilon G, Gill T, Marette A, Bazinet L (2016) Enhancement of glucose uptake in muscular cell by peptide fractions separated by electrodialysis with filtration membrane from salmon frame protein hydrolysate. J Funct Foods 22:337–346

Sabeena Farvin KH, Andersen LL, Nielsen HH, Jacobsen C, Jakobsen G, Johansson I, Jessen F (2014) Antioxidant activity of cod (*Gadus morhua*) protein hydrolysates: in vitro assays and evaluation in 5% fish oil-in-water emulsion. Food Chem 149:326–334

Sae-leaw T, O'Callaghan YC, Benjakul S, O'Brien NM (2016) Antioxidant, immunomodulatory and antiproliferative effects of gelatin hydrolysates from seabass (*Lates calcarifer*) skins. Int J Food Sci Tech 51:1545–1551

Saidi S, Deratani A, Belleville M-P, Ben Amar R (2014) Antioxidant properties of peptide fractions from tuna dark muscle protein by-product hydrolysate produced by membrane fractionation process. Food Res Int 65:329–336

Sai-Ut S, Benjakul S, Sumpavapol P, Kishimura H (2015) Antioxidant activity of gelatin hydrolysate produced from fish skin gelatin using extracellular protease from *Bacillus amyloliquefaciens* H11. J Food Process Pres 39:394–403

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

Sampath Kumar NS, Nazeer RA, Jaiganesh R (2012) Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*). Amino Acids 42:1641–1649

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 Tech 4:1399–1406

Sarmadi BH, Ismail A (2010) Antioxidative peptides from food proteins: a review. Peptides 31:1949–1956

Sayari N, Sila A, Haddar A, Balti R, Ellouz-Chaabouni S, Bougatef A (2016) Valorisation of smooth hound (*Mustelus mustelus*) waste biomass through recovery of functional, antioxidative and antihypertensive bioactive peptides. Environ Sci Pollut Res 23:366–376

Senphan T, Benjakul S (2014) Antioxidative activities of hydrolysates from seabass skin prepared using protease from hepatopancreas of Pacific white shrimp. J Funct Foods 6:147–156

Shackelford RE, Kaufmann WK, Paules RS (2000) Oxidative stress and cell cycle checkpoint function1. Free Radic Bio Med 28:1387–1404

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

Sheriff SA, Sundaram B, Ramamoorthy B, Ponnusamy P (2014) Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of *Rastrelliger kanagurta* by proteolytic enzymes. Saudi J Biol Sci 21:19–26

Sila A, Bougatef A (2016) Antioxidant peptides from marine by-products: isolation, identification and application in food systems: a review. J Funct Foods 21:10–26

Sila A, Hedhili K, Przybylski R, Ellouz-Chaabouni S, Dhulster P, Bougatef A, Nedjar-Arroume N (2014) Antibacterial activity of new peptides from barbel protein hydrolysates and mode of action via a membrane damage mechanism against *Listeria monocytogenes*. J Funct Foods 11:322–329

Sohal RS (2002) Role of oxidative stress and protein oxidation in the aging process. Free Radic Bio Med 33:37–44

Suarez-Jimenez G, Burgos-Hernandez A, Ezquerra-Brauer J (2012) Bioactive peptides and depsipeptides with anticancer potential: sources from marine animals. Mar Drugs 10:963–986

Tang W, Zhang H, Wang L, Qian H, Qi X (2015) Targeted separation of antibacterial peptide from protein hydrolysate of anchovy cooking wastewater by equilibrium dialysis. Food Chem 168:115–123

Udenigwe CC, Aluko RE (2012) Food protein-derived bioactive peptides: production, processing, and potential health benefits. J Food Sci 77:11–24

Valavanidis A, Vlahogianni T, Dassenakis M, Scoullos M (2006) Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf 64:178–189

Vermeirssen V, Camp JV, Verstraete W (2004) Bioavailability of angiotensin I converting enzyme inhibitory peptides. Brit J Nutr 92:357–366

Wald M, Schwarz K, Rehbein H, Bußmann B, Beermann C (2016) Detection of antibacterial activity of an enzymatic hydrolysate generated by processing rainbow trout by-products with trout pepsin. Food Chem 205:221–228

Wang W, Li Z, Liu J, Wang Y, Liu S, Sun M (2013) Comparison between thermal hydrolysis and enzymatic proteolysis processes for the preparation of tilapia skin collagen hydrolysates. Czech J Food Sci 31:1–4

Wang TY, Hsieh CH, Hung CC, Jao CL, Chen MC, Hsu KC (2015) Fish skin gelatin hydrolysates as dipeptidyl peptidase IV inhibitors and glucagon-like peptide-1 stimulators improve glycaemic control in diabetic rats: a comparison between warm- and cold-water fish. J Funct Foods 19:330–340

Weng W, Tang L, Wang B, Chen J, Su W, Osako K, Tanaka M (2014) Antioxidant properties of fractions isolated from blue shark (*Prionace glauca*) skin gelatin hydrolysates. J Funct Foods 11:342–351

Wijesekara I, Kim S-K (2010) Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: prospects in the pharmaceutical industry. Mar Drugs 8:1080–1093

Wiriyaphan C, Chitsomboon B, Yongsawadigul J (2012) Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. Food Chem 132:104–111

World Health Organization (2010) Causes of death 2008: data sources and methods. World Health Organization, Geneva. Available at http://www.who.int/healthinfo/global_burden_disease/cod_2008_sources_methods.pdf

Zavareze RE, Telles AC, Mello El Halal SL, Rocha M, Colussi R, Assis LM, Castro LAS, Dias ARG, Prentice-Hernández C (2014) Production and characterization of encapsulated antioxidative protein hydrolysates from Whitemouth croaker (*Micropogonias furnieri*) muscle and byproduct. LWT-Food Sci Technol 59:841–848